Food and Chemical Toxicology 124 (2019) 300–315

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Alpha-mangostin: Anti-inflammatory and antioxidant effects on established collagen-induced arthritis in DBA/1J mice

Diana Rocio Herrera-Aco[a], Omar Noel Medina-Campos[b], José Pedraza-Chaverri[b], Edda Sciutto-Conde[c], Gabriela Rosas-Salgado[d], Gladis Fragoso-González[c,*]

[a] Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Ciudad Universitaria s/n, Ciudad de México, 04650, Mexico
[b] Facultad de Química, Universidad Nacional Autónoma de México, Ciudad Universitaria s/n, Ciudad de México, 04650, Mexico
[c] Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Ciudad Universitaria s/n, Ciudad de México, 04650, Mexico
[d] Facultad de Medicina, Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, 62350, Mexico

ARTICLE INFO

Keywords:
α-mangostin
Inflammation
Oxidative stress
Collagen-induced arthritis
Rheumatoid arthritis

ABSTRACT

Rheumatoid arthritis (RA) is an autoimmune disease that causes physical disability in people worldwide. Despite progress made in RA treatment in the past decade, new drugs with high efficacy but few long-term adverse effects are still needed.

This study focused on evaluating the therapeutic potential of α-mangostin on established collagen-induced arthritis (CIA) in DBA/1J mice. Arthritic DBA/1J mice were orally administered with two doses of α-mangostin (10 and 40 mg/kg) daily, for 33 days. Alpha-mangostin significantly decreased the clinical score in the short term at both doses and decreased the histopathological score at the higher dose. This improvement was accompanied by a reduction on serum levels of anti-collagen IgG2a autoantibodies and of the production of LIX/CXCL5, IP-10/CXCL10, MIG/CXCL9, RANTES/CCL5, IL-6 and IL-33 in the joints of CIA mice. Alpha-mangostin also exhibited an anti-oxidant effect decreasing the NADPH oxidase activity and lipid peroxidation and preserving the levels of reduced glutathione in the arthritic joints. In vitro this xanthone demonstrated modulatory properties on LPS-activated dendritic cells, although in Th1 and Th17-polarized lymphocytes promotes a pro-apoptotic phenotype.

Altogether this study illustrates the capacity of α-mangostin to ameliorate the early clinical and histological signs of established CIA by reducing the inflammatory and oxidative responses.

1. Introduction

Alpha-mangostin is one of the major xanthones isolated from mangosteen (*Garcinia mangostana* Linn), a tropical tree that grows in the southeast of Asia. Different parts of mangosteen have been used in the traditional medicine to treat several health conditions such as infected wounds, diarrhea, abdominal pain, dysentery and fever (Pedraza-Chaverri et al., 2008; Jang et al., 2012; Lee et al., 2013). This xanthone has shown to possess diverse biological activities, i.e. antineoplastic (Shan et al., 2014), antioxidant (Márquez-Valadez et al., 2009), antibacterial (Pedraza-Chaverri et al., 2008; Al-Massarani et al., 2013), anti-inflammatory (Pedraza-Chaverri et al., 2008; Jang et al., 2012; Lee et al., 2013), cardioprotector (Sampath and Vijayaraghavan, 2007), antidiabetic (Nelli et al., 2013), antimycotic (Ibrahim et al., 2016) and antiparasitic (Ibrahim et al., 2016). Regarding its antioxidant properties, this xanthone has proved to diminish protein oxidation and

Abbreviations: 7-AAD, 7-Amino-Actinomycin D; CAT, catalase; CFA, complete Freund's adjuvant; CIA, collagen-induced arthritis; COX-2, cyclooxygenase-2; DMSO, dimethyl sulfoxide; EU, endotoxin units; Foxp3, forkhead box P3; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GST, glutathione-S-transferase; H_2O_2, hydrogen peroxide; HE, hematoxylin-eosin; IFA, incomplete Freund adjuvant; IFN-γ, interferon gamma; IL, interleukin; iNOS, induced nitric oxide synthase; IP-10, interferon gamma-induced protein 10; LIX, LPS-inducible CXC chemokine; LPS, lipopolysaccharide; mBMDCs, murine bone marrow-derived dendritic cells; MCP-1, monocyte chemoattractant protein-1; MDA, malondialdehyde; MIG, monokine induced by gamma interferon; MIP-1α, macrophage inflammatory protein 1 alpha; MIP-1β, macrophage inflammatory protein 1 beta; MT, Masson trichrome; NF-κB, nuclear transcription factor κB; NO, nitric oxide; NOX, nicotinamide-adenine-dinucleotide phosphate oxidase; O_2^-, superoxide anion; PD-L1, programmed death-ligand 1; PGE_2, prostaglandin E_2; RA, rheumatoid arthritis; RANTES, regulated on activation normal T expressed and secreted; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; TGF-β, transforming growth factor β; TNF-α, tumor necrosis factor- α; Treg, regulatory T cell

* Corresponding author.
E-mail address: gladis@unam.mx (G. Fragoso-González).

https://doi.org/10.1016/j.fct.2018.12.018
Received 10 August 2018; Received in revised form 23 November 2018; Accepted 13 December 2018
Available online 14 December 2018
0278-6915/

lipid peroxidation both *in vitro* and *in vivo*. Moreover, α-mangostin has the capacity to preserve the activity of several antioxidant enzymes (glutathione-S-transferase (GST), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) and one of the main intracellular antioxidant systems, reduced glutathione (GSH) in some animal models of disease (Sampath and Vijayaraghavan, 2007). Concerning the anti-inflammatory properties of α-mangostin, its oral and intraperitoneal administration (50 mg/kg) has shown to exhibit anti-inflammatory activity in rats with hind paw edema induced by carrageenan administration, cotton pellet-induced granuloma and granuloma with bag techniques (Gopalakrishnan et al., 1980). In addition to the preceding, α-mangostin has demonstrated to inhibit the primary and secondary responses of arthritis induced by complete Freund's adjuvant (CFA) administration (Gopalakrishnan et al., 1980). α-mangostin has also proved to be a blocking agent of histaminergic and serotonergic receptors (Chairungsrilerd et al., 1996) and an inhibitor of several mediators of inflammation such as nitric oxide (NO), prostaglandin E_2 (PGE_2), induced nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), tumor necrosis factor- α (TNF-α), interleukin (IL)-4 (Chen et al., 2008) and human lipoxygenase-12 (LOX-12) (Deschamps et al., 2007).

Rheumatoid arthritis is an autoimmune disease which represents about 1% of world's population (Iwamoto et al., 2008). The age of onset is between 40 and 50 years although it can occur at all ages (Alamanos and Drosos, 2005). The etiology of RA involves a combination of genetic and environmental factors. The latter ones comprise infectious processes and smoking which result in the development of inflammatory processes that cause damage to the joint tissue, in part due to the generation of oxidative stress (Kobayashi et al., 2008). The pathology begins with a significant inflammatory process, mainly in the peripheral synovial joints. This process becomes chronic and over time can provoke structural damage to the cartilage, bones and ligaments (Goronzy and Weyand, 2005). Regarding the inflammatory cells which play an essential role in the RA, the major players are the T helper lymphocytes (Th1 and Th17). Both types of lymphocytes are producers of cytokines which perpetuate and exacerbate the inflammatory joint process and are mostly responsible for the increased production of metalloproteinases and the recruitment and activation of cells of the innate immune system (macrophages and dendritic cells) as well as the activation of cells at the local level (type B synoviocytes and osteoclasts) (Smith and Haynes, 2002; Lubberts, 2010; Li et al., 2011). In addition to this, some of the cytokines produced in RA, such as TNF-α can cause the activation of the enzyme nicotinamide-adenine-dinucleotide phosphate oxidase (NOX) which is involved in the production of reactive oxygen species (ROS). ROS are responsible in conjunction with reactive nitrogen species (RNS) for the development of oxidative stress in RA and the depletion of GSH that occurs at the intracellular level. This latter event perpetuates inflammation by inducing activation of the nuclear transcription factor κB (NF-κB) (Filippin et al., 2008). Different animal models have been used to study this disease; however, one of most commonly employed at present is collagen-induced arthritis because this model resembles several aspects that occur in human RA (Vincent et al., 2012).

To date, a cure for this disease has not been found, and treatment is limited to reduce the symptomatology of RA. Besides, most of the currently available treatments have several disadvantages, such as the generation of long-term adverse effects or their high cost (Mijnheer et al., 2013). For this reason, there is a need to find new anti-inflammatory molecules that exhibit minimal adverse effects.

In the present study, the effectiveness of α-mangostin to reduce the clinical signs and the inflammatory and oxidant processes in DBA/1J mice with established CIA was evaluated. Mechanisms underlying this efficacy are further explored.

2. Materials and Methods

2.1. Reagents

RPMI-1640 medium, L-glutamine, β-mercaptoethanol and fetal bovine serum (FBS) were purchased from Gibco (Gaithersburg, MD, USA). Gentamicin was obtained from Tornel (Naucalpan, MEX, Mexico). HEPES was purchased from Promega (Madison, WI, USA). Recombinant murine granulocyte macrophage colony-stimulating factor (rmGM-CSF) was obtained from Peprotech (Rocky Hill, NJ, USA). Lipopolysaccharide (LPS), dimethyl sulfoxide (DMSO), formaldehyde, CFA, incomplete Freund adjuvant (IFA) and all reagents used to determine oxidative stress and the activity of antioxidant enzymes were purchased from Sigma-Aldrich Chemical (St. Louis, MO, USA). Ethylenediamine tetra-acetic acid (EDTA) was obtained from Spectrum (New Brunswick, NJ, USA). Bovine type II collagen solution was obtained from Chondrex (Redmond, WA, USA). Methotrexate was purchased from Teva (North Wales, PA, USA). All the antibodies used for flow cytometry in mBMDCs cultures, Annexin V, anti-CD3 antibodies and recombinant cytokines IL-6, IL-1β, TNF-α, IL-2, IL-12 and transforming growth factor β (TGF-β) were purchased from Biolegend (San Diego, CA, USA). 7-Amino-Actinomycin D (7-AAD) and anti−IFN−γ, anti-IL-4 and anti-CD28 antibodies were obtained from BD Pharmingen (San Diego, CA, USA). The recombinant cytokine IL-23 was purchased from R & D Systems (Minneapolis, MN, USA). All the antibodies used for flow cytometry in Th1 and Th17 cultures were purchased from eBioscience (San Diego, CA, USA).

2.2. Extraction and isolation of α-mangostin

α-mangostin was obtained from the pericarp of *Garcinia mangostana* as previously described (Pedraza-Chaverrí et al., 2009). Briefly, the dried pericarp of *G. mangostana* was extracted by maceration with CH_2Cl_2−MeOH (1:1) at room temperature for 3 weeks. After filtration and evaporation of the solvent under reduced pressure, the combined crude organic extract was obtained. The extract was subjected to chromatography over a silica gel column and eluted with hexane/CH_2Cl_2 (1:1) to obtain seven primary fractions (F1−F7). α-mangostin crystallized spontaneously from fraction F7 as a major component, as a yellow solid: melting point 179–180 °C; UV (MeOH) λ_{max} 320 (3.5) nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H (ppm) 13.8, 6.8, 6.25, 5.25, 4.10, 3.8, 3.40, 1.82, 1.79 and 1.65, which was consistent with previously reported data (Ee et al., 2006; Ji et al., 2007).

2.3. Animals

Male DBA/1J, BALB/c and C57BL/6 mice of 7–8 weeks of age were obtained from Biomedical Research Institute. Five mice were housed in each cage and were kept under standard conditions with a controlled temperature at 23 °C and a 12-h light/dark cycle. Animals were fed with standard laboratory chow and water *ad libitum*. All experiments followed the guidelines in the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and experimental protocols were reviewed and approved by the Ethical Committee for the Care and Use of Laboratory Animals (Protocol Number 186) at the Biomedical Research Institute at the Universidad Nacional Autónoma de México (UNAM).

2.4. Endotoxin assay

The chromogenic Limulus Amebocyte Lysate (LAL) assay from Charles River (San Diego, CA, USA) was used according to the supplier's instructions to determine the endotoxin level of the α-mangostin. Briefly, this test consisted of mixing 100 µl of the LAL reagent containing a chromophore (*para* nitroaniline, pNA) with 100 µl of 1 mg/ml α-mangostin stock solution. The reaction was incubated at 37 °C for 1 h,

and readings were done at 405 nm (every minute for 1 h) in a spectrophotometer (Biotek Synergy HT, Winoosky, VT, USA). The results of each reading were compared against a standard endotoxin curve and expressed as EU/ml.

2.5. Experimental groups

Five experimental groups of 6 mice each were established: the control group (without CIA), the CIA group plus vehicle (corn oil where α-mangostin was diluted), the CIA group plus methotrexate at 0.5 mg/kg and the CIA group plus α-mangostin at two different doses selected according to previous reports using α-mangostin as an anti-inflammatory molecule (10 and 40 mg/kg) (Gopalakrishnan et al., 1980; Chen et al., 2008; Nguemfo et al., 2009; Jang et al., 2012; Nava Catorce et al., 2016). Treatments were initiated from day 35, once the signs of arthritis were evident in mice (redness and/or swelling in digits or other parts of the paws), and were given orally daily by an orogastric catheter in a final volume of 100 µl per mouse. Treatments were administered for 33 days.

2.6. Induction of collagen-induced arthritis

Induction of CIA was performed according to the protocol provided by Chondrex, Inc. with minor modifications. Briefly, 0.1 ml emulsion of bovine type II collagen solution (2 mg/ml in 0.05 M acetic acid) and CFA containing 1 mg/ml *Mycobacterium tuberculosis* was injected subcutaneously at the base of the tail. A booster injection with 60 µl emulsion of collagen and IFA was administered at 1.5 cm from the base of the tail on day 21.

2.7. Clinical assessment of arthritis

To determine the severity of arthritis, visual inspections of the animals were conducted by two investigators blinded to the arthritis treatment three times per week from day 21 counting from the first immunization, which allowed to establish clinical severity and joint inflammation based on the degree of affection of the paw joints according to a previously described subjective scale (Cho et al., 2009). Briefly, the degree of edema and inflammation presented by each of the four limbs is individually registered and assigned a numeric value: 0 = no edema or swelling, 1 = slight edema and erythema limited to the foot or ankle, 2 = slight edema and erythema from the ankle to the tarsal bone, 3 = moderate edema and erythema from the ankle to the tarsal bone, and 4 = edema and erythema from the ankle to the entire leg. The arthritic score for each mouse was expressed as the sum of the scores of four limbs. The maximum clinical score obtained by each mouse was 16 points. The mean arthritis index for each experimental group was determined by summing the scores of each mouse and dividing the result by the number of mice in the group.

2.8. Histopathological assessment of arthritis

A hind limb of each mouse was fixed with 10% formalin for 72 h, decalcified with a solution of 10% EDTA for 7 days and embedded in paraffin. Hematoxylin-eosin (HE) and Masson Trichrome (MT) stainings were performed to characterize the inflammatory phenomenon as well as the destruction of articular cartilage. The degree of inflammation and damage to the cartilage present in the joint was evaluated, according to the following criteria, previously reported (Camps et al., 2005): 0 = no inflammation, 1 = slight thickening of the lining layer or some infiltrating cells in the sublining layer, 2 = slight thickening of the lining layer plus some infiltrating cells in the sublining layer, 3 = thickening of the lining layer, influx of cells in the sublining layer, and presence of cells in the synovial space, and 4 = synovium highly infiltrated with many inflammatory cells.

