Anti-inflammatory and membrane stabilizing properties of methyl jasmonate in rats

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[ABSTRACT] The present investigation was carried out to evaluate anti-inflammatory and membrane stabilizing properties of methyl jasmonate (MJ) in experimental rat models of acute and chronic inflammation. The effects of MJ on acute inflammation were assessed using carrageenan-induced rat’s paw edema model. The granuloma air pouch model was employed to evaluate the effects of MJ on chronic inflammation produced by carrageenan in rats. The number of white blood cells (WBC) in pouch exudates was estimated using light microscopy. The levels of biomarkers of oxidative stress, such as malondialdehyde (MDA), glutathione (GSH) and activity of antioxidant enzymes in the exudates, were determined using spectrophotometry. The membrane stabilizing property of MJ was assessed based on inhibition of hemolysis of rat red blood cells (RBC) exposed to hypotonic medium. Our results indicated that MJ (25–100 mg·kg⁻¹, i.p.) produced significant anti-inflammatory activity in carrageenan-induced paw edema in rats (P < 0.05). MJ reduced the volume of pouch exudates and the number of WBC in carrageenan-induced granulomatous inflammation. It also exhibited potent antioxidant and membrane stabilizing activities. In conclusion, these findings suggest the therapeutic potentials of methyl jasmonate in disease conditions associated with inflammation and its anti-inflammatory activity may be related to its antioxidant and membrane stabilizing activities.

KEY WORDS| Methyl jasmonate; Granuloma air pouch model; Anti-inflammatory; Antioxidant; Membrane stabilizing property

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Introduction
Inflammation is a response of body tissues to injury, which helps limit the degree of damage and promote wound healings [1]. There are several components through which inflammatory reaction mediates tissue injury and worsens symptoms associated with chronic diseases of inflammation origin. The main components involved in the pathogenesis of chronic diseases of inflammatory origin include edema formation, WBC infiltrations, and release of chemical mediators including reactive oxygen species (ROS) [2-5]. Edema, which limits body function, is due to synergism between several inflammatory mediators that increase vascular permeability accompanied by accumulation of fluid in the interstitial tissues [1, 4]. Increased vasodilatation encourages WBC infiltrations to the site of injury, whose activity leads to the release of inflammatory mediators and other cytotoxic products including ROS [1-2, 4]. The activity of WBC-mediated release of ROS plays a key role in the onset and progression of chronic inflammatory diseases like rheumatoid arthritis (RA).

The carrageenan-induced paw edema model, which is mediated through the release of histamine, serotonin, bradykinin, and prostaglandins, is widely used for evaluation of acute inflammation [1, 4]. The granuloma air pouch model of chronic inflammation closely mimics the pathology of RA and shares similar features with the disease, such as patterns of tissue destructions, infiltrations of WBC, and released mediators [5, 5-7]. Thus, inhibition of infiltrations of WBC, stabilization of lysosomal membrane and prevention of WBC-mediated release of ROS may be effective approaches
to the treatment of RA. However, the drugs currently used for the management of inflammatory conditions especially RA, have limited efficacies and are also compromised by incidence of adverse effects after prolonged use [2-3, 8]. Thus, the current research effort is centered on the development of safer drugs of plant origin that could be efficacious for the treatment of chronic inflammatory disorders.

Methyl jasmonate (MJ) is a naturally occurring bioactive compound originally isolated from the essential oil of *Jasminum grandiflorum*, but now obtained commercially through pharmaceutical synthesis [9]. MJ has won international recognition over the years, as a potential source of new drug against several neoplastic diseases [10]. Moreover, in vivo studies have also confirmed that MJ does not cause significant local or systemic adverse effects, regardless of the route of exposure in humans and experimental animals [12].

