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BIOMEDICAL SCIENCES

Quercetin and ibuprofen combination displayed anti-inflammatory effects and also extenuates the enteric neurons damage of arthritic rats

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Abstract: This study aimed to investigate the antioxidant and anti-inflammatory properties of quercetin on the cellular components of the Enteric Nervous System in the ileum of rats with arthritis. Rats were distributed into five groups: control (C), arthritic (AIA), arthritic treated with ibuprofen (AI), arthritic treated with quercetin (AQ) and arthritic treated with both ibuprofen and quercetin (AIQ). The ileum was processed for immunohistochemical techniques for HuC/D, calcitonin gene-related peptide, and vasoactive intestinal polypeptide. Measurements in histological sections, chemiluminescence assays, and total antioxidant capacity were also performed. Rheumatoid arthritis resulted in a decrease in neuronal density, yet neuroplasticity mechanisms were evident through observed changes in varicosities size and neuronal area compared to the control group. Reduced paw edema and neuroprotective effects were predominantly noted in both plexuses, as evidenced by the increased density preservation of HuC/D-IR neurons in the AIQ group. The increase of lipoperoxidation levels and paw edema volume in the AQ group was observed compared to the arthritic, whereas the AIQ group mainly showed similar results to those observed in the control. The enteropathy associated with arthritis proved to be significant in the field of gastroenterology, and the combination of quercetin and ibuprofen demonstrated promising anti-inflammatory and neuroprotective effects.

Key words: Enteric nervous system, ileum, neuroplasticity, quercetin, rheumatoid arthritis.

INTRODUCTION

Rheumatoid arthritis (RA) affects approximately 1% of the world's population, and it can cause irreversible changes in the body (Taneja 2014). RA is recognized as a chronic inflammatory disease that is not only limited to the bones and joints but can also affect several body systems. It is characterized as an autoimmune, multisystem disease, which includes the digestive system and consequently the enteric nervous system (ENS) (Veselinovic et al. 2014, Smolen et al. 2016, Quinonez-Flores et al. 2016).

The digestive system becomes sensitized in arthritic individuals, who may experience symptoms such as gastroesophageal reflux, abdominal pain, diarrhea, and constipation. These symptoms may be directly related to the disease itself or the medications used, ultimately affecting the enteric nervous system (Smolen & Aletaha 2015).

In the traditional treatment of RA drugs have been used to reduce joint pain, stiffness, and swelling., mainly methotrexate, disease-modifying antirheumatic drugs (DMARDs), and non-steroidal anti-inflammatory drugs (NSAIDs, e.g. ibuprofen) (Jeyadevi et al. 2013, Ji et al. 2013, Smolen & Aletaha 2015). Frequent use of these medicines is associated with gastrointestinal side effects (Jeyadevi et al. 2013). Biologic drug therapies have been used for severe cases of RA, which are agents that inhibit the synthesis of inflammatory cytokines and regulate lymphocyte activity (Vivar & Van Vollenhoven 2014). Co-administration of antioxidants with traditional antirheumatic drugs already present on the market may result in promising beneficial effects (Helmy et al. 2001).

In this context, quercetin, a flavonoid and polyphenolic compound that stands out due to its widespread consumption in the human diet, through found in fruits (e.g. apples and grapes), and vegetables (e.g. broccoli and onions) cereals, nuts, wines, and teas, is renowned for its anti-inflammatory and antioxidant properties (Liu et al. 2015). It is known for its anti-inflammatory and antioxidant properties, which can be beneficial both for alleviating the side effects of traditional medication for rheumatoid arthritis and as a means to reduce systemic damage caused to the gastrointestinal tract, primarily due to effects related to exacerbated oxidative stress (Wang et al. 2021).

Recent studies have highlighted the anti-inflammatory, antioxidant, hypertensive, anti-obesity, anti-hypercholesterolemic, anti-atherosclerotic, and anti-tumor activities of quercetin (Hasnat et al. 2024). It has been shown to alleviate arthritis symptoms by suppressing the release of inflammatory cytokines, reducing levels of cyclooxygenase (COX-2) induced by lipopolysaccharide, and inhibiting bone resorption through the suppression of nuclear factor-kappa β (NF-kβ) and AP-1 activity. In the pathophysiology of the disease, the increase of oxidative stress and the production of pro-inflammatory cytokines has been reported in chronic degenerative diseases, additionally, quercetin prevents the recruitment of macrophages, neutrophils and synoviocyte proliferation (Parthiban et al. 1995, Obrosova 2002, Veselinovic et al. 2014, El-Said et al. 2022).

Enteric neurodegeneration is poorly reported in RA (Cojocaru et al. 2011). The gastrointestinal tract is innervated by neurons of the Enteric Nervous System (ENS), which are responsible for the control of motility, absorption, secretion, maintenance of mucosal integrity, and immune function (Furness 2008). Peripheral neuropathy has been already reported as extra-articular manifestations induced by RA, which leads to sensory and motor alterations (Albani et al. 2006, Agarwal et al. 2008, Sim et al. 2014). In a study using the RA model, segments of the jejunum and ileum of Holztman arthritic rats induced by Complete Freud's Adjuvant (CFA) did not display quantitative changes, although morphometric changes were observed in the myenteric neuronal population (Souza et al. 2011).

The vasoactive intestinal polypeptide (VIP) has anti-inflammatory, antioxidant, and anti-apoptotic effects and stimulates smooth muscle relaxation and secretion of fluids and electrolytes. This neurotransmitter is able to regulate various transduction and transcription pathways, leading to a decrease of inflammation by the inhibition of the production of a broad spectrum of proinflammatory cytokines (Furness 2008, Nezami & Srinivasan 2010). Furthermore, VIP exerts an important control of defense cells (e.g. neutrophils, macrophages and lymphocytes). This polypeptide is also involved in neuroplasticity and neuroprotection mechanisms and is considered a neurotrophic

factor, which stimulates the synthesis of other growth factors and inhibits cellular apoptosis induced by oxidative stress (Delgado et al. 2004, Ekblad & Bauer 2004, Gonzalez-Rey et al. 2005, Brenneman 2007, White et al. 2010, Morell et al. 2012, Deng et al. 2016).

Calcitonin gene-related peptide (CGRP) is a vasodilator neuropeptide that exerts a crucial role in nociceptive transmission pathways and downregulation of inflammatory responses, expressing antioxidant and anti-inflammatory properties. CGRP also stimulates smooth muscle relaxation and is expressed by different immune cells (Furness 2008, Chandrasekharan et al. 2013, Holzmann 2013, Wu et al. 2015).

Quercetin has important biological effects (e.g. anti-inflammatory and antioxidant activities) (Nabavi et al. 2015, Caparroz-Assef et al. 2007). Building upon these findings, we can hypothesize in our study that quercetin, with its potential properties, may aid in the treatment of rheumatoid arthritis by alleviating the effects of the inflammatory disease itself and the side effects caused by traditional medication.

The objective of this study was to evaluate the effects of quercetin on rheumatic inflammatory parameters and on the morphology of ENS structures in the ileum of arthritic rats using a dosage of 50 mg/kg and combined treatment of 17.5 mg/kg of ibuprofen with 50 mg/kg of quercetin.

MATERIALS AND METHODS

Animals and experimental designs

Experimental procedures were carried out following the international norms of ethical conduct and were previously approved by the Committee of Ethical Conduct on the Use of Animals in Experimentation of the Universidade Estadual de Maringá - Paraná - Brazil, CEUA nº 4462180216.

We used twenty-five 56-day-old male Holtzman rats (*Rattus norvegicus*) obtained from the Central Bioterium of the Universidade Estadual de Maringá. Rats were kept in controlled environmental conditions of temperature (22° ± 2°C) and illumination (cycle 12/12 hours light-dark). All animals received standard balanced Nuvital feed (Nuvital®, Colombo, Paraná, Brazil) and water *ad libitum* in the Sectorial Bioterium of the Department of Pharmacology and Therapy.

After 3 days of adaptation, the 56-day-old rats were weighing 183.2 ± 13.8 g. The animals were randomly distributed into 5 groups: control (C), adjuvant-induced arthritic without treatment (AIA), arthritic treated with ibuprofen (AI), arthritic treated with quercetin (AQ) and combined treatment with ibuprofen and quercetin (AIQ). Animals were maintained during 60 days of the experimental period and the treatments were performed every day using gavage. Animals were weighed on alternating days to calculate the daily dose to be administered.

RA was induced by intradermal injection of 0.1 mL CFA of 5% heat-killed suspension of *Mycobacterium tuberculosis* in the plantar region of the left hind paw. Control animals were submitted to the same procedure but received an intradermal injection containing only the vehicle mineral oil (Nujol®, Schering-Plow, São Paulo, Brazil) (Bracht et al. 2012, Costa et al. 2016). Treatments were carried out via gavage with quercetin administered at the dose of 50 mg/kg (Kaur et al. 2011, Phillips & Powley 2007), diluted in water and ibuprofen 17.5 mg/kg diluted in carboxymethylcellulose 10% (Kaur et al. 2011). Control animals received gavage only using the diluents. In this study, a dosage of 17.5 mg/kg of ibuprofen was chosen to align with previous research as a positive control, representing a conventional anti-inflammatory medication commonly used by humans (Wisniewski-Rebecca

et al. 2015, Aguiar et al. 2017, Barbosa et al. 2017, da Rocha et al. 2017).

Evaluation of chronic inflammatory response

Body weight

Body weight was measured on alternating days in the morning throughout the experimental period.

Total and differential leukocyte quantification

Blood was collected from the tail of each animal one day prior to induction and at the end of the experiment. Samples were diluted 1:20 in Turk's liquid and total leukocytes were quantified using an optical microscope (Motic China Group, Shanghai, China) in a 10x objective lens with a Neubauer chamber and then, multiplied by a correction factor of 50x. Differential quantification was performed using a blood smear and stained by the May-Grünwald-Giemsa method and quantified by optical microscopy in a 100x objective lens using immersion oil.

Paw edema

The increase of paw volume (µL) was measured from hind paws to tibiotarsal joints using a digital plethysmograph (Ugo Basili®, Milan, Italy) on days 1st, 3rd, 10th, 14th, 21st, 28th, 35th, 42nd, 49th and 56th, after RA induction.