2.9. Measurement of IgG subtypes

A blood sample of all experimental mice was obtained through orbital sinus puncture, and the sera were separated by centrifugation at 3500 × *g* for 10 min and stored at −70 °C until processing. The presence of anti-collagen II IgG1 and IgG2a antibodies was determined using the Mouse Anti-Type II ELISA Collagen IgG Subtype Assay Kit with TMB (Chondrex, Inc.) according to the instructions described by the manufacturer.

2.10. Preparation of tissue homogenates

The kidneys, liver and joints were obtained and stored at −70 °C until processing. Liver and kidney samples were homogenized in a polytron for 10 s in a cold phosphate buffer (50 mM, pH 7.4) in a 1:10 w/v ratio, while the joint samples were pulverized with liquid nitrogen using a mortar. For the latter, one part was homogenized in a cold phosphate buffer (50 mM, pH 7.4) in a 1:5 w/v ratio and the other was homogenized in a cold lysis buffer (20 mM Tris base pH 7.4, 250 mM NaCl, 2 mM EDTA pH 7.4, 1% Triton X-100 and 10% glycerol) containing 1 mM PMSF in a 1:4 w/v ratio. The homogenates were centrifuged at 10,000 × *g* at 4 °C for 30 min, and the supernatants were obtained to quantify the total proteins according to a previously described method (Lowry et al., 1951). Finally, the homogenates in cold phosphate buffer were used to determine the activity of the antioxidant enzymes [CAT, glutathione reductase (GR), GPx, GST and SOD], GSH content and to perform measurement of oxidative stress markers (lipid peroxidation, oxidized proteins and NOX activity). The homogenates in cold lysis buffer containing 1 mM PMSF were used to determine the cytokines and chemokines present in the joints.

2.11. Measurement of prostaglandin E_2 (PGE_2) in serum and joints

PGE_2 levels were determined in serum and joints of the different experimental groups using a Prostaglandin E_2 Express EIA Kit (Cayman Chemical, Ann Arbor, MI, USA). The assay was performed according to the supplier's instructions, and the results were expressed in ng of PGE_2/ml serum and ng of PGE_2/mg protein in the joint homogenates.

2.12. Measurement of cytokines and chemokines in joints

2.12.1. Determination of cytokines and chemokines in joint homogenates

EMD Millipore's MILLIPLEX MAP Mouse Cytokine/Chemokine Magnetic Bead Panel (Merck Millipore, Burlington, MA, USA) was employed according to the supplier's instructions to determine the cytokines and chemokines present in joint homogenates of each experimental group. The Luminex analyzer (MAGPIX) (EMD Millipore) and Luminex xPONENT acquisition software were used to analyze the data obtained using 5-parameter logistic regression. The IL-1β and IL-18 levels present in the joints were determined using the Mouse IL-1β ELISA MAX Standard Kit (Biolegend) and the Mouse IL-18 Platinum ELISA Kit (eBioscience) according to the instructions provided by the manufacturer.

2.12.2. Immunohistochemistry

Briefly, to evaluate IL-33 expression in joint tissues, 3 µm tissue sections were blocked with 4% goat serum and 1% bovine serum albumin (BSA) plus 0.1% Triton X-100 in PBS for 1 h at 37 °C. After blocking, the tissues were stained with rabbit anti-mouse IL-33 antibody (1:100 dilution) (Santa Cruz Biotechnology, Dallas, Texas, USA) overnight at 4 °C. The next day, the samples were incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (1:100 dilution) (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA) for 1 h at 37 °C followed by incubation with 3,3′-diaminobenzidine (DAB) and counterstained with hematoxylin. To determine the area of expression of IL-33 in the joint tissues, each tissue was evaluated

with an Olympus BX-500 microscope and analyzed with Infinity Software v 6.3.0.

2.13. Determination of in vivo oxidative stress and antioxidant activity

2.13.1. Determination of GPx, GR and GST activity

The activity of GPx was measured indirectly following a previously described method (Lawrence and Burk, 1976). This method is based on the decrease in absorbance at 340 nm due to the disappearance of NADPH, which is used by the GR to regenerate the GSH employed by the GPx to reduce hydroperoxides. The results were expressed in U/mg protein, where 1 U is the amount of enzyme required to oxidize 1 μmol of NADPH/min.

The activity of GR was determined using a previously reported assay (Carlberg and Mannervik, 1975). This method consists of monitoring the disappearance of NADPH at 340 nm, which is used as a cofactor by the GR to generate GSH from glutathione oxidized (GSSG). The results were expressed in U/mg protein where 1 U of the enzyme is equal to 1 μmol of oxidized NADPH/min.

The activity of GST was obtained using a previously described method (Habig et al., 1974). In this method, GST catalyzes the conjugation of GSH with the 1-chloro-2,4-dinitrobenzene (CDNB) and the final adduct formed absorbs at 340 nm. The results were expressed as μmoles of GSH-CDNB conjugate formed/min/mg protein using an extinction coefficient of 9.6 mM^{-1} cm^{-1}.

2.13.2. Determination of CAT and SOD activity

Catalase activity was assessed following a previous methodology (Aebi, 1984). The fundament of this method is based on the decrease in the absorbance of hydrogen peroxide (H_2O_2) due to its degradation by the enzyme present in the sample. The degradation of H_2O_2 is monitored at 240 nm every 15 s for 30 s. The results were expressed as *k*/mg protein where *k* is used as the unit of catalase activity.

The activity of SOD was measured by the ability of this enzyme to avoid the reduction of nitro blue tetrazolium (NBT) according to a previously described assay (Oberley and Spitz, 1984). Briefly, the xanthine-xanthine oxidase system is used to generate superoxide anions ($O_2^{\cdot-}$), which reduce the NBT to formazan that is detected at 560 nm. If the SOD is present in the sample, the generated $O_2^{\cdot-}$ is transformed into H_2O_2 and this avoids the formazan generation. The results were expressed as units of SOD/mg protein where 1 unit of the enzyme is defined as the quantity of SOD required to produce 50% inhibition of NBT reduction.

2.13.3. Determination of NOX activity

To evaluate the NOX activity, a chemiluminescence (CL) technique was used. Reaction mixture (final volume 200 μl) contained 75 mM phosphate buffer (pH 7.4), 10 mM ethylene glycol tetraacetic acid (EGTA), 300 mM sucrose, 20 mM lucigenin and 2 mM NADPH. The reaction was started by adding 20 μl of the sample of the supernatants of the homogenates of the different tissues presumably containing NOX. After the reaction, ROS were generated by NADPH oxidase, which reacted with lucigenin that captures the high energy electrons. The luminescence generated by lucigenin was directly proportional to the activity of NOX, and the maximum activity was obtained 15 min after the start of the reaction. The results were expressed in U of CL (mV.s).

2.13.4. Determination of GSH

To estimate the GSH level present in different tissues, a previously described fluorimetric method was used (Fernández-Checa and Kaplowitz, 1990). This assay is based in the formation of a stable, fluorescent adduct of monochlorobimane (itself nonfluorescent) with GSH in a reaction catalyzed by GST. The fluorescence is measured at excitation wavelength of 385 nm and an emission wavelength of 478 nm and the results were expressed in μmol/mg protein.

2.13.5. Estimation of lipid peroxidation

Measurement of the malondialdehyde (MDA) levels (an end product of lipid peroxidation) expressed as nmol/mg protein in different tissues was determined using a previously described colorimetric assay (Gérard-Monnier et al., 1998). This test is based on the detection of a colorful compound formed between MDA and 1-methyl-2-phenylindole in acid medium, which is detected at 586 nm.

2.13.6. Estimation of oxidized proteins

To evaluate the oxidized proteins, a previously described colorimetric method was employed (Reznick and Packer, 1994). This assay is based on the reactivity of the carbonyl groups originated by the action of ROS on proteins with 2,4-dinitrophenylhydrazine (DNPH) to form a protein-hydrazone complex which absorbs at 370 nm. Protein carbonyl contents were calculated using an absorption coefficient of 22 000 $M^{-1}cm^{-1}$. The results were expressed as nmol of carbonyls/mg protein.

2.14. Bone marrow-derived dendritic cells culture

Murine bone marrow-derived dendritic cells (mBMDCs) were generated from the bone marrow cells of the tibia and femur of 7–8 week old male BALB/c mice following the procedures previously described, with minor modifications (Lutz et al., 1999). Briefly, at day 0, 6×10^6 bone marrow cells were cultured in 60 mm Petri dishes containing 6 ml RPMI-1640 medium supplemented with 2 mM L-glutamine, 50 μM β-mercaptoethanol, 5% heat-inactivated FBS, gentamicin (20 μg/ml) and 10 mM HEPES. Bone marrow cells were differentiated to mBMDCs by the addition of rmGM-CSF (20 ng/ml) to the culture medium for 6 days. At day 6 all medium was removed, and mBMDCs were fed with 3 ml culture medium containing rmGM-CSF (4 ng/ml). Additionally, mBMDCs were stimulated with LPS (100 ng/ml) and different doses of α-mangostin dissolved in DMSO (0.5, 1, 3, 5, 7 and 10 μg/ml) for 4 or 24 h. After 4 or 24 h of stimulation with LPS and α-mangostin, the cells were harvested and evaluated by flow cytometry using a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) after staining cells with FITC-CD11c, PE-CD86, PerCpCy5.5-CD80, APC-CD40, PE-programmed death-ligand 1 (PD-L1) and Biotin-IA/IE antibodies. Finally, the cells were analyzed using the FCS Express software. Cell culture supernatants of each condition were obtained and stored at −70 °C to measure soluble factors.

2.15. Th1 and Th17 polarization and stimulation

Spleen cells were obtained from C57BL/6J mice and erythrocytes were lysed. The remaining cells (splenocytes) were placed in a ratio of 2×10^6 lymphocytes per well in commercial 24-well plates, and anti-CD3 antibody (5 μg/ml) and anti-CD28 antibody (1 μg/ml) were added to complete their activation. Cells were maintained in culture for 6 days with a mixture of cytokines that favored differentiation into Th17 or Th1 cells. For Th17 cells-polarizing conditions, splenocytes were stimulated with TGF-β (5 ng/ml), IL-6 (20 ng/ml), IL-23 (10 ng/ml), IL-1β (5 ng/ml) and TNF-α (5 ng/ml) as well as anti−IFN−γ (10 μg/ml) and anti-IL-4 (10 μg/ml) antibodies to avoid their polarization towards Th1 and Th2, respectively. For Th1 cells-polarizing conditions, splenocytes were stimulated with IL-12 (10 ng/ml) and IL-2 (10 ng/ml) as well as anti-IL-4 antibody (10 μg/ml) to avoid their polarization towards Th2. The effect of different concentrations of α-mangostin (0.5, 1, 3 and 5 μg/ml) on cultures of cells polarized towards Th17 or Th1 was evaluated by culturing the cells under polarizing conditions towards Th17 or Th1 concurrently with the different concentrations of α-mangostin during 6 days. Six days post-culture the cells were treated for 6 h with Cell Activation Cocktail with brefeldin A (Biolegend). After this time, the cells were harvested, and the percentages of $CD4^+CD25^{high}Foxp3^+$ and $CD4^+$ $IFN\text{-}\gamma^+$ cells were evaluated by flow cytometry using a FACSCalibur flow cytometer and FCS Express software in the Th1-

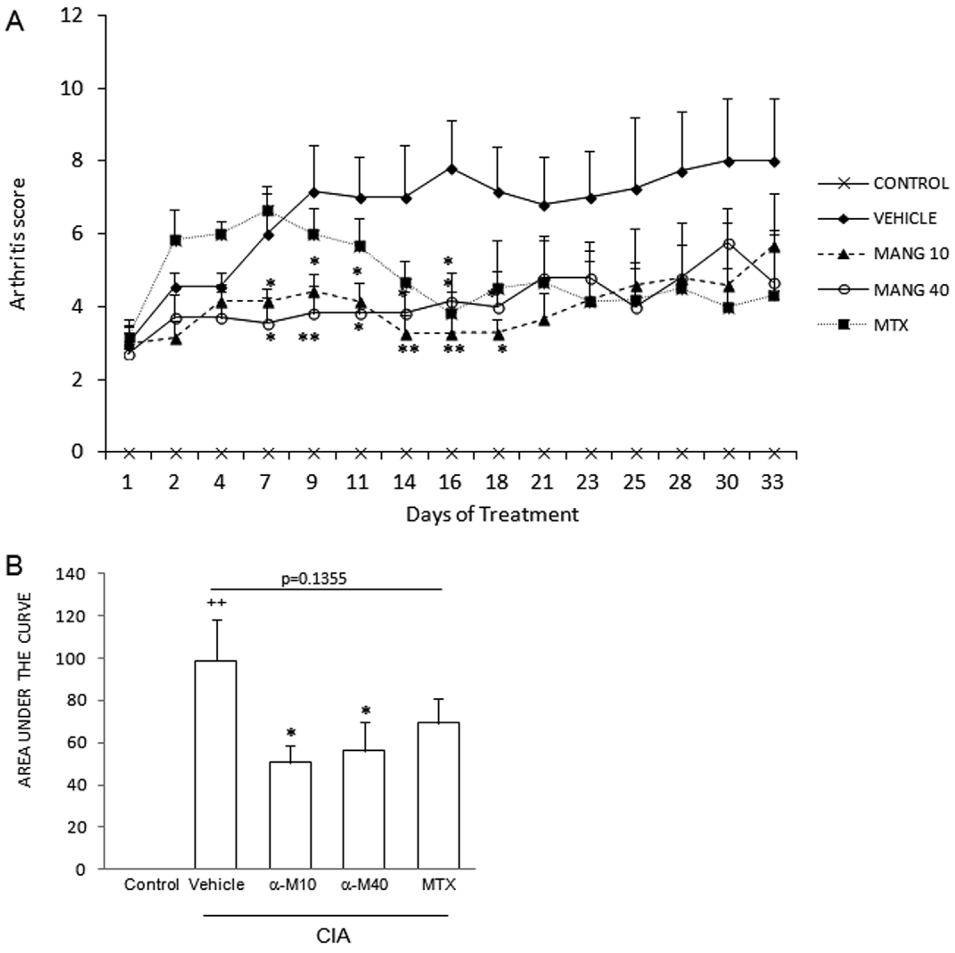

Fig. 1. Therapeutic effects of α-mangostin on arthritis score from day 1–33 of treatment after CIA was established (A). Area under the curve of each experimental group that represents the overall behavior of each group during 33 days of treatment (B). Control, non-arthritic mice; Vehicle, arthritic mice treated with vehicle (corn oil); Mang 10, arthritic mice treated with 10 mg/kg of α-mangostin; Mang 40, arthritic mice treated with 40 mg/kg of α-mangostin; MTX, arthritic mice treated with methotrexate. Data are represented as mean ± SEM. n = 6. Group curve is significantly different $^{++}p < 0.01$ versus control group; *p < 0.05, **p < 0.01 versus vehicle group (by ANOVA, Tukey test and LS means contrast test).

polarized cultures. The percentages of $CD4^+CD25^{high}Foxp3^+$ and $CD4^+$ IL-17^+ cells were evaluated in the Th17-polarized cultures. Supernatants from each experimental condition were obtained and stored at −70 °C until used.

2.16. Cell viability

Cell viability was measured using the Annexin V/7-AAD Apoptosis Assay. Briefly, mBMDCs and Th1 and Th17 cells of each experimental group were harvested and 1×10^6 cells/ml were resuspended in 1X Binding Buffer (10 mM HEPES/NaOH pH 7.4, 140 mM NaCl and 2.5 mM $CaCl_2$). Subsequently, 100 µl of this solution (1×10^5 cells) were stained with FITC Annexin V and 7-AAD. The cells were incubated for 15 min at RT (25 °C) in the dark and analyzed by flow cytometry within 1 h.