The notable absence of toxicity of MJ *in vitro* and *in vivo* studies indicates that it could be used safely in cancer chemotherapy, [10, 12] and deserves further pharmacological investigations as a novel therapeutic agent for the treatment of other ailments as well. In recent years, there is an increasing interest in the development of MJ as a therapeutic agent for the treatment of inflammatory disorders [10]. This interest stems from the observation that MJ shares a similar chemical structure with anti-inflammatory prostaglandins [13]. Based on this observation, the anti-inflammatory potentials of MJ and its derivatives have been investigated in culture macrophage cells [13-14]. The results of these studies have confirmed that MJ and its derivatives have significant anti-inflammatory property by decreasing the release of pro-inflammatory cytokines due to inhibition of the NF-B pathway [13-14]. Moreover, the rise in the levels of MJ in plants following injury [10-11] also indicates a crucial role in the fight against inflammatory processes. In our previous studies, we reported that MJ significantly reduced nociceptive responses associated with inflammatory conditions in animal models of pain [15]. However, literature search revealed that no studies have been carried out to establish anti-inflammatory property of MJ *in vivo* animal models of inflammation. Thus, the present study was designed to examine the effects of MJ on animal models of acute and chronic inflammation that closely mimic the pathology of RA, while also describing its effect on hyposaline-induced rat RBC hemolysis.

**Materials and Methods**

**Animals**

Male Wistar rats weighing 160–200 g were obtained from the Central Animal House, University of Ibadan, Ibadan. They were kept in cages at room temperature of 22 ± 2 °C under light/dark (12 : 12) cycle and had access to commercial food pellets and water *ad libitum*. They were acclimatized for one week before commencement of the experiments. The experimental procedures were carried out in compliance with National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No.85-23, revised 1985). All efforts were made to minimize the suffering of the animals.

**Drugs and chemicals**

Methyl jasmonate (Sigma Aldrich, Germany), acetylsalicylic acid (aspirin, Sigma, Germany), carrageeanan (Sigma, Germany), indomethacin (Sigma, Germany), 5, 50 di-thio-bis-2-nitrobenzoic acid (DTNB, Sigma, Germany), triochloroacetic acid (TCA, Sigma, Germany), thiobarbituric acid (TBA, Sigma, Germany), sodium carbonate (British Drug House, Medspeak, United Kingdom), potassium carbonate (BDH), sodium chloride (BDH), Lurk solution (Sigma, Germany) and methylene blue (Sigma, Germany) were used in the study.

**Preparation of methyl jasmonate solution**

MJ of 95% purity was prepared according to the procedure previously described by Umukoro and Olugbemide [15]. Briefly, MJ was dissolved in 95% ethanol and this 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.

**Carrageenan-induced rat paw edema model**

The effects of MJ on acute inflammation was evaluated using the carrageenan-induced rat paw edema model, according to the method previously described [16]. The rats were randomly distributed into five treatment groups (6 per group). The first 3 groups were treated with MJ (25, 50 or 100 mg·kg⁻¹, i.p.), whereas the fourth and fifth groups received aspirin (100 mg·kg⁻¹, i.p.) and vehicle (10 mL·kg⁻¹ of 1% ethanol, i.p.) respectively. At 30 min after treatment, the right hind paw volume of each rat was determined before induction of acute inflammation with sub-plantar injection of 0.1 mL of 1% carrageenan [16]. The paw volumes were then measured using a plethysmometer (Ugo Basile) at 0 (before injection of carrageenan) and 3 h post-carrageenan administration. The increase in paw volume was determined as the difference between the paw volume at 0 and 3 h. The percentage inhibition was calculated as previously described [1].

**Assay of inflammatory exudates**

The inflammatory exudates were produced using the granuloma air pouch model of chronic inflammation according to the technique previously described [17] and as reported by Boris and Stevenson [18]. The rats were randomly divided into 5 groups (6 per group) and anesthetized with ether in a transparent glass box. Thereafter, the dorsal intrascapular region was shaved and disinfected. Air pouch was then created at the shaved portion of the back by injection of 20 mL of sterile air. Then, 1 mL of 20% carrageean suspension in sesame oil was injected into the pouch at 30 min after treatment with MJ (25–100 mg·kg⁻¹), aspirin
(25 mg·kg\(^{-1}\)) or vehicle (10 mg·kg\(^{-1}\) of 1% ethanol) intraperitoneally. Treatments were continued daily for 4 days after induction of chronic inflammation with carrageenan. On the 5\(^{th}\) day, the animals were sacrificed under ether anesthesia and the inflammatory exudates were obtained and measured using a 5-mL syringe. The inflammatory fluid was divided into different portions for biochemical assays and estimation of the number of WBC.

