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Research article

Chondroprotective effect of melatonin and strontium ranelate in animal model of osteoarthritis

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ABSTRACT

Purpose: To analyze the action of strontium ranelate (SR) and melatonin in isolation or in association in knees, liver and kidneys of rats Wistar with induced osteoarthritis (OA).
Methods: Thirty male rats were induced to OA through an anterior cruciate ligament transection (ACLT), and treated with melatonin and SR in isolation or in association. Morphological, histopathological, histochemical and morphometric analysis were realized of the structure of the articular capsule, as well as histopathological analysis of liver and kidneys from the animals.
Results: The experimental model was successful. The association of the drugs presented chondroprotective pharmacodynamics. However, more successful results were identified from analysis of animals in which received melatonin in isolation, regarding biochemical parameters of glutamic oxalacetic transaminase. The prepared slide samples of liver and kidneys from groups submitted to the isolated use of SR and melatonin or the association of these drugs presented no differences, when compared to the control group.
Discussion: The administration of the drugs presented chondroprotective effect and prevented from the aggravation of articulate damages, and was not capable of modifying the histology of liver or kidneys. This finding suggests a safe association for the treatment of OA, however it requires further investigation in order to expand therapeutic perspectives regarding improvements of the quality of life of individuals in our society.

1. Introduction

Osteoarthritis (OA) of the knee is the most frequent osteoarticular disease worldwide [1]. A disease with inflammatory and degenerative characteristics in which causes the destruction of articular cartilage, with complex etiology [2, 3]. In addition to the pain, there is a significant reduction on the range of motion and muscular strength, which results in functional limitation and consequent interference in daily basis activities [4, 5, 6], in which the clinical treatment is always indicated through modifications in the lifestyle and use of medication [7]. Among the available drugs for treatment, there are analgesics in which do not interfere on the course of the disease; and anti-inflammatories, with controversial use as a result of its side effects [8].

In this manner, the strontium ranelate (SR), medication indicated for the control of postmenopausal osteoporosis, demonstrated beneficial action in the articular cartilage and the subchondral bone, with possible efficiency for the treatment of OA [9]. The use of this drug reduced the amount of type II collagen biomarkers (CTX-II) in the urine, which explains the decreased degeneration, known to restrain the bone resorption, while enhancing bone formation, improving the structure of the osteoarticular system [10, 11, 12, 13, 14]. Nevertheless, with no apparent cause, in face of so many benefits, it is not known the causes for

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market withdrawal of this medication. There are no major impacts or severe side effects related to strontium ranelate (SR).

Regarding this context, the melatonin presented satisfactory results for the treatment of OA. According to Armijo *et al.* [15], low levels of melatonin in menopause could be an important factor for the development and the maintenance of osteoporosis, since replacement in female rats leads to enhancements of the bone mineral density and the articular cartilage thickness [16]. The melatonin is widely mentioned for reducing specific reactions of oxygen and nitrogen, having anti-inflammatory effects, stimulating antioxidant enzyme activity and decreasing the action of pro-oxidant enzymes [16, 17, 18].

However, no reports in the literature were found indicating the associated administration of those drugs for the treatment of OA, requiring to consider possible effects on the osteomioarticular structures of the knees, intensely affected by OA, as well as in filtering organs, such as liver and kidneys, since they will be requested by the organism when these drugs are metabolized. Therefore, it is crucial that researches are applied for clarifying the effects of those drugs and its pharmacological combination and benefits for the ones affected by OA.

2. Materials and methods

2.1. Study design

It is an experimental case-control research, which was submitted to the Ethics Committee in the Animal Use (CEUA) of the School of Medicine (FCM) – UNIFACISA, and approved under nº 01.0001.2012 CIAEP/CONCEA, protocol nº 0037/22052014.