2.17. Analysis of secreted inflammatory mediators

The cytokine levels of TNF-α, IL-6, IL-12 (p70), IFN-γ and IL-10 were measured in the supernatants of cultured dendritic using ELISA MAX™ Standard Sets kits (Biolegend) according to the provider's instructions. The Mouse TGF-β 1 ELISA Ready-SET-Go! ® (eBioscience Inc.) was used to measure TGF-β in culture supernatants. IFN-γ, IL-10 and TGF-β were measured in the supernatants of Th1-polarized cultures and IL-17A, IL-10 and TGF-β in the Th17-polarized cultures by ELISA. The Mouse IL-17 A ELISA MAX™ Standard Sets kit (Biolegend) was used for the measurement of IL-17A in accordance with the provider's instructions.

2.18. Statistical analysis

Each *in vitro* experiment was performed at least in triplicate. Data were analyzed using the JMP 5.0.1 statistical software program and expressed as mean ± standard error of the mean (SEM). The analysis of variance (ANOVA) followed by Tukey test for multiple comparisons and LS means contrast test were used to analyze parametric data. The Kruskal-Wallis test was used to analyze non-parametric data. P values < 0.05 were considered to be significant.

3. Results

3.1. Levels of endotoxin in α-mangostin

Because endotoxins can activate cells of the innate immune system and promote the secretion of proinflammatory cytokines, the endotoxin level in the isolated α-mangostin was measured to avoid any interference in the observed effects. The endotoxin level of 1 mg/ml α-mangostin was determined by the chromogenic LAL assay and was found lower than 0.05 EU/ml (equivalent to 0.005 ng/ml of LPS) which was within the endotoxin level allowed for sterile water by Food and Drug Administration (FDA). Additionally, it has been proven that these levels of endotoxin do not affect cytokine secretion and the expression of activation markers in some phenotypes of human DCs (Schwarz et al., 2014).

3.2. Therapeutic effect of α-mangostin on established CIA

To evaluate the therapeutic potential of α-mangostin in a model of CIA developed in mice, two oral doses 10 and 40 mg/kg were tested.

Methotrexate, a drug frequently used to ameliorate the symptoms of rheumatoid arthritis in human patients was employed as a reference drug. Treatments were administered from day 35 when the signs of arthritis were evident, and the CIA was established in mice. Each treatment was administered daily for 33 consecutive days. An arthritis score was used to determine the clinical severity of arthritis and joint inflammation. The arthritis score exhibited continuous increases in vehicle-treated mice from day 1 to day 16 of treatment; afterward, it remained relatively stable. A significant decrease in the arthritis score was found in α-mangostin-treated mice regardless of the dose used from day 7 to day 18 of treatment compared to vehicle-treated mice. No significant differences between both doses during this time were found. After the 18th day of treatment, none of the doses of α-mangostin demonstrated significant differences with vehicle-treated mice. In contrast, methotrexate did not significantly reduce the arthritis score in the first days of treatment until the 16th day of treatment (Fig. 1A).

The area under the curve of each of the experimental groups from day 1 to day 33 of treatment was estimated to evaluate the overall behavior of each of the treatments over time. Both doses of α-mangostin exhibited a significant decrease in the area under the curve with respect to vehicle-treated mice ($p < 0.05$). However, they did not show significant differences between them (Fig. 1B). Mice treated with methotrexate did not show significant differences with vehicle-treated mice although it did demonstrate a tendency to decrease the area under the curve in the last days of treatment ($p = 0.0877$) (data not shown). These results could mean that α-mangostin might have an early effect on the arthritis signs whereas methotrexate has a late effect in the arthritis control.

3.3. Histological changes in knee joints of CIA mice treated with α-mangostin

To determine if α-mangostin was able to control inflammation and damage to joint cartilage in CIA mice, histological sections of the knee joint of each of the experimental groups were evaluated according to the Materials and Methods section. The knee joints of vehicle-treated mice revealed synovial hyperplasia, massive infiltration of inflammatory cells within the joint space, extensive cartilage and bone erosions and pannus formation. The inflammatory infiltrate in vehicle-treated mice was composed mainly of mononuclear cells (macrophages and lymphocytes) and fibroblasts, although some neutrophils within the joint space were also found (Fig. 2A). The higher dose of α-mangostin significantly reduced the histological score. However, the lower dose of α-mangostin and methotrexate did not present a significant effect on the histopathological changes found in vehicle-treated mice (Fig. 2B).

3.4. Effect of α-mangostin on the production of anti-collagen II IgG1 and IgG2a antibodies

Anti-CII IgG antibodies are critical in the development of CIA, and it has been shown to have a high correlation with the severity of arthritis in CIA mice (Williams et al., 1998). Therefore, the effect of α-mangostin on the production of anti-CII IgG antibodies was evaluated. Both anti-CII IgG1 and anti-CII IgG2a antibodies were significantly increased in the vehicle-treated mice compared to those in the control group. Moreover, levels of anti-CII IgG2a antibodies showed a 5-fold increase compared to levels of anti-collagen IgG1 antibodies. A significant decrease in anti-CII IgG2a antibodies was found only in mice treated with the higher dose of α-mangostin (Fig. 3A). However, none of the treatments affected the production of anti-CII IgG1 antibodies (Fig. 3B).

3.5. Effect of α-mangostin on joint inflammatory mediators

It has been demonstrated by different research groups that cytokines, chemokines and other inflammatory mediators such as PGE_2 play a key role in inflammation and joint destruction that occurs in RA (Feldmann and Maini, 2008; Hoxha, 2018). Chemokines are mostly involved in the recruitment of inflammatory cells to the joint, while cytokines and PGE_2 are also involved in the destruction and damage to the joint (Hoxha, 2018; Iwamoto et al., 2008; Choy, 2012; Caselli et al., 2018). We explored whether α-mangostin could modify the production of these cytokines and chemokines in the CIA joint and the PGE_2 release at peripheral and local level. PGE_2 levels in both serum and joints were found to be increased in the vehicle-treated mice compared to those in the control group. Treatments with either the different doses of α-mangostin or methotrexate showed a decrease in the production of PGE_2 in serum and joints, this reduction being more noticeable in the latter. However, these reductions were not significant in any of treatments (Fig. 3C and D). Moreover, it was found that CIA mice treated only with vehicle had a significant increase in the production of the cytokines IL-6, IL-1β, IL-18 and IL-33 and the chemokines LIX (CXCL5), IP-10 (CXCL10), MCP-1 (CCL2), MIP-1α (CCL3), MIP-1β (CCL4), MIG (CXCL9) and RANTES (CCL5) compared to control mice. As observed in Fig. 4A, the dose of 10 mg/kg of α-mangostin exhibited a significant decrease in the production of IL-6 and the chemokines LIX, IP-10 and RANTES ($p < 0.05$) while the dose of 40 mg/kg not only significantly decreased the IL-6 and these three chemokines but also significantly decreased the MIG chemokine ($p < 0.05$). Although no significant differences were found between the two doses of α-mangostin employed, the dose of 40 mg/kg demonstrated significance slightly higher than the dose of 10 mg/kg in reducing the production of IL-6 and these chemokines. On the other hand, methotrexate only showed an efficient reduction in the production of LIX and RANTES chemokines ($p < 0.05$ and $p < 0.01$, respectively) (Fig. 4A), whereas the levels of IL-1β and IL-18 were not affected by any of the treatments used (Fig. 4B and C). The expression of IL-33 was found to be significantly increased in the joint of vehicle-treated mice compared to those in the control group ($p < 0.05$) being this expression more evident in the fibroblasts and the mononuclear cells of the inflammatory infiltrate (Fig. 5A). The treatments with both doses of α-mangostin and methotrexate decreased the expression of IL-33 in joint tissues significantly with respect to vehicle-treated mice (Fig. 5A and B).

3.6. Effect of α-mangostin on oxidative stress and the activity of antioxidant enzymes

It has been documented that the production of ROS and RNS may be increased and the activity of some of the enzymes of the antioxidant defense system reduced in patients with RA. This imbalance in redox equilibrium can cause damage to cartilage and extracellular matrix components as well as activate inflammatory pathways such as NF-κB (Hitchon and El-Gabalawy, 2004; Mirshafiey and Mohsenzadegan, 2008). We evaluated the effect of α-mangostin on oxidative stress and the preservation of the antioxidant defense system in the joints, liver and kidney of CIA mice. The levels in the activity of NOX and MDA (an end product of lipid peroxidation) were found increased in the joints of vehicle-treated mice ($p < 0.05$). In addition, GSH levels (one of the central antioxidant defense systems) were found to be decreased at the joint level in this same group compared to control mice ($p < 0.05$). The dose of 10 mg/kg of α-mangostin was able effectively to decrease the NOX activity and the generation of MDA and demonstrated to preserve the levels of GSH in the joints of CIA mice ($p < 0.05$). The dose of 40 mg/kg of α-mangostin only decreased lipid peroxidation in the joints of CIA mice ($p < 0.05$). On the other hand, methotrexate was also able to decrease MDA production and preserve GSH levels significantly in the joints of CIA mice ($p < 0.05$). The GPx, GST and CAT activities did not exhibit significant differences between the different experimental groups at the joint level (Fig. 6). No significant differences were found in the levels of MDA, GSH and NOX, GPx, GST, SOD, GR and CAT activities in the liver and kidney of mice of the different experimental groups. Nevertheless, a significant increase in the number of

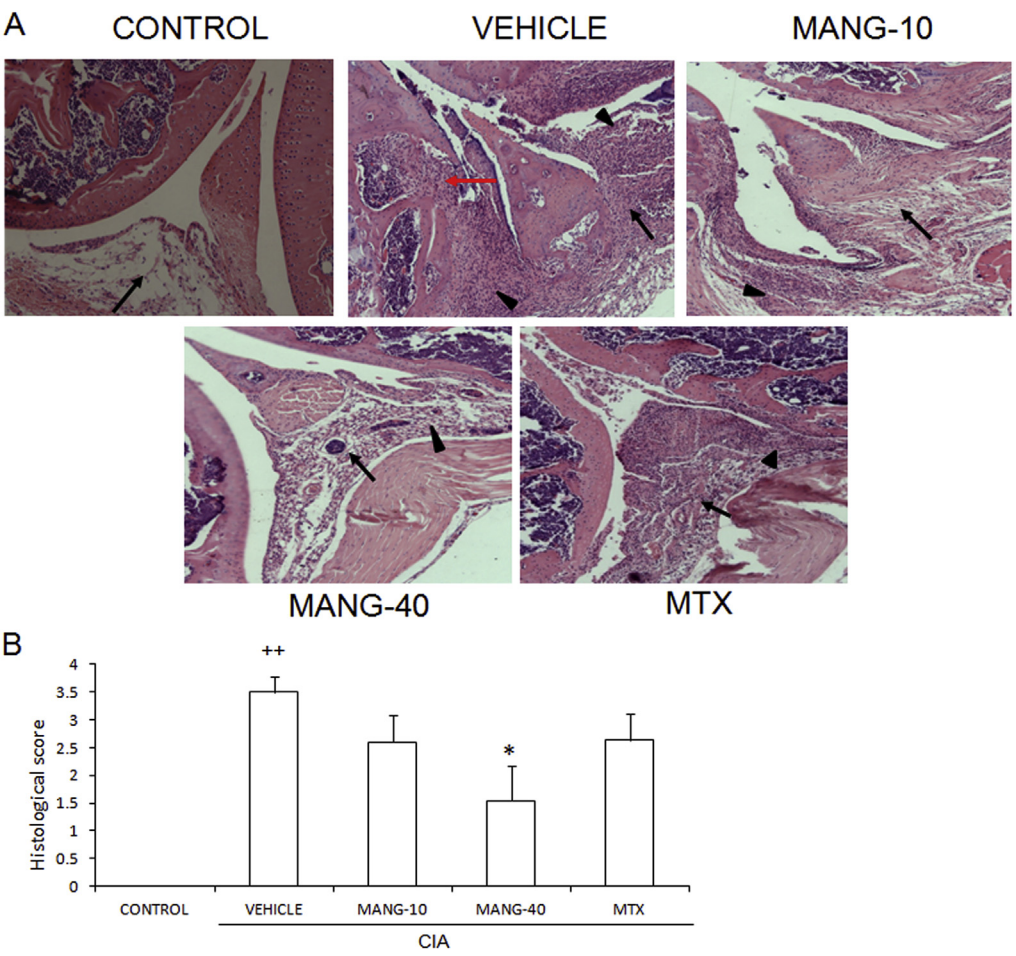

Fig. 2. Histological analysis of knee joints on day 68 (hematoxylin and eosin stained joint tissue sections). Original magnification 10X. Photomicrographs are representative of histopathology of six mice (n = 6) per experimental group. Black arrows indicate the synovial membrane, head arrows indicate the inflammatory infiltrate and synovial hyperplasia into the joint and red arrows indicate the cartilage and bone destruction by pannus (A). Histological scores obtained by the evaluation of formation of pannus, synovial hyperplasia, amount of inflammatory infiltrate and magnitude of damage to articular cartilage and bone in the joints of each experimental group (B). Control, non-arthritic mice; Vehicle, arthritic mice treated with vehicle (corn oil); Mang 10, arthritic mice treated with 10 mg/kg of α-mangostin; Mang 40, arthritic mice treated with 40 mg/kg of α-mangostin; MTX, arthritic mice treated with methotrexate. Data are represented as mean ± SEM. n = 6. Group is significantly different, $^{++}p < 0.01$ versus control group; $^{*}p < 0.05$ versus vehicle group (by ANOVA and LS means contrast test). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

oxidized proteins was found in the liver of mice treated with methotrexate ($p < 0.05$) (data not shown).

3.7. Cytotoxicity of α-mangostin

α-mangostin has been shown to possess anti-inflammatory effects on several cells of the innate immune system including various macrophage lines (Tewtrakul et al., 2009). In order to get a deeper understanding about the mechanisms involved in the anti-inflammatory capacity of α-mangostin on CIA, we decided to study its effect on activated dendritic cells and polarized Th1 and Th17 lymphocytes which play a central role in the immunopathogenesis of RA. Because several studies have demonstrated that α-mangostin possesses cytotoxic effects on different cell types (Liu et al., 2015) including several tumor cell lines (Nakagawa et al., 2007; Doi et al., 2009), we first investigated its cytotoxic effect on LPS activated mBMDCs and lymphocytes activated and polarized towards Th1 and Th17. α-mangostin did not demonstrate cytotoxic effects on mBMDCs activated with LPS for 4 and 24 h at any of the concentrations evaluated (0.5–10 μg/ml). However, it did reveal cytotoxic effects on lymphocytes activated and polarized towards Th1 and Th17, inducing a significant decrease in cell viability ($p < 0.05$) approximately of 37% with the dose of 5 μg/ml (data not shown).

3.8. Effect of α-mangostin on mBMDCs activated with LPS

Once it was determined that α-mangostin has no cytotoxic effects on mBMDCs activated with LPS, we explored the ability of α-mangostin to modify the expression of activation molecules as well as the production of proinflammatory and anti-inflammatory cytokines in these mBMDCs. The mBMDCs activated with LPS showed a significant increase both in the percentage of CD86 expression ($p < 0.05$) and mean fluorescence intensity (MFI) for CD86 ($p < 0.01$) with respect to unstimulated mBMDCs. α-mangostin significantly decrease the percentage of $CD11c^+$ $CD86^+$ cells in doses between 7 and 10 μg/ml with respect to mBMDCs activated with LPS that only received the vehicle ($p < 0.05$). In addition, MFI for CD86 was also significantly reduced in $CD11c^+$ cells at the same doses (Fig. 7).

