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Application of Formononetin for the Treatment of Knee Osteoarthritis Induced by Medial Meniscectomy in a Rodent Model

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Abstract: Formononetin suppresses catabolic effects in primary rat chondrocytes induced by IL-1β, which makes it a promising candidate for in vivo studies on the treatment and prevention of osteoarthritis (OA). The goal of this study is to investigate the effects of the oral administration of formononetin in a rodent model of OA. OA was induced by medial meniscectomy in the right knee joint of rats. The animals were assigned into four groups ($n = 6$): Vehicle (treated with saline), FNT10 (formononetin, 10 mg/kg), Ibuprofen (10 mg/kg), and Sham (simulated surgery, treated with saline). The treatment of the animals was performed daily by the oral route. After six weeks, the knee joints were removed and histologically processed. Histological sections stained in Safranin-O were used to assess the histological grading of the articular cartilage damage. An analysis of the immunohistochemical expression of type II collagen and IL-1β was also performed. The oral administration of formononetin significantly reduced cartilage-matrix-loss width ($p < 0.01$), degeneration scores ($p < 0.05$), and the total articular cartilage-wear depth ($p < 0.01$) in comparison with Group Vehicle. Type II collagen immunoexpression was intense and homogeneous in FNT10, comparable to that of Sham, scarce and irregularly distributed in Vehicle, and homogeneous but less intense in Ibuprofen. Furthermore, formononetin significantly reduced the immunohistochemical expression of IL-1β in joint chondrocytes ($p < 0.01$), but ibuprofen did not ($p > 0.05$). From this study, the oral administration of formononetin was found to attenuate OA-associated pathological damage in rodents, likely because of IL-1β expression downregulation in chondrocytes. These findings suggest that formononetin is a potential therapeutic for treatment.

Keywords: osteoarthritis; Wistar rats; flavonoid; pathology; immunohistochemistry

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1. Introduction

Formononetin—7-hydroxy-3(4-methoxyphenyl) chromone ($C_{16}H_{12}O_4$) is an isoflavonoid that can occur in dietary products such as beans, carrot, cauliflower, green peas, iceberg lettuce, and red potatoes [1], and it has been identified as the major chemical marker of Brazilian red propolis [2–5]. Formononetin has been previously demonstrated to inhibit inflammatory pain in mouse models [6,7], and more recently, studies have also demonstrated anti-inflammatory activity in experimental LPS-induced inflammation models [8,9]. Formononetin has also been proved to reduce the catabolic effects induced by IL-1β in primary rat chondrocytes, such as an increased expression of matrix metalloproteinase (MMP 13, MMP1, and MMP-3), as well as to down-regulate catabolic oxidative stress mediators, such as nitric oxide, inducible nitric

oxide synthase, cyclooxygenase-2, and prostaglandin E2. These effects led to an increase in the content of proteoglycans and the formation of the pericellular matrix in the culture of IL-1 β-treated chondrocytes, without affecting cell viability [10].

Osteoarthritis (OA) is a multi-factorial degenerative synovial joint disorder, often age related and/or trauma induced, that is highly prevalent in the world population, affecting approximately 300 million people in 2017 [11]. Pain, joint effusion, and difficulty in walking are the most common symptoms found in clinical evaluations [12]. In the advanced stages of the disease, patients with OA may present joint contractures, muscular atrophy, and deformity of the limbs. Arthrosis can affect any joint; however, the knees are the most frequently affected [13].

The degenerative process of the articular cartilage can be triggered by mechanical and biological routes. In the biomechanical path, injury and chondral wear are influenced by the level of joint lubrication and the degree of friction between adjacent joint surfaces. On the other hand, the biochemical pathway depends on the activity of pro-inflammatory substances, which accelerate the mechanisms of tissue degradation of the joint [14]. Although these two metabolic pathways can be separately triggered, there is evidence that they interfere with each other in the pathophysiology of arthrosis [15]. Chondrocytes play a central role in maintaining cartilage homeostasis. They control the production and degradation of the components of the extracellular matrix (ECM) according to the conditions of the environment in the joint [16]. They are able to respond to different biochemical and biomechanical stimuli, with a loss of the metabolic balance of the ECM, resulting in cartilage degeneration and the worsening of the evolutionary process of arthrosis [17]. Although OA is characterized by a range of articular changes, such as synovial inflammation of variable intensity, thickening of the subchondral bone, osteophytes formation, degeneration of the ligaments, and hypertrophy of the joint capsule, the loss of cartilage homeostasis with the consequent degradation of the central articular cartilage is considered the main feature of the disease [18]. The loss of cartilage homeostasis results in the degradation of the extracellular matrix rich in collagen and proteoglycans (ECM), fibrillation and erosion of the joint surface, cell death, and dystrophic tissue calcification [19].