Euthanasia, sample collection, and processing of tissues

After sixty days, animals, which were previously kept for 12 hours of fasting, received administration of vincristine (Fracaro et al. 2016) (0.5 mg/kg body weight; Eurofarma®, São Paulo, Brazil) via penile vein 2 hours before euthanasia in order to block the formation of microtubules and cell proliferation (Binet et al. 1990) Subsequently, the euthanasia was performed under intraperitoneal anesthesia using thiopental 40 mg/kg body weight (Abbott Laboratories, Chicago, IL, USA). The celiotomy was performed to remove the small intestine and then, its length and the ileal width were measured, and the total area of the small intestine was calculated in cm (Veselinovic et al. 2014, Vicentini et al. 2016).

The ileum was rinsed in phosphate-buffered saline (PBS; 0.1 M; pH 7.4), filled with Zamboni's fixative solution and then incubated for 18 hours at 4 °C. The samples were opened along the mesenteric border and washed in 80% alcohol until removal of the fixative. Tissues were sequentially dehydrated using a series of alcohols (95% and 100%), diaphanized in xylol, rehydrated with descending series of alcohols (100%, 90%, 80% and 50%) and then, stored in PBS containing 0.08% sodium azide (Sigma-Aldrich, Inc., St. Louis, MO, USA) at 4 °C.

Tissues were cut into pieces of approximately 1 cm^2 and microdissected in stereoscopic binocular loupes Stemi DV4 (Zeiss, Jena, Germany) in order to obtain whole-mount preparations of the muscular tunica and the submucosa.

Immunohistochemistry

Whole-mount preparations of the submucosal and muscular layers were washed in PBS with 0.5% Triton X-100 (Sigma, St. Louis, MO, USA) (2 x 10 minutes) and incubated in the same solution that also contained 2% bovine serum albumin (BSA) and 10% normal donkey serum (blocking solution) for 2 hours at room temperature (RT).

The tissues were incubated in the following primary antibodies: mouse anti-HuC/D (1:400; Molecular Probes, Eugene, OR, USA), rabbit anti-VIP (1:300; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and rabbit anti-CGRP (1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA) in a solution that contained PBS, 0.5% Triton X-100, 2% BSA

and 10% normal donkey serum for 2 hours in an incubator at 37 °C.

Samples were kept at RT for 46 hours under constant shaking. Intestinal samples were rinsed using PBS and 0.5% Triton X-100 and then, the tissues were incubated in the following secondary antibodies for 6 hours at RT: Alexa fluor 488 donkey anti-mouse (1:400; Molecular Probes, Eugene, OR, USA) against HuC/D, Alexa fluor 568 goat anti-rabbit (1:250; Molecular Probes, Eugene, OR, USA) against VIP and Alexa Fluor 488 donkey anti-rabbit (1:500; Molecular Probes, Eugene, OR, USA) against CGRP. Subsequently, whole-mount preparations were washed in PBS, the slides were mounted using 10% buffered glycerol and stored at 4 ºC.

Quantitative analysis of the enteric neurons HuC/D-IR and VIP-IR

Analysis was performed by random sampling in the intermediate region of the ileal circumference (60° - 120° and 240° - 300°, considering 0° as the mesenteric border) (Zanoni et al. 2005). Thirty images per animal were captured using Olympus® BX40 fluorescence optical microscope (Olympus, Tokyo, Japan) with 20x objective lens.

For the quantitative analysis of the myenteric and submucosal plexuses, all neurons present per image were counted. Quantification and determination of the image area were performed using Image Pro® Plus 4.5 image analysis software (Media Cybernetics, Inc. Silver Spring, MD, USA). The density for the total populations and subpopulations of neurons was expressed as the total number of neurons per cm^2.

A correction factor was calculated by comparison between the area of the intestinal segments of all experimental groups in relation to control area (Vicentini et al. 2016) (Table I). All density data were multiplied by a correction factor.

Morphometric analysis of neurons and varicosities

The measurements of the area size of neuronal body and varicosities in the nerve fibers were performed using the same images for neuronal

Table I. Physiological parameters.

Parameters			Experimental groups		
	C	AIA	AI	AQ	AIQ
IA (cm^2)	192 ± 41.0	162 ± 8.7	163 ± 12.1	138 ± 4.9	148 ± 5.8
CF	Standard	0.84	0.85	0.72	0.77
ITL/mm^3	9.2 ± 0.44	10.9 ± 0.73	10.7 ± 0.89	10.5 ± 0.92	10.2 ± 0.61
FTL/mm^3	10.2 ± 0.67	20.7 ±1.93*	19.4 ± 2.11*	27.4± 2.63*,#	17.0 ± 1.31*
IML (%)	96.4 ± 1.44	91.2 ± 1.02	91.2 ± 0.37	92.8 ± 1.74	89.0 ± 3.49*
FML (%)	79.6 ± 2.20	71.0 ± 3.72	63.6 ± 4.70*	66.8 ± 2.82*	67.6 ± 2.54*
IPL (%)	3.6 ± 1.44	8.8 ± 1.02	8.8 ± 0.37	7.2 ± 1.74	11.0 ± 3.49*
FPL (%)	20.4 ± 2.20	29.0 ± 3.72	36.4 ± 4.70*	33.2 ± 2.82*	32.4 ± 2.54*

Intestinal area (IA, expressed in cm^2), correction factors (CF) for neuronal density, quantification of initial (ITL) and final (FTL) total leukocytes (mm^3), differential quantification of initial mononuclear leukocytes (IML) final mononuclear leukocytes (FML) and initial polymorphonuclear leukocytes (IPL) and final polymorphonuclear leukocytes (FPL) in percentage. Experimental groups: control (C), adjuvant-induced arthritic without treatment (AIA), arthritic treated with 17.5 mg kg^{-1} ibuprofen (AI), arthritic treated with 50 mg kg^{-1} quercetin (AQ); arthritic treated with 50 mg kg^{-1} quercetin and 17.5 mg kg^{-1} ibuprofen (AIQ). n = 5 rats per group. Values expressed as mean ± standard error. * p < 0.05 compared to control, # p < 0.05 compared to AIA.

quantification. The areas (μm^2) of 100 cell bodies were obtained for HuC/D-IR neurons from each animal in both plexuses, but VIPergic neuronal area was only measured in the submucosal plexus using 20x objective lens using Image Pro® Plus 4.5 program. For the morphometric evaluation of the VIP-IR and CGRP-IR varicosities in both plexuses, the areas of 400 varicosities were measured per animal of images obtained using 40x objective lens using Image Pro® Plus 4.5 program.

Intestinal mucosa

The ileum was carefully washed for stool removal and opened along the mesenteric border. Samples were adhered on carton paper with the mucosal surface facing upwards and then, immersed in 10% buffered formalin for 6 hours at RT.

After fixation, dehydration and paraffin inclusion were performed, 2 µm thick semi-serial sections were obtained on a Leica RM2265 microtome (Leica Biosystems Nussloch GmbH, Nussloch, Germany) and stained using hematoxylin-eosin and finally, mounted with Permount.

The crypt depth and villus height were measured in histological sections, in which the crypt depth included the length between the junction crypt-villus and the base of the crypt, and villus height was measured from the junction until the top of villi. Morphometry was performed measuring 10 units of crypts and villi per animal using the software Image Pro® Plus (Media Cybernetics, MD, USA).

Oxidative state study

After collection, the intestinal segments were immediately washed, frozen in liquid nitrogen and then, stored in a freezer at -80 °C.

The level of lipoperoxidation of the samples was measured by chemiluminescence induced by tert-butyl hydroperoxide (Gonzalez Flecha et al. 1991), only modified by the addition of hemin (Zamburlini et al. 1995). The analysis was performed in relative light units (RLU) in the luminometer according to the consumption of the t-butyl hydroperoxide oxidizing agent that was added to the reaction. Determination of the total antioxidant capacity was also performed measuring the levels of total antioxidants of the tissue, mainly of low molecular weight antioxidants (Repetto et al. 1996), using vitamin D analogous (trolox) antioxidant as standard. A homogenate was obtained from the ileum using 10mM monobasic phosphate buffer (pH 7.4, 0.9% NaCl), which was prepared at a concentration of 5 mg mL^{-1} for chemiluminescence induced by tert-butyl hydroperoxide and 15 mg mL^{-1} for total antioxidant capacity.

Statistical analysis

The results were submitted a randomized block design in the programs Statistica 7, followed by the Fisher's Test with a level of significance of 5%. The graphics were created in GraphPad Prism 6.1 and expressed as mean ± standard error of the mean.

RESULTS

Physiological and inflammatory parameters

The initial body weight of the animals was similar between all groups ($p > 0.05$; Figure 1e). The arthritic animals demonstrated a decreased final body weight in relation to the control ($p < 0.05$; Figure 1e and 1f). In the AIA and AQ groups, softer and diarrheic feces were found, mainly at the end of the experimental period.

There was no difference between the groups ($p > 0.05$; Table I) in the initial leukocyte quantification, whereas there was an increase of 103% for AIA, and 67% for AIQ and 90% for AI in the final total leukocytes compared to control

