

Anti-Inflammatory and Anti-Hyperalgesic Effects of *Ximenia americana* Bark Extract in a Murine Model of Rheumatoid Arthritis

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The aim of this study was to evaluate the anti-hyperalgesic and anti-inflammatory profiles of the hydroethanolic extract of *Ximenia americana* bark (HEXA) in a murine model of rheumatoid arthritis (RA). The RA model was induced through intra-articular injection of Complete Freund's Adjuvant (CFA). ELISA was used to analyze the production of inflammatory mediators, and nociception was assessed using the Von-Frey method. HEXA significantly reduced CFA-induced mechanical hyperalgesia ($p < 0.01$) and ipsilateral knee edema ($p < 0.001$). These effects were asso-

ciated with reduced levels of IL-6 and TNF- α ($p < 0.01$) and increased IL-10 concentrations ($p < 0.01$) in the knee joint. HEXA also reduced total protein levels ($p < 0.0001$) and myeloperoxidase (MPO) enzyme activity ($p < 0.0001$) compared to untreated controls. Liver enzyme analysis revealed no signs of toxicity after chronic treatment. HEXA demonstrated significant anti-hyperalgesic and anti-inflammatory activity in a CFA-induced arthritis model, supporting its potential as a candidate for further investigation in inflammatory joint diseases.

1. Introduction

Rheumatoid arthritis (RA) is a chronic clinical condition resulting from the dysregulation of pro-inflammatory cytokines (such as TNF- α , IL-1 β , and IL-6), increased expression of

eicosanoid-producing enzymes (such as cyclooxygenase-2 and 5-lipoxygenase), adhesion molecules, and matrix metalloproteinases, as well as hyperproliferation of synovial fibroblasts.^[1] RA is a systemic autoimmune disease that predominantly affects the joints, producing chronic inflammation that causes damage to joints and extra-articular organs.^[2]

There is currently no cure for RA, and the effectiveness of treatments varies significantly between patients, suggesting an undefined pathogenic diversity.^[3] Nonpharmacological treatments include surgery and physical rehabilitation.^[4] However, the most common therapeutic options involve nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying antirheumatic drugs (DMARDs), which can be administered orally, topically, or intra-articularly. However, chronic RA is often associated with poor treatment adherence and high dropout rates due to adverse effects or insufficient efficacy,^[5,6] highlighting the need to develop new therapeutic approaches. Yet, finding new alternative approaches to mitigate chronic arthritis has been a significant challenge due to low financial cost and improved therapeutic benefits.^[7] In this context, drug development targeting key cytokines and their signaling pathways can represent a promising approach in RA pharmacology. Importantly, evidence indicates that TNF- α , IL-6, and IL-17 and their receptors are key drug targets in RA.^[8]

Nutraceuticals have garnered significant attention for their potential in the treatment of inflammatory diseases, particularly arthritis. These food-derived compounds offer a promising alternative to conventional pharmaceuticals due to their natural origin and reduced side effect profile.^[5] Various fruits have long been recognized for their therapeutic potential in manag-

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ing inflammatory conditions, including arthritis.^[9] Among these, the genus *Ximenia* stands out for its notable anti-inflammatory properties which have been shown to decrease the production of pro-inflammatory cytokines.^[10]

Other plant-derived natural products have also demonstrated pronounced anti-inflammatory activity due to their ability to inhibit inflammatory mediator production (such as cytokines, chemokines, nitric oxide (NO), and eicosanoids) and regulate the profile of helper T cells.^[11,12] Evidence has shown that the ability of natural products to modulate inflammatory parameters is associated with the presence of flavonoids and terpenes, secondary metabolites with significant immunomodulatory, anti-inflammatory, and analgesic effects.^[11,13,14] Natural products have also shown promise in the development of innovative therapeutic strategies for managing RA, as they can serve as sources of novel chemical compounds that may act on significant therapeutic targets in autoimmune diseases.^[15,16] Furthermore, there is evidence that medications which inhibit/modulate cytokines such as TNF- α , IL-1 β , and IL-6, which are increased in RA patients, promote significant therapeutic benefits in RA due to their ability to reduce hyperalgesia and inhibit disease progression.^[3,15] Therefore, medicinal plants and their secondary metabolites are important sources of substances to alleviate the main symptoms of RA and other inflammatory chronic pain conditions.^[17]

Ximenia americana (wild plum) is a regionally important, edible plant belonging to the *Oleaceae* family, and is valued for its nutritional, medicinal, and cosmetic uses, particularly in tropical and subtropical regions. It has a pantropical distribution, being found in Africa, South and Central America, Asia, and Oceania. In Brazil, it is found in a number of biomes, including the Caatinga, a unique semi-arid biome found exclusively in northeastern Brazil, which contains a great diversity of medicinal plant species. It is widely used in several countries as a food, medicine, and a source of essential oils with many industrial applications.^[18] Additionally, the scientific literature has reported that the stem bark has gastroprotective,^[19] acaricidal,^[20] antibacterial, antiparasitic,^[21] antifungal, antioxidant, antiproliferation, anti-inflammatory,^[22] antidiabetic, and hepatoprotective properties,^[18,23] highlighting the therapeutic potential of this species, which has been used in traditional medicine for managing inflammatory conditions,^[24] such as RA.^[18] In a previous study, our group validated this anti-inflammatory effect, by demonstrating an anti-edematogenic effect in acute and chronic inflammation models, and demonstrated the ability of inflammatory mediators, including arachidonic acid metabolites, serotonin, and histamine, that are associated with the production of pro-inflammatory cytokines (TNF- α and IL -1 β).^[22,25]

Therefore, this study aimed to address the lack of knowledge regarding the activity of the ethanolic extract of *X. americana* bark (HEXA) in relation to the hyperalgesia involved in the pathogenesis of rheumatoid arthritis. Specifically, the goal was to evaluate the anti-hyperalgesic and anti-inflammatory profiles of HEXA in a mouse model of arthritis, thereby aiming to create better scientific evidence for its use in traditional medicine.

2. Materials and Methods

2.1. Plant Material and Extract Preparation

The bark of *X. americana* was collected at the "Sítio Lambedor" located in the municipality of Farias Brito (Caatinga region), Ceará State, Brazil, at the geographic coordinates: SAD 69 - Latitude 06° 57' 26.30" and Longitude 39° 32' 17.40"). A voucher specimen was registered at the Herbarium Caririense Dárdano de Andrade Lima under number 10976. The National Genetic Heritage and Associated Traditional Knowledge Management System (SISGEN) under number A989D0B approved the pharmacological use of the species in this study.