The percentage and MFI of CD40 were significantly increased in LPS-activated mBMDCs compared to non-stimulated mBMDCs ($p < 0.01$). α-mangostin proved to be capable of significantly decrease both the percentage and MFI of CD40 in LPS-activated mBMDCs from the dose of 5 μg/ml. The higher dose of α-mangostin proved to be the best in decreasing the percentage of CD40 in $CD11c^+$ cells and better than the 5 μg/ml dose in decreasing the MFI of CD40 in $CD11c^+$ cells ($p < 0.01$) (Fig. 8). The expression of CD80, IA/IE and PD-L1 in $CD11c^+$ cells were also examined, but no changes were found with any of the doses of α-mangostin evaluated (data not shown).

To determine the effect of α-mangostin on the production of proinflammatory and anti-inflammatory cytokines in mBMDCs activated with LPS, the production of IFN-γ, TNF-α, IL-6, IL-12 (p70), IL-10 and TGF-β was evaluated. mBMDCs activated with LPS exhibited a significant increase in the production of IL-12 (p70), TNF-α, IL-6 and IL-10 after 4 h of stimulation ($p < 0.01$) and a significant increase of IFN-γ after of 24 h of stimulation ($p < 0.05$) with respect to unstimulated mBMDCs. α-mangostin significantly decreased the production of IL-12 (p70) at doses of 3 μg/ml, and increased the production of IL-10 at doses of 5 μg/ml with regard to the LPS activated BMDCs that only received DMSO after 4 h of stimulation ($p < 0.01$). A decrease in the levels of INF-γ was also found from the dose of 3 μg/ml of α-mangostin but was not statistically significant ($p = 0.07$). The secretion of TNF-α,

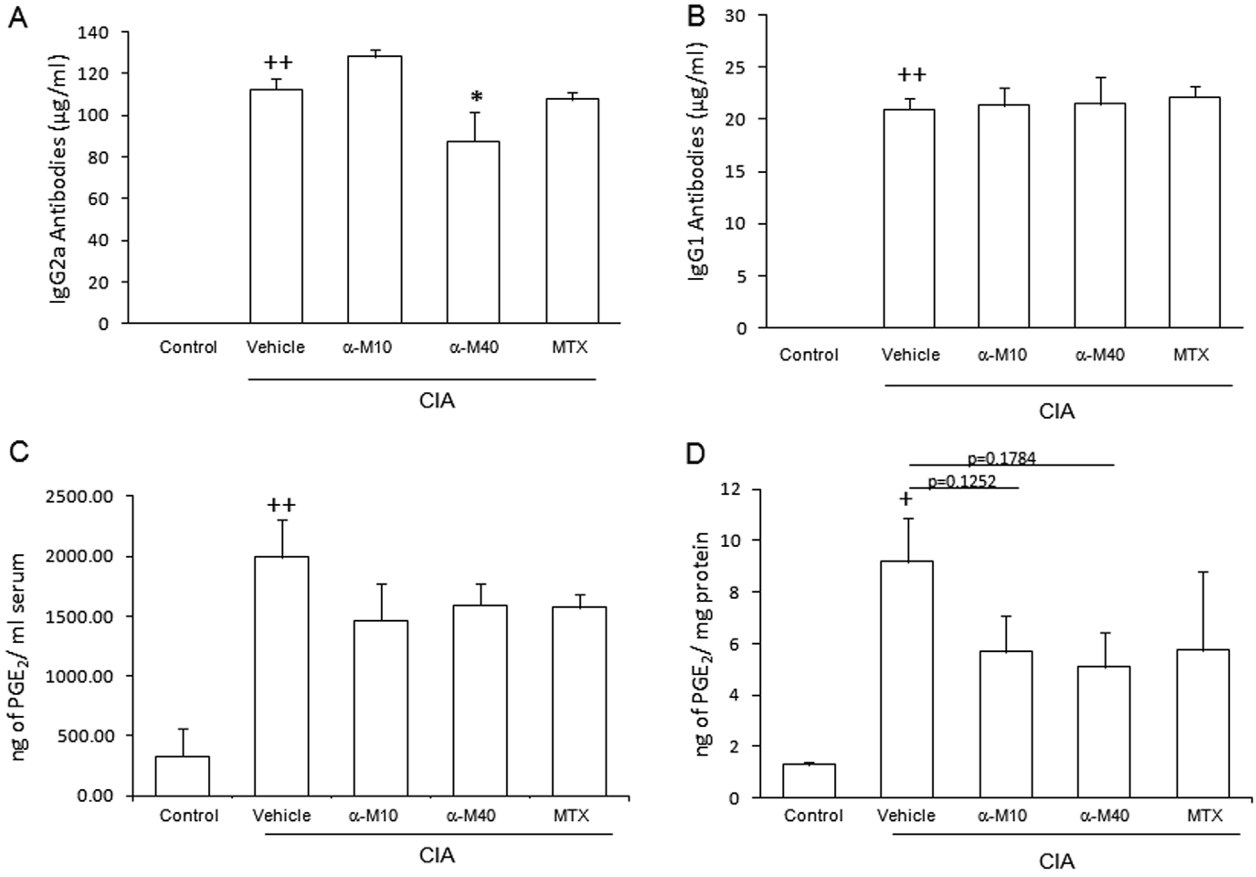

Fig. 3. Serum antibody levels of IgG2a (A) and IgG1 (B) anti-CII on day 68. The antibodies concentration was expressed in µg/ml and was determined by ELISA. PGE_2 levels in serum (C) and joints (D) of mice of the different experimental groups. PGE_2 levels were expressed in ng/ml of serum or ng/mg of protein and were determined by a competitive enzyme immunoassay. Control, non-arthritic mice; Vehicle, arthritic mice treated with vehicle (corn oil); Mang 10, arthritic mice treated with 10 mg/kg of α-mangostin; Mang 40, arthritic mice treated with 40 mg/kg of α-mangostin; MTX, arthritic mice treated with methotrexate. Data are represented as mean ± SEM. n = 6. Group is significantly different, $^{+}p < 0.05$, $^{++}p < 0.01$ versus control group; $^{*}p < 0.05$ versus vehicle group (by ANOVA and LS means contrast test).

IL-6 and TGF-β did not present changes in any doses of α-mangostin tested (Fig. 9). These results indicate that α-mangostin possess immunomodulatory effects on mBMDCs activated with LPS, which are due to the decrease in the expression of costimulatory molecules, the decrease in cytokines that polarize towards Th1 cells (IL-12 p70) and the increase in the anti-inflammatory cytokine IL-10.

3.9. Effect of α-mangostin on Th1 and Th17 polarization

Several studies have shown that some polyphenols possess the ability to prevent proliferation or modify the production of cytokines in lymphocytes (Roseghini et al., 2007). At present, it is unknown whether α-mangostin, which belongs to the polyphenols group, is capable of modifying the polarization of lymphocytes from one phenotype to another. Therefore, the capacity of α-mangostin to avoid the polarization of lymphocytes towards a Th1 or Th17 phenotype favoring polarization towards a regulatory phenotype (Treg) was studied. α-mangostin demonstrated no effects on the polarization of Th1 and Th17 lymphocytes in doses of 0.5–3 µg/ml, nor did it show increases in the regulatory phenotype in any of these doses. Doses starting at 5 µg/ml of α-mangostin exhibited decreases in the populations of both Th17 and Th1 lymphocytes, as well as a decrease in the production of some cytokines such as IL-17A and IL-10. However, these decreases were probably due to a proapoptotic effect of this molecule on activated lymphocytes, since cell viability was reduced by 37% at this dose as was mentioned in section *3.7* (data not shown). These data show that α-mangostin is not capable of modifying the polarization of Th1 or Th17 cells towards a regulatory phenotype in the doses tested; however, it is capable of inducing apoptosis in activated T lymphocytes and polarized towards a Th1 or Th17 phenotype.

4. Discussion

α-mangostin has been shown to possess anti-inflammatory properties in several animal models of disease including arthritis (Ibrahim et al., 2016). The results obtained in the present study demonstrate the effectiveness of α-mangostin to decrease the clinical signs, inflammation and joint destruction in DBA/1J mice with established CIA. A higher effectiveness was exhibited by the higher dose of α-mangostin (40 mg/kg) because it significantly decreased the infiltration of inflammatory cells, synovial hyperplasia and the cartilage and bone damage in CIA mice. This effect was only observed during the first 18 days of treatment. This is particularly interesting since the therapeutic response to methotrexate an anti-inflammatory drug frequently used for arthritis treatment begins after 3–6 weeks of starting treatment and reaches its maximal improvement after 12 weeks or more (Weinblatt, 2013). Thus, it is feasible that the combination of both treatments may improve the current treatment. Previous evidence also point to the potential of α-mangostin in the arthritis treatment. However, most studies conducted to demonstrate its anti-inflammatory capacity in arthritis have used non-autoimmune arthritis models or have administered α-mangostin at the time of induction of disease or even before inducing it when arthritis is still not established, and the inflammatory and joint destruction processes are not yet clinically evident

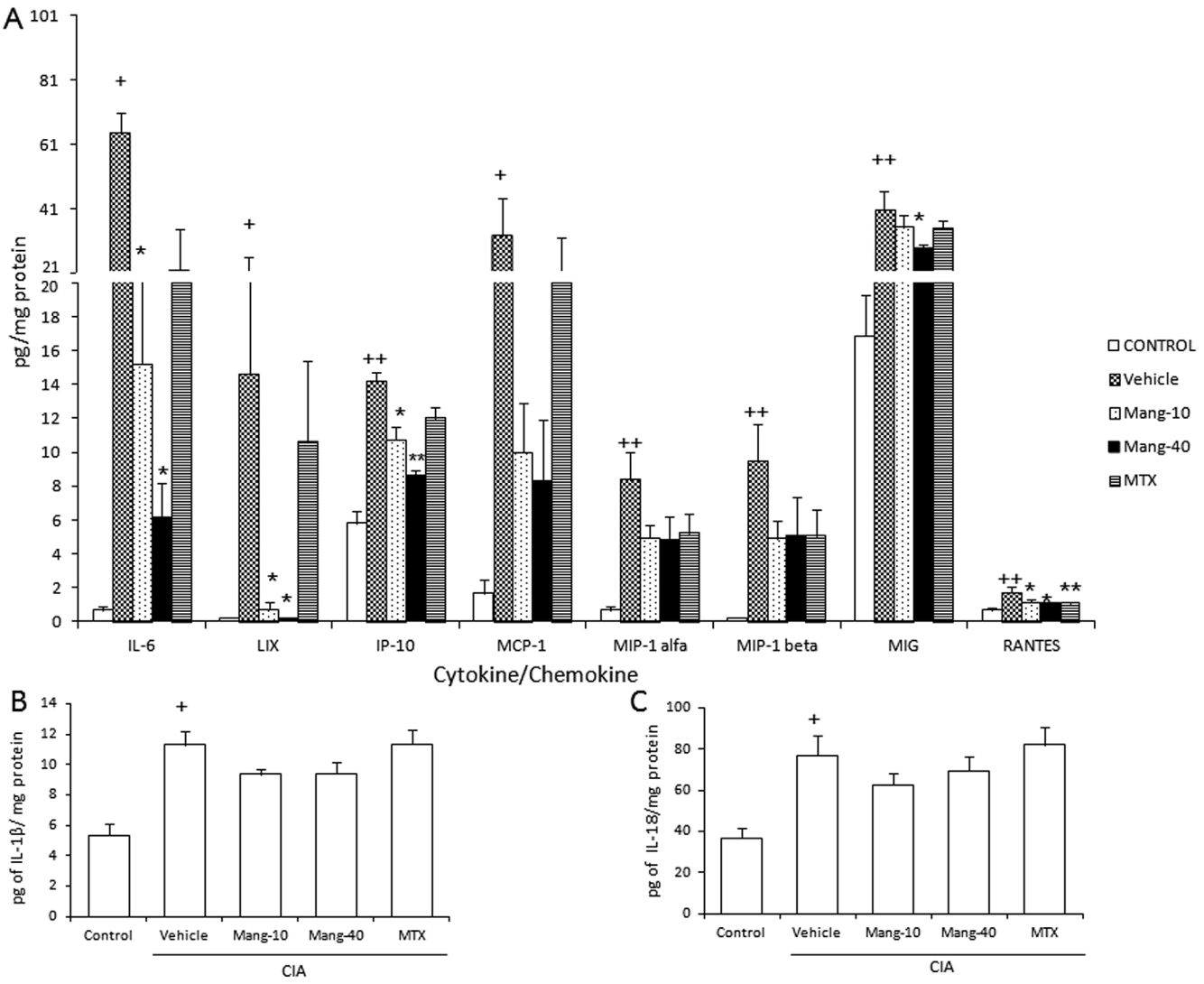

Fig. 4. Cytokines and chemokines levels in joint homogenates of CIA mice after 33 days of treatment. The concentration was expressed in pg/mg of protein and was determined by Luminex xMAP Technology (A) and ELISA test (B and C). Control, non-arthritic mice; Vehicle, arthritic mice treated with vehicle (corn oil); Mang 10, arthritic mice treated with 10 mg/kg of α-mangostin; Mang 40, arthritic mice treated with 40 mg/kg of α-mangostin; MTX, arthritic mice treated with methotrexate. Data are represented as the mean ± SEM. n = 6. The group is significantly different, $^{+}p < 0.05$, $^{++}p < 0.01$ versus control group; *p < 0.05, **p < 0.01 versus vehicle group (by ANOVA, Tukey test and LS means contrast test).

(Gopalakrishnan et al., 1980; Ibrahim et al., 2016; Lee et al., 2013).

Rheumatoid arthritis is an autoimmune disease in which both humoral immunity and cellular immunity are involved. The generation of autoantibodies such as rheumatoid factor, anti-citrullinated protein antibodies and antibodies against specific components of the joint (i.e. collagen type II and some peptidoglycans) play a critical role in the pathophysiology of the disease (Duskin and Eisenberg, 2010; Trouw et al., 2013; Frisenda et al., 2013). Indeed, these antibodies can even appear before the clinical manifestations of the disease and give rise to immune complexes which can be placed in other tissues besides the joint tissue and in this way exacerbate the inflammation and joint destruction (Schaeverbeke et al., 2012; Boissier et al., 2012). α-mangostin was shown to decrease the generation of anti-collagen II IgG2a antibodies at the dose of 40 mg/kg. This might indicate that part of mechanisms by which α-mangostin had anti-inflammatory activity in murine CIA may be due to it affects the humoral response during disease, which is reflected in decreasing of autoantibodies production and probably in the generation of immune complexes. Furthermore, its effect in reducing only the generation of anti-collagen II IgG2a antibodies and not anti-collagen IgG1 antibodies could point towards an effect of α-mangostin on Th1 responses.

For many years, several inflammatory mediators have been identified in the synovial fluid of RA patients including high levels of prostaglandins (PGs). PGE_2 is a metabolite of the arachidonic acid pathway, which is produced by chondrocytes and synovial fibroblasts from RA patients (Hoxha, 2018). The binding of PGE_2 to its E-type prostanoid receptor 4 exerts proinflammatory activity in the rheumatic synovium because it modifies the cytokine production, i.e., IL-6, IL-23 and vascular endothelial growth factor, promotes Th17 cells differentiation by stimulating IL-23 secretion by activated DCs and increases type II collagen degradation (Hoxha, 2018; Jia et al., 2014; Konya et al., 2013). As observed, α-mangostin showed a tendency to decrease the production of PGE_2 in joints, which might be at least partially limiting the damage to specific proteins of the joint and modulating certain proinflammatory effects that are orchestrated by the PGE_2 pathway in CIA mice as mentioned above. In fact, a study has recently published the use of a PGE_2 receptor 4 antagonist which has demonstrated to be an effective treatment in reducing inflammation and joint damage in mice and rats with CIA (Caselli et al., 2018).