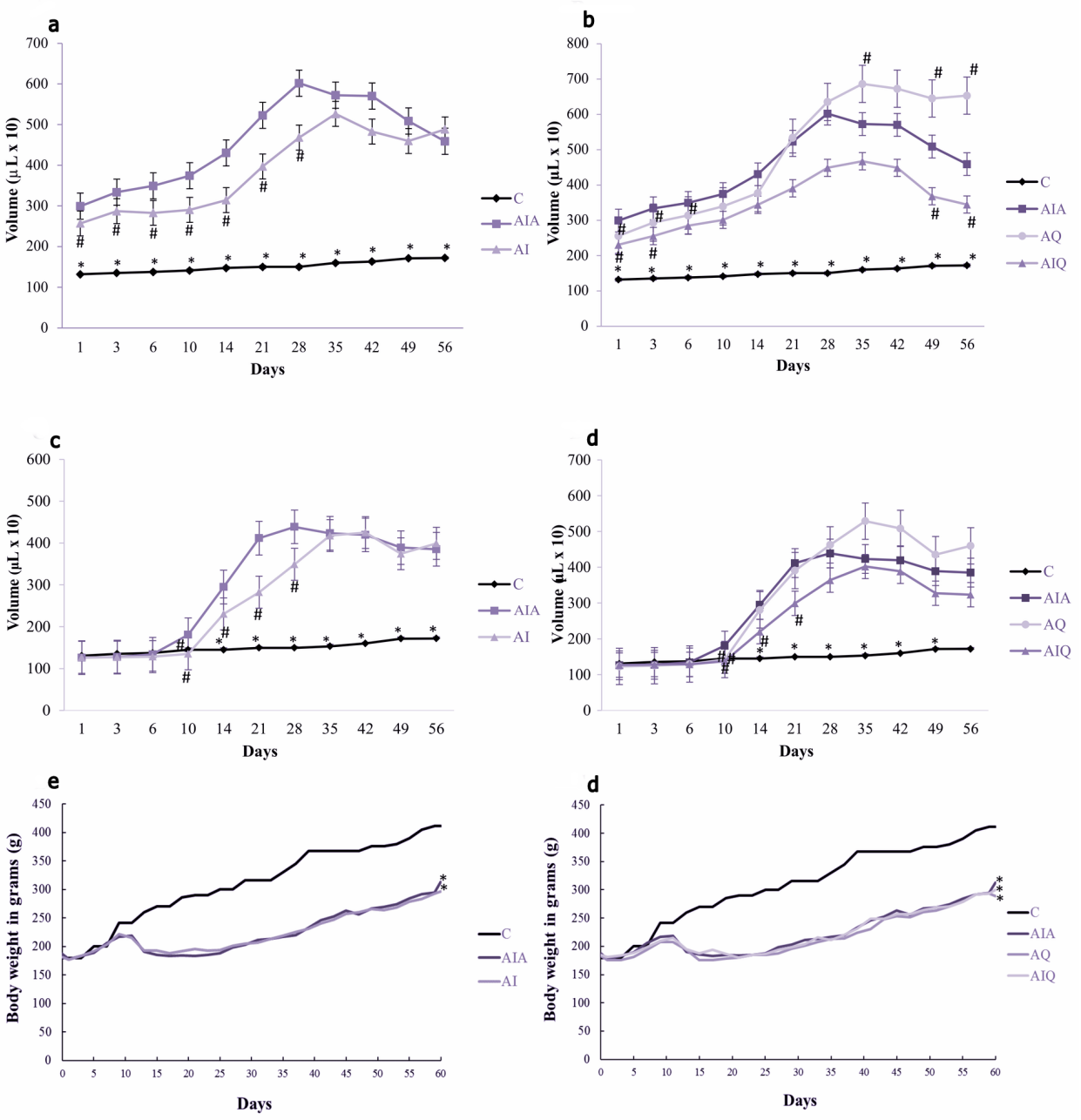

Figure 1. Evolution of physiological parameters. Paw edema in the left hind (induction site) induced by Freund's complete adjuvant (a and b) and right hind (contralateral paw) (c and d) in Holtzman rats and evolution of body weight during the experimental period (e and f) in the following experimental groups: control (C), adjuvant-induced arthritic without treatment (AIA), arthritic treated with 17.5 mg kg^{-1} ibuprofen (AI), arthritic treated with 50 mg kg^{-1} quercetin (AQ) and arthritic treated with 50 mg kg^{-1} quercetin and 17.5 mg kg^{-1} ibuprofen (AIQ). Figures a, c and e illustrate the treatment effects of ibuprofen (AI group) and figures b, d and f show the treatment effects of quercetin (AQ) and the combination of ibuprofen and quercetin (AIQ). The results were expressed as paw volume increase (µL) from 1st up to 56th day. Each point was expressed as mean ± standard error and body weight was expressed in grams (g). n = 5 animals per group; * $p < 0.05$ compared C to the other groups; # $p < 0.05$ compared to AIA.

at the end of the experiment ($p < 0.05$; Table I). In AQ, there was almost a three-fold increase in the final total leukocytes than control ($p < 0.05$; Table I).

The quantification of initial mononuclear leukocytes demonstrated a small difference between the AIQ compared to control ($p < 0.01$; Table I). The final polymorphonuclear leukocytes increased 78%, 63% and 59% in the AI, AQ and AIQ groups compared to the control, respectively ($p < 0.05$). Comparing AIA to the control, there was a 42% increase of these final polymorphonuclear leukocytes in arthritic rats, even being non-significant result for polymorphonuclear leukocytes ($p > 0.05$). However, the final mononuclear leukocytes showed significant reductions of 20%, 16% and 15% in the AI, AQ and AIQ groups in relation to control, respectively ($p < 0.05$; Table I). When we compare the treated groups (AI, AQ and AIQ) with the untreated arthritic group (AIA) a significant increase of 35.6% was observed only in the AQ group in relation to AIA in the final leukocytes total parameter, the other parameters presented in the table showed no significant difference for groups AI, AQ AIQ in relation to AIA.

Evolution of the paw edema

Figures 1a and 1b illustrate the evolution of edema in the left hind paw (injection site) for the C, AIA, AI, AQ and AIQ groups. At the day of induction (1st day), all arthritic animals displayed an increased paw volume in relation to C group ($p < 0.05$; Figure 1a and 1b). Treatment with ibuprofen displayed a beneficial reduction on left paw edema from 1st ($p < 0.01$) until 28th day ($p < 0.008$; Figure 1a). For the arthritic animals treated with quercetin (AQ), the reduction of left paw inflammation occurred significantly on the 1st ($p < 0.008$), 3rd ($p < 0.04$) and 6th ($p < 0.03$) days, whereas there was a significant increase of this edema compared to AIA (Figure 1b) on the 35th ($p < 0.03$), 49th ($p < 0.025$) and 56th ($p < 0.002$) days. The AIQ group displayed an improvement of the inflammatory status in the left hind paw that remained from the 1st ($p < 0.000$) until 28th day ($p < 0.003$). On 35th and 42nd days, the values were not statistically different for AIQ in relation to the AIA, although there was again an attenuation of the edema on 49th ($p < 0.02$) and 56th ($p < 0.04$; Figure 1b) days.

In the evolution of right paw edema (contralateral), paw swelling was observed in all arthritic animals from the 10th day until the end of experimental period ($p < 0.05$; Figures 1c and 1d). The anti-inflammatory effect of ibuprofen was beneficial and significant from 10th ($p < 0.0001$) until the 28th day ($p < 0.04$). However, a reduction of paw edema was not observed in AI due to similar values to the AIA from 35th day until the end of the experimental period ($p > 0.05$; Figure 1c). The administration of quercetin alone was able to attenuate the edema until the 10th day ($p < 0.001$), although similar results to those observed in the AIA were seen in the other days until the end of the experiment ($p > 0.05$; Figure 1d). However, the combined treatment of quercetin and ibuprofen displayed beneficial effects on the edema evolution from 10th ($p < 0.0001$; Figure 1d) until 21st day ($p < 0.003$; Figure 1d). From 28th until 56th day, the results were similar to those observed in AIA ($p > 0.05$; Figure 1d).

Quantitative analysis of the HuC/D-IR and VIP--IR neurons

There was a decrease intestinal area in 16%, 15%, 28% and 23% for AIA, AI, AQ and AIQ groups compared to the control, respectively.

The density of HuC/D-IR enteric neurons decreased 38% in the myenteric plexus for AIA in comparison to control ($p < 0.05$; Table II). However, an increased neuronal density of 33%, 17% and 31% was observed for AI, AQ and AIQ

Table II. Neuronal density of myenteric and submucosal plexuses in ileum of arthritic rats.

Groups	Myenteric Plexus	Submucosal plexus		
	HuC/D	HuC/D	VIP	VIP (%)
C	17391 ± 728	12821 ± 507	4161 ± 163	32.5
AIA	10762 ± 460*	6322 ± 259*	3210 ± 128*	50.8
AI	14314 ± 592*#	6337 ± 229*	3397 ± 125*	53.6
AQ	12637 ± 478*#	6357 ± 225*	3263 ± 114*	51.3
AIQ	14065 ± 536*#	8382 ± 305*#	3791 ± 142#	45.2

VIPergic neurons and overall neuronal population (HuC/D protein) (neurons/cm^2) and relative proportion (%) between HuC/D- and VIP-IR submucosal neurons. Groups: control (C), adjuvant-induced arthritic without treatment (AIA), arthritic treated with 17.5 mg kg^{-1} ibuprofen (AI), arthritic treated with 50 mg kg^{-1} quercetin (AQ); arthritic treated with 50 mg kg^{-1} quercetin and 17.5 mg kg^{-1} ibuprofen (AIQ). n = 5 rats per group. Values expressed as mean ± standard error. * p < 0.05 compared to control, # p < 0.05 compared to AIA.

groups compared to AIA group, respectively (p < 0.05; Table II).

In the submucosal plexus, the HuC/D-IR neurons were more affected by RA than myenteric plexus, where a reduction of approximately 50% of enteric neurons in the AIA (p < 0.05; Table II) was observed. Neuroprotective effects were observed on the HuC/D-IR submucosal neurons in AIQ due to the neuronal preservation of 24% compared to AIA (p < 0.0001; Table II).

The VIP-IR submucosal neurons demonstrated a reduction of 23% compared to C (p < 0.0001; Table II). In AIQ, there was a 15% increase in the VIPergic density (p < 0.002; Table II) in relation to AIA, displaying similar values to those observed to control (p > 0.05; Table II). The relative proportion of VIP is shown in Table I and the immunostainings for HuC/D-IR and VIP-IR neurons are shown in Figures 2a and 3a.

Morphometric analysis of the HuC/D-IR neurons

The neuronal body area of the HuC/D-IR myenteric neurons was similar between C, AIA and AI groups (p > 0.05; Figure 2b). in AQ and AIQ, there was a reduction of neuronal body size compared to AIA (p < 0.05; Figure 2b). Reductions in neuronal body size for HuC/D-IR submucosal neurons were seen in all groups compared to control (p < 0.03; Figure 2c). The treatment with ibuprofen (AI) for the HuC/D-IR submucosal neurons increased their cell body area in comparison to untreated arthritic animals (p < 0.03; Figure 2c).

Representative photomicrographs of the immunostaining of HuC/D-IR neurons are shown in Figure 2a.