**Estimation of white blood cell counts in inflammatory exudates**

The number of WBC in the inflammatory exudates was estimated according to the method previously described \[21\]. The inflammatory fluid (0.2 mL) was mixed with 0.38 mL of Lurk solution and 1% methylene blue was added to the mixture. After thorough mixing, two drops of the mixture were filled on both sides of improved Neubauer counting chamber using a pipette. The cells were allowed to settle on the hemocytometer and were then viewed under a high-power (40 ×) microscope and counted manually.

**Estimation of the levels of reduced glutathione (GSH) in inflammatory exudates**

The effects of MJ on the concentrations of reduced GSH in the inflammatory exudates were determined according to the procedure of Moron et al. \[20\]. Equal volume (0.4 mL) of exudates and 20% trichloroacetic acid (TCA) (0.4 mL) were mixed. The mixture was centrifuged using a cold centrifuge at 4 °C for 20 min. The supernatant (0.25 mL) was added to 2 mL of 0.6 mmol·L\(^{-1}\) DTNB. The final volume was made up to 3 mL with phosphate buffer (0.2 mol·L\(^{-1}\), pH 8.0). The absorbance was read at 412 nm using a spectrophotometer and the concentrations of GSH in the fluid were expressed as µmol·mL\(^{-1}\).

**Estimation of malondialdehyde (MDA) concentrations in inflammatory exudates**

Aliquot of 0.4 mL of the exudates was mixed with 1.6 mL of Tris-KCl buffer, to which 0.5 mL of 30% TCA was added. Then 0.5 mL of 0.75% TBA was added and placed in a water bath for 45 min at 50 °C. This was then cooled in ice and centrifuged at 3 000 r·min\(^{-1}\) for 15 min. The clear supernatant was collected and the absorbance at 532 nm was measured against a reference blank of distilled water. The MDA level was calculated according to the method previously described \[21\] using a molar extinction coefficient of 1.56 × 10\(^{4}\) mol·L\(^{-1}\)·cm\(^{-1}\) and expressed in unit/mg protein.

**Estimation of protein level in test samples**

Protein was measured in inflammatory fluid for SOD and catalase according to the method of Gornall et al. \[22\]. The inflammatory fluid (0.2 mL) was serially diluted 10 times with distilled water (1.8 mL of distilled water). This was done to reduce the level of protein in the samples to the sensitivity range of Buret test. The diluted sample (1 mL) was added to 3 mL of Buret reagent. The mixture was then incubated at room temperature for 30 min and the absorbance was read at 540 nm using distilled water as blank. The protein content of the samples was extrapolated from the standard curve and multiplied by 10 to get the actual amount in the sample.

**Assay of catalase activity in inflammatory exudates**

Catalase activity was determined according to the method previously described \[23\]. The inflammatory fluid (0.2 mL) was diluted with 3.8 mL of distilled water to give a 1 : 20 dilution. The assay mixture was consisted of 5 mL of 0.1 mol·L\(^{-1}\) phosphate buffer (pH 7.4) and 4 mL of H\(_2\)O\(_2\) solution (800 µmol·L\(^{-1}\)) in a 10 mL flat bottom flask. Then, 1 mL of the diluted sample was rapidly mixed with the reaction mixture by a gently swirling motion at room temperature. Thereafter, 1 mL of the reaction mixture was taken and added to 2 mL of dichromate acetic acid reagent [5% solution of K\(_2\)Cr\(_2\)O\(_7\)] with glacial acetic acid (1 : 3 by volume) in a cuvette at an intervals of 60 s over a 180-s period. The absorbance was read at 570 nm and catalase activity was calculated by extrapolating the remaining H\(_2\)O\(_2\) from the standard curve.

**Assay of superoxide dismutase (SOD) activity**

The level of SOD activity was determined by the method of Misra and Fridovich \[24\]. Inflammatory exudate (0.2 mL) was diluted in 1.8 mL of distilled water to make a 1 in 10 dilution. The diluted sample (0.2 mL) was added to 2.5 mL of 0.05 mol·L\(^{-1}\) carbonate buffer (pH 10.2) and the reaction was started by adding 0.3 mL of freshly prepared 0.3 mmol·L\(^{-1}\) adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette (blank) contained 2.5 mL buffer, 0.3 mL of substrate (adrenaline) and 0.2 mL of water. The increase in absorbance at 480 nm was monitored every 30 s for a period of 150 s. One unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline.