The experimental procedures for the induction of OA, pharmacological treatments and euthanasia were performed by a veterinary doctor inside the facilities of FCM/Higher Education and Development Center – CESED. The remaining procedures for histological, histochemical and morphometric analysis, as well as the data analysis were performed at the Rural Federal University of Pernambuco, in partnership with the Animal Morphology and Physiology Department. Plasma measurements were performed at a specific laboratory of veterinary analysis. The histological analysis was realized at the Pathological Anatomy Laboratory of the Alcides Carneiro Teaching Hospital from Federal University of Campina Grande and the microscopic and the photomicrographic analysis of the slides were developed by the Microscopy Laboratory of the Northeastern Strategic Technologies Center, at Federal University of Pernambuco.

2.2. Animals and experimental groups

Thirty male rats Wistar were used, obtained from the bioterium of CESED, aged nine to twelve weeks, weighing 250g–320g, kept inside collective cages (n = 3 per cage), with *ad libitum* water and food, and temperature and humidity of 22 ± 2 °C and 55%–65%, respectively. The animals were daily supervised to identify alterations of the tissue or presence of joint swelling and measurement of the weight.

The sample consisted of thirty animals randomly distributed into five groups, arranged as follow:

Group I (GI) – Control: not induced to OA;
Group II (GII) – Placebo: Induced to OA, without pharmacological treatments;
Group III (GIII) – Treatment with SR after the induction to OA;
Group IV (GIV) – Treatment with melatonin after the induction to OA;
Group V (GV) – Treatment with SR and melatonin after the induction to OA;

2.3. Induction to osteoarthritis

Osteoarthritis was induced through an anterior cruciate ligament transection (ACLT) in accordance with the modified methodology by Silva Júnior [19] and Scott *et al.* [20]. Therefore, all animals were anesthetized intramuscularly with Ketamine Hydrochloride (80 mg/kg) and Xylazine (20 mg/kg). After trichotomy and antisepsis of the region, it was performed a longitudinal incision on the skin over the right knee, followed by a lateral parapatellar incision, and the patellar tendon was medially flipped, providing access to the articular cavity. Once visualized the ACL, it was carefully sectioned with microsurgical scissors, avoiding injuries to adjacent structures.

The free movement of the femur in relation to the tibia in the posteroanterior direction ("Anterior Drawer Test") confirmed the rupture of the ligament. Following this procedure, the patellar tendon was relocated and the incision was sutured with a 5-0 absorbable thread, while the skin was sutured with Nylon® 4/0. There was no dehiscence or signs of infection in the surgical wounds of the animals.

2.4. Treatment with SR

After seven days from the induction to OA, it was administered 50 mg/kg in a single daily dose, orally, following the adapted methodology by Pelletier *et al.* [21] for ten days.

2.5. Treatment with melatonin

The melatonin (Sigma, St. Louis, MO, USA) was administered at the dose of 200μg/100g of the body weight of the animal, through subcutaneous injections at the beginning of the evening (18h). The drug was dissolved in ethanol (0,02mL) and diluted in 0,9% Sodium Chloride (0,2mL) for rats from Groups III and V, in accordance with the proposed methodology by Prata-Lima *et al.* [22].

2.6. Collection of blood for plasma analysis

Through mechanical contention of the animal inside a PVC pipe, one mL of blood was collected through the puncture with catheter (24G) from the lateral tail vein, and then placed inside microtubes with sodium heparin (20μL), homogenized and maintained at room temperature. To obtain the plasma, the samples were submitted to centrifugation in temperature of -4 °C and velocity of 3.000rpm for 10 min, subsequently in temperature of -20 °C.

All biochemical analysis were quantified by spectrometry, automatic ultraviolet analyzer (UV). For dosages of Glycose UV (490 a 520nm); Total cholesterol (Cholesterol) UV (490 a 540nm) and Triglycerides (Trigl) UV (490 a 540 nm) by Labtest kits. For the dosages of Urea UV (340nm); High-density lipoprotein (HDL) and Low-density lipoprotein (LDL) UV (600–700nm); Glutamic-oxalacetic transaminase (GOT) and Glutamic-pyruvic transaminase (GPT) UV (340nm); Creatinine UV (405 a 415 nm) e Uric acid UV (490 a 510nm) were utilized Biotecnica kits.