Morphometric analysis of immunostaining for VIP

The neuronal body area of the VIP-IR submucosal neurons was similar between C, AIA, AI and AIQ groups (p > 0.05; Figure 3d). The size of neuronal bodies in AQ were increased 25% in comparison to AIA (p < 0.00001; Figure 3a). The VIP-IR varicosities in the arthritic animals (AIA) displayed an increase of their size in the myenteric and submucosal plexuses in comparison to the control (p < 0.0001; Figures 3e and 3f). In the myenteric plexus, all treatments reduced the varicosity sizes in 25%, 19% and 26% for AI, AQ and AIQ groups in relation to AIA, respectively (p < 0.00001; Figure 3e). Similar results were seen in the submucosal plexus, in

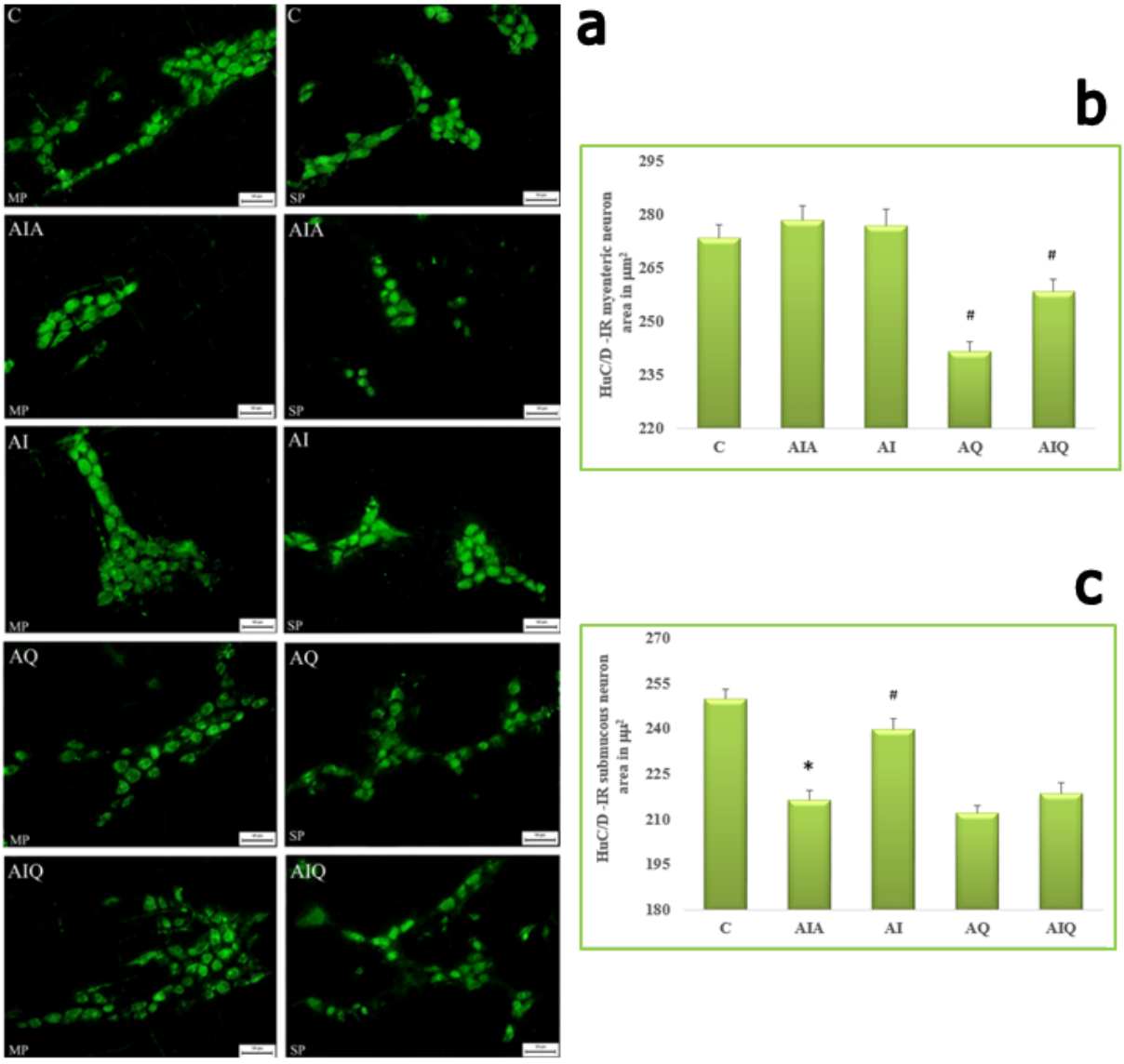

Figure 2. Morphometry of HuC/D-IR neurons. Representative photomicrographs of immunostaining HuC/D-IR neurons in the myenteric (MP) and submucosal (SP) plexuses (a) of the ileum. Morphometric analysis of the neuronal bodies in the myenteric (b) and submucosal (c) plexuses of the ileum. Experimental groups: control (C), adjuvant-induced arthritic without treatment (AIA), arthritic treated with 17.5 mg kg^{-1} ibuprofen (AI), arthritic treated with 50 mg kg^{-1} quercetin (AQ); arthritic treated with 17.5 mg kg^{-1} ibuprofen and 50 mg kg^{-1} quercetin (AIQ). n = 5 animals per group. * p < 0.000001 was compared to C; # p <0.007 compared to AIA. Calibration bar = 50 µm.

which AI, AQ and AIQ demonstrated reductions in varicosity sizes of 30%, 22% and 11% in relation to AIA, respectively (p < 0.00001; Figure 3c).

Representative photomicrographs of morphometric analysis of the VIP immunostainings illustrate the VIPergic submucosal neurons (Figure 3a), and VIP-IR varicosities in myenteric (Figure 3b) and submucosal plexuses (Figure 3c).

Morphometric analysis of immunostaining for CGRP

The CGRP-IR varicosities in myenteric plexus of the arthritic animals (AIA) were larger than

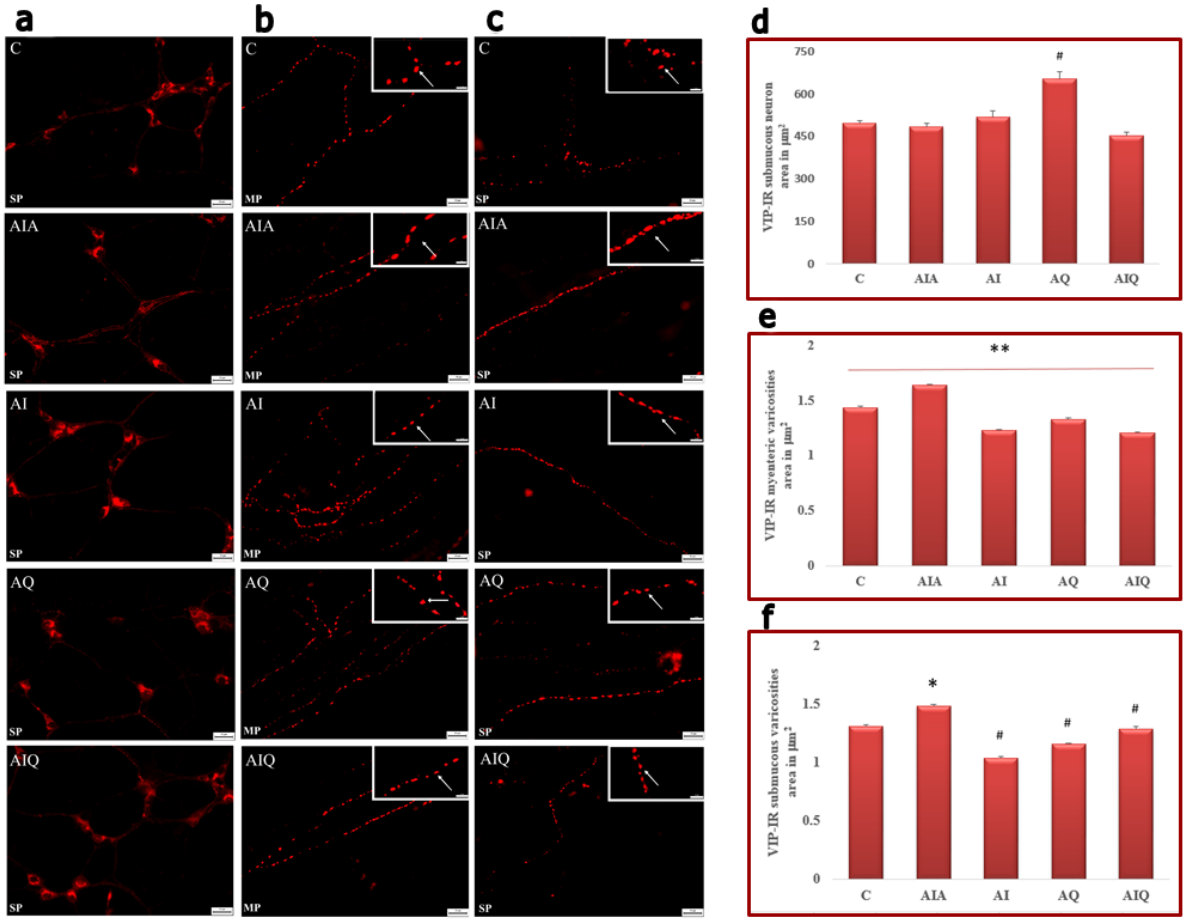

Figure 3. Morphometry of VIP-IR neurons and varicosities. Representative photomicrographs of VIP-IR neuronal bodies in the submucosal plexus (SP) (a) and VIP-IR varicosities in the myenteric (MP) (b) and submucosal (c) plexuses of the ileum. Morphometric analysis of VIP-IR submucosal neurons (d), morphometric analyses of VIP-IR varicosities in the myenteric (e) and submucosal (f) plexuses. Experimental groups: control (C), adjuvant-induced arthritic without treatment (AIA), arthritic treated with 17.5 mg kg^{-1} ibuprofen (AI), arthritic treated with 50 mg kg^{-1} quercetin (AQ); arthritic treated with 17.5 mg kg^{-1} ibuprofen and 50 mg kg^{-1} quercetin (AIQ). n = 5 animals per group. * p < 0.001 compared to C; ** p < 0.001 indicates that all groups were compared to each other; # p < 0.001 compared to AIA. The calibration bar used for neurons and varicosities was 50 µm and 25 µm, respectively.

control (p < 0.05; Figure 4b). The treatment with quercetin (AQ) reduced varicosity area in relation to the control (p < 0.01, Figure 4a and 4b). Treatment with ibuprofen alone (AI) showed similar areas compared to untreated arthritic animals (AIA) (p > 0.05), whereas AQ demonstrated a 15% smaller varicosity area but AIQ displayed a 15% larger size of these CGRP-IR varicosities than those results observed in AIA (p < 0.05; Figure 4b).

In the submucosal plexus, AIA displayed smaller varicosity areas compared to the control (p < 0.05; Figure 4c). The AQ group demonstrated similar areas to those observed in the AIA (p > 0.05; Figure 4c). The treatments with ibuprofen (AI and AIQ) promoted increased areas of CGRP-IR varicosities compared AIA group (p < 0.05; Figure 4c).