The plant bark was dried and transferred to a container, soaked in a mixture of distilled water, and absolute ethanol (1:1) for 72 h. The hydroethanolic extract obtained was then filtered and transferred to a rotary evaporator (27–35 rpm; 45 °C) for ethanol removal. After removing the solvent, the extract was kept in a water bath for ethanol evaporation for 24 h before being freeze dried.

2.2. Animals

Female and male mice (20–30 g) were provided by the Animal Care Unit of the Regional University of Cariri (URCA). The protocols were approved by the Animal Experimentation and Use Committee of URCA under process number CEUA N° 00152/2022.2. All animals were maintained with food (Labina, Purina, Brazil) and water ad libitum in a climatized room at 24 ± 2 °C on a light/dark cycle of 12 h. This study was carried out following the recommendations of the National Council for the Control of Animal Experiments (CONCEA) and the Guide for the Care and Use of Laboratory Animals of the National Institute of Health-USA (NIH, 1996). The euthanasia of animals was performed by overdose of anesthetic using sodium pentobarbital injected into the peritoneal cavity. The dosage was carefully calculated based on the animal's weight (250 mg/kg) to ensure the rapid induction of sleep, leading to respiratory and cardiac depression, in accordance with the Guide for the Care and Use of Laboratory Animals and the National Council for the Control of Animal Experiments (CONCEA).

2.3. Complete Freund's Adjuvant (CFA)-Induced Arthritis Model

A previously developed murine model of arthritis induced by the administration of CFA was used.^[26,27] Mice were anesthetized using a mixture of ketamine/ xylazine (100/5 mg/kg) administered intraperitoneally (i.p.). Four intra-articular injections of 10 μ L of CFA (1 mg/mL *Mycobacterium tuberculosis* in paraffin oil, Sigma, EUA) were administered unilaterally at days 0, 7, 14, and 21 into the right knee joint. The challenge was injected through the patellar ligament into the articular space, using the femoral condyles as a guide. Control mice were challenged with the same volume of saline.

2.4. Determination of Knee Diameter

The diameter of the knee joint was measured using a digital electronic caliper (MTX, TollsWord). Measurements of the knee were performed in the anterior–posterior (AP) and later–lateral (LL), in both the ipsilateral and contralateral positions. The following equation calculated the area: $V = \frac{4 \cdot \pi \cdot r_{\text{m}}^3}{3}$, where the radius equals the mean of the AP and LL divided by two. The percentage of increase in joint edema was calculated by the difference between contralateral and ipsilateral knee volume, divided by contralateral knee volume, multiplied by 100.^[28]

2.5. Mechanical Hyperalgesia

Mechanical hyperalgesia was tested in mice using the method previously described by Cunha et al.,^[29] using a digital analgesiometer (digital Von–Frey; Insight, São Paulo, Brazil). The mice were placed in transparent cages (12 × 10 × 17 cm) with wire mesh floors and acclimatized in a quiet room for 1 h before the measurements started. The investigator was trained to apply the tip perpendicularly to the central area of the hind paw with a gradual increase in pressure. The endpoint was characterized by the withdrawal of the paw, followed by precise flinching movements. After this response, the pressure intensity was automatically recorded. The stimulus intensity was obtained by averaging three measurements with minimal intervals of 3 min.

2.6. TNF- α , IL-6, and IL-10 Cytokines

After all treatments and analyses of the arthritis protocol were completed, the mice were anesthetized (ketamine/xylazine 100/5 mg/kg i.p.), the intra-articular space was washed three times with 10 μ L of 10 μ M EDTA in phosphate-buffered saline (PBS), and synovial fluid was collected. After centrifugation (10,000 rpm for 5 min), the supernatant was diluted, and the concentrations of TNF- α , IL-6, and IL-10 were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (eBioscience). Readings were performed at 450 nm in a spectrophotometer (ASYS), and concentrations were obtained by interpolation from a standard curve. All results were expressed as picograms (pg) of cytokine per milliliter (mL).

2.7. Evaluation of Myeloperoxidase (MPO) Activity and Quantification of Total Proteins

Joint samples were thawed and centrifuged at 4,500 rpm for 12 min at 4° C, and the supernatant was then collected. MPO activity levels were determined using a previously described technique,^[30] which used 0.0005% hydrogen peroxide as a substrate for MPO. One unit of MPO was defined as the amount capable of converting 1 μ mol of hydrogen peroxide to water in 1 min at 22° C. The variation in optical density of the mixture of samples with the o-dianisidine solution as a function of reaction

time was quantified by ELISA at a wavelength of 450 nm. The results were expressed as g/dL of lavage.

Total protein quantification was performed by combining the homogenate with the reagent developed by Biotechnica (Varginha, MG, Brazil; CEP 37026–470), based on the reaction of copper ions in an alkaline medium. The resulting violet-colored complex absorbance is proportional to the concentration of proteins in the sample analyzed. The samples were then incubated at 37 °C for 10 min and analyzed by ELISA at 550 nm.

2.8. Hepatotoxic Potential

Serum levels of aspartate transaminase (AST) and alanine transaminase (ALT) were used as biochemical markers for early acute hepatic damage to investigate the potential toxicity of HEXA. After 25 days of treatment, the animals were anesthetized, blood was collected, and serum was obtained by centrifugation at 3000 rpm for 10 min. The concentrations of AST and ALT were determined using a diagnostic kit from Labtest (Lagoa Santa, MG, Brazil).^[31]

2.9. Statistical Analysis

The data obtained were evaluated by one-way or two-way analyses of variance (ANOVA) followed by Tukey's post-hoc test. The differences were considered significant when $p < 0.05$. All analyses were performed using the GraphPad Prism (5.0) software (San Diego, CA, USA).

3. Results and Discussion

Rheumatoid arthritis (RA) is characterized by chronic inflammation of the joints associated with an autoimmune reaction triggered by several epigenetic and external factors,^[32] leading to swelling, pain, progressive deformity, destruction of the synovial membrane, and tissue remodeling.^[33,34]

Current hormonal or steroidal anti-inflammatory drugs do not entirely alleviate the main symptoms of RA and cannot produce lasting gains for patients.^[5,6] Worryingly, the repeated use of these medications is associated with several well-documented number of adverse effects, such as renal, hepatic, and cardiovascular toxicity.^[35] Therefore, there has been increasing interest in natural products, particularly medicinal plants with anti-inflammatory properties that have been successfully used in folk medicine to treat a variety of autoimmune diseases, including RA, with strong supporting scientific evidence.^[36]