Despite the extent of the inflammatory response in OA being often low-grade, increased attention has been paid to the role of inflammation in the pathogenesis of OA. Inflammation seems to occur due to the release of danger-associated molecular patterns (DAMPs), resulting from tissue degradation, such as multiple alarmins (e.g., the calgranulins S100A8 and S100A9), the degradation products of collagen, fibronectin (cartilage extracellular matrix proteins) and hyaluronic acid (a low-molecular-weight proteoglycan of cartilage), and free fatty acids in response to intense mechanical stress [20]. DAMPs induce the release of a range of inflammatory cytokines, such as TNF-α and IL-1β, from a variety of cell subsets, promoting synovitis and altering chondrocyte differentiation, function, and viability, leading to the progression of the disease [21]. Therefore, chemical compounds with anti-inflammatory properties are potential treatment agents for osteoarthritis.

Based on the evidence that formononetin might play a role in minimizing the inflammation-induced deleterious effects in IL-1β-treated chondrocytes, the goal of this study was to investigate the effects of this isoflavonoid in a rodent model of OA.

2. Materials and Methods

2.1. Ethics

The ethical principles of the National Animal Experiment Control Council (CONCEA, Brasilia, Brazil) for experiments in animals were applied in this study, which was approved by the Ethics Committee for Animal Experimentation (approval 010915).

2.2. Experimental Animals and Groups

The twenty-four male Wistar rats (*Rattus norvegicus albinus*, 220 ± 20 g) used in this study were housed in plastic cages with beddings of wood-shavings, which were replaced daily, under a controlled temperature at 22 ± 2 °C and a 12 h light/dark cycle, with water

and food (Labina®; Purina, São Paulo, Brazil) ad libitum. The animals were randomly assigned into four groups, according to the treatment applied (Table 1).

Table 1. Design of the experimental groups according to the surgical procedure used to induce osteoarthritis and treatment applied.

Group (n = 6)	Surgical Procedures	Treatment [1]
Sham	Simulated surgery [2]	Vehicle
Vehicle	Medial meniscectomy	Vehicle
FNT10	Medial meniscectomy	Formononetin (10 mg/Kg)
Ibuprofen	Medial meniscectomy	Ibuprofen (10 mg/kg)

[1] Oral administration, 24/24 h, over the time-course of the experiment. [2] Simulated surgeries with no alteration of the anatomical structures.

2.3. Experimental Model of Osteoarthritis

The surgical procedure for osteoarthritis induction was performed as previously described by Bendele (2001) [22]. Briefly, the animals were subjected to dissociative anesthesia with an intraperitoneal administration of 0.10 mL/100 g of 10% ketamine (Ketamine®, Rhobifarma Ind. Ltd. Pharmaceuticals, Hortolândia, São Paulo, Brazil) and 0.25 mL/100 mg of xylazine (Anasedan® Purina) and placed in the supine position with paw fixation using adhesive tape. The right knee was subjected to trichotomy and antisepsy with polyvinylpyrrolidone iodine. A 3-cm longitudinal incision was made on the medial region of the right knee, followed by a medial capsule incision. The lateral displacement of the extensor mechanism without transection of the patellar ligament was delicately performed to ensure the best visualization. The medial collateral ligament was exposed and sectioned 3 mm from the joint line, and the medial meniscus was partially removed. Subsequently, the extensor mechanism was anatomically repositioned, and the capsule and skin were sutured. In Group Sham, the surgical procedure was limited to the skin incision and suturing, without any deeper interference from the knee structures. The treatment of the animals (Table 1) was performed daily for 6 weeks. Euthanasia of the animals occurred 2 weeks after the end of treatment, using a lethal anesthetic dose (3× anesthetic dose).

2.4. Tissue Preparation for Histological Analysis

The knees were dissected and fixed with 10% formalin during the 24 h. The samples were decalcified using 20% ethylenediaminetetraacetic acid (EDTA) during 7 days on a plate shaker with daily solution renovation. The knees were dissected, fixed in formaldehyde (10%, pH 7.4), dehydrated, diaphanized, and frontally embedded in paraffin. Histological sections (5 µm thick) were obtained from each paraffin-embedded sample from the center of the medial tibial plateau, so that every three serial sections followed an interval of 200 µm, until a total of 18 sections were achieved. One histological section of each serial group of slides was stained using the Safranin-O technique. The other two sections of each serial group of slides were subjected to further immunohistochemical procedures. All the histological slides were scanned for further morphometric analysis using ImageJ® 1.49v software (National Institutes of Health, Bethesda, MD, USA).