Representative photomicrographs of immunostaining for CGRP-IR varicosities are shown in Figure 4a.

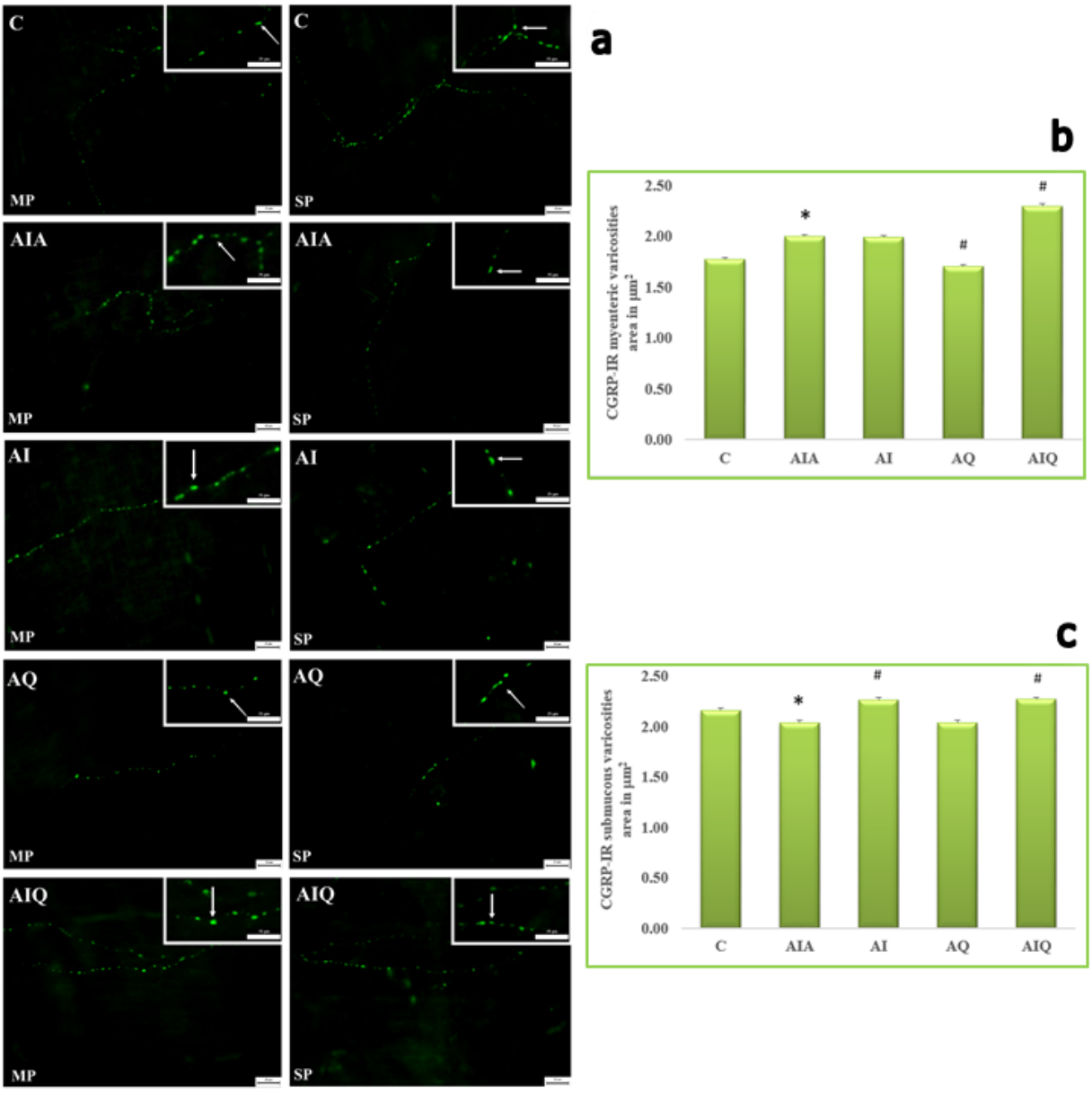

Figure 4. Morphometry of CGRP-IR varicosities. Representative photomicrographs of in the myenteric (MP) and submucosal plexuses (SP) (a) of the ileum. Morphometric analysis myenteric (b) and submucosal (c) plexuses. Experimental groups: control (C), adjuvant-induced arthritic without treatment (AIA), arthritic treated with 17.5 mg kg^{-1} ibuprofen (AI), arthritic treated with 50 mg kg^{-1} quercetin (AQ); arthritic treated with 17.5 mg kg^{-1} ibuprofen and 50 mg kg^{-1} quercetin (AIQ). n = 5 animals per group. * p < 0.01 compared to C; # p < 0.0001 compared to AIA. Calibration bar = 25 µm.

Intestinal mucosa

An increase in villus height (p < 0.0005; Figure 5b) and crypt depth (p < 0.00001; Figure 5c) was observed in the arthritic animals (AIA) in relation to control. In animals treated with ibuprofen alone (AI), there was an increase of 20% villus height (p < 0.00006; Figure 5b) and reduction of 16% crypt depth (p < 0.03; Figure 5c) in comparison to AIA. The villus height and crypt depth in AQ animals were lower in relation to

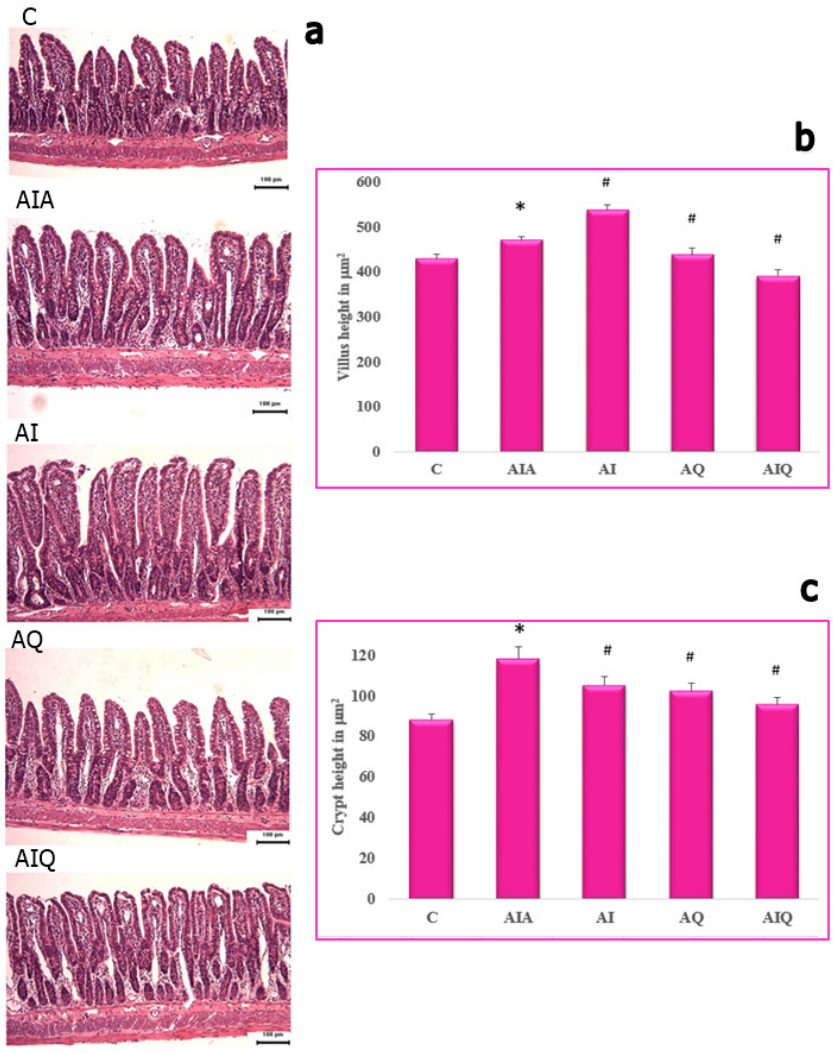

Figure 5. Intestinal mucosa. Photomicrographs of the intestinal wall of the ileum (a), villus height (b) and crypt depth (c). Experimental groups: control (C), adjuvant-induced arthritic without treatment (AIA), arthritic treated with 17.5 mg kg^{-1} ibuprofen (AI), arthritic treated with 50 mg kg^{-1} quercetin (AQ); arthritic treated with 17.5 mg kg^{-1} ibuprofen and 50 mg kg^{-1} quercetin (AIQ). n = 5 animals per group. * $p < 0.005$ compared to C; # $p < 0.001$ compared to AIA. Calibration bar = 100 µm.

AIA ($p < 0.05$; Figures 5b and 5c). The villus height was 16% lower in AIQ ($p < 0.00001$, Figure 5b) compared to that observed in AIA, whereas the crypt depth was similar to C ($p > 0.05$; Figure 5c). Representative photomicrographs of the intestinal mucosa are shown in Figure 5a.

Oxidative status

In tert-butyl hydroperoxide-induced chemiluminescence technique, the AIA animals demonstrated no significant differences in relation to control ($p > 0.05$; Figure 6a and 6b). The highest mean values were observed in AQ compared to other groups ($p < 0.05$; Figure 6b). The combination of ibuprofen and quercetin (AIQ) demonstrated more similar results to those observed in the control than the other groups ($p > 0.05$).

The total tissue antioxidant capacity showed similarity between all groups ($p > 0.05$; Figure 6c).