In addition to PGE_2, other inflammatory mediators such as several

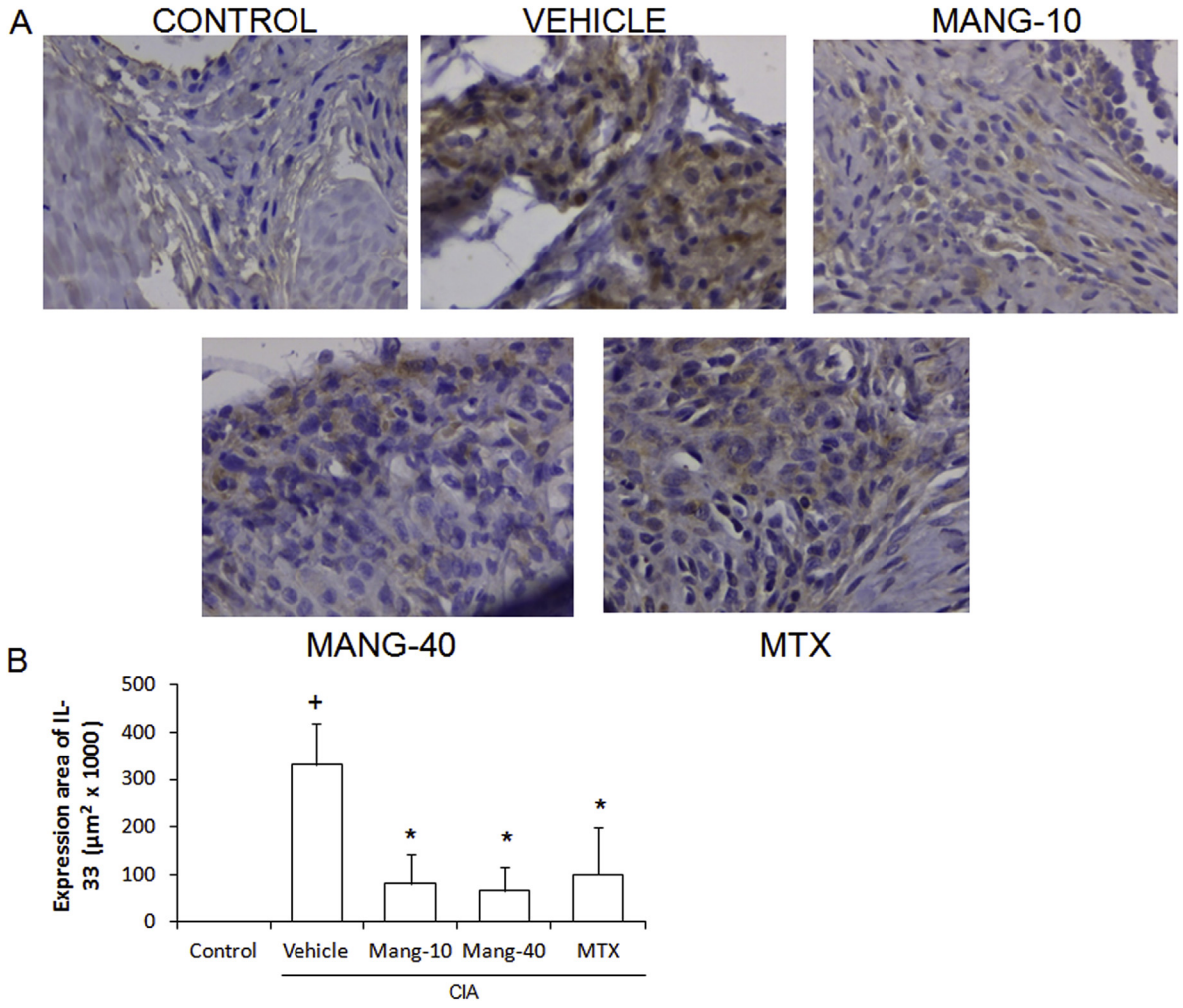

Fig. 5. Expression of IL-33 cytokine in joint tissues of CIA mice after 33 days of treatment. The IL-33 presence was determined by immunochemistry (A) and was expressed in surface area (μm^2) (B). Control, non-arthritic mice; Vehicle, arthritic mice treated with vehicle (corn oil); Mang 10, arthritic mice treated with 10 mg/kg of α-mangostin; Mang 40, arthritic mice treated with 40 mg/kg of α-mangostin; MTX, arthritic mice treated with methotrexate. Data are represented as the mean ± SEM. n = 4. The group is significantly different, $^{+}p < 0.05$ versus control group; $^{*}p < 0.05$ versus vehicle group (by ANOVA, Tukey test and LS means contrast test).

cytokines and chemokines have been implicated in the pathogenesis of RA. It has been demonstrated that the imbalance between proinflammatory and anti-inflammatory cytokines in favor of the former can promote self-immunity and participate in the maintenance of inflammation and joint destruction during RA (McInnes et al., 2007). Some of these proinflammatory cytokines (i.e., TNF-α, IL-1 and IL-6) are so important in the development of pathology, that development of monoclonal antibodies to block their function has significantly decreased pain, signs and joint damage in RA patients (Devine et al., 2011). In our CIA model, IL-6, IL-1β, IL-18 and IL-33 cytokines were significantly increased in the synovial joint; however, only IL-6 and IL-33 were significantly reduced by the treatment with α-mangostin. Since IL-6 is a pleiotropic cytokine which has several functions in RA, such as collaborating in the production of autoantibodies by promoting the maturation of B cells, participating in the differentiation of Th17 cells, stimulating the production of IL-8 and MCP-1 by endothelial cells (allowing neutrophils and monocytes recruitment), inducing the synoviocyte proliferation and the osteoclast differentiation and increasing the production of matrix metalloproteinases (MMPs) (Srirangan and Choy, 2010; Yoshida and Tanaka, 2014); its reduction could be related to the anti-inflammatory effect observed in the clinical and histopathological score. Moreover, it has been demonstrated that the decrease in IL-6 levels during the first 12 months of treatment is related to better clinical results and the blocking of IL-6 signaling by monoclonal antibodies such as tocilizumab rapidly improves the RA disease activity (Mijnheer et al., 2013; Yoshida and Tanaka, 2014). Therefore, molecules such as α-mangostin that affect the production of this cytokine could have a beneficial effect on RA.

On the other hand, IL-33 has recently emerged as a cytokine involved in the pathophysiology of RA since it is able to activate NF-κB and MAPK pathways in macrophages inducing the production of proinflammatory cytokines such as IL-1β and IL-6, and chemokines such as MCP-1 among others (Chen et al., 2017). Furthermore, it has been shown that exogenous administration of IL-33 promotes the production of pro-inflammatory cytokines and chemokines which exacerbates the inflammatory process in CIA mice (Chen et al., 2017; Carrasco et al., 2015). Conversely, antibodies that block the signaling pathway of IL-33 (T1/ST2) have been shown to decrease the severity of CIA in mice (Palmer et al., 2009). In the light of the above results, the decrease in the production of this cytokine in CIA mice by α-mangostin could contribute to control the inflammatory process and joint damage that occur in this disease.

Several cell types have been identified in the inflammatory process and joint destruction that occurs in RA, many of which are recruited from the periphery to synovial joints, i.e., monocytes, T cells, B cells, neutrophils and dendritic cells, among others through small molecules

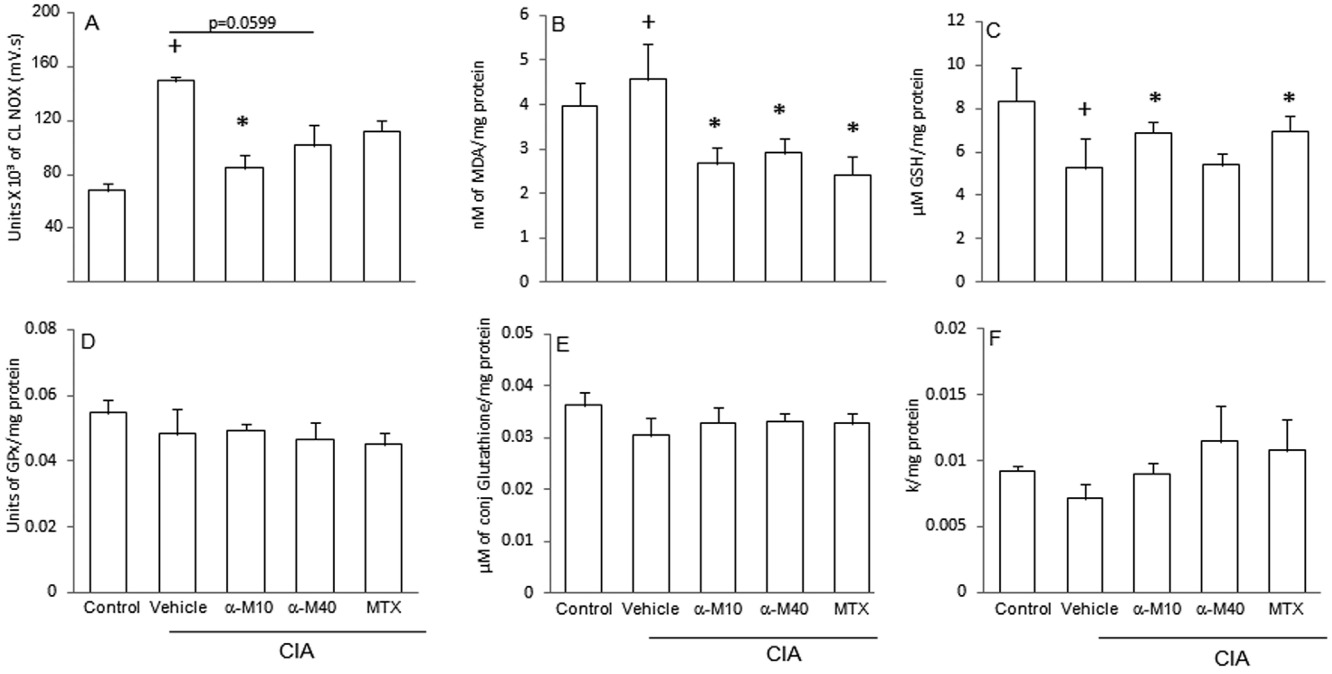

Fig. 6. Effects of α-mangostin on oxidative stress and the preservation of antioxidant defense systems in CIA mice joints. Results of (A) NOX activity expressed as units of chemiluminescence (CL) x 10^3 (mV.s), (B) lipid peroxidation in nmoles of MDA/mg protein, (C) GSH levels in µmoles of GSH/mg protein, (D) GPx activity in units of GPx/mg protein, (E) GST activity in µmoles of conjugated glutathione/mg protein and (F) CAT activity in k unit/mg protein. Control, non-arthritic mice; Vehicle, arthritic mice treated with vehicle (corn oil); Mang 10, arthritic mice treated with 10 mg/kg of α-mangostin; Mang 40, arthritic mice treated with 40 mg/kg of α-mangostin; MTX, arthritic mice treated with methotrexate. Data are represented as the mean ± SEM. n = 6. The group is significantly different, $^{+}p < 0.05$ versus control group; $^{*}p < 0.05$ versus vehicle group (by ANOVA, Tukey test and LS means contrast test).

called chemokines (Iwamoto et al., 2008). Several chemokines have been found present in the joints of RA patients, i.e. IL-8/CXCL8, RANTES/CCL5, MCP-1/CCL2, IP-10/CXCL10, MIG/CXCL9, epithelial neutrophil-activating peptide 78 (ENA-78)/CXCL5, growth-related oncogene (GROα)/CXCL1, connective tissue activating peptide III (CTAP-III)/CXCL7, MIP-1α/CCL3 and MIP-1β/CCL4, among others (Iwamoto et al., 2008; Koch, 2005). Although the principal function of these chemokines is the recruitment of inflammatory cells to the rheumatic synovium, some of them may also exhibit other functions such as promoting angiogenesis (Koch, 2005; Rump et al., 2017). α-mangostin was shown to significantly reduce the production of LIX/CXCL5, IP-10/CXCL10, MIG/CXCL9 and RANTES/CCL5. LIX/CXCL5 is one of the main chemokines involved in the recruitment of neutrophils in mice and plays a significant role in the production of TNF-α in macrophages and mast cells through the binding with its receptor (CXCR2). Moreover, in humans, ENA-78/CXCL5 has been shown to be increased in the synovium of RA patients where it participates significantly in angiogenesis (Vieira et al., 2009). On the other hand, IP-10/CXCL10 is expressed mainly in infiltrating macrophage-like cells and fibroblast-like synoviocytes in joints of RA patients where it recruits mainly T cells that express its receptor (CXCR3). Furthermore, there is also evidence that this chemokine may be partly responsible for bone erosion that occurs in RA since it mediates the expression of RANKL in $CD4^+$ T cells which is involved in the osteoclast differentiation (Antonelli et al., 2014). In addition to IP-10/CXCL10, MIG/CXCL9 is another cytokine implied in the recruitment of activated T cells and Th1 cells, is expressed in synovial fibroblasts of RA patients, induced by IFN-γ and plays an essential role in the early infiltration of plasma cells into rheumatic synovium (Iwamoto et al., 2008; Tsubaki et al., 2005). Moreover, this chemokine in concert with IP-10/CXCL10 and RANTES/CCL5 have been shown to increase the proteolytic activity of fibroblast-like synoviocytes by increasing its collagenase and gelatinase activity (Iwamoto et al., 2008). On the other hand, RANTES/CCL5 is a chemokine expressed by activated T cells, fibroblast-like synoviocytes and mononuclear cells into synovium of RA patients (Mellado et al., 2015). This chemokine promotes the expression of iNOS and the IL-6 and MMP-3 production in chondrocytes and also inhibits the synthesis of proteoglycans in these same cells, contributing therefore to the degradation of articular cartilage (Iwamoto et al., 2008). In light of the above, the decrease of LIX/CXCL5, IP-10/CXCL10, MIG/CXCL9 and RANTES/CCL5 by α-mangostin could not only be impacting the recruitment of inflammatory cells to the synovium but also the processes of angiogenesis and bone and cartilage destruction that take place in the joints of RA patients.