2.5. Pathological Analysis of Histological Slides Stained in Safranin-O

To assess the histological grading of the articular cartilage degeneration, the modified Mankin score for histological assessment of osteoarthritis in rats described by Gerwin et al. (2010) [23] was used. The pathological criteria used in this study are described as follows:

Cartilage-matrix-loss width (CLW). The width of the area of collagen-matrix loss was measured along the surface (0% depth), as well as at the level of the midzone (50% depth), and tidemark (100% depth). Only areas of complete cartilage-matrix loss were measured, and any floating debris was ignored.

Cartilage degeneration score (CDS). For the assessment of cartilage degeneration severity, the medial tibial plateau was divided into three zones of equal width: zone 1 (Z1) on the

outside (the medial edge of joint), a central zone 2 (Z2), and an inner zone 3 (Z3) on the inside (adjacent to the central cruciate ligaments). The severity of cartilage degeneration in each zone was scored from 0 to 5, according to the following criteria: 0—no degradation; 1—minimal degeneration, representing 5–10% of the total cartilage area affected by matrix or chondrocyte loss; 2—mild degeneration, corresponding to 11–25% affected; 3—moderate degeneration, representing 26–50% affected; 4—marked degeneration, corresponding to 51–75% affected; and 5—severe degeneration, representing more than 75% affected. The final score was obtained by adding the three zones with a maximum value of 15.

Total articular-cartilage-wear depth (CWD). This was assessed by determining the wear-depth percentage of the three-width zones of the medial tibial plateau (Z1 to Z3), dividing the depth of the area of cartilage degeneration by the cartilage thickness (both in micrometers), from the projected cartilage surface to the tidemark. The software Image J was used to determine the depth of the area of degeneration and the cartilage thickness in micrometers.

2.6. Immunohistochemical Procedures

Six histological sections (5 μm thick) from each animal were mounted on previously silanized glass slides, dewaxed in xylol, and washed in decreasing concentrations of ethyl alcohol (100%, 95%, 90%, 80%, and 70%). The enzymatic blocking of endogenous peroxidase activity was performed with 3% hydrogen peroxide and methyl alcohol (for 10 min in a dark room). The immunodetection procedure of the antigens was carried out incubating the primary antibodies, as described in Table 2. Prior to the incubation, the antigenic recovery of both antigens was carried out using moist heat under pressure in a solution of 10 mM citrate buffer/pH 6.0. The sections were incubated with secondary antibodies (SABC—streptavidin–biotin complex, catalog number SA1022) at 37 °C for 30 min. The reaction was revealed by incubating the sections with diaminobenzidine chromogen (DAB, Ventana Medical Systems, Tucson, AZ, USA, 30 min), and counter-staining was performed with Meyer's hematoxylin. Both steps were performed with an interval of 4 min each. The analysis of the type II collagen immunoexpression pattern was descriptive. The mean number of cells positive for IL-1β was counted in 36 histological fields per joint surface (400×, analytical area corresponding to 0.025 mm^2).

Table 2. Target antigens and antibodies used in the immunohistochemical study.

Antigen	Clone	Dilution	Incubation
Collagen type II	2B1.5 (Invitrogen)	1:800	12 h
IL-1β	AAR15G (Serotec)	1:250	12 h

2.7. Statistical Analysis

All data obtained in this study were subjected to an analysis of normal distribution using the Shapiro–Wilk test. The homogeneity of variances (homoscedasticity) was tested using Bartlett's test. The CLW-, CWD-, and IL-1β-positive cell data were expressed as mean ± standard error mean. The differences between groups were assessed using ANOVA and Bonferroni's multiple comparison test. The CDS data were expressed as median and interquartile range. The differences between the groups were assessed using the Kruskal–Wallis test and Dunn's multiple comparison test. The confidence level (alpha) was 5% and the confidence interval used was 95%.

3. Results and Discussion

As shown in Figure 1, intense and homogeneous patterns of impregnation by Safranin-O were observed in the tibial articular cartilage in Group Sham along the articular surface and depth, involving typical and well-distributed chondrocytes. Group Vehicle exhibited poor impregnation, especially in the joint-wear zone. Chondrocytes were swollen, reduced in number, and irregularly distributed in the cartilage. The loss of cartilage matrix, leaving a moth-eaten appearance in the articular surface, is notorious. Group FNT10 showed