DISCUSSION

The RA model displayed significant reductions of body weight, including in the chronic phase when an adaptation to the disease occurs. The RA-induced cachexia has been reported in up to two thirds of arthritic patients due to the

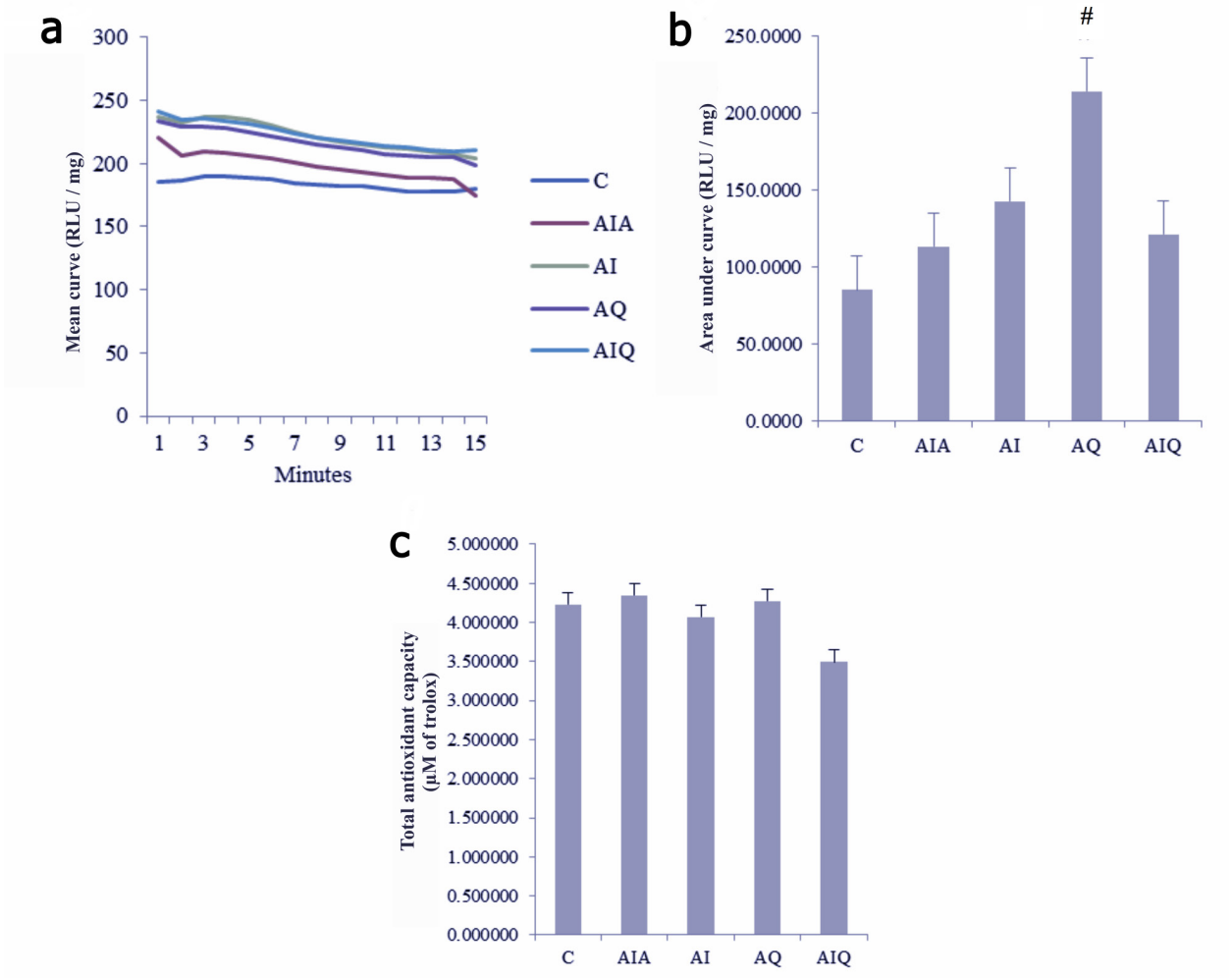

Figure 6. Tissue oxidative state. Chemiluminescence technique induced by tert-butyl hydroperoxide is illustrated by the mean curve (a) and area under the curve (b). Figure c shows the total antioxidant capacity (TAC). Experimental groups: control (C), adjuvant-induced arthritic without treatment (AIA), arthritic treated with 17.5 mg kg^{-1} ibuprofen (AI), arthritic treated with 50 mg kg^{-1} quercetin (AQ); arthritic treated with 17.5 mg kg^{-1} ibuprofen and 50 mg kg^{-1} quercetin (AIQ). n = 5 animals per group. # $p < 0.05$ compared to AIA.

production of pro-inflammatory cytokines such as TNF-α and IL-1, which play an important role in energy and protein metabolism (Roubenoff 2009).

Regarding the effects of RA on the quantification of leukocytes and paw inflammation in both paws, the increased number of total leukocytes and the enhance of inflammation process of the right and left hind paw in the AIA is in line with the literature (Tracey et al. 2008, Rodrigues Silva et al. 2008, Oliveira de Melo et al. 2011). However, the highest number of systemic total leukocytes at the end of the experiment observed in AQ may explain the highest inflammation process in the paw edema, even higher than AIA, which was mainly found at the second month of the experimental period.

In these both analyzes, ibuprofen, which is often used by humans, yielded its expected anti-inflammatory effects in the most days during the experiment, reducing the paw edema and total leukocytes at the end of the experiment. The significant reduction in the number of total

leukocytes observed in the AIQ group, more significantly than in the AI group, suggests that the anti-inflammatory effect of ibuprofen may have been potentiated by quercetin. Studies have demonstrated an immunomodulatory effect of quercetin in vivo and in vitro (Nair et al. 2002, Sallam et al. 2022). Furthermore, the combination of quercetin and ibuprofen exhibited the highest anti-inflammatory effects in both right and left hind paw edema, particularly evident during the second month of the rheumatoid arthritis experiment. These collective results suggest promising anti-inflammatory effects of this combined therapy, potentially enhancing the prognosis for arthritic patients (Rodrigues Silva et al. 2008).

The arthritic disease (AIA group) displayed negative impact on ENS due to the significant reduction of the HuC/D-IR neurons for both plexuses, as well as VIP-IR submucosal neurons in this work. Several diseases affect the ENS, although this animal model of severe and chronic RA displayed to be relevant in the gastroenterology field as an enteropathy and neurodegenerative disease, which has been barely reported in the literature. Presence of peripheral neuropathy and gastrointestinal symptoms (e.g. abdominal pain, nausea, vomiting and diarrhea) in arthritic patients confirms the extra-articular manifestations of the disease (Cojocaru et al. 2011, Sim et al. 2014, Albani et al. 2006, Agarwal et al. 2008, Choi & Kim 2010, Craig & Cappelli 2018, Pagnoux et al. 2005). Furthermore, RA causes an activation of pro-inflammatory pathways resulting in joint and systemic inflammation, which may affect multiple organ systems, and neuro-inflammatory events have been already observed in the brain (Fuggle et al. 2014).

Rheumatological manifestations are frequent in inflammatory bowel diseases (Rodríguez-Reyna et al. 2009), indicating a certain correlation between these two autoimmune diseases. The submucosal plexus was much more affected by disease than myenteric plexus. The neuronal loss in the AIA probably occurred due to a neurodegenerative effect as a consequence of the systemic inflammatory response of the RA and due to the low production of growth factors, decreased neuronal activity and the production of its neurotransmitters (Liu et al. 2010, Pereira et al. 2011). Furthermore, the submucosal plexus displayed reductions of the area size of the HuC/D-IR neuronal bodies and CGRP-IR varicosities, which may indicate lower production of neurotransmitters. However, the increased varicosity area of VIP for both plexuses and CGRP in the myenteric plexus in AIA may indicate neuronal plasticity with increased release of the neurotransmitters (Vicentini et al. 2016, de Souza et al. 2017), since VIP and CGRP have antioxidant and anti-inflammatory properties (Duan et al. 2017) to compensate the neuronal damage against the disease.

Ibuprofen was used in this study as a positive control since it is considered an anti-inflammatory drug that has been traditionally used by the worldwide population. In general, neuroprotective effects were observed in quantitative and morphometric parameters using all markers (VIP, CGRP and HuC/D) for AI. In recent studies, quercetin supplementation has reduced the diabetes-induced neuronal damages, thus promoting neuroprotection on the duodenum (Lopes et al. 2012), cecum (Ferreira et al. 2013) and jejunum (Vieira-Frez et al. 2017).

In the AQ animals, the quantitative analysis demonstrated that the treatment with quercetin alone was not able to protect the general and VIPergic neuronal populations in the submucosal plexus. Negative effects of quercetin were observed through that majority of morphometric analysis, which displayed

decreased areas of neuronal bodies, except for VIPergic neuronal area, and CGRP- and VIP-IR varicosities which may be related to a decreased release of neurotransmitters, thus demonstrating negative neuroplastic effects possibly due to the pro-oxidant action of quercetin (Harwood et al. 2007). The increased VIPergic somatic area in the submucosal plexus would reflect more productions of neurotransmitters as a compensatory mechanism against VIPergic neuronal loss induced by the disease (Tunçel et al. 2012).

However, this VIPergic hypertrophy of the submucosal neurons associated with the reductions of VIP-IR varicosities may be explained by defective axonal transport of neuronal proteins that are synthesized inside the neuronal body due to the accumulation of these proteins (de Souza et al. 2017). In general, the highest inflammation process observed by paw edema and final leukocyte quantification at the end of the experiment in AQ may explain the lack of neuroprotection by quercetin on the ileum. Despite this inflammatory effect is unexpected in the chronic phase in AQ, these data can be explained by pro-oxidant effects observed by the chemiluminescence technique due the increased oxidative stress in this group. Increased reactive oxygen species levels induces cellular damage on lipids, proteins and nucleic acids and, whether this oxidant imbalance is associated with antioxidant deficiency persistence, cellular redox signaling pathways are affected (Quinonez-Flores et al. 2016). Furthermore, only animals of AIA and AQ groups displayed the presence of diarrhea and softer feces during the experiment, demonstrating that the rheumatic enteropathy affects the functioning of GI tract and quercetin treatment was not able to avoid this effect.

In this study, the combination of quercetin and ibuprofen exhibited the highest neuronal density preservation for HuC/D-IR neuronal population for both plexuses and VIP-IR submucosal neurons. The treatments with ibuprofen (AI) and quercetin (AQ) presented lower neuroprotective effects in this study. In contrast to AQ, the increased CGRP-IR varicosity areas observed in both ganglionated plexuses in AIQ may indicate a neuroprotective activity, since CGRP exerts a crucial role in inflammatory diseases due to its anti-inflammatory and antioxidant properties, leading to a suppression of inflammatory process in the intestine by inhibition the synthesis of inflammatory mediators (Wu et al. 2015, Liu et al. 2015, Tunçel et al. 2012, Raud et al. 1991, Russell et al. 2014).

VIP exerts direct and indirect effects on the permeability of the intestinal epithelium through regulation of the epithelial barrier function, which may reflect mucosal susceptibility to inflammatory process. VIPergic submucosal neurons directly innervate the intestinal epithelial crypt and promote intestinal secretion of ions and fluids. The increase of villus height and crypt depth observed in AIA may have occurred to offset the reduction of VIP-IR submucosal neurons and likely due to the increase of the VIP-IR varicosity area (Chandrasekharan et al. 2013), enhancing the production and release of VIP.