Because inflammation and oxidative stress are closely interlinked in certain diseases including RA, ROS and RNS are other critical elements considered to be involved in the damage to bone and cartilage in RA patients (Mateen et al., 2016). Several studies have shown that total ROS, $O_2^{\cdot-}$, H_2O_2 and hydroxyl radical are increased in patients with RA since some cells such as monocytes and neutrophils rise their production. On the other hand, certain antioxidant defenses, i.e., β-carotene, vitamin E, and SH groups are diminished, while others, such as SOD, CAT, GPx, and GR activities and GSH levels have had variable results. The above induces the generation of an environment in favor of oxidative stress, which finally causes damage to lipids, proteins and DNA in RA patients (Quinonez-Flores et al., 2016). In addition to being directly involved in damage to the joint components, ROS and RNS can also have an effect on several cells of the immune system, i.e., T lymphocytes can become refractory to the induction of cell death and fibroblast-like synoviocytes can generate mutations of p53 gene (Quinonez-Flores et al., 2016; Hitchon and El-Gabalawy, 2004; Griffiths, 2005). In the CIA model, an increase of NOX (an $O_2^{\cdot-}$-generating enzyme) and a decrease of GSH was observed. α-mangostin was shown to decrease the activity of NOX and lipid peroxidation, as well as to increase the levels of GSH, one of the principal intracellular antioxidant defense systems. These findings point to a protective effect of α-mangostin against the oxidative stress generated in CIA mice, which could not only be limiting the direct damage to the joint tissue but also

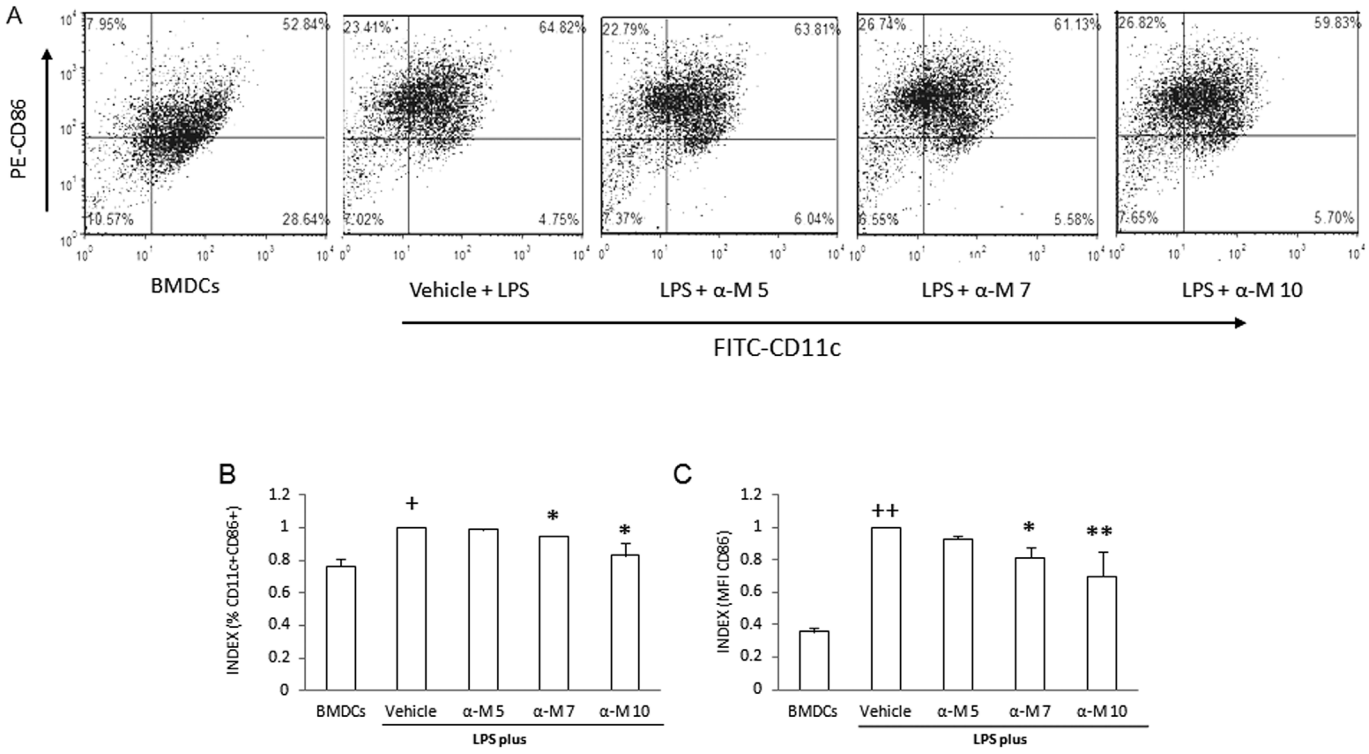

Fig. 7. Effect of α-mangostin on mBMDCs activated with LPS. The effect was evaluated by flow cytometry, and data were analyzed using the FCS Express software. Representative dot plots of the effect of α-mangostin on the expression of CD86 in $CD11c^+$ cells (mBMDCs) activated with LPS for 4 h after staining cells with PE-CD86 and FITC-CD11c antibodies (A). Index of the percentage of $CD11c^+$ $CD86^+$ cells of each experimental group (B). Index of mean fluorescence intensity (MFI) for CD86 in $CD11c^+$ cells of each experimental group (C). Data are representative of three independent experiments. BMDCs, murine bone marrow-derived dendritic cells without stimulus; Vehicle + LPS, BMDCs treated with lipopolysaccharide (LPS) and dimethyl sulfoxide (DMSO); LPS + α-M 5, BMDCs treated with LPS and 5 μg/ml of α-mangostin; LPS + α-M 7, BMDCs treated with LPS and 7 μg/ml of α-mangostin; and LPS + α-M 10, BMDCs treated with LPS and 10 μg/ml of α-mangostin. Data are represented as mean ± SEM. The group is significantly different, $^+p < 0.05$, $^{++}p < 0.01$ versus non-stimulated mBMDCs; $^*p < 0.05$, $^{**}p < 0.01$ versus vehicle group (by Kruskal-Wallis test for Index of the percentage of $CD11c^+$ $CD86^+$ cells and by ANOVA, Tukey test and LS means contrast test for CD86 MFI).

the inflammatory process due to the actions of ROS and RNS on the aforementioned immune cells.

α-mangostin has been shown to possess anti-inflammatory effects on several cell types that play essential roles in RA. For example, *in vitro* α-mangostin is able to inhibit NO and PGE_2 release and iNOS expression in LPS-stimulated murine macrophage RAW 264.5 cells and is capable of inducing apoptosis in human rheumatoid fibroblast-like synoviocyte MH7A cells (Chen et al., 2008; Zhang et al., 2018). However, its effect on activated dendritic cells as well as on Th1 and Th17 lymphocytes, critical players in the disease process of RA, has not been completely clarified. In this work, it was demonstrated that α-mangostin is able to decrease the expression of co-stimulatory molecules such as CD86 and CD40 in LPS-activated BMDCs, which might have an impact on its capability to present antigens to autoreactive T cells. In addition, it was also able to decrease the production of IL-12 p70 and showed a tendency to decrease the production of IFN-γ in these cells, which could be limiting Th1 responses. On the other hand, α-mangostin also increased IL-10 production in LPS-activated BMDCs, an anti-inflammatory cytokine that has been related with the control of several inflammatory pathologies (Lyer and Cheng, 2013; Siqueira Mietto et al., 2015; Ip et al., 2017). All these aforementioned α-mangostin effects on activated DCs indicate that this xanthone can promote a regulatory dendritic cells phenotype, which could be useful to reduce inflammation and consequently the joint damage that occurs in RA patients. Because, it has been proven that DCs in synovial joint tissues of patients with RA are raised, and these DCs show an increased expression of activation markers as well as an enhanced capability to induce the proliferation of T lymphocytes and their production of cytokines (i.e., IFN-γ, IL-17 and IL-4) under stimulation (Yu and Langridge, 2017).

Th1 and Th17 lymphocytes are the main subsets of T cells involved in RA. Th17 cells have been found increased in peripheral blood, bone marrow and synovial fluid of RA patients and it is known that they participate importantly in joint destruction through the production of IL-17A which promotes osteoclastogenesis and is involved in the recruitment of neutrophils to the joint (Gaffen, 2009; Sarkar et al., 2010; Kotake et al., 2017). Th1 cells have also been found increased in the synovial fluid and bone marrow of patients with RA and have been associated with osteoclastogenesis due to an increase in the expression of receptor activator of nuclear factor-κB ligand (RANKL) (Li et al., 2017). On the other hand, it is well known that T cells with a regulatory phenotype (Tregs) are able to control Th1 and Th17 cell responses (Li et al., 2007; Kleinewietfeld and Hafler, 2013; Shrestha et al., 2015). In this study we investigated whether α-mangostin has the ability to favor the generation of regulatory T cells in an environment that promotes polarization towards Th17 or Th1 cells because Th1 and Th17 lymphocytes polarization has been shown to be favored in the synovial joints of RA patients, and several polyphenols have been demonstrated to decrease the generation of these Th17 and Th1 cells through the induction of regulatory T cells in some animal models of arthritis (Park et al., 2011; Xuzhu et al., 2012; Rogers et al., 2010). Our results show that while α-mangostin was not able to modify the polarization in both Th1 and Th17 cells towards a regulatory phenotype, it was able to induce apoptosis of these two activated cellular subtypes. This could be of importance since the generation of apoptosis of both Th1 and Th17 cells could contribute to the reduction of inflammation and joint damage generated by these cells. In fact, some other polyphenols such as resveratrol that have anti-inflammatory properties in different diseases have been shown to generate apoptosis of activated T lymphocytes as

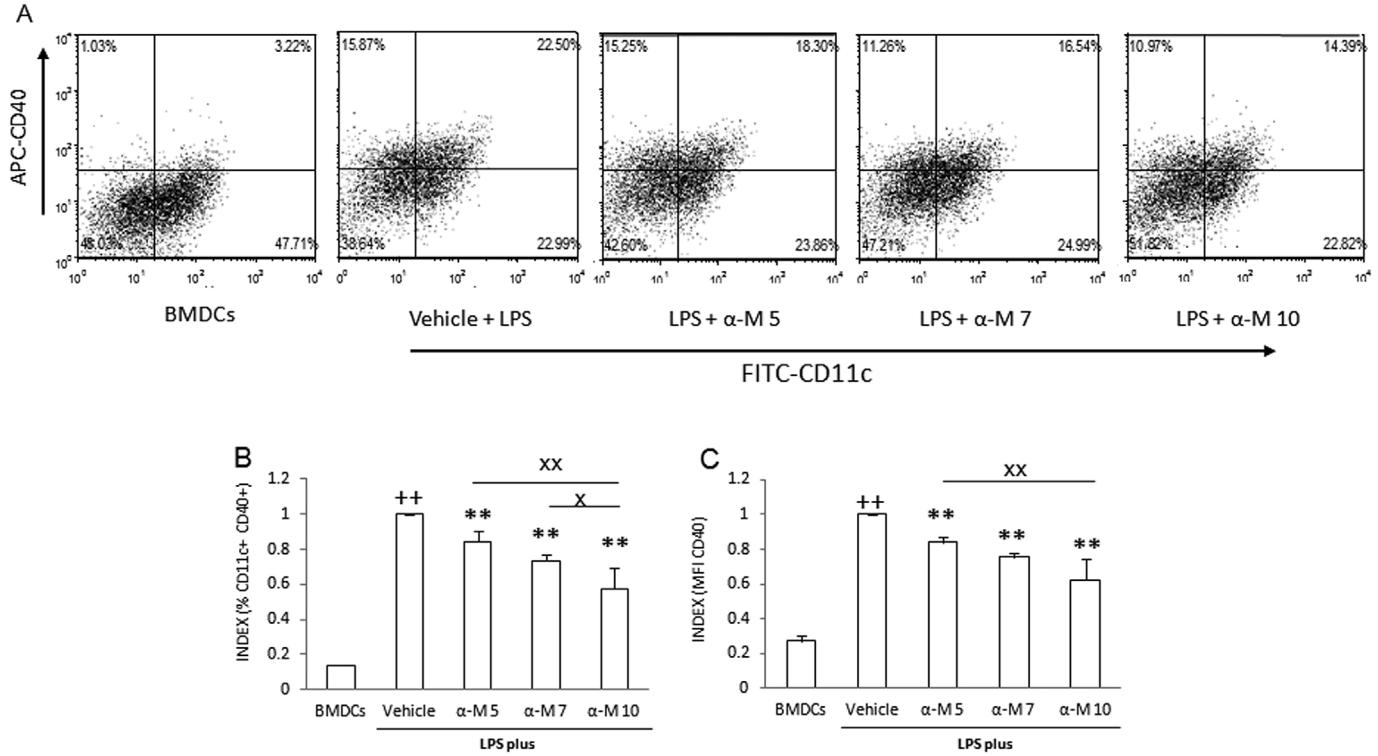

Fig. 8. Effect of α-mangostin on mBMDCs activated with LPS. The effect was evaluated by flow cytometry, and data were analyzed using the FCS Express software. Representative dot plots of the effect of α-mangostin on the expression of CD40 in $CD11c^+$ cells (mBMDCs) activated with LPS for 4 h after staining cells with APC-CD40 and FITC-CD11c antibodies (A). Index of the percentage of $CD11c^+$ $CD40^+$ cells of each experimental group (B). Index of mean fluorescence intensity (MFI) for CD40 in $CD11c^+$ cells of each experimental group (C). Data are representative of three independent experiments. BMDCs, murine bone marrow-derived dendritic cells without stimulus; Vehicle + LPS, BMDCs treated with lipopolysaccharide (LPS) and dimethyl sulfoxide (DMSO); LPS + α-M 5, BMDCs treated with LPS and 5 μg/ml of α-mangostin; LPS + α-M 7, BMDCs treated with LPS and 7 μg/ml of α-mangostin; and LPS + α-M 10, BMDCs treated with LPS and 10 μg/ml of α-mangostin. Data are represented as mean ± SEM. $^{++}p < 0.01$ versus non-stimulated mBMDCs; $^{**}p < 0.01$ for significant differences in a group versus vehicle group or $^{x}p < 0.05$, $^{xx}p < 0.01$ for significant differences between groups (by ANOVA, Tukey test and LS means contrast test).

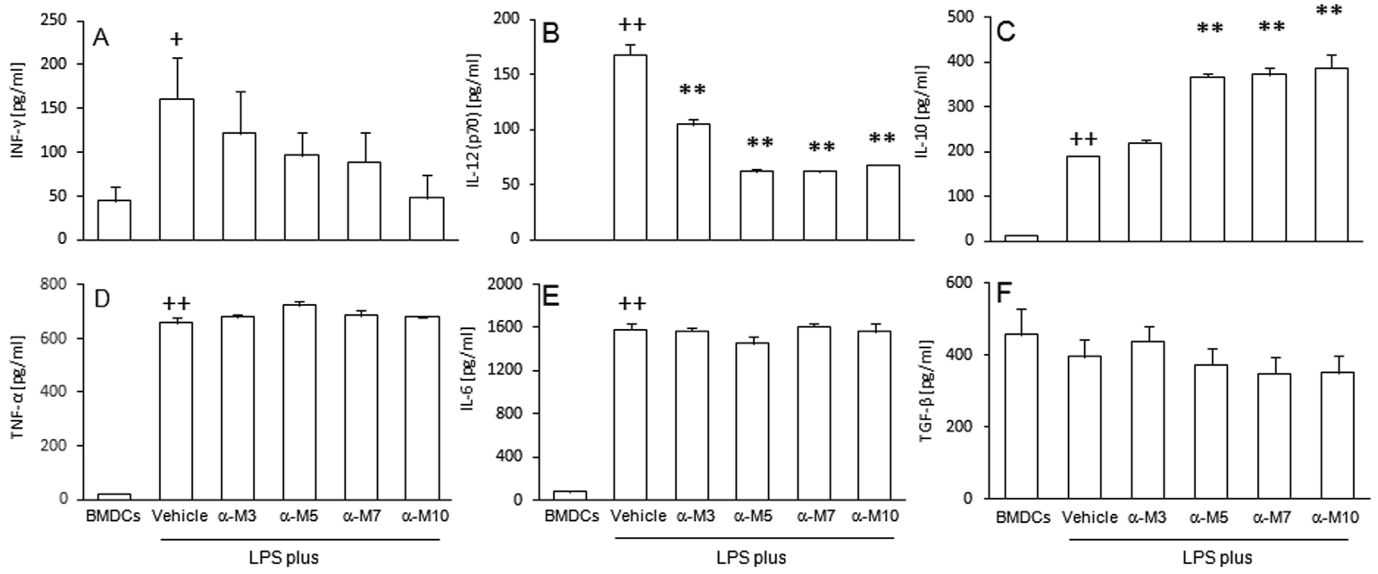

Fig. 9. Effect of α-mangostin on cytokines production in culture supernatants of mBMDCs activated with LPS for 4 or 24 h. The concentration was expressed in pg/ml and was determined by ELISA. Cytokine production was measured after 4 h of activation with LPS for every case except for INF-γ (24 h). (A) interferon-γ (INF-γ) production, (B) interleukin-12 (p70) (IL-12 p70) production, (C) interleukin-10 (IL-10) production, (D) tumor necrosis factor-α (TNF-α) production, (E) interleukin-6 (IL-6) production, and (F) transforming growth factor-β (TGF-β) production. Data are representative of three independent experiments. BMDCs, murine bone marrow-derived dendritic cells without stimulus; Vehicle + LPS, BMDCs treated with lipopolysaccharide (LPS) and dimethyl sulfoxide (DMSO); LPS + α-M 3, BMDCs treated with LPS and 3 μg/ml of α-mangostin; LPS + α-M 5, BMDCs treated with LPS and 5 μg/ml of α-mangostin; LPS + α-M 7, BMDCs treated with LPS and 7 μg/ml of α-mangostin; and LPS + α-M 10, BMDCs treated with LPS and 10 μg/ml of α-mangostin. Data are represented as mean ± SEM. The group is significantly different, $^{+}p < 0.05$, $^{++}p < 0.01$ versus non-stimulated mBMDCs; $^{**}p < 0.01$ versus vehicle group (by ANOVA, Tukey test and LS means contrast test for all cytokines except for IL-10 (Kruskal-Wallis test)).

part of their mechanism of action (Radkar et al., 2007).