more intense impregnation by Safranin-O than that of Group Vehicle, but not as homogeneous as that of Group Sham. A stronger intensity of staining was observed two-thirds deep into the articular cartilage, in opposition to the most superficially deep in the first-third. No morphological sign of intracellular chondrocyte edema was found in either group. The oral administration of formononetin significantly reduced the mean of cartilage-matrix-loss width (1.04 ± 0.16 µm) in comparison with Group Vehicle (1.57 ± 0.09 µm) ($p < 0.01$). No significant difference was observed comparing the vehicle with ibuprofen groups (1.24 ± 0.05 µm). The greatest degeneration scores were obtained in Group Vehicle (4.20 ± 0.48), and the oral administration of formononetin significantly reduced the scores of cartilage degeneration (2.66 ±0.42; $p < 0.05$). However, no significant difference was observed comparing the group treated with ibuprofen (3.60 ± 0.49) with the vehicle and FNT10 groups ($p > 0.05$). Furthermore, the treatment with formononetin (FNT10) significantly reduced the cartilage-wear depth (43.80 ± 3.64%) in comparison with that of Group Vehicle (65.00 ± 4.84%) ($p < 0.01$), which was not observed with Group Ibuprofen (45.75 ± 1.77%) ($p > 0.05$). As no change in the articular surface of the joint was observed in Group Sham, this group was excluded from this quantitative analytical procedure.