Quercetin has been mainly studied for its anti-inflammatory and antioxidant properties (Caparroz-Assef et al. 2007). Slight anti-inflammatory effects were observed in the acute phase of the experiment regarding the paw edema. However, the significant inflammatory process in the chronic phase probably occurred due to either its prolonged administration of quercetin or its dose. The administration of quercetin at high doses may cause oxidative effects that would lead to cellular apoptosis in long-term experiment due to the accumulation of quercetin in the brain and liver. For molecules

that are highly antioxidant, pro-oxidant effects may also occur (Choi & Kim 2010, Harwood et al. 2007).

Other studies have been demonstrated pro-oxidant and hepatotoxic effects of free quercetin at a dose of 100 mg/kg during 30 days of treatment (Jain et al. 2014). Quercetin at dose of 150 mg/kg demonstrated no significant anti-inflammatory effects by assessment of paw edema in RA model during 30 days in other study (Gardi et al. 2015), similar to the initial results (acute phase) observed in our study. However, quercetin has already shown significant anti-inflammatory effects in arthritic rats evaluating the paw diameter with quercetin administration at a high dose 160 mg/kg during treatment period from the 9th until 21st day of the disease, in the acute phase that shows a higher severity period of disease than chronic stage (Ansari et al. 2014).

Probably the relationship of pro-oxidant effect and inflammatory action is related to dose depending on the time of use. Our study was a chronic treatment so the dosage may have been excessive for the long time it was used. This same dosage can have an anti-inflammatory effect in shorter periods, in chronicity it did not show the same action.

Therefore, this study showed that the arthritic enteropathy led to remarkable alterations on ENS through the remarkable reduction of neuronal density that has been rarely reported in the literature and deserves the gastroenterologist's attention for arthritic patients. Furthermore, the combined treatment with ibuprofen and quercetin (AIQ) displayed beneficial effects on the reduction of inflammation and neuroprotective effects, preventing neurodegeneration in the ileum.

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REFERENCES

AGARWAL V, SINGH R, WICLAF, CHAUHAN S, TAHLAN A, AHUJA CK, GOEL D & PAL L. 2008. A clinical, electrophysiological, and pathological study of neuropathy in rheumatoid arthritis. Clin Rheumatol 27: 841-844.

AGUIAR RP, ALDAWSARI FS, WIIRZLER LAM, SILVA-FILHO SE, SILVA-COMAR FMS, BERSANI-AMADO CA, VELAZQUEZ-MARTINEZ CA & CUMAN RKN. 2017. Synthesis and Biological Evaluation of New Tyrosol-Salicylate Derivatives as Potential Anti-Inflammatory Agents. Curr Pharm Des 23(44): 6841-6848.

ALBANI G, RAVAGLIA S, CAVAGNA L, CAPORALI R, MONTECUCCO C & MAURO A. 2006. Clinical and electrophysiological evaluation of peripheral neuropathy in rheumatoid arthritis. J Peripher Nerv Syst 11: 174-175.

ANSARI M, NEHA HA & KHAN HA. 2014. Quercetin alleviate oxidative stress and inflammation through up-regulation of antioxidant machinery and down- regulation of COX and NF-kB expression in collagen induced rheumatoid arthritis. Int J Drug Dev Res 6: 215-230.

BARBOSA CP, BRACHT L, AMES FQ, DE SOUZA SILVA-COMAR FM, TRONCO RP & BERSANI-AMADO CA. 2017. Effects of Ezetimibe, Simvastatin, and their combination on inflammatory parameters in a rat model of adjuvant-induced arthritis. Inflammation 40(2): 717-724.

BINET S, CHAINEAU E, FELLOUS A, LATASTE H, KRIKORIAN A, COUZINIER J-P & MEININGER V. 1990. Immunofluorescence study of the action of navelbine, vincristine and vinblastine on mitotic and axonal microtubules. Int J Cancer 46: 262-266.

BRACHT L, BARBOSA CP, CAPARROZ-ASSEF SM, CUMAN RKN, ISHII-IWAMOTO EL, BRACHT A & BERSANI-AMADO CA. 2012. Effects of simvastatin, atorvastatin, ezetimibe, and ezetimibe + simvastatin combination on the inflammatory process and on the liver metabolic changes of arthritic rats. Fundam Clin Pharmacol 26: 722-734.

BRENNEMAN DE. 2007. Neuroprotection: a comparative view of vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide. Peptides (NY) 28: 1720-1726.

CAPARROZ-ASSEF SM, BERSANI-AMADO CA, KELMER-BRACHT AM, BRACHT A & ISHII-IWAMOTO EL. 2007. The metabolic changes caused by dexamethasone in the adjuvant-induced arthritic rat. Mol Cell Biochem 302: 87-98.

CHANDRASEKHARAN B, NEZAMI BG & SRINIVASAN S. 2013. Emerging neuropeptide targets in inflammation: NPY and VIP. Am J Physiol Gastrointest Liver Physiol 304.

CHOI EJ & KIM GH. 2010. Quercetin accumulation by chronic administration causes the caspase-3 activation in liver and brain of mice. Biofactors 36: 216-221.

COJOCARU M, COJOCARU IM, SILOSI I & VRABIE CD. 2011. Gastrointestinal Manifestations in Systemic Autoimmune Diseases. Maedica (Bucur) 6: 45.

COSTA LG, GARRICK JM, ROQUÈ PJ & PELLACANI C. 2016. Mechanisms of Neuroprotection by Quercetin: Counteracting Oxidative Stress and More. Oxid Med Cell Longev 2016: 10 p.

CRAIG E & CAPPELLI LC. 2018. Gastrointestinal and Hepatic Disease in Rheumatoid Arthritis. Rheum Dis Clin North Am 44: 89-111.

DA ROCHA BA, RITTER AM, AMES FQ, GONÇALVES OH, LEIMANN FV, BRACHT L, NATALI MR, CUMAN RK & BERSANI-AMADO CA. 2017. Acetaminophen-induced hepatotoxicity: Preventive effect of trans anethole. Biomed Pharmacother 86: 213-220.

DE SOUZA SRG, NETO MHM, PERLES JVCM, FREZ FCV, ZIGNANI I, RAMALHO FV, HERMES-ULIANA C, BOSSOLANI GDP & ZANONI JN. 2017. Antioxidant Effects of the Quercetin in the Jejunal Myenteric Innervation of Diabetic Rats. Front Med (Lausanne) 227: 102675.

DELGADO M, POZO D & GANEA D. 2004. The significance of vasoactive intestinal peptide in immunomodulation. Pharmacol Rev 56: 249-290.

DENG K, YU L, ZHENG X, ZHANG K, WANG W, DONG P, ZHANG J & REN M. 2016. Target of Rapamycin Is a Key Player for Auxin Signaling Transduction in Arabidopsis. Front Plant Sci 7: 291.

DUAN JX, ZHOU Y, ZHOU AY, GUAN XX, LIU T, YANG HH, XIE H & CHEN P. 2017. Calcitonin gene-related peptide exerts anti-inflammatory property through regulating murine macrophages polarization in vitro. Mol Immunol 91: 105-113.

EKBLAD E & BAUER AJ. 2004. Role of vasoactive intestinal peptide and inflammatory mediators in enteric neuronal plasticity. Neurogastroenterol Motil 16(Suppl. 1): 123-128.

EL-SAID KS, ATTA A, MOBASHER MA, GERMOUSH MO, MOHAMED TM & SALEM MM. 2022. Quercetin mitigates rheumatoid arthritis by inhibiting adenosine deaminase in rats. Mol Med 28(1): 24.

FERREIRA PEB, LOPES CRP, ALVES AMP, ALVES ÉPB, LINDEN DR, ZANONI JN & BUTTOW NC. 2013. Diabetic neuropathy: an evaluation of the use of quercetin in the cecum of rats. World J Gastroenterol 19: 6416-6426.

FRACARO L, FREZ FCV, SILVA BC, VICENTINI GE, DE SOUZA SRG, MARTINS HA, LINDEN DR, GUARNIER FA & ZANONI JN. 2016. Walker 256 tumor-bearing rats demonstrate altered interstitial cells of Cajal. Effects on ICC in the Walker 256 tumor model. Neurogastroenterol Motil 28: 101-115.

FUGGLE NR, HOWE FA, ALLEN R & SOFAT N. 2014. New insights into the impact of neuro-inflammation in rheumatoid arthritis. Front Neurosci 8: 357.

FURNESS JB. 2008. The Enteric Nervous System, Blackwell Publishing ed., p. 1-28.

GARDI C ET AL. 2015. Quercetin reduced inflammation and increased antioxidant defense in rat adjuvant arthritis. Arch Biochem Biophys 583: 150-157.

GONZALEZ FLECHA B, LLESUY S & BOVERIS A. 1991. Hydroperoxide-initiated chemiluminescence: an assay for oxidative stress in biopsies of heart, liver, and muscle. Free Radic Biol Med 10: 93-100.

GONZALEZ-REY E, CHORNY A, FERNANDEZ-MARTIN A, VARELA N & DELGADO M. 2005. Vasoactive intestinal peptide family as a therapeutic target for Parkinson's disease. Expert Opin Ther Targets 9: 923-929.

HARWOOD M, DANIELEWSKA-NIKIEL B, BORZELLECA JF, FLAMM GW, WILLIAMS GM & LINES TC. 2007. A critical review of the data related to the safety of quercetin and lack of evidence of in vivo toxicity, including lack of genotoxic/carcinogenic properties. Food Chem Toxicol 45: 2179-2205.

HASNAT H ET AL. 2024. Flavonoids: A treasure house of prospective pharmacological potentials. Heliyon 10: e27533.

HELMY M, SHOHAYEB M, HELMY MH & EL-BASSIOUNI EA. 2001. Antioxidants as adjuvant therapy in rheumatoid disease. A preliminary study. Arzneimittelforschung 51: 293-298.

HOLZMANN B. 2013. Modulation of immune responses by the neuropeptide CGRP. Amino Acids 45: 1-7.

JAIN AK, THANKI K & JAIN S. 2014. Novel self-nanoemulsifying formulation of quercetin: Implications of pro-oxidant

activity on the anticancer efficacy. Nanomedicine 10: e959-e969.

JEYADEVI R, SIVASUDHA T, RAMESHKUMAR A, ANANTH DA, ASEERVATHAM GSB, KUMARESAN K, KUMAR LD, JAGADEESWARI S & RENGANATHAN R. 2013. Enhancement of anti arthritic effect of quercetin using thioglycolic acid-capped cadmium telluride quantum dots as nanocarrier in adjuvant induced arthritic Wistar rats. Colloids Surf B Biointerfaces 112: 255-263.