One plant in which there has been growing interest is *X. americana* (wild plum), which is a medicinal species widely used to treat a variety of inflammatory conditions, including acute manifestations such as edema and gingivitis, and chronic inflammations such as joint inflammation.^[37,38] In the northeastern region of Brazil, particularly in the Caatinga region, traditional communities utilize this plant to treat a range of ailments, especially those of an inflammatory and painful nature, which are alleviated through the use of homemade remedies prepared

from this notable medicinal plant.^[39] Our research group has conducted extensive studies on HEXA bark. These investigations have shown that the extract has a phytochemical profile rich in flavonoids, tannins, saponins, and alkaloids. High performance liquid chromatography (HPLC) analysis undertaken by da Silva et al.^[25] and de Menezes et al.^[21] identified gallic acid, quercetin, quercitrin, catechin, rutin, kaempferol, chlorogenic acid, caffeic acid, and ellagic acid as the bioactive compounds in the *X. americana* extracts^[25] which aligns with findings from other studies.^[40–42]

The effects of *X. americana* in acute and chronic anti-inflammatory responses have been recently reported by da Silva et al.,^[10,22] who demonstrated that the extract could inhibit leukocyte recruitment and decrease serum levels of cytokines such as TNF- α and IL-1 β in a carrageenan-induced peritonitis model. It is known that TNF- α and IL-1 β play a fundamental role in the pathogenesis of RA as their levels are elevated in the synovial tissue, synovial fluid, and serum of RA patients.^[43,44] Patients with RA have increased levels of TNF- α , IL-1 β , and IL-6 regardless of therapy and medications that modulate these cytokines appear to hold promise in controlling the most troublesome symptoms of RA.^[45] Furthermore, evidence shows that TNF- α can stimulate the production of other pro-inflammatory cytokines, including IL-1 β , IL-6, and IL-8, and granulocyte-macrophage colony-stimulating factor (GM-CSF). Therefore, modulating TNF- α -dependent responses can bring promising results in drug development targeting the control of RA.^[44,46,47]

In this study, we used a CFA-induced arthritis model which triggered a chronic inflammatory process characterized by elevated concentrations of cytokines such as IL-1 β , IL-6, and TNF- α .^[48] Untreated CFA-challenged mice presented significantly increased knee edema in the ipsilateral joint compared with the contralateral knee joints or in the knee joints of saline-injected mice, which was maintained until day 25 after the start of CFA administration, with no visual evidence of knee edema in the contralateral hindlimb. The mice were treated daily with HEXA or vehicle from day 1 to day 25, and the development of the arthritis-like condition or the possible attenuation of this inflammatory state was monitored. The anti-inflammatory effect of chronic treatment with HEXA at 50 mg/kg was determined on days 4, 11, 18, and 25 after the first CFA administration (Figure 1a).

HEXA treatment significantly reduced the diameter of CFA-induced ipsilateral knee edema ($p < 0.001$), which is potentially associated with the inhibition of pro-inflammatory cytokines, mainly TNF- α and IL-1 β , as shown in Figure 1. Consistent evidence has shown the involvement of these cytokines in edema development in the knee joint.^[22] According to Kannan et al.,^[49] CFA stimulates the production of IL-1 β , IL-6, and TNF- α , corroborating our findings (Figure 1b,c).

Quercitrin has a direct impact on the production of cytokines in the inflammatory process,^[50] reducing inflammatory cells^[51] and inhibiting the nuclear factor kappa B (NF- κ B) pathway and nitric oxide production (NO).^[52]

Caffeic acid has demonstrated antioxidant, anti-inflammatory, antitumor, and immunomodulatory properties. Treatment of rheumatoid arthritis synoviocytes (RA-FLS) with

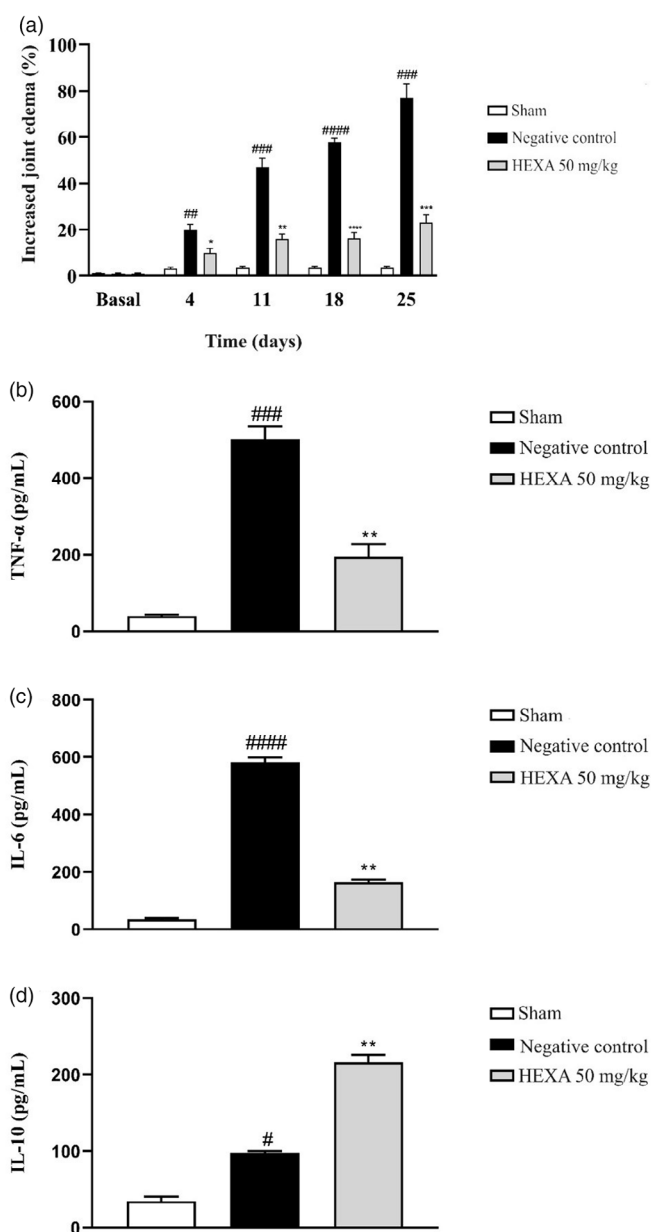


Figure 1. Anti-inflammatory effect of HEXA on CFA-induced inflammation. (a) Edema ($n = 6$). (b) IL-6 levels in synovial fluid ($n = 6$). (c) TNF- α levels in synovial fluid ($n = 6$). (d) IL-10 levels in synovial fluid ($n = 6$). Statistical analysis: two-way ANOVA followed by Bonferroni test or one-way ANOVA followed by Tukey's test. ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ compared to the vehicle group; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, and #### $p < 0.0001$ compared to the Sham group (inactive group).

caffeic acid showed the induction of apoptosis and a significant reduction in the cytokines IL-6 and TNF- α , in addition to prostaglandin E₂ (PGE₂), and MMP-1.^[53] According to the authors, inhibition of the phosphorylation of I κ B and IKK kinases in the NF- κ B pathway appears to be the main pharmacological mechanism involved. The inhibition of cytokines such as IL-1 β , TNF- α , and IL-6 by caffeic acid^[54–59] may be related to the activities presented.