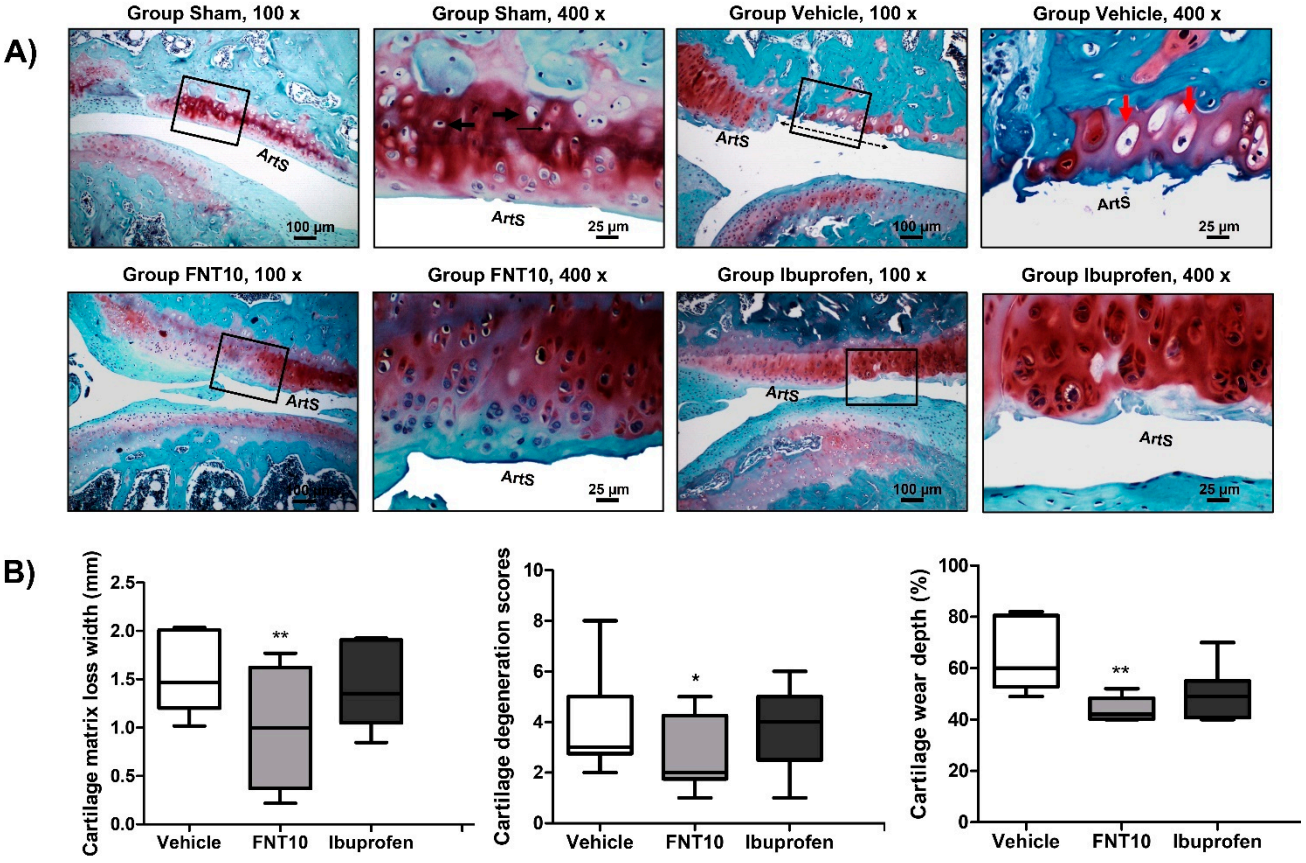

Figure 1. (**A**) Histological sections of the experimental groups stained with Safranin-O. Group Sham presented intense and homogeneous patterns of impregnation with Safranin-O involving typical chondrocytes (black arrows) along the articular surface (ArtS). Group Vehicle showed poor impregnation with Safranin-O, a clear loss of cartilage matrix (double-headed dotted arrow), and swollen chondrocytes (red arrows). Note the moth-eaten appearance of the articular surface. Group FNT presented a more regular appearance of the wear zone of the tibial joint surface compared with that of Group Ibuprofen. (**B**) Assessment of the mean cartilage-matrix-loss width, cartilage degeneration scores, and percentage of cartilage-wear depth in the experimental groups treated with oral administrations of vehicle only (Vehicle), formononetin at 10 mg/kg (FNT), and ibuprofen at 10 mg/kg. Differences in comparison with Vehicle are expressed as * $p < 0.05$ and ** $p < 0.01$ (ANOVA and Bonferroni's multiplex comparison test).

The pattern of immunoreactivity for the anti-collagen II antibodies, evidenced by a brown-colored matrix staining in the epiphyseal cartilage area, is shown in Figure 2. A homogeneous immunoreactivity along the articular surface, as well as along the entire length of the epiphyseal cartilage, was observed in Group Sham. Group Vehicle showed a marked reduction in the expression of collagen II, expressed as a sparse and irregular immunoreactivity pattern, limited to some focal areas of the specimens. The group treated with formononetin showed an irregular immunoreactivity pattern, with strong staining in the deepest two-thirds of the epiphyseal cartilage but showed progressive loss of antigenic expression in the most superficially deep one-third. The group treated with ibuprofen presented an immunostaining pattern similar to that of Group FNT10.

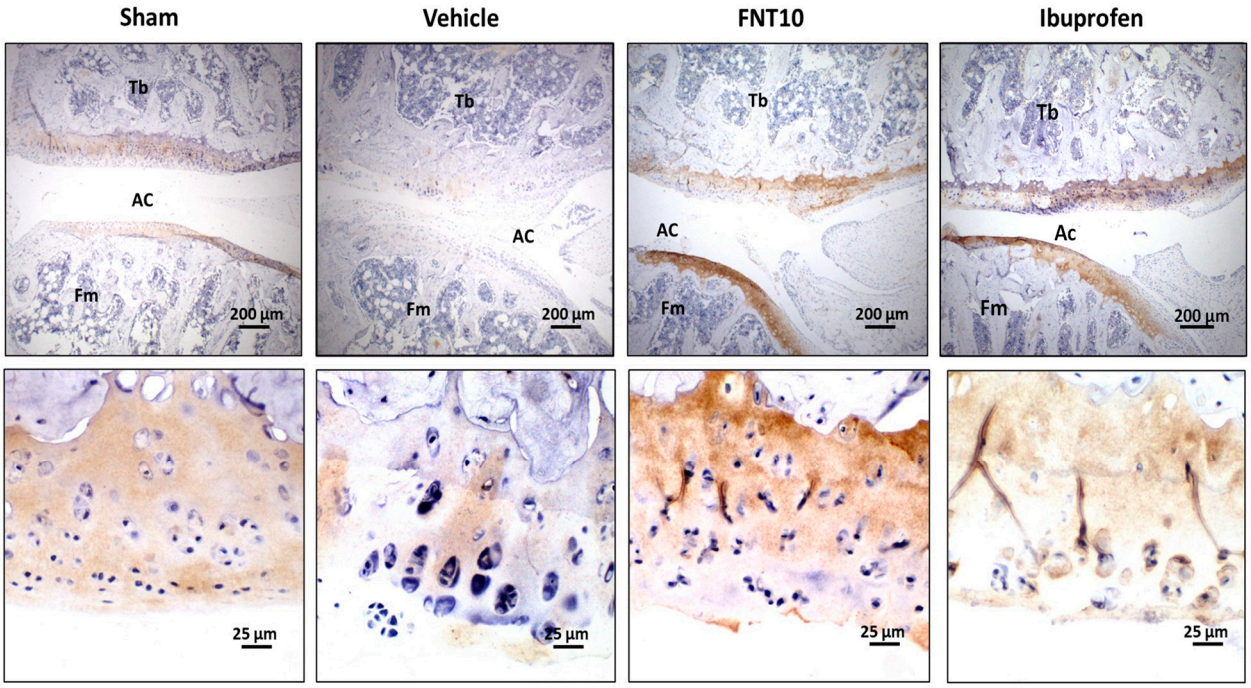

Figure 2. Immunohistochemical expression of collagen type II in the tibial articular surfaces in the experimental groups. Upper row of pictures shows a panoramic view of the tibial–femoral joint (40×), whereas the lower row presents detailed images of the tibial surfaces (400×). Group Sham showed a homogeneous immunoreactivity along the articular surface and the epiphyseal cartilage, whereas Group Vehicle presented a sparse and irregular immunoreactivity pattern, limited to some focal areas of the specimens. The groups treated with formononetin at 10 mg/kg (Group FNT) and ibuprofen showed strong staining at the deepest two-thirds of the epiphyseal cartilage, and progressive loss of immunoreactivity in the most superficial first-third. Legend: AC—articular cavity; Tb—tibial bone; Fm—femoral bone.