In conclusion, our results demonstrate that α-mangostin possess anti-inflammatory and antioxidant capacity on established CIA in mice that effectively improve the onset of the disease during the first 18 days. Therefore, α-mangostin could be combined with methotrexate or another disease-modifying antirheumatic drug (DMARD) to cover early stages of RA and improve the quality of life of RA patients. Mechanisms involved in the α-mangostin effectiveness begin to be revealed.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

The authors thank Beatriz Hernández, MSc Evelyn Pulido, Raquel Guerrero and DSc Armando Pérez for their support in carrying out the histological techniques, to Jorge García and Isabel Herrera for their assistance in the handling of laboratory animals and tissue extraction and to Rubí Zavala and Monica Martínez for animal care. This work was supported by the National Council for Science and Technology (CONACYT CB-2012-169011) and Programa de Investigación para el Desarrollo y la Optimización de Vacunas, Inmunomoduladores y Métodos Diagnósticos del Instituto de Investigaciones Biomédicas, UNAM. The authors gratefully acknowledge the financial assistance of CONACYT to Diana Rocio Herrera-Aco from Programa de Doctorado en Ciencias de la Producción y de la Salud Animal, UNAM, who received the fellowship254086.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2018.12.018.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.fct.2018.12.018.

References

Aebi, H., 1984. [13] Catalase in Vitro. Methods Enzymol. 105, 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3.

Al-Massarani, S.M., El Gamal, A.A., Al-Musayeib, N.M., Mothana, R.A., Basudan, O.A., Al-Rehaily, A.J., Farag, M., Assaf, M.H., El Tahir, K.H., Maes, L., 2013. Phytochemical, antimicrobial and antiprotozoal evaluation of Garcinia Mangostana pericarp and α-mangostin, its major xanthone derivative. Molecules 18, 10599–10608. https://doi.org/10.3390/molecules180910599.

Alamanos, Y., Drosos, A.A., 2005. Epidemiology of adult rheumatoid arthritis. Autoimmun. Rev. 4, 130–136. https://doi.org/10.1016/j.autrev.2004.09.002.

Antonelli, A., Ferrari, S.M., Giuggioli, D., Ferrannini, E., Ferri, C., Fallahi, P., 2014. Chemokine (C-X-C motif) ligand (CXCL)10 in autoimmune diseases. Autoimmun. Rev. 13, 272–280. https://doi.org/10.1016/j.autrev.2013.10.010.

Boissier, M.C., Semerano, L., Challal, S., Saidenberg-Kermanac'h, N., Falgarone, G., 2012. Rheumatoid arthritis: From autoimmunity to synovitis and joint destruction. J. Autoimmun. 39, 222–228. https://doi.org/10.1016/j.jaut.2012.05.021.

Camps, M., Rückle, T., Ji, H., Ardissone, V., Rintelen, F., Shaw, J., Ferrandi, C., Chabert, C., Gillieron, C., Françon, B., Martin, T., Gretener, D., Perrin, D., Leroy, D., Vitte, P.-A., Hirsch, E., Wymann, M.P., Cirillo, R., Schwarz, M.K., Rommel, C., 2005. Blockade of PI3Kgamma suppresses joint inflammation and damage in mouse models of rheumatoid arthritis. Nat. Med. 11, 936–943. https://doi.org/10.1038/nm1284.

Carlberg, I., Mannervik, B., 1975. Purification and characterization of the flavoenzyme glutathione reductase from rat liver. J. Biol. Chem. 250, 5475–5480. https://doi.org/5480.

Carrasco, T.G., Morales, R.A., Pérez, F., Terraza, C., Yáñez, L., Campos-Mora, M., Pino-Lagos, K., 2015. Alarmin' immunologists: IL-33 as a putative target for modulating T cell-dependent responses. Front. Immunol. 6, 4–11. https://doi.org/10.3389/fimmu.2015.00232.

Caselli, G., Bonazzi, A., Lanza, M., Ferrari, F., Maggioni, D., Ferioli, C., Giambelli, R., Comi, E., Zerbi, S., Perrella, M., Letari, O., Di Luccio, E., Colovic, M., Persiani, S., Zanelli, T., Mennuni, L., Piepoli, T., Rovati, L.C., 2018. Pharmacological characterisation of CR6086, a potent prostaglandin E2receptor 4 antagonist, as a new potential disease-modifying anti-rheumatic drug. Arthritis Res. Ther. 20, 1–19. https://doi.org/10.1186/s13075-018-1537-8.

Chairungsrilerd, N., Furukawa, K., Ohta, T., Nozoe, S., Ohizumi, Y., 1996. Pharmacological properties of alpha-mangostin, a novel histamine H1 receptor antagonist. Eur. J. Pharmacol. 314, 351–356.

Chen, L.G., Yang, L.L., Wang, C.C., 2008. Anti-inflammatory activity of mangostins from Garcinia mangostana. Food Chem. Toxicol. 46, 688–693. https://doi.org/10.1016/j.fct.2007.09.096.

Chen, S., Chen, B., Wen, Z., Huang, Z., Ye, L., 2017. IL-33/ST2-mediated inflammation in macrophages is directly abrogated by IL-10 during rheumatoid arthritis. Oncotarget 8, 32407–32418. https://doi.org/10.18632/oncotarget.16299.

Cho, M. La, Heo, Y.J., Park, M.K., Oh, H.J., Park, J.S., Woo, Y.J., Ju, J.H., Park, S.H., Kim, H.Y., Min, J.K., 2009. Grape seed proanthocyanidin extract (GSPE) attenuates collagen-induced arthritis. Immunol. Lett. 124, 102–110. https://doi.org/10.1016/j.imlet.2009.05.001.

Choy, E., 2012. Understanding the dynamics: pathways involved in the pathogenesis of rheumatoid arthritis. Rheumatology 51, v3–v11. https://doi.org/10.1093/rheumatology/kes113.

Deschamps, J.D., Gautschi, J.T., Whitman, S., Johnson, T.A., Gassner, N.C., Crews, P., Holman, T.R., 2007. Discovery of platelet-type 12-human lipoxygenase selective inhibitors by high-throughput screening of structurally diverse libraries. Bioorg. Med. Chem. 15, 6900–6908. https://doi.org/10.1016/j.bmc.2007.08.015.

Devine, E.B., Alfonso-Cristancho, R., Sullivan, S.D., 2011. Effectiveness of biologic therapies for rheumatoid arthritis: an indirect comparisons approach. Pharmacotherapy 31, 39–51. https://doi.org/10.1592/phco.31.1.39.

Doi, H., Shibata, M.A., Shibata, E., Morimoto, J., Akao, Y., Iinuma, M., Tanigawa, N., Otsuki, Y., 2009. Panaxanthone isolated from pericarp of Garcinia mangostana L. suppresses tumor growth and metastasis of a mouse model of mammary cancer. Anticancer Res. 29, 2485–2495. https://doi.org/VL - 29.

Duskin, A., Eisenberg, R.A., 2010. The role of antibodies in inflammatory arthritis. Immunol. Rev. 233, 112–125. https://doi.org/10.1111/j.0105-2896.2009.00856.x.

Ee, G.C.L., Daud, S., Taufiq-Yap, Y.H., Ismail, N.H., Rahmani, M., 2006. Xanthones from Garcinia mangostana (Guttiferae). Nat. Prod. Res. 20, 1067–1073. https://doi.org/10.1080/14786410500463114.

Feldmann, M., Maini, S.R.N., 2008. Role of cytokines in rheumatoid arthritis: An education in pathophysiology and therapeutics. Immunol. Rev. 223, 7–19. https://doi.org/10.1111/j.1600-065X.2008.00626.x.

Fernández-Checa, J.C., Kaplowitz, N., 1990. The use of monochlorobimane to determine hepatic GSH levels and synthesis. Anal. Biochem. 190, 212–219. https://doi.org/10.1016/0003-2697(90)90183-A.

Filippin, L.I., Vercelino, R., Marroni, N.P., Xavier, R.M., 2008. Redox signalling and the inflammatory response in rheumatoid arthritis. Clin. Exp. Immunol. 152, 415–422. https://doi.org/10.1111/j.1365-2249.2008.03634.x.

Frisenda, S., Perricone, C., Valesini, G., 2013. Cartilage as a target of autoimmunity: A thin layer. Autoimmun. Rev. 12, 591–598. https://doi.org/10.1016/j.autrev.2012.10.003.

Gaffen, S.L., 2009. The role of interleukin-17 in the pathogenesis of rheumatoid arthritis. Curr. Rheumatol. Rep. 11, 365–370. https://doi.org/10.1007/s11926-009-0052-y.

Gérard-Monnier, D., Erdelmeier, I., Régnard, K., Moze-Henry, N., Yadan, J.-C., Chaudière, J., 1998. Reactions of 1-Methyl-2-phenylindole with Malondialdehyde and 4-Hydroxyalkenals. Analytical Applications to a Colorimetric Assay of Lipid Peroxidation. Chem. Res. Toxicol. 11, 1176–1183. https://doi.org/10.1021/tx9701790.

Gopalakrishnan, C., Shankaranarayanan, D., Kameswaran, L., Nazimudeen, S.K., 1980. Effect of mangostin, a xanthone from Garcinia mangostana Linn. in immunopathological & inflammatory reactions. Indian J. Exp. Biol. 18, 843–846.

Goronzy, J.J., Weyand, C.M., 2005. Rheumatoid arthritis. Immunol. Rev. 204, 55–73. https://doi.org/10.1111/j.0105-2896.2005.00245.x.

Griffiths, H.R., 2005. ROS as signalling molecules in T cells–evidence for abnormal redox signalling in the autoimmune disease, rheumatoid arthritis. Redox Rep. 10, 273–280. https://doi.org/10.1179/135100005X83680.

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130–7139. https://doi.org/10.14026/j.cnki.0253-9705.2010.23.013.

Hitchon, C.A., El-Gabalawy, H.S., 2004. Oxidation in rheumatoid arthritis. Arthritis Res. Ther. 6, 265–278. https://doi.org/10.1186/ar1447.

Hoxha, M., 2018. A systematic review on the role of eicosanoid pathways in rheumatoid arthritis. Adv. Med. Sci. 63, 22–29. https://doi.org/10.1016/j.advms.2017.06.004.

Ibrahim, M.Y., Hashim, N.M., Mariod, A.A., Mohan, S., Abdulla, M.A., Abdelwahab, S.I., Arbab, I.A., 2016. α-Mangostin from Garcinia mangostana Linn: An updated review of its pharmacological properties. Arab. J. Chem. 9, 317–329. https://doi.org/10.1016/j.arabjc.2014.02.011.

Ip, W.K.E., Hoshi, N., Shouval, D.S., Snapper, S., Medzhitov, R., 2017. Anti-inflammatory effect of IL-10 mediated by metabolic reprogramming of macrophages. Science 356, 513–519. https://doi.org/10.1126/science.aal3535.

Iwamoto, T., Okamoto, H., Toyama, Y., Momohara, S., 2008. Molecular aspects of rheumatoid arthritis: Chemokines in the joints of patients. FEBS J. 275, 4448–4455. https://doi.org/10.1111/j.1742-4658.2008.06580.x.

Jang, H.Y., Kwon, O.K., Oh, S.R., Lee, H.K., Ahn, K.S., Chin, Y.W., 2012. Mangosteen xanthones mitigate ovalbumin-induced airway inflammation in a mouse model of asthma. Food Chem. Toxicol. 50, 4042–4050. https://doi.org/10.1016/j.fct.2012.08.037.

Ji, X., Avula, B., Khan, I.A., 2007. Quantitative and qualitative determination of six xanthones in Garcinia mangostana L. by LC-PDA and LC-ESI-MS. J. Pharmaceut. Biomed. Anal. 43, 1270–1276. https://doi.org/10.1016/j.jpba.2006.10.018.