Interleukin-10 (IL-10), a regulatory cytokine, can inhibit the production of several cytokines, including those significantly

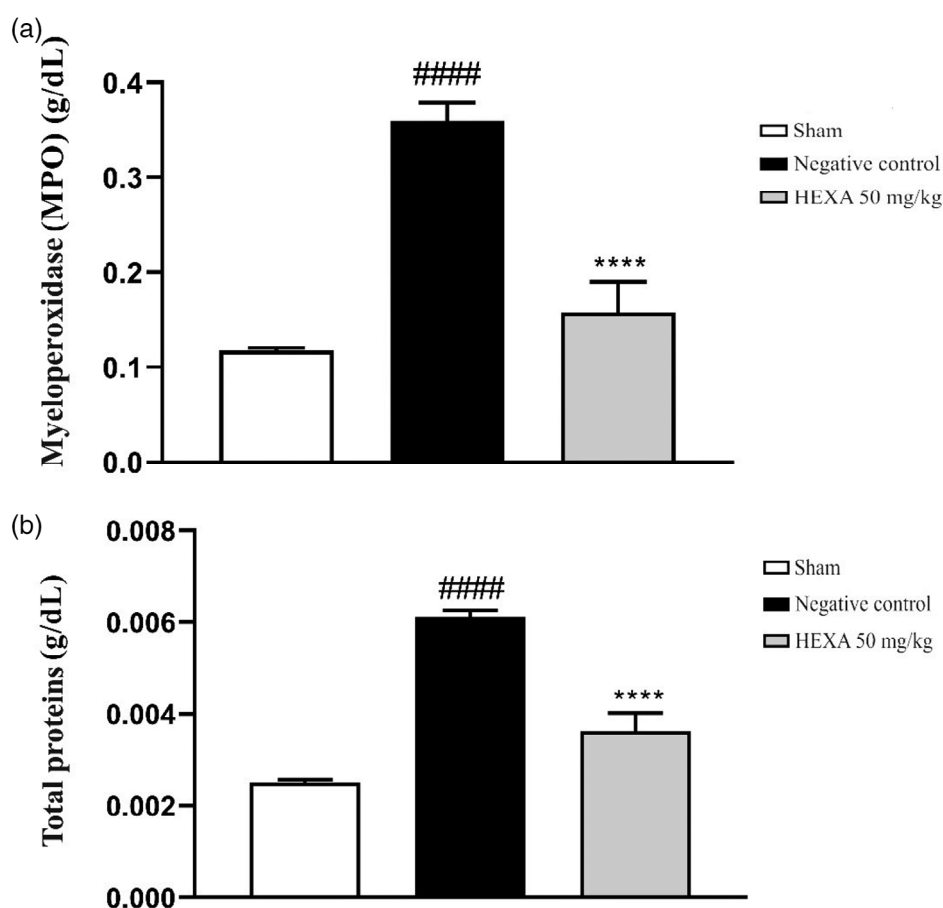


Figure 2. Effect of (a) HEXA on myeloperoxidase (MPO) enzyme activity and (b) protein accumulation in mouse joints in the CFA-induced arthritis model. Statistical analysis: One-way ANOVA followed by Tukey's test ($n = 6/\text{group}$). **** $p < 0.001$ compared to the negative control group and ##### $p < 0.0001$, compared to the Sham group.

involved in RA pathogenesis (TNF- α , IL-1 β , and IL-6), in a variety of cell types, such as macrophages and neutrophils.^[60] Additionally, IL-10 can promote self-regulation,^[61,62] inhibit the generation of reactive oxygen species (intermediates), and antagonize the effects of TNF- α .^[63,64] Furthermore, studies have shown that IL-10 production is increased in inflammatory processes, such as anemia and RA. According to Ginting et al.,^[63] quercitrin increased the cytokine IL-10 which is linked to the decrease in inflammatory cytokines, for instance, TNF- α , IL-1 β , and IL-6, which corroborates our results. Recently, da Silva et al.^[10,22] demonstrated the chronic effect of HEXA against CFA and related the presence of quercetin, where previous studies revealed that quercetin increases the levels of pro-inflammatory cytokines, such as IL-10 (immunoregulatory, anti-inflammatory, and/or pro-inflammation in the pathogenesis of RA). According to Leyva-López et al.,^[14] the expression of NF- κ B downregulated by quercetin is linked to the upregulation of IL-10. In this sense, the author proposed that quercitrin has anti-inflammatory activity similar to quercetin (Figure 1d).

The present work also evaluated the levels of MPO in the joint tissues, which is a parameter of neutrophil activation. In RA, the activation of neutrophils and macrophages leads to increased superoxide generation, resulting in cell oxidative damage due to increased lipid peroxidation and reduced antioxidant

capabilities.^[65] The results of this study demonstrated that CFA administration caused an increase in MPO activity, which was significantly reduced by the treatment with HEXA 50 mg/kg when compared to the untreated control group (Figure 2a). Studies demonstrate that the antioxidant potential of *Ximenia americana* can be attributed mainly to the presence of phenolic compounds such as quercetin and rutin, which demonstrated antioxidant effects associated with the regulation of glutathione synthesis and oxidative stress caused by heme-oxygenase (HO) and superoxide dismutase (SOD).^[23,66,67]

A study by De Barros et al.^[68] associated the reduction in gastric MPO activity with the reduction of inflammatory damage to quercitrin, demonstrating that it is a flavonoid that participates in the action of reducing inflammatory damage in the development of ulcers. Our results are in agreement with the anti-inflammatory activity presented by HEXA, as Tang et al.^[51] demonstrated that quercitrin decreases neutrophil migration. Neutrophil inhibition and MPO reduction may be associated with the ability of caffeic acid to modulate neutrophil activation by inhibiting the 5-LOX/LTB4 pathway.^[69]

ROS are known to play a role in the progression of RA, influenced by oxidative stress, aging, and environmental factors. Increased ROS production can cause damage to the joint matrix through lipid peroxidation in the membrane, in addition to acti-