The cell cytoplasmic immunoreactivity for the anti-interleukin 1β antibodies is demonstrated in Figure 3A. Group Sham showed an intense immunostaining pattern in bone marrow cells, but only an occasional one in epiphyseal chondrocytes. Group Vehicle exhibited strong positivity in epiphyseal chondrocytes along the entire length of the epiphyseal cartilaginous plaque on the joint surface, suggesting significant inflammation throughout the chondral space. Positive immunostaining in epiphyseal chondrocytes was also observed in the FNT10 and ibuprofen groups, but this was less intense than in Group Vehicle.

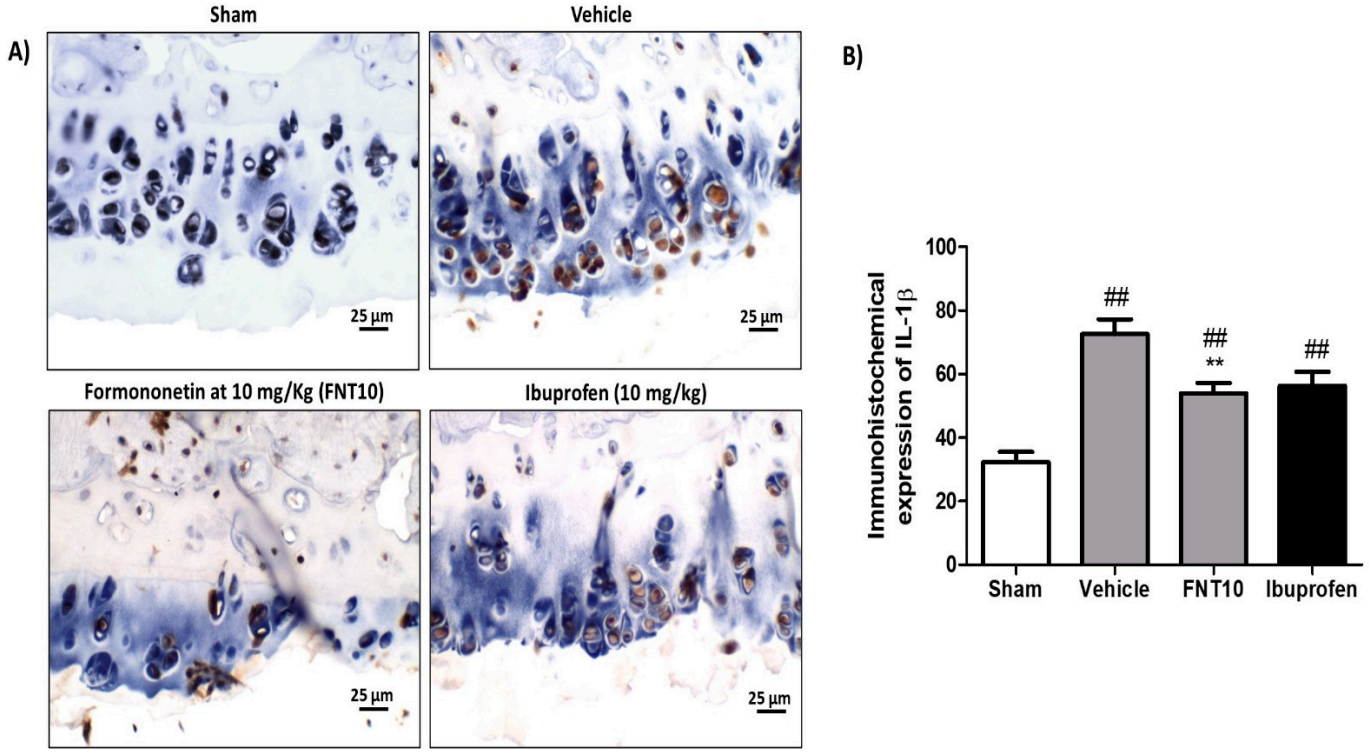

Figure 3. (**A**) Immunohistochemical expression pattern of cell positivity for IL-1β in the experimental groups treated with oral administration of vehicle only (Vehicle), formononetin at 10 mg/kg (FNT), and ibuprofen at 10 mg/kg (LSAB, 400×). (**B**) Assessment of the mean number of IL-1β-positive cells for histological field (0.025 mm^2). Differences in comparison with Vehicle are expressed as ** $p < 0.01$, whereas differences in comparison with Sham are expressed as $^{\#\#}$ $p < 0.011$ (ANOVA and Bonferroni's multiplex comparison test).