Jia, X.Y., Chang, Y., Sun, X.J., Dai, X., Wei, W., 2014. The role of prostaglandin E2 receptor signaling of dendritic cells in rheumatoid arthritis. Int. Immunopharm. 23,

163–169. https://doi.org/10.1016/j.intimp.2014.08.024.
Kleinewietfeld, M., Hafler, D.A., 2013. The plasticity of human Treg and Th17 cells and its role in autoimmunity. Semin. Immunol. 3425, 305–312. https://doi.org/10.1016/j.smim.2013.10.009.
Kobayashi, S., Momohara, S., Kamatani, N., Okamoto, H., 2008. Molecular aspects of rheumatoid arthritis: Role of environmental factors. FEBS J. 275, 4456–4462. https://doi.org/10.1111/j.1742-4658.2008.06581.x.
Koch, A.E., 2005. Chemokines and their receptors in rheumatoid arthritis: Future targets? Arthritis Rheum. 52, 710–721. https://doi.org/10.1002/art.20932.
Konya, V., Marsche, G., Schuligoi, R., Heinemann, A., 2013. E-type prostanoid receptor 4 (EP4) in disease and therapy. Pharmacol. Ther. 138, 485–502. https://doi.org/10.1016/j.pharmthera.2013.03.006.
Kotake, S., Yago, T., Kobashigawa, T., Nanke, Y., 2017. The Plasticity of Th17 Cells in the Pathogenesis of Rheumatoid Arthritis. J. Clin. Med. 6, 67. https://doi.org/10.3390/jcm6070067.
Lawrence, R.A., Burk, R.F., 1976. Glutathione peroxidase activity in selenium-deficient rat liver. Biochem. Biophys. Res. Commun. 71, 952–958. https://doi.org/10.1016/0006-291X(76)90747-6.
Lee, L.T., Tsai, Y.F., Hu, N.Y., Wang, C.W., Huang, K.K., Hsiao, J.K., Shih, Y.C., Munekazu, I., 2013. Anti-arthritis effect of mangostins from G.Mangostana. Biomed. Prev. Nutr. 3, 227–232. https://doi.org/10.1016/j.bionut.2012.10.002.
Li, M.O., Wan, Y.Y., Flavell, R.A., 2007. T Cell-Produced Transforming Growth Factor-β1 Controls T Cell Tolerance and Regulates Th1- and Th17-Cell Differentiation. Immunity 26, 579–591. https://doi.org/10.1016/j.immuni.2007.03.014.
Li, Q., Han, D., Cong, B., Shan, B., Zhang, J., Chen, H., Ma, C., Liyanage, S.S., 2011. Cholecystokinin octapeptide significantly suppresses collagen-induced arthritis in mice by inhibiting Th17 polarization primed by dendritic cells. Cell. Immunol. 272, 53–60. https://doi.org/10.1016/j.cellimm.2011.09.007.
Li, S., Yin, H., Zhang, K., Wang, T., Yang, Y., Liu, X., Chang, X., Zhang, M., Yan, X., Ren, Y., Pan, W., Zhang, L., 2017. Effector T helper cell populations are elevated in the bone marrow of rheumatoid arthritis patients and correlate with disease severity. Sci. Rep. 7, 4776. https://doi.org/10.1038/s41598-017-05014-8.
Liu, Y., Park, J.M., Chang, K.H., Chin, Y.W., Lee, M.Y., 2015. α- and γ-mangostin cause shape changes, inhibit aggregation and induce cytolysis of rat platelets. Chem. Biol. Interact. 240, 240–248. https://doi.org/10.1016/j.cbi.2015.08.021.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275. https://doi.org/10.1016/0304-3894(92)87011-4.
Lubberts, E., 2010. Th17 cytokines and arthritis. Semin. Immunopathol. 32, 43–53. https://doi.org/10.1007/s00281-009-0189-9.
Lutz, M.B., Kukutsch, N., Ogilvie, A.L., Rößner, S., Koch, F., Romani, N., Schuler, G., 1999. An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. J. Immunol. Methods 223, 77–92. https://doi.org/10.1016/S0022-1759(98)00204-X.
Lyer, S.S., Cheng, G., 2013. Role of Interleukin 10 Transcriptional Regulation in Inflammation and autoimmune disease. Natl. Inst. Heal. 10, 54–56. https://doi.org/10.1038/nmeth.2250.Digestion.
Márquez-Valadez, B., Lugo-Huitrón, R., Valdivia-Cerda, V., Miranda-Ramírez, L.R., Pérez-De La Cruz, V., González-Cuahutencos, O., Rivero-Cruz, I., Mata, R., Santamaría, A., Pedraza-Chaverrí, J., 2009. The natural xanthone alpha-mangostin reduces oxidative damage in rat brain tissue. Nutr. Neurosci. 12, 35–42. https://doi.org/10.1179/147683009X388850.
Mateen, S., Moin, S., Zafar, A., Khan, A.Q., 2016. Redox signaling in rheumatoid arthritis and the preventive role of polyphenols. Clin. Chim. Acta 463, 4–10. https://doi.org/10.1016/j.cca.2016.10.007.
McInnes, I.B., McInnes, I.B., Schett, G., Schett, G., 2007. Cytokines in the pathogenesis of rheumatoid arthritis. Nat. Rev. Immunol. 7, 429–442. https://doi.org/10.1038/nri2094.
Mellado, M., Martínez-Muñoz, L., Cascio, G., Lucas, P., Pablos, J.L., Rodríguez-Frade, J.M., 2015. T cell migration in rheumatoid arthritis. Front. Immunol. 6, 384. https://doi.org/10.3389/fimmu.2015.00384.
Mijnheer, G., Prakken, B.J., van Wijk, F., 2013. The effect of autoimmune arthritis treatment strategies on regulatory T-cell dynamics. Curr. Opin. Rheumatol. 25, 260–267. https://doi.org/10.1097/BOR.0b013e32835d0ee4.
Mirshafiey, A., Mohsenzadegan, M., 2008. The role of reactive oxygen species in immunopathogenesis of rheumatoid arthritis. Iran. J. Allergy, Asthma Immunol. 7, 195–202. https://doi.org/07.04/ijaai.195202.
Nakagawa, Y., Iinuma, M., Naoe, T., Nozawa, Y., Akao, Y., 2007. Characterized mechanism of α-mangostin-induced cell death: Caspase-independent apoptosis with release of endonuclease-G from mitochondria and increased miR-143 expression in human colorectal cancer DLD-1 cells. Bioorg. Med. Chem. 15, 5620–5628. https://doi.org/10.1016/j.bmc.2007.04.071.
Nava Catorce, M., Acero, G., Pedraza-Chaverri, J., Fragoso, G., Govezensky, T., Gevorkian, G., 2016. Alpha-mangostin attenuates brain inflammation induced by peripheral lipopolysaccharide administration in C57BL/6J mice. J. Neuroimmunol. 15, 20–27. https://doi.org/10.1016/j.jneuroim.2016.05.008.
Nelli, G.B., K, A.S., Kilari, E.K., 2013. Antidiabetic effect of alpha-mangostin and its protective role in sexual dysfunction of streptozotocin induced diabetic male rats. Syst. Biol. Reprod. Med. 59, 319–328. https://doi.org/10.3109/19396368.2013.820369.
Nguemfo, E.L., Dimo, T., Dongmo, A.B., Azebaze, A.G.B., Alaoui, K., Asongalem, A.E., Cherrah, Y., Kamtchouing, P., 2009. Anti-oxidative and anti-inflammatory activities of some isolated constituents from the stem bark of Allanblackia monticola Staner L.C (Guttiferae). Inflammopharmacology 17, 37–41. https://doi.org/10.1007/s10787-008-8039-2.
Oberley, L.W., Spitz, D.R., 1984. [61] Assay of Superoxide Dismutase Activity in Tumor Tissue. Methods Enzymol. 105, 457–464. https://doi.org/10.1016/S0076-6879(84)05064-3.
Palmer, G., Talabot-Ayer, D., Lamacchia, C., Toy, D., Seemayer, C.A., Viatte, S., Finckh, A., Smith, D.E., Gabay, C., 2009. Inhibition of interleukin-33 signaling attenuates the severity of experimental arthritis. Arthritis Rheum. 60, 738–749. https://doi.org/10.1002/art.24305.
Park, M.K., Park, J.S., Cho, M. La, Oh, H.J., Heo, Y.J., Woo, Y.J., Heo, Y.M., Park, M.J., Park, H.S., Park, S.H., Kim, H.Y., Min, J.K., 2011. Grape seed proanthocyanidin extract (GSPE) differentially regulates Foxp3 + regulatory and IL-17 + pathogenic T cell in autoimmune arthritis. Immunol. Lett. 135, 50–58. https://doi.org/10.1016/j.imlet.2010.09.011.
Pedraza-Chaverri, J., Cárdenas-Rodríguez, N., Orozco-Ibarra, M., Pérez-Rojas, J.M., 2008. Medicinal properties of mangosteen (Garcinia mangostana). Food Chem. Toxicol. 46, 3227–3239. https://doi.org/10.1016/j.fct.2008.07.024.
Pedraza-Chaverrí, J., Reyes-Fermín, L.M., Nolasco-Amaya, E.G., Orozco-Ibarra, M., Medina-Campos, O.N., González-Cuahutencos, O., Rivero-Cruz, I., Mata, R., 2009. ROS scavenging capacity and neuroprotective effect of alpha-mangostin against 3-nitropropionic acid in cerebellar granule neurons. Exp. Toxicol. Pathol. 61, 491–501. https://doi.org/10.1016/j.etp.2008.11.002.
Quinonez-Flores, C.M., Gonzalez-Chavez, S.A., Del Rio Najera, D., Pacheco-Tena, C., 2016. Oxidative Stress Relevance in the Pathogenesis of the Rheumatoid Arthritis: A Systematic Review. BioMed Res. Int. 2016, 6097417 14 pages. https://doi.org/10.1155/2016/6097417.
Radkar, V., Hardej, D., Lau-Cam, C., Billack, B., 2007. Evaluation of resveratrol and piceatannol cytotoxicity in macrophages, T cells, and skin cells. Arh. Hig. Rada. Toksikol. 58, 293–304. https://doi.org/10.2478/v10004-007-0020-8.
Reznick, A.Z., Packer, L., 1994. Oxidative damage to proteins: Spectrophotometric method for carbonyl assay. Methods Enzymol. 233, 357–363. https://doi.org/10.1016/S0076-6879(94)33041-7.
Rogers, N.M., Kireta, S., Coates, P.T.H., 2010. Curcumin induces maturation-arrested dendritic cells that expand regulatory T cells in vitro and in vivo. Clin. Exp. Immunol. 162, 460–473. https://doi.org/10.1111/j.1365-2249.2010.04232.x.
Roseghini, R., Rocha, D.S., Clarêncio, J., Costa, S.L., Costa, M.F.D., Tardy, M., Nascimento, R., Schaer, R., Velozo, E., Meyer, R., Freire, S., 2007. Flavonoid rutin alters the viability and function of mitogen-stimulated splenocytes and thymocytes compared with non stimulated cells. Immunopharmacol. Immunotoxicol. 29, 271–285. https://doi.org/10.1080/08923970701512940.
Rump, L., Mattey, D.L., Kehoe, O., Middleton, J., 2017. An initial investigation into endothelial CC chemokine expression in the human rheumatoid synovium. Cytokine 97, 133–140. https://doi.org/10.1016/j.cyto.2017.05.023.
Sampath, P.D., Vijayaraghavan, K., 2007. Cardioprotective effect of α-mangostin, a xanthone derivative from Mangosteen on tissue defense system against isoproterenol-induced myocardial infarction in rats. J. Biochem. Mol. Toxicol. 21, 336–339. https://doi.org/10.1002/jbt.20199.
Sarkar, S., Cooney, L.A., Fox, D.A., 2010. The role of T helper type 17 cells in inflammatory arthritis. Clin. Exp. Immunol. 159, 225–237. https://doi.org/10.1111/j.1365-2249.2009.04016.x.
Schaeverbeke, T., Truchetet, M. Élise, Richez, C., 2012. When and where does rheumatoid arthritis begin? Jt. Bone Spine 79, 550–554. https://doi.org/10.1016/j.jbspin.2012.09.004.
Schwarz, H., Schmittner, M., Duschl, A., Horejs-Hoeck, J., 2014. Residual endotoxin contaminations in recombinant proteins are sufficient to activate human CD1c+ dendritic cells. PLoS One 9. https://doi.org/10.1371/journal.pone.0113840.
Shan, T., Cui, X., Li, W., Lin, W., Lu, H., Li, Y., Chen, X., Wu, T., 2014. α-Mangostin suppresses human gastric adenocarcinoma cells in vitro via blockade of Stat3 signaling pathway. Acta Pharmacol. Sin. 35, 1065–1073. https://doi.org/10.1038/aps.2014.43.
Shrestha, S., Yang, K., Guy, C., Vogel, P., Neale, G., Chi, H., 2015. Treg cells require the phosphatase PTEN to restrain TH1 and TFH cell responses. Nat. Immunol. 16, 178–187. https://doi.org/10.1038/ni.3076.
Siqueira Mietto, B., Kroner, A., Girolami, E.I., Santos-Nogueira, E., Zhang, J., David, S., 2015. Role of IL-10 in Resolution of Inflammation and Functional Recovery after Peripheral Nerve Injury. J. Neurosci. 35, 16431–16442. https://doi.org/10.1523/JNEUROSCI.2119-15.2015.
Smith, J.B., Haynes, M.K., 2002. Rheumatoid Arthritis—A Molecular Understanding. Ann. Intern. Med. 136, 908–922. https://doi.org/10.7326/0003-4819-136-12-200206180-00012.
Srirangan, S., Choy, E.H., 2010. The role of Interleukin 6 in the pathophysiology of rheumatoid arthritis. Ther. Adv. Musculoskelet. Dis. 2, 247–256. https://doi.org/10.1177/1759720X10378372.
Tewtrakul, S., Wattanapiromsakul, C., Mahabusarakam, W., 2009. Effects of compounds from Garcinia mangostana on inflammatory mediators in RAW264.7 macrophage cells. J. Ethnopharmacol. 121, 379–382. https://doi.org/10.1016/j.jep.2008.11.007.
Trouw, L.A., Huizinga, T.W.J., Toes, R.E.M., 2013. Autoimmunity in rheumatoid arthritis: different antigens—common principles. Ann. Rheum. Dis. 72, ii132–ii136. https://doi.org/10.1136/annrheumdis-2012-202349.
Tsubaki, T., Takegawa, S., Hanamoto, H., Arita, N., Kamogawa, J., Yamamoto, H., Takubo, N., Nakata, S., Yamada, K., Yamamoto, S., Yoshie, O., Nose, M., 2005. Accumulation of plasma cells expressing CXCR3 in the synovial sublining regions of early rheumatoid arthritis in association with production of Mig/CXCL9 by synovial fibroblasts. Clin. Exp. Immunol. 141, 363–371. https://doi.org/10.1111/j.1365-2249.2005.02850.x.
Vieira, S.M., Lemos, H.P., Grespan, R., Napimoga, M.H., Dal-Secco, D., Freitas, A., Cunha, T.M., Verri, W.A., Souza, D.A., Jamur, M.C., Fernandes, K.S., Oliver, C., Silva, J.S., Teixeira, M.M., Cunha, F.Q., 2009. A crucial role for TNF-α in mediating neutrophil influx induced by endogenously generated or exogenous chemokines, KC/CXCL1 and

LIX/CXCL5. Br. J. Pharmacol. 158, 779–789. https://doi.org/10.1111/j.1476-5381.2009.00367.x.

Vincent, T.L., Williams, R.O., Maciewicz, R., Silman, A., Garside, P., Bevan, S., Chanalaris, A., Chapman, V., Cope, A., Cruwys, S., Dell'Accio, F., Gaskin, P., Gilroy, D., Glasson, S., Hegen, M., McDougall, J., Moore, A., du Sert, N.P., Perretti, M., Pitsillides, A., Robinson, V., Seed, M., Thompson, S., Walsh, D.A., Williams, N., 2012. Mapping pathogenesis of arthritis through small animal models. Rheumatol. (United Kingdom) 51, 1931–1941. https://doi.org/10.1093/rheumatology/kes035.

Weinblatt, M.E., 2013. Methotrexate in rheumatoid arthritis: a quarter century of development. Trans. Am. Clin. Climatol. Assoc. 124, 16–25.

Williams, P.J., Jones, R.H.V., Rademacher, T.W., 1998. Correlation between IgG anti-type II collagen levels and arthritic severity in murine arthritis. Autoimmunity 27, 201–207. https://doi.org/10.3109/08916939808993831.

Xuzhu, G., Komai-Koma, M., Leung, B.P., Howe, H.S., McSharry, C., McInnes, I.B., Xu, D., 2012. Resveratrol modulates murine collagen-induced arthritis by inhibiting Th17 and B-cell function. Ann. Rheum. Dis. 71, 129–135. https://doi.org/10.1136/ard.2011.149831.

Yoshida, Y., Tanaka, T., 2014. Interleukin 6 and rheumatoid arthritis. BioMed Res. Int. 2014, 698313 12 pages. https://doi.org/10.1155/2014/698313.

Yu, M.B., Langridge, W.H.R., 2017. The function of myeloid dendritic cells in rheumatoid arthritis. Rheumatol. Int. 37, 1043–1051. https://doi.org/10.1007/s00296-017-3671-z.

Zhang, J., Chen, Q., Wang, S., Li, T., Xiao, Z., Lan, W., Huang, G., Cai, X., 2018. α-Mangostin, A Natural Xanthone, Induces Apoptosis and ROS Accumulation in Human Rheumatoid Fibroblast-Like Synoviocyte MH7A Cells. Curr. Mol. Med. 17, 375–380. https://doi.org/10.2174/1566524018666171205123220.