**Evaluation of membrane stabilizing activity of MJ**

Membrane stabilizing activity of MJ was evaluated using inhibition of rat red blood cell hemolysis induced by hypotonic medium according to the method previously described by Shinde et al. \[25\]. The blood samples were collected by cardiac puncture from the rats and mixed with equal volume of sterilized Alsever solution; the mixture was then centrifuged at 3 000 r·min\(^{-1}\) for 10 min. The packed cells were washed with sodium phosphate saline buffer (0.1 mol·L\(^{-1}\), pH 7.2) thrice and a suspension in 10% PBS was then made. The RBC suspension (0.5 mL) was incubated with 5 mL of either hypotonic solution (50 mmol·L\(^{-1}\) NaCl) in 10 mmol·L\(^{-1}\) sodium phosphate buffered saline (pH 7.4) or isotonic-buffered solution of MJ (0.05, 0.10, and 0.25 mg·mL\(^{-1}\)) or indomethacin (0.5 mg·mL\(^{-1}\)). The control sample was consisted of 0.5 mL of RBC mixed with hypotonic-buffered saline solution alone. The mixtures were incubated for 10 min at room temperature and centrifuged for 10 min at 3 000 r·min\(^{-1}\) and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition of hemolysis or membrane stabilization was calculated as described by Shinde et al. \[25\].
**Statistical analysis**

The data were analyzed using Graph Pad Prism software version 4.0 and expressed as mean ± SEM. Statistical analysis was done using one-way ANOVA, followed by Newman-Keuls post hoc test. P values less than 0.05 were considered statistically significant.

**Results**

**Methyl jasmonate reduces paw edema volume induced by carrageenan in rats**

The effects of MJ on acute inflammation induced by carrageenan in rats are shown in Table 1. One-way ANOVA revealed that there were significant differences among the treatment groups [F(4, 20) = 115.40, P < 0.0001]. Post-hoc analysis by Newman-Keuls Multiple Comparison Test showed that the increase in paw edema volume produced by carrageenan was significantly (P < 0.05) reduced by MJ (50–100 mg·kg⁻¹, i.p.). As shown in Table 1, aspirin (100 mg·kg⁻¹) also inhibited the inflammatory edema in comparison with vehicle in a significant manner (P < 0.05). However, MJ (25 mg·kg⁻¹) did not produce significant inhibition of acute inflammation caused by carrageenan when compared with vehicle (P > 0.05, Table 1).

**Table 1 Effects of methyl jasmonate on carrageenan-induced paw edema in the rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg·kg⁻¹)</th>
<th>Paw Oedema Volume (mL)</th>
<th>Inhibition of Oedema (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>–</td>
<td>0.53 ± 0.01</td>
<td>–</td>
</tr>
<tr>
<td>MJ</td>
<td>25</td>
<td>0.41 ± 0.01</td>
<td>22.64</td>
</tr>
<tr>
<td>MJ</td>
<td>50</td>
<td>0.21 ± 0.02*</td>
<td>60.38</td>
</tr>
<tr>
<td>MJ</td>
<td>100</td>
<td>0.16 ± 0.02*</td>
<td>69.81</td>
</tr>
<tr>
<td>Aspirin</td>
<td>25</td>
<td>0.19 ± 0.01*</td>
<td>64.15</td>
</tr>
</tbody>
</table>

Values represent the Mean ± SEM (n = 6). *P < 0.05 vs vehicle (ANOVA followed by Newman-Keuls Multiple Comparison Test)

**Methyl jasmonate reduces inflammatory exudates and inhibits white blood cell infiltrations**

The effects of MJ on the volume of inflammatory fluid and WBC infiltrations produced by carrageenan in the granuloma air pouch model of chronic inflammation are shown in Tables 2 and 3. One-way ANOVA showed that there were significant differences among treatment groups: inflammatory fluid volume [F(4, 20) = 15.29, P < 0.0001] and WBC counts [F(4, 20) = 126.30, P < 0.0001]. Post-hoc analysis by Newman-Keuls Multiple Comparison Test showed that MJ (50 and 100 mg·kg⁻¹) produced a significant (P < 0.05) decrease in the concentrations of MDA in the inflammatory fluid in a dose-dependent fashion. Aspirin (25 mg·kg⁻¹) also produced a significant (P < 0.05) decrease in the concentrations of MDA when compared with vehicle (P < 0.05).