As demonstrated in Figure 3B, the mean number of IL-1β-positive cells in Group Vehicle (72.52 ± 4.60 cells/0.025 mm^2), FNT10 (53.92 ± 3.30 cells/0.025 mm^2), and Ibuprofen (50.62 ± 3.01 cells/0.025 mm^2) was significantly greater than in Group Sham (32.33 ± 3.24 cells/0.025 mm^2) ($p < 0.001$), but only FNT was significantly reduced in comparison with Group Vehicle ($p > 0.01$). Moreover, no significant difference was observed between any treated groups (FNT10 and Ibuprofen; $p > 0.05$).

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used as a primary drug for the pharmacological treatment of osteoarthritis [24]. Ibuprofen and paracetamol are effective anti-inflammatory drugs in the treatment of patients with OA, but as ibuprofen has been shown to be more effective than paracetamol in both single and multiple doses [25], this drug was used as a positive control in the current study. However, although these compounds may relieve OA-related pain and swelling, they are not able to improve the damage to articular cartilage [26] and frequently promote side effects, especially in the upper gastrointestinal and cardiovascular systems [27,28]. Therefore, other safe and effective compounds to manage the signs and symptoms of OA and inhibit cartilage degeneration, but with fewer side effects, have been actively studied.

In the current study, the possible influences of formononetin on OA-associated pathological changes of the articular cartilage of the knee joint were assessed using a rat articular cartilage degeneration model [23]. The use of formononetin was based on its ability to suppress the loss of proteoglycan and pericellular matrix content in IL-1 β-treated chondrocytes, because of the selective inhibition of matrix metalloproteinase (MMP 13, MMP1, and MMP-3) and downregulation of oxidative stress mediators [10]. The dose of 10 mg/kg was based both on a previous study on the anti-inflammatory and antinociceptive activities of

formononetin administered by the oral route [6] and on a pilot test in experimental OA (data not shown) to provide a rational use of experimental animals.

Safranin-O dye was chosen for the histological analysis. This dye is based on the combination of an anion (e.g., keratan sulfate or chondroitin sulfate) and a cationic chromophyll, which stains red the proteoglycans distributed in the cartilaginous tissue; hence, the intensity of staining is proportional to the concentration of PG in the tissue [29]. The reduced and irregular pattern of stain observed in Group Vehicle in opposition to the homogeneous pattern seen in Group Sham has also been reported in previous studies [23,30], suggesting that the experimental model was successful. On the other hand, the groups treated with the oral administration of formononetin showed a more intense pattern of dye impregnation, especially in the deepest third of the tissue, which is suggestive of a possible chondroprotective effect.

To confirm the protective effect of the oral administration of formononetin on joint cartilage, the grading system recommended by the Osteoarthritis Research Society International (OARSI) for histological assessments of osteoarthritis in rats was used in the current study. The simple semi-quantitative score method used in this system is accurate, reproducible, and practical for most studies, as it permits a more complete assessment of the cartilage histopathology [23,31]. Based on the OARSI histopathological grading system, formononetin significantly attenuated the cartilage-matrix-loss width, degeneration scores, and wear depth, supporting the hypothesis that the isoflavonoid can prevent, at least partially, the joint damage induced by medial meniscectomy.

The content of proteoglycan in the joints is closely related to the type II collagen content [32]. Type II collagen is the main molecular component of the extracellular framework of adult articular cartilage [33], and previous studies have demonstrated that the pathological degenerative changes observed in the articular cartilage in OA are associated with the reduction of the tissue contents of type II collagen [32], which can be observed both in experimental models [23,30] and in clinical trials [34].

The similarities in the immunohistochemical expression of type II collagen and the content of proteoglycan assessed by Safranin-O impregnation observed in the current study fully supported this theory and seemed to suggest that the chondroprotective effects played by formononetin might be related to a possible modulation of type II collagen catabolism. Previous studies have demonstrated increased levels of metalloproteinase (MMP)- 3 and MMP-13, enzymes that degrade the extracellular substrates of the cartilage matrix and collagen type II, respectively, in OA [35,36]. As formononetin has been previously demonstrated to effectively reduce the expression of MMP-3 and MMP-13 and increase proteoglycan content by antagonizing the IL-1β-induced catabolic effects in chondrocytes in vitro [10,37], we investigated the immunohistochemical expression of IL-1β in the chondrocytes of the articular joint in vivo.