Plasmatic dosages are relevant for identifying possible severe damage not detectable at tissue level, since the physiological effects when taking the drugs are immediate. Overdoses of glycose might lead to glycation of collagen and to reduce the capacity of recovery of the cartilage, in which already occurs slowly in the articular cartilage. Liver and kidneys are the main filtering organs of circulating drugs in our organism, it processes lipids and it produces urea, which is metabolized by the kidneys, along with other biochemical components such as alkaline phosphatase, uric acid and creatinine. Furthermore, the melatonin is an important regulator of carbohydrates and lipids, besides being an excellent antioxidant. Therefore, it is fundamental to complement biochemical tests for diagnosis of severe damages in which respond to pathological and pharmacological actions.

2.7. Morphological analysis of articulations, histopathological, histochemical and morphometric of the cartilaginous and bone tissues

After the end of the pharmacological treatment, the animals were euthanized. For this, rats were anesthetized intramuscularly with Ketamine Hydrochloride (80 mg/kg) and Xylazine (20 mg/kg), and then

collected femurs, liver and kidneys from each animal, with fixation of this material in 10% buffered formaldehyde.

Subsequently, samples of the bone tissue were processed according to routine techniques for inclusion in paraffin and production of slides, stained with hematoxylin and eosin (H&E stain) and Von Kossa (method to detect calcium), analyzed with a light microscope (OLYMPUS® BX-49), and observed with a photomicroscope (OLYMPUS® BX-50). To quantify the alteration of pixels from the material stained with the Von Kossa method, the Gimp 2.6 *software* (GNU Image Manipulation Program, UNIX platforms) was utilized. The results obtained were submitted through a statistical test and a comparative graph between the groups was produced.

For the measurements of the space between the articulations, the articular cartilage and the associated structures, the same software mentioned above was utilized with the scale tool converted to *μm.* The data measured were also submitted to statistical analysis.

However, the ideal is to perform magnetic resonance imaging (MRI) for identifying the thickness of tissue and articular spaces. Unfortunately, this research has limited resources and some techniques, such as pigments and molecular markers for glycoproteins, proteoglycans and collagens were not available. In order to preserve and to standardize morphometric analysis, the material was fixed for microscopic evaluation without opening of the epithelium lining and the muscular tissue. Consequently, it was impossible to verify the damage as suggested by (CIRS II), the articular aspect was observed only through the processing for impregnation in paraffin for histopathological analysis and coloring. Therefore, it is suggested that new researches are developed in the future for specific genetic and molecular analysis.

2.8. Histopathological analysis of hepatic and renal tissues

Livers and kidneys from the animals were processed according to routine techniques for inclusion in paraffin, and H&E stained. The data were analyzed from direct observation of slides as well as photomicrographs obtained from the electronic microscopy (Leica DM 500 Axion Vision, version 2.0.0, camera Leica ICC 50 HD, Software Axion Vision), comparing the morphologies of liver and kidneys between the groups, in order to identify the condition of preservation of the tissues and the cellular properties.

2.9. Statistical analysis

The data were submitted through Kruskal-Wallis non-parametric test, with the comparison of the mean values with the *post hoc* test in a paired manner. A 95% level of significance was assumed. The PASW Statistic 18 software was utilized.

3. Results

The induction to OA was confirmed by the free movement of the femur in relation to the tibia in the posteroanterior direction after the rupture of the ACL. Throughout the experimental procedures it was not perceived any formation of edema, infection or weaknesses of the animal, however, the mobility of the member with the broken ACL was reduced, as a result of the surgical incision, the purpose of the methodology applied to this article.

Clinical and behavioral aspects of the animals were not modified after the induction to OA and neither with the treatment proposed, therefore stayed healthy and eating as routine.