JI JJ, LIN Y, HUANG SS, ZHANG HL, DIAO YP & LI K. 2013. Quercetin: A Potential Natural Drug for Adjuvant Treatment of Rheumatoid Arthritis. Afr J Tradit Complement Altern Med 10(3): 418-421.

KAUR S, BIJJEM KRV & SHARMA PL. 2011. Anti-inflammatory and antihyperalgesic effects of the combination of ibuprofen and hemin in adjuvant-induced arthritis in the Wistar rat. Inflammopharmacology 19: 265-272.

LIU P ET AL. 2015. Quercetin ameliorates hypobaric hypoxia-induced memory impairment through mitochondrial and neuron function adaptation via the PGC-1α pathway. Restor Neurol Neurosci 33: 143-157.

LIU W, YUE W & WU R. 2010. Effects of diabetes on expression of glial fibrillary acidic protein and neurotrophins in rat colon. Auton Neurosci 154: 79-83.

LOPES CRP, FERREIRA PEB, ZANONI JN, ALVES AMP, ALVES ÉPB & BUTTOW NC. 2012. Neuroprotective effect of quercetin on the duodenum enteric nervous system of streptozotocin-induced diabetic rats. Dig Dis Sci 57: 3106-3115.

MORELL M, SOUZA-MOREIRA L & GONZALEZ-REY E. 2012. VIP in neurological diseases: more than a neuropeptide. Endocr Metab Immune Disord Drug Targets 12: 323-332.

NABAVI SF, RUSSO GL, DAGLIA M & NABAVI SM. 2015. Role of quercetin as an alternative for obesity treatment: you are what you eat! Food Chem 179: 305-310.

NAIR MP, KANDASWAMI C, MAHAJAN S, CHADHA KC, CHAWDA R, NAIR H, KUMAR N, NAIR RE & SCHWARTZ SA. 2002. The flavonoid, quercetin, differentially regulates Th-1 (IFNgamma) and Th-2 (IL4) cytokine gene expression by normal peripheral blood mononuclear cells. Biochim Biophys Acta 1593(1): 29-36. DOI: 10.1016/s0167-4889(02)00328-2. PMID: 12431781.

NEZAMI BG & SRINIVASAN S. 2010. Enteric nervous system in the small intestine: pathophysiology and clinical implications. Curr Gastroenterol Rep 12: 358-365.

OBROSOVA IG. 2002. How does glucose generate oxidative stress in peripheral nerve? Int Rev Neurobiol 50: 3-35.

OLIVEIRA DE MELO J, PEDROCHI F, BAESSO ML, HERNANDES L, TRUITI MCT, BARONI S & BERSANI-AMADO CA. 2011. Evidence of deep percutaneous penetration associated with anti-inflammatory activity of topically applied Helicteres gardneriana extract: a photoacoustic spectroscopy study. Pharm Res 28: 331-336.

PAGNOUX C, MAHR A, COHEN P & GUILLEVIN L. 2005. Presentation and outcome of gastrointestinal involvement in systemic necrotizing vasculitides: analysis of 62 patients with polyarteritis nodosa, microscopic polyangiitis, Wegener granulomatosis, Churg-Strauss syndrome, or rheumatoid arthritis-associated vasculitis. Medicine 84: 115-128.

PARTHIBAN A, VIJAYALINGAM S, SHANMUGASUNDARAM KR & MOHAN R. 1995. Oxidative stress and the development of diabetic complications--antioxidants and lipid peroxidation in erythrocytes and cell membrane. Cell Biol Int 19: 987-994.

PEREIRA RVF, TRONCHINI EA, TASHIMA CM, ALVES EPB, LIMA MMH & ZANONI JN. 2011. L-glutamine supplementation prevents myenteric neuron loss and has gliatrophic effects in the ileum of diabetic rats. Dig Dis Sci 56: 3507-3516.

PHILLIPS RJ & POWLEY TL. 2007. Innervation of the gastrointestinal tract: patterns of aging. Auton Neurosci 136: 1-19.

QUINONEZ-FLORES CM, GONZALEZ-CHAVEZ SA, 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. DOI: 10.1155/2016/6097417.

RAUD J, LUNDEBERG T, BRODDA-JANSEN G, THEODORSSON E & HEDQVIST P. 1991. Potent anti-inflammatory action of calcitonin gene-related peptide. Biochem Biophys Res Commun 180: 1429-1435.

REPETTO M, REIDES C, GOMEZ CARRETERO ML, COSTA M, GRIEMBERG G & LLESUY S. 1996. Oxidative stress in blood of HIV infected patients. Clin Chim Acta 255: 107-117.

RODRIGUES SILVA D, BARONI S, SVIDZINSKI AE, BERSANI-AMADO CA & CORTEZ DAG. 2008. Anti-inflammatory activity of the extract, fractions and amides from the leaves of Piper ovatum Vahl (Piperaceae). J Ethnopharmacol 116: 569-573.

RODRÍGUEZ-REYNA TS, MARTÍNEZ-REYES C & YAMAMOTO-FURUSHO JK. 2009. Rheumatic manifestations of inflammatory bowel disease. World J Gastroenterol 15: 5517-5524.

ROUBENOFF R. 2009. Rheumatoid cachexia: a complication of rheumatoid arthritis moves into the 21st century. Arthritis Res Ther 11(2): 108.

RUSSELL FA, KING R, SMILLIE SJ, KODJI X & BRAIN SD. 2014. Calcitonin gene-related peptide: physiology and pathophysiology. Physiol Rev 94: 1099-1142.

SALLAM AA, AHMED MM, EL-MAGD MA, MAGDY A, GHAMRY HI, ALSHAHRANI MY & ABOU EL-FOTOH MF. 2022. Quercetin-Ameliorated, Multi-Walled Carbon Nanotubes-Induced Immunotoxic, Inflammatory, and Oxidative Effects in Mice. Molecules 27(7): 2117.

SIM MK, KIM DY, YOON J, PARK DH & KIM YG. 2014. Assessment of peripheral neuropathy in patients with rheumatoid arthritis who complain of neurologic symptoms. Ann Rehabil Med 38: 249-255.

SMOLEN JS & ALETAHA D. 2015. Rheumatoid arthritis therapy reappraisal: strategies, opportunities and challenges. Nat Rev Rheumatol 11: 276-289.

SMOLEN JS, ALETAHA D & MCINNES IB. 2016. Rheumatoid arthritis. Lancet 388: 2023-2038.

SOUZA IDS, RIBEIRO JS, BERSANI-AMADO CA & JACQUELINE NZ. 2011. Analysis of myosin-V immunoreactive myenteric neurons from arthritic rats. Arq Gastroenterol 48: 205-210.

TANEJA V. 2014. Arthritis susceptibility and the gut microbiome. FEBS Lett 588: 4244-4249.

TRACEY D, KLARESKOG L, SASSO EH, SALFELD JG & TAK PP. 2008. Tumor necrosis factor antagonist mechanisms of action: a comprehensive review. Pharmacol Ther 117: 244-279.

TUNÇEL N, KORKMAZ OT, TEKIN N, ŞENER E, AKYÜZ F & INAL M. 2012. Antioxidant and anti-apoptotic activity of vasoactive intestinal peptide (VIP) against 6-hydroxy dopamine toxicity in the rat corpus striatum. J Mol Neurosci 46: 51-57.

VESELINOVIC M, BARUDZIC N, VULETIC M, ZIVKOVIC V, TOMIC-LUCIC A, DJURIC D & JAKOVLJEVIC V. 2014. Oxidative stress in rheumatoid arthritis patients: relationship to diseases activity. Mol Cell Biochem 391(1-2): 225-232.

VICENTINI GE, FRACARO L, DE SOUZA SRG, MARTINS HA, GUARNIER FA & ZANONI JN. 2016. Experimental Cancer Cachexia Changes Neuron Numbers and Peptide Levels in the Intestine: Partial Protective Effects after Dietary Supplementation with L-Glutamine. PLoS ONE 11(9): e0162998.

VIEIRA-FREZ FC, MARTINS-COLOMBO-PERLES JV, ROBERT-LINDEN D, GIBBONS SJ, AMILCAR-MARTINS H, ALMEIDA-BRITO-ROMUALDO D, DE-SOUZA SR, DAION-PIOVEZANA-BOSSOLANI G & ZANONI JN. 2017. Restoration of density of interstitial cells of Cajal in the jejunum of diabetic rats after quercetin supplementation. Rev Esp Enferm Dig 109: 190-195.

VIVAR N & VAN VOLLENHOVEN RF. 2014. Advances in the treatment of rheumatoid arthritis. F1000Prime Rep 6.

WANG Y, CHEN S, DU K, LIANG C, WANG S, OWUSU BOADI E, LI J, PANG X, HE J & CHANG YX. 2021. Traditional herbal medicine: Therapeutic potential in rheumatoid arthritis. J Ethnopharmacol 279: 114368.

WHITE CM, JI S, CAI H, MAUDSLEY S & MARTIN B. 2010. Therapeutic potential of vasoactive intestinal peptide and its receptors in neurological disorders. CNS Neurol Disord Drug Targets 9(5): 661-666.

WISNIEWSKI-REBECCA ES, ROCHA BA, WIIRZLER LA, CUMAN RK, VELAZQUEZ-MARTINEZ CA & BERSANI-AMADO CA. 2015. Synergistic effects of anethole and ibuprofen in acute inflammatory response. Chem Biol Interact 242: 247-253.

WU Y, HAO GM, HE J, LV TT, WANG HL, MAO YQ, WANG X, WANG W & HAN J. 2015. Lentivirus mediated over expression of CGRP inhibited oxidative stress in Schwann cell line. Neurosci Lett 598: 52-58.

ZAMBURLINI A, MAIORINO M, BARBERA P, PASTORINO AM, ROVERI A, COMINACINI L & URSINI F. 1995. Measurement of lipid hydroperoxides in plasma lipoproteins by a new highly-sensitive "single photon counting" luminometer. Biochim Biophys Acta 1256: 233-240.

ZANONI JN, DE FREITAS P, PEREIRA RVF, DOS SANTOS PEREIRA MA & DE MIRANDA NETO MH. 2005. Effects of supplementation with ascorbic acid for a period of 120 days on the myosin-V and NADPHd positive myenteric neurons of the ileum of rats. Anat Histol Embryol 34: 149-153.

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