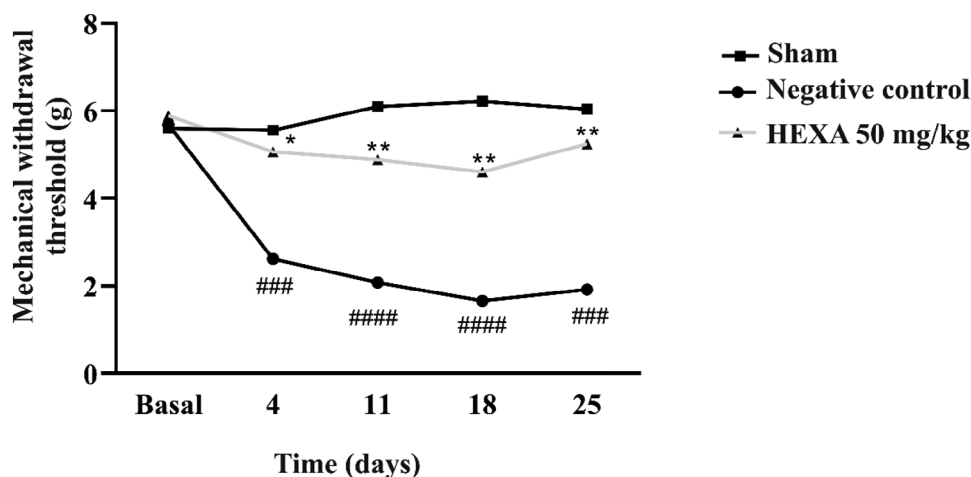


Figure 3. Antihyperalgesic effect of HEXA on mechanical hyperalgesia in a CFA-induced arthritis model. Statistical analysis: Two-way ANOVA followed by Bonferroni test. * $p < 0.05$ and ** $p < 0.01$ compared to the vehicle; ### $p < 0.001$ and #### $p < 0.001$, compared to the Sham group.

ating matrix metalloproteinase, which in turn can induce RA in articular synoviocytes.^[23] Our results corroborate the findings of Misra et al.^[70] who used fractions of *Camellia sinensis* var. *assamica* to demonstrate its effects against ROS.

Protein accumulation in inflammatory infiltrates is a crucial marker of edema and is significantly associated with the pathophysiological changes observed in RA.^[71] HEXA significantly reduced protein accumulation in the CFA-stimulated animals compared to the untreated control group (Figure 2b), corroborating the findings of Silva et al.,^[10,22] who observed a significant reduction in edema development following HEXA treatment.

HEXA treatment also significantly reduced mechanical hyperalgesia. Von-Frey filaments, which are broadly used to analyze mechanical sensitivity in pain-related models, were used in the current study.^[72] CFA injection into the ipsilateral joint in mice induces edema and stimulates painful responses, which may be associated with paw withdrawal latency due to noxious mechanical stimuli. Mechanical hypernociception was verified on the 4th, 11th, 18th, and 25th day after CFA injection, with HEXA treatment significantly reducing mechanical hyperalgesia ($p < 0.001$) compared to the vehicle from the fourth day, with peak effects on days 11 and 18. Studies indicate that inflammatory hypernociception is closely linked to inflammatory mediators such as cytokines and bradykinin (BK), which is generated when plasma components reach extravascular tissue.^[73,74] Recent studies have shown that HEXA inhibited inflammatory responses in the sub-acute phase by regulating cytokine production.^[75] Additionally, da Silva et al.^[10,22] showed that the anti-inflammatory effects of HEXA in a sub-chronic model of paw edema induced by CFA are partially due to the inhibition of inflammatory mediators such as IL-1 β , TNF- α , IL-6, and PGE₂. Therefore, it is suggested that the effect of HEXA on inflammatory hypernociception may be due to the inhibition of cytokines involved in the inflammatory pathway (Figure 3).

Both quercitrin and quercetin are flavonoids and their analgesic and anti-inflammatory activities are related, in part, to their NF- κ B inhibitory effects. However, it is reasonable to speculate that the inhibitory effect of flavonoids on COX-2

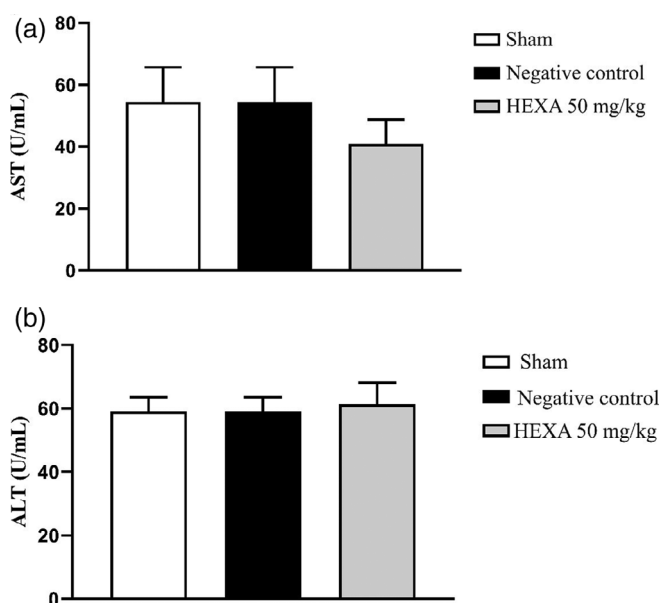


Figure 4. Effects of HEXA treatment on the activity of liver enzymes: (a) TGO and (b) TGP in the serum of mice 29 days after CFA-induced arthritis. Results are expressed as the mean $\pm$ SEM ($n = 6$ /group).

and COX-1 is responsible, at least in part, for their analgesic effect.^[76] Quercitrin is widely reported in the literature to be able to inhibit the NF- κ B pathway,^[51] and block the action of bradykinin (BK), a bioactive peptide that can sensitize nerve endings, increasing pain perception.^[77] In addition, it can also inhibit the action of COX-1, COX-2,^[52,78–80] and the production or action of PGE₂,^[10,22,50,52,81] the findings which corroborate our results.

To address potential toxicity following HEXA treatment, a biochemical evaluation of hepatotoxicity was carried out through the liver damage markers AST and ALT. It was observed that HEXA administration induced no changes in these parameters, thus showing no evident sign of toxicity (Figure 4a,b). This result corroborates the observation of Ballo et al.,^[75] who found no evidence of hepatotoxicity in mice treated with HEXA at 250 and

150 mg/kg in combination with *Terminalia macroptera* extract daily for 28 days.