**Table 2 Effects of methyl jasmonate on the volume of exudates in carrageenan-induced granulomtous inflammation in the rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg·kg⁻¹)</th>
<th>Volume of Exudates (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>–</td>
<td>1.26 ± 0.11</td>
</tr>
<tr>
<td>MJ</td>
<td>25</td>
<td>0.62 ± 0.1*</td>
</tr>
<tr>
<td>MJ</td>
<td>50</td>
<td>0.52 ± 0.06*</td>
</tr>
<tr>
<td>MJ</td>
<td>100</td>
<td>0.38 ± 0.08*</td>
</tr>
<tr>
<td>Aspirin</td>
<td>25</td>
<td>0.48 ± 0.09*</td>
</tr>
</tbody>
</table>

Values represent the Mean ± SEM (n = 6). *P < 0.05 vs vehicle (ANOVA followed by Newman-Keuls Multiple Comparison Test)

**Fig. 1** Effects of methyl jasmonate (MJ; 25, 50 or 100 mg·kg⁻¹, i.p.) or acetylsalicylic acid (ASA; 25 mg·kg⁻¹, i.p.) on malondialdehyde (MDA) level in inflammatory exudates in rats. Data represent the Mean ± SEM (n = 6). *P < 0.05 vs control (ANOVA followed by Newman-Keuls Multiple Comparison test)
**Methyl jasmonate increases glutathione level in inflammatory fluid**

The effects of MJ on the concentrations of reduced GSH in the inflammatory exudates induced by carrageenan in the granuloma air pouch model of chronic inflammation are presented in Fig. 2. One-way ANOVA showed that there were significant differences among the treatment groups [F(4, 20) = 13.10, P < 0.0001]. Post-hoc analysis by Newman-Keuls Multiple Comparison Test revealed that MJ (25, 50, and 100 mg·kg⁻¹) produced a significant (P < 0.05) increase in the concentrations of GSH in the inflammatory exudates in comparison with vehicle, which suggested its free radicals scavenging property. As shown in Fig. 2, aspirin (25 mg·kg⁻¹) significantly increased the concentrations of GSH in the exudates.

**Methyl jasmonate increases activity of antioxidant enzymes in inflammatory fluid**

The effects of MJ on activity of antioxidant enzymes in the inflammatory exudates are shown in Figs. 3 and 4. One-way ANOVA revealed that there were significant differences among the treatment groups: catalase activity [F(4, 20) = 6.79, P = 0.0013] and SOD activity [F(4, 20) = 4.72, P = 0.0076]. Post-hoc analysis by Newman-Keuls Multiple Comparison Test showed that MJ (25, 50, and 100 mg·kg⁻¹, i.p.) produced a significant (P < 0.05) increase in the activity of catalase and SOD in the exudates when compared with vehicle (Figs. 3 and 4).

**Methyl jasmonate inhibits rat red blood cell lysis induced by hypotonic medium**

Table 4 shows the effects of MJ on rat red blood cell hemolysis induced by hypotonic solution. One-way ANOVA revealed that there were significant differences among the treatment groups [F(4, 20) = 123.20, P < 0.0001]. Post-hoc analysis by Newman-Keuls Multiple Comparison Test showed that MJ (0.05, 0.10, and 0.25 mg·mL⁻¹) resulted in a significant (P < 0.05) inhibition of rat RBC lysis induced by hypotonic medium when compared with control, which suggested its membrane-stabilizing property. Indomethacin at a concentration of 0.5 mg·mL⁻¹ also demonstrated a significant membrane stabilizing activity (P < 0.05, Table 4).