We found that the immunohistochemical expression of IL-1β was significantly reduced by the oral administration of formononetin. The increased release of IL-1β was associated with the overexpression of cartilage-degrading enzymes and progressive reduction of type II collagen, with a subsequent breakdown of articular cartilage [30]. These data seemed to point at a possible relationship between the reduction of IL-1β expression induced by formononetin and the attenuation of the degenerative damage observed in the articular cartilage in the experimental OA model. However, other in vivo models showed a conflicting role for this molecule, because although early studies using therapeutic approaches in animal models showed a benefit, many other murine studies failed to demonstrate protection where the ligands (IL-1α/β), the cytokine activator (the IL-1-converting enzyme), or the receptor (IL-1R) have been knocked out [38]. Therefore, further investigations are still necessary to clarify the precise mechanisms underlying the chondroprotective effects of formononetin in OA experimental models. In addition, formononetin has been previously proved to exert an anti-inflammatory effect in carrageenan-induced hind paw edema and peritonitis models in rodents [6]. Although anti-inflammatory or analgesic tests were not performed in the current study, these data make possible to suppose that formononetin

could be helpful to prevent histological degenerative changes of injured joints, as to relieve OA-associated pain and swelling.

The oral administration of this drug was proved to promote anti-inflammatory and analgesic effects as well as to reduce joint stiffness in rats with adjuvant arthritis [39]. More recently, ibuprofen was demonstrated to reduce gross arthritis scores in experimental OA induced with bovine type II collagen and incomplete Freund's adjuvant, possibly due to the inhibition of COX2 and iNOS mRNA level expressions [40]. However, no preventive effect on the OA-related histological damages was exerted by ibuprofen in the current study. Two reasons might explain the apparent contrast between our data and those previously reported. The first is the differences in the experimental models of OA induction, because the joint injury was induced by an intra-articular injection of adjuvants in opposition to the medial meniscectomy conducted in the current study. Adjuvant-induced OA leads to extensive swelling and pain, but since it is a typical acute model, degenerative changes in the cartilage are not supposed to be produced, whereas medial meniscectomy is an aggressive model of OA that leads to stiffness and severe degenerative changes [41]. For this reason, ibuprofen is expected to be more effective in adjuvant-induced OA than in medial-meniscectomy-induced OA. Second, the OA severity assessment in those previous studies was based on macroscopic features, such as joint swelling and stiffness, whereas the pathological degenerative changes were the focus of the current study. As a NSAID, ibuprofen's ability to reduce pain and swelling is well established, but our results suggested that this drug was ineffective to prevent the histological degenerative damages of the joint caused by medial meniscectomy. These findings might be related to its apparent inability to inhibit IL-1β immunohistochemical expression in joint chondrocytes, as observed in the current study. Treatment with ibuprofen had no inhibitory effect on TNF-α or IL-1β levels in periapical exudates in humans [42], which supports our findings.

On the other hand, the inhibition of IL-1β-induced nitric oxide (NO) and prostaglandin E2 (PGE2) production by ibuprofen has been previously demonstrated, likely resulting from a modulation of via RhoA signaling, a regulatory factor involved in a variety of cell functions, such as the regulation of cytoskeletal dynamics, transcription, and cell-cycle progression [43]. Although ibuprofen exerted no protective effect on the cartilage joint degeneration, these data point to a possible protective effect of this drug on the chondrocyte phenotype from IL-1β stimulation. Hence, further investigations are necessary to fully clarify the precise effects of ibuprofen and other NSAIDs on OA induced by medial meniscectomy.

4. Conclusions

This study supported the conclusion that the oral administration of formononetin attenuates the OA-associated articular pathological changes induced by medial meniscectomy in rodents and that this biological effect might be related to a downregulation of IL-1β expression in chondrocytes. These findings suggested that formononetin may be a potential therapeutic for the treatment of OA.

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Institutional Review Board Statement: This work raises ethical issues as part of the experiments performed on animals. The animal study was approved by the Ethics Committee on Animal Research

of Tiradentes University (CEUA/UNIT) through opinion no. 010915. The study was conducted within the ethical and legal standards, by qualified personnel certified with the FELASA Cat-C certificate, in support of Law No. 11,794 of 08.10.2008, which establishes procedures for their scientific use; Environmental Crimes Law No. 9.605 of 12 February 1998; and the Ethical Principles for the Use of Laboratory Animals of the Brazilian Society of Laboratory Animal Science (SBCAL/COBEA). The 3R principles have been followed by applying the EU Directive 2010/63/EU transferred to the national Decreto-Lei 113/2013 (in Portugal), together with the 2001/83/EC, 86/609/EEC (on the protection of animals used for experimental and other scientific research and the Amsterdam protocol on animal protection and welfare of 1997 FP7, decision number 1982/2006EC).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data and materials are available from authors upon request.

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