4. Conclusion

This study indicates that HEXA can modulate the inflammatory and hyperalgesic responses triggered by CFA in a murine RA model without evident hepatotoxicity. These effects are potentially associated with reduced MPO activity, reduced edema development, and modulation of pro-inflammatory (TNF- α and IL-6) and regulatory (IL-10) activity. The present findings support the ethnopharmacological use of *X. americana* and indicate that this species has the potential to be used in the development of antiarthritic drugs.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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- [1] D. Khanna, G. Sethi, K. S. Ahn, M. K. Pandey, A. B. Kunnumakkara, B. Sung, A. Aggarwal, B. B. Aggarwal, *Curr. Opin. Pharmacol.* **2007**, *7*, 344–351.
- [2] A. F. Radu, S. G. Bungau, *Cells* **2021**, *10*, 2857.
- [3] F. Zhang, A. H. Jonsson, A. Nathan, N. Millard, M. Curtis, Q. Xiao, M. Gutierrez-Arcelus, W. Apruzzese, G. F. M. Watts, D. Weisenfeld, S. Nayar, J. Rangel-Moreno, N. Meednu, K. E. Marks, I. Mantel, J. B. Kang, L. Rumker, J. Mears, K. Slowikowski, K. Weinand, D. E. Orange, L. Geraldino-Pardilla, K. D. Deane, D. Tabechian, Accelerating Medicines Partnership: RA/SLE Network, S. Raychaudhuri, *Nature* **2023**, *623*, 616–624.
- [4] M. Zhang, W. Hu, C. Cai, Y. Wu, J. Li, S. Dong, *Mater. Today Bio.* **2022**, *14*, 100223.
- [5] M. Her, A. Kavanaugh, *Nat. Rev. Gastroenterol. Hepatol.* **2015**, *12*, 363–368.
- [6] R. B. Müller, J. von Kempis, S. R. Haile, M. H. Schiff, *Seminars in Arthritis and Rheumatism* **2015**, *45*, 28–34.
- [7] C. Faustino, L. Pinheiro, N. Duarte, *Life* **2023**, *13*, 1514.
- [8] S. H. Venkatesha, S. Dudics, B. Acharya, K. D. Moudgil, *Int. J. Mol. Sci.* **2015**, *16*, 887–906.
- [9] N. C. Mesa, I. A. Alves, F. M. P. Vilela, e D. M. Silva, L. A. P. Forero, D. M. A. Novoa, J. de Carvalho da Costa, *Food Res. Int.* **2023**, *170*, 113013.
- [10] B. A. F. da Silva, R. T. Pessoa, R. H. S. da Costa, M. R. C. de Oliveira, A. G. B. Ramos, M. G. de Lima Silva, L. Y. S. da Silva, C. R. Medeiros, S. G. L. Florencio, J. Ribeiro-Filho, H. D. M. Coutinho, A. Raposo, S. Yoo, H. Han, I. R. A. de Menezes, L. J. Quintans Júnior, *Biomed. Pharmacother.* **2023**, *166*, 115249.
- [11] J. S. S. Quintans, S. Shanmugam, L. Heimfarth, A. A. S. Araújo, J. R. G. Almeida, S. da, L. Picot, L. J. Quintans-Júnior, *Food Chem. Toxicol.* **2019**, *123*, 233–257.
- [12] N. A. Zeferino, S. A. Laurentino, N. B. De Barros, C. A. B. De Oliveira, T. S. Martins, *Brazilian J. Develop.* **2021**, *7*, 91805–91819.
- [13] G. R. Gandhi, M. T. S. L. Neta, R. G. Sathiyabama, J. d. S. S. Quintans, A. M. de Oliveira e Silva, A. A. d. S. Araújo, N. Narain, L. J. Q. Júnior, R. Q. Gurgel, *Phytomedicine* **2018**, *44*, 74–84.
- [14] N. Leyva-López, E. P. Gutierrez-Grijalva, D. L. Ambriz-Perez, J. Basilio Heredia, *Int. J. Mol. Sci.* **2016**, *17*, 921.
- [15] A. M. S. Carvalho, L. Heimfarth, K. A. Santos, A. G. Guimarães, L. Picot, J. R. G. S. Almeida, J. S. S. Quintans, L. J. Quintans-Júnior, *Phytomedicine* **2019**, *57*, 137–147.
- [16] M. Choudhary, V. Kumar, H. Malhotra, S. Singh, *J. Intercult. Ethnopharmacol.* **2015**, *4*, 147.
- [17] D. Sharma, P. Chaubey, V. Suvarna, *J. Food Biochem.* **2021**, *45*, <https://doi.org/10.1111/jfbc.13673>.
- [18] G. A. Kefelegn, B. Desta, *Sci. World J.* **2021**, *8*, 8880021, <https://doi.org/10.1155/2021/8880021>.
- [19] R. T. Pessoa, I. S. Alcântara, L. Y. S. da Silva, R. H. S. da Costa, T. M. Silva, C. D. de Moraes Oliveira-Tintino, A. G. B. Ramos, M. R. C. de Oliveira, A. O. B. P. B. Martins, B. C. G. V. de Lacerda, E. M. de Andrade, J. Ribeiro-Filho, C. M. Gonçalves Lima, H. D. M. Coutinho, I. R. A. de Menezes, *Analytica* **2023**, *4*, 141–158.
- [20] R. H. S. da Costa, A. O. B. P. B. Martins, M. R. C. de Oliveira, I. S. Alcântara, F. F. Ferreira, F. F. C. dos Santos, G. de Araújo Delmondes, F. A. B. da Cunha, H. D. M. Coutinho, I. R. A. de Menezes, *Biologia* **2022**, *77*, 1667–1674.
- [21] I. R. A. de Menezes, R. H. S. da Costa, A. Augusti Boligon, M. Rolón, C. Coronel, C. Vega, H. D. Melo Coutinho, M. S. da Costa, S. R. Tintino, R. L. Silva Pereira, T. R. de Albuquerque, J. R. G. da Silva Almeida, L. J. Quintans-Júnior, *Comparat. Immunol. Microbiol. Infect. Dis.* **2019**, *64*, 40–46.
- [22] B. A. F. da Silva, R. T. Pessoa, R. H. S. da Costa, M. R. C. de Oliveira, A. G. B. Ramos, M. G. de Lima Silva, L. Y. S. da Silva, C. R. Medeiros, S. G. L. Florencio, J. Ribeiro-Filho, H. D. M. Coutinho, A. Raposo, S. Yoo, H. Han, I. R. A. de Menezes, L. J. Quintans Júnior, *Biomed. Pharmacother.* **2023**, *166*, 115249.
- [23] W. B. Bakrim, A. D. R. Nurcahyanti, M. Dmirieh, I. Mahdi, A. M. Elgamal, M. A. El Raey, M. Wink, M. Sobeh, *Oxidat. Med. Cell. Longev.* **2022**, *2022*, 3486257, <https://doi.org/10.1155/2022/3486257>.
- [24] V. De Carvalho Nilo Bitu, V. De Carvalho Nilo Bitu, E. F. F. Matias, W. P. De Lima, A. Da Costa Portelo, H. D. M. Coutinho, I. R. A. De Menezes, *J. Ethnopharmacol.* **2015**, *172*, 265–272.