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**Fig. 2.** Effects of methyl jasmonate (MJ; 25, 50 or 100 mg·kg⁻¹, i.p.) or acetylsalicylic acid (ASA; 25 mg·kg⁻¹, i.p.) on glutathione (GSH) concentration in inflammatory exudates in rats. Values represent the Mean ± SEM (n = 6). *P < 0.05 vs control (ANOVA followed by Newman-Keuls Multiple Comparison Test)

**Fig. 3.** Effects of methyl jasmonate (MJ; 25, 50 or 100 mg·kg⁻¹, i.p.) or acetylsalicylic acid (ASA; 25 mg·kg⁻¹, i.p.) on superoxide dismutase (SOD) activity in inflammatory exudates in rats. Data represent the Mean ± SEM (n = 6). *P < 0.05 vs control (ANOVA followed by Newman-Keuls Multiple Comparison Test)

**Fig. 4.** Effects of methyl jasmonate (MJ; 25, 50 or 100 mg·kg⁻¹, i.p.) or acetylsalicylic acid (ASA; 25 mg·kg⁻¹, i.p.) on the activity of catalase in exudative fluid in rats. Each column represents the Mean ± SEM (n = 6). *P < 0.05 vs control (ANOVA followed by Newman-Keuls Multiple Comparison Test)

**Table 4.** Membrane stabilizing property of methyl jasmonate in hypotonic solution-induced red blood cell lysis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (mg·mL⁻¹)</th>
<th>Optical Density</th>
<th>Inhibition of RBC haemolysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>–</td>
<td>0.21 ± 0.002</td>
<td>–</td>
</tr>
<tr>
<td>Methyl jasmonate</td>
<td>0.05</td>
<td>0.08 ± 0.002²</td>
<td>61.90</td>
</tr>
<tr>
<td>Methyl jasmonate</td>
<td>0.10</td>
<td>0.067 ± 0.001¹</td>
<td>68.10</td>
</tr>
<tr>
<td>Methyl jasmonate</td>
<td>0.25</td>
<td>0.055 ± 0.002²</td>
<td>73.81</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.50</td>
<td>0.05 ± 0.01¹</td>
<td>76.19</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM for 4 sets of experiments. *P < 0.05 vs vehicle (ANOVA followed by Newman-Keuls Multiple Comparison Test)
Discussion

The results from the present study showed that MJ produced a significant suppression of edema associated with acute inflammation induced by carrageenan in rats. In the granuloma air pouch model of chronic inflammation, MJ demonstrated a significant inhibition of exudative fluid formation induced by carrageenan in rats. It also suppressed WBC infiltrations, as shown by the decrease in their number in the inflammatory exudates. It further reduced the activity of ROS in the inflammatory fluid, as evidenced by a decrease in the concentrations of MDA and elevation of the levels of endogenous antioxidant defense system. These findings suggested that MJ might play a beneficial role in the treatment of ailments associated with both acute and chronic inflammatory conditions.

Acute inflammation produced by carrageenan is mediated through the stepwise release of histamine, bradykinin and serotonin in the early phase and prostaglandins in the late phase [1, 4, 6]. These mediators are known to produce edema through vasodilatation and increased vascular permeability, which promote fluid accumulation in the interstitial tissues [1,4,26]. However, prostaglandins have been reported to be weak in producing inflammation but are better at potentiating the effects of other mediators [4]. The late appearance of prostaglandins during carrageenan-induced edema further confirms that they are better in potentiating and amplifying inflammatory responses produced by earlier released mediators [4]. This observation has made inhibition of prostaglandins biosynthesis an important target for development of drugs with anti-inflammatory activity. Moreover, compounds, which inhibit the biosynthesis of prostaglandins, like non-steroidal anti-inflammatory drugs (NSAIDs), are known to reduce acute inflammation induced by carrageenan in rats [1, 27]. Thus, the findings that MJ inhibited the edematous-like effect of carrageenan in rats suggested its anti-inflammatory effects.