- [25] B. A. F. da Silva, R. H. S. da Costa, C. N. Fernandes, L. H. I. Leite, J. Ribeiro-Filho, T. R. Garcia, H. D. M. Coutinho, A. G. Wanderley, I. R. A. de Menezes, *Food Chem. Toxicol.* **2018**, *119*, 199–205.
- [26] S. D. Gaudie, D. S. McQueen, C. J. Clarke, I. P. Chessell, *J. Neurosci. Methods* **2004**, *139*, 281–291.
- [27] J. R. Ghilardi, K. T. Freeman, J. M. Jimenez-Andrade, K. A. Coughlin, M. J. Kaczmarzka, G. Castaneda-Corral, A. P. Bloom, M. A. Kuskowski, P. W. Mantyh, *Arthritis Rheum.* **2012**, *64*, 2223–2232.
- [28] L. J. Quintans-Júnior, S. R. Gandhi, F. R. S. Passos, L. Heimfarth, E. W. M. Pereira, B. S. Monteiro, K. S. dos Santos, M. C. Duarte, L. S. Abreu, Y. M. Nascimento, J. F. Tavares, M. S. Silva, I. R. A. Menezes, H. D. M. Coutinho, Á. A. N. Lima, G. Zengin, J. S. S. Quintans, *J. Ethnopharmacol.* **2020**, *258*, 112938.
- [29] T. M. Cunha, W. A. Verri, G. G. Vivancos, I. F. Moreira, S. Reis, C. A. Parada, F. Q. Cunha, S. H. Ferreira, *J. Med. Biol. Res.* **2004**, *37*, 401–407.
- [30] P. P. Bradley, R. D. Christensen, G. Rothstein, *Blood*, **60**, n.d., <https://ashpublications.org/blood/article-pdf/60/3/618/588739/618.pdf>.
- [31] S. S. Mizokami, N. S. Arakawa, S. R. Ambrosio, A. C. Zarpelon, R. Casagrande, T. M. Cunha, S. H. Ferreira, F. Q. Cunha, W. A. Verri, *J. Nat. Prod.* **2012**, *75*, 896–904.
- [32] M. Frank-Bertoncelj, K. Klein, S. Gay, *Epigenomics* **2017**, *9*, 493–504.
- [33] F. Pandolfi, L. Franza, V. Carusi, S. Altamura, G. Andriollo, E. Nucera, *Int. J. Mol. Sci.* **2020**, *21*, 5238.
- [34] T. Zhao, Y. Wei, Y. Zhu, Z. Xie, Q. Hai, Z. Li, D. Qin, *Front. Immunol.* **2022**, *13*, 1007165, <https://doi.org/10.3389/fimmu.2022.1007165>.
- [35] S. Dudics, D. Langan, R. R. Meka, S. H. Venkatesha, B. M. Berman, C. T. Che, K. D. Moudgil, *Int. J. Mol. Sci.* **2018**, *19*, 2508, <https://doi.org/10.3390/ijms19092508>.
- [36] M. Bilal, M. Qindeel, L. V. Nunes, M. T. S. Duarte, L. F. R. Ferreira, R. N. Soriano, H. M. N. Iqbal, *Marine Drugs* **2021**, *19*, 10, <https://doi.org/10.3390/md19010010>.
- [37] D. B. James, A. O. Owolabi, H. Ibiyeye, J. Magaji, & Y. A. Ikugiyi, *Afr. J. Biotechnol.* **2008**, *7*, 4274–4278, <https://doi.org/10.5897/AJB08.760>.
- [38] V. A. Maikai, P. I. Kobo, B. V. O. Maikai, *Afr. J. Biotechnol.* **2010**, *9*, 7744–7746.
- [39] K. E. S. D. Silva-Leite, A. M. S. Assreuy, L. F. Mendonça, L. E. A. Damasceno, M. G. R. De Queiroz, P. A. S. Mourão, A. F. Pires, M. G. Pereira, *Revista Brasileira de Farmacognosia* **2017**, *27*, 339–345.
- [40] B. Kumudhaveni, R. Radha, M. Thirumal, R. Vijayabharathi, *Ancient Sci. Life* **2024**, *38*, 101–110.
- [41] T. O. Olanrewaju, P. O. Odumosu, K. O. Eyong, *GSC Biol. Pharma. Sci.* **2019**, *2*, 108–117.
- [42] V. T. Uchôa, C. M. M. Sousa, A. A. Carvalho, A. E. G. Sant’Ana, M. H. Chaves, *J. Appl. Pharma. Sci.* **2016**, *6*, 091–096.
- [43] Y. L. Liu, H. M. Lin, R. Zou, J. C. Wu, R. Han, L. N. Raymond, P. F. Reid, Z. H. Qin, *Acta Pharmacol. Sin.* **2009**, *30*, 219–227.
- [44] J. M. Teixeira, H. B. Abdalla, R. T. Basting, B. D. Hammock, M. H. Napimoga, J. T. Clemente-Napimoga, *Int. Immunopharmacol.* **2020**, *87*, 106841, <https://doi.org/10.1016/j.intimp.2020.106841>.
- [45] S. Siebert, A. Tsoukas, J. Robertson, I. McInnes, *Pharmacol. Rev.* **2015**, *67*, 280–309.
- [46] M. Boshtam, S. Asgary, S. Kouhpayeh, L. Shariati, H. Khanahmad, *Inflammation* **2017**, *40*, 340–349.
- [47] X. Ma, S. Xu, *Biomed. Rep.* **2013**, *1*, 177–184.
- [48] A. Billiau, P. Matthys, *J. Leukocyte Biol.* **2001**, *70*, 849–860.
- [49] K. Kannan, R. A. Ortmann, D. Kimpel, *Pathophysiology* **2005**, *12*, 167–181.
- [50] M. Ding, J. Zhao, L. Bowman, Y. Lu, X. Shi, *Int. J. Oncol.* **2010**, *36*, 59–67, <https://doi.org/10.3892/ijo.00000475>.
- [51] J. Tang, P. Diao, X. Shu, L. Li, L. Xiong, *Biomed Res. Int.* **2019**, *2019*, 7039802, <https://doi.org/10.1155/2019/7039802>.
- [52] D. Camuesco, M. Comalada, M. E. Rodríguez-Cabezas, A. Nieto, M. D. Lorente, A. Concha, A. Zarzuelo, J. Gálvez, *Br. J. Pharmacol.* **2004**, *143*, 908–918.
- [53] W. Wang, W. Sun, L. Jin, *Int. Immunopharmacol.* **2017**, *48*, 61–66.
- [54] M. S. Cho, W. S. Park, W. K. Jung, Z. J. Qian, D. S. Lee, J. S. Choi, D. Y. Lee, S. G. Park, S. K. Seo, H. J. Kim, J. Y. Won, B. C. Yu, I. W. Choi, *Pharma. Biol.* **2014**, *52*, 926–932.