The effects of MJ on chronic inflammation were also evaluated using the granuloma air pouch technique based on the volume of inflammatory exudates formation, WBC counts and levels of antioxidant biomarkers in the fluids. The carrageenin-induced granuloma air pouch is a model of chronic inflammation, which involves the injection of suspension of carrageenan into the subcutaneous air bleb that has been raised on the back of rats [6]. The granuloma air pouch model provides a suitable cavity for eliciting a chronic type of inflammatory reactions as found in the synovial cavity during RA [6, 28]. The pathological features in the air pouch model are similar to those of RA in many ways, including infiltration of inflammatory cells, increased oxidative stress, patterns of tissue destructions, and progression of the disease [2-3, 5]. Activated leukocytes, especially the mononuclear phagocytic cells, are the major players in the pathology of tissue [5, 7, 29] like the type seen during carrageenan-induced granulomatous inflammation. These cells release a variety of inflammatory mediators and cytotoxic products, including ROS/lysosomal enzymes, during the process of phagocytosis of the deposit of carrageenan [6]. These mediators further drive the ongoing inflammatory process by promoting infiltration of WBC to the site of inflammation [2-3, 8].

The release of ROS by activated WBC further causes tissue damage through lipid peroxidation, which results in progressive tissue destructions characterized by granulomatous inflammation. ROS also propagates inflammation by stimulating release of cytokines, such as IL-1, TNF-α, and interferon-γ, which are responsible for the recruitment of additional inflammatory cells [2-3, 30]. Thus, free radicals are important mediators that provoke or sustain inflammatory responses and their neutralization by antioxidants might be helpful for the treatment of chronic inflammation like RA [3, 28, 30].

Also, it is noteworthy that currently available anti-inflammatory drugs can only alleviate the symptoms of RA but not the course of the disease [19, 29, 31]. Thus, current research efforts have focused on the development of compounds with potent antioxidant property that could be used as adjuncts in the therapy of chronic inflammatory disorders [2, 29, 31]. This approach affords protection of cartilages or joint tissues against the deleterious effects of ROS and therefore halts the progression of the disease [2, 29, 31]. In the present study, MJ was found to suppress the volume of exudates and the number of WBC in the inflammatory fluid. The increased activity of ROS in the inflammatory fluid was inhibited by MJ, as evidenced by a significant reduction in the concentrations of MDA and elevation of the levels of GSH in the fluid. Also, MJ increased the levels of catalase and superoxide dismutase, which further suggested its antioxidant property. Thus, the finding that MJ significantly modified the components of granuloma air pouch model of chronic inflammation suggested that it may play a crucial role in the management of chronic inflammatory disorders like RA.

The in vitro anti-inflammatory activity of MJ was investigated using rat RBC membrane stabilization method. The RBC membrane stability test is based on the finding that a number of non-steroidal anti-inflammatory agents inhibit RBC lysis, presumably by stabilizing the membrane of the cells [10]. The erythrocyte membrane is considered typically as a model of the lysosomal membrane, which plays an important role in inflammation [5, 25]. The compounds which prevent the lysis of membrane caused by the release of hydrolytic enzymes contained within the lysosomes may relieve some symptoms of inflammation [5, 25]. The release of hemoglobin from RBC subjected to hypotonic stress has been shown to be prevented by anti-inflammatory drugs because of their membrane stabilizing properties [10, 25, 32]. It is our opinion that the membrane stabilizing activity shown by MJ in the present study may have a significant contribution to its anti-inflammatory effects. This suggestion is corroborated by the finding that MJ like indomethacin, exhibited significant
inhibition of RBC lysis, which suggested protection of lysosomal membrane against injurious stimuli that are involved in the initiation and propagation of chronic inflammation. Moreover, prevention of RBC lysis has been described as a biochemical index and as an in vitro model for the evaluation of compounds with anti-inflammatory property [1, 25, 32]. Thus, compounds with membrane stabilizing property are expected to demonstrate anti-inflammatory activity via prevention of the release of lysosomal phospholipases, which are the prime initiators of the inflammatory reaction [1, 25, 32].

Conclusion

The results from the present study demonstrated that methyl jasmonate had in vivo anti-inflammatory effect on both acute and chronic animal models of inflammation in rats. These effects may be related to the stabilization of lysosomal membrane and inhibition of WBC-mediated release of inflammatory mediators including reactive oxygen species, which are the major culprits involved in the pathology of chronic inflammatory diseases like RA.

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