- [55] S. R. Kim, Y. R. Jung, D. H. Kim, H. J. An, M. K. Kim, N. D. Kim, H. Y. Chung, *Arch. Pharmacol. Res.* **2014**, *37*, 539–547.
- [56] Y. Seon Song, E.-H. Park, G. Min Hur, Y. Sue Ryu, Y. Sup Lee, J. Yeol Lee, Y. Man Kim, C. Jin, Caffeic acid phenethyl ester inhibits nitric oxide synthase gene expression and enzyme activity, n.d., www.elsevier.com/locate/canlet.
- [57] W. S. Yang, D. Jeong, Y. S. Yi, J. G. Park, H. Seo, S. H. Moh, S. Hong, J. Y. Cho, *Mediators Inflamm.* **2013**, *2013*, 518183, <https://doi.org/10.1155/2013/518183>.
- [58] M. Zhang, J. Zhou, L. Wang, B. Li, J. Guo, X. Guan, Q. Han, H. Zhang, *Biol. Pharm. Bull.* **2014**, *37*, 347–354.
- [59] D. Zielińska, H. Zieliński, J. M. Laparra-Llopis, D. Szawara-Nowak, J. Honke, J. A. Giménez-Bastida, *Nutrients* **2021**, *13*, 1–15.
- [60] F. Bazzoni, N. Tamassia, M. Rossato, M. A. Cassatella, *Eur. J. Immunol.* **2010**, *40*, 2360–2368.
- [61] M. Luiz, B. Júnior, R. D. Lopes, M. Cerqueira, L. Seelaender, A. C. Lopes, Anti-inflammatory Effect of Physical Training in Heart Failure: Role of TNF- α and IL-10, n.d.
- [62] K. Yamaoka, T. Otsuka, H. Niino, H. Nakashima, Y. Tanaka, S. Nagano, E. Ogami, Y. Niho, N. Hamasaki, & K. Izuhara, *J. Interferon Cytokine Res.* **2004**, *19*, 629–637.
- [63] C. N. Ginting, I. N. E. Lister, E. Girsang, M. S. Mutia, Y. E. P. Lubis, A. Amalia, R. Rizal, W. Widowati, *J. Phys.: Conf. Ser.* **2019**, *1374*, 012033.
- [64] N. Nozaki, S. Yamaguchi, M. Yamaoka, M. Okuyama, H. Nakamura, H. Tomoike, *J. Mol. Cell. Cardiol.* **2003**, *30*, 2003–2012.
- [65] A. R. Abdollahi, F. Firouzian, R. Haddadi, A. Nourian, *Inflammopharmacology* **2021**, *29*, 107–121.
- [66] A. O. Ademosun, G. Oboh, F. Bello, P. O. Ayeni, *J. Evidence-Based Complement. Alternat. Med.* **2016**, *21*, NP11–NP17.
- [67] M. Kobori, Y. Takahashi, Y. Akimoto, M. Sakurai, I. Matsunaga, H. Nishimuro, K. Ippoushi, H. Oike, M. Ohnishi-Kameyama, *J. Function. Foods* **2015**, *15*, 551–560.
- [68] M. De Barros, L. Mota Da Silva, T. Boeing, L. B. Somensi, B. J. Cury, L. De Moura Burci, J. R. Santin, S. F. De Andrade, F. D. Monache, V. Cechinel-Filho, *Naunyn-Schmiedeberg’s Arch. Pharmacol.* **2016**, *389*, 403–417.
- [69] C. m. Yu, Y. Wang, S. c. Ren, Z. I. Liu, C. I. Zhu, Q. Liu, H. r. Li, C. y. Sun, X. y. Sun, J. Xie, J. f. Wang, X. m. Deng, *Int. Immunopharmacol.* **2023**, *125*, <https://doi.org/10.1016/j.intimp.2023.111143>.
- [70] S. Misra, A. M. A. Ikbal, D. Bhattacharjee, M. Hore, S. Mishra, S. Karmakar, A. Ghosh, R. Srinivas, A. Das, S. Agarwal, K. D. Saha, P. Bhardwaj, I. B. Ubhadia, P. Ghosh, S. De, O. N. Tiwari, D. Chattopadhyay, P. Palit, *J. Food Biochem.* **2022**, *46*, e14487.
- [71] V. F. A. M. Derksen, C. F. Allaart, A. H. M. Van Der Helm-Van Mil, T. W. J. Huizinga, R. E. M. Toes, D. Van Der Woude, *Rheumatology (United Kingdom)* **2023**, *62*, 407–416.
- [72] T. Martinov, M. Mack, A. Sykes, D. Chatterjea, *J. Visual. Exp.: JoVE* **2013**, *82*, 512212, <https://doi.org/10.3791/51212>.
- [73] S. H. Ferreira, B. B. Lorenzetti, S. Poole, *Br. J. Pharmacol.* **1993**, *110*, 1227–1231.
- [74] B. B. Garrido-Suárez, G. Garrido, A. B. Menéndez, N. Merino, O. Valdés, N. de la Paz, A. Romero, L. Delgado, M. D. Fernández, O. Piñeros, R. Delgado-Hernández, *J. Integrat. Med.* **2023**, *21*, 34–46.
- [75] M. Ballo, F. L. Da, S. Bah, R. Sanogo, E. N. H. Youl, *Biomed. Pharmacol. J.* **2023**, *16*, 1081–1091.
- [76] C. R. Ferraz, T. T. Carvalho, M. F. Manchope, N. A. Artero, F. S. Rasquel-Oliveira, V. Fattori, R. Casagrande, W. A. Verri, *Molecules* **2020**, *25*, 762.
- [77] B. -H. Kim, I. J. Lee, H. -Y. Lee, B. Y. Hwang, S. -B. Han, & Y. Kim, *Nitric Oxide* **2007**, *17*, 134–142.
- [78] J. A. Manthey, *Microcirculation* **2000**, *7*, S29–S34, <https://doi.org/10.1080/mic.7.S1.S29.S34>.
- [79] P. Rathee, H. Chaudhary, S. Rathee, D. Rathee, V. Kumar, K. Kohli, *Inflamm. Allerg. Drug Targets* **2009**, *8*, 229–235.
- [80] A. E. Rotelli, T. Guardia, A. O. Juárez, N. E. De La Rocha, L. E. Pelzer, *Pharmacol. Res.* **2003**, *48*, 601–606.
- [81] L. W. Chen, W. C. Ko, *Med. Drug Disc.* **2021**, *12*, 100106.

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