Biochemical assessments presented differences regarding dosages of total cholesterol (Cholesterol) H [x^2 (4) = 15,884; p < 0,04] between GII and GIV; creatinine (Creat) H [x^2 (4) = 16,969; p < 0,01] between GI and GIV and between GII and GIV; glutamic-oxalacetic transaminase (GOT) H [x^2 (4) = 17,930; p < 0,01] between GI and GIV and between GIV and GV; urea [x^2 (4) = 16.170; p < 0,03] between GII and GV, between GIII and GV and between GIV and GV; and high-density lipoprotein (HDL) [x^2 (4) = 18,437; p < 0,01] between GII and GIV and between GII and GV (see Table 1 for details).

At the same table, it is possible to identify the reference data of the groups, also quantified by plasma analysis, which did not diverge: uric acid [x^2 (4) = 5,861; p > 0,05]; glutamic-pyruvic transaminase (GPT) [x^2 (4) = 13,631; p > 0,05]; triglycerides (Trigl) [x^2 (4) = 9,622; p > 0,05]; low-density lipoprotein (LDL) [x^2 (4) = 6,02; p > 0,05]; and average weight (Weight A) [x^2 (4) = 7,388; p > 0,05] of the animals.

The bone tissue, the articular cartilage and the constituent cells of tissues were presented well preserved in all experimental groups. Morphological analysis demonstrated the integrity of the superficial, transitional (mid), deep (radial) and calcified layers of the articular cartilage, and the conservation of the basophilia of the cartilage matrix of the femurs of rats, identified by the appropriate infiltration of the pigment (Image "A" of Figure 1). Furthermore, it was not observed any lymphocytic infiltration, pyknosis, necrosis or fibrillation in any area of the studied tissue.

Morphometric analysis of the bone tissue was submitted to standardization and measurement by image software, and revealed significant differences regarding the dimensions of cartilages and the femoropatellar articular spaces. There was reduction of the measure of the articular capsule from GII, when compared to GI, [x^2 (4) = 16,520; p > 0,05]. The distance between cartilages differed from GII when

Table 1. Plasmatic levels of animals per experimental group of Uric acid, Total cholesterol (Cholesterol), Creatinine, Glycose, Glutamic-oxalacetic transaminase (GOT), Glutamic-pyruvic transaminase (GPT), Triglycerides (Trigl), Urea, High-density lipoprotein (HDL), Low-density lipoprotein (LDL) and Average weight (Weight A) from rats of experimental groups after the treatment.

Groups	I	II	III	IV	V
	Mean ± Standard deviation	Mean ± Standard deviation	Mean ± Standard deviation	Mean ± Standard deviation	Mean ± Standard deviation
Uric acid	1.0 ± 0.2	1.1 ± 0.2	1.3 ± 0.5	0.8 ± 0.1	1.1 ± 0.2
Cholesterol	37.8 ± 1.1	31.6 ± 1.5*	33.6 ± 5.6	41 ± 1*	40 ± 2.9
Glycose	124.6 ± 4.9	114.4 ± 7.0	120.4 ± 17.3	111.6 ± 7.7	109.6 ± 19.4
HDL	30.4 ± 0.5	27 ± 0.7*ø	29.4 ± 4.1	35.4 ± 1.1*	34.4 ± 2.5ø
GOT	80.4 ± 10.8ø	114 ± 8.9	123.6 ± 28.6	73.6 ± 11.6*	126.6 ± 17.6*
GPT	59.4 ± 0.5	63.8 ± 2.5	69.8 ± 5.7	56.6 ± 7.8	69.6 ± 7.8
Trigl.	55.2 ± 2.5	23 ± 4.5	48.6 ± 19.6	60.4 ± 22.5	45.8 ± 22.4
Urea	34.2 ± 0.8	29.6 ± 3.9*	28 ± 7.5ø	29 ± 4.5∞	44.6 ± 4.9*ø∞
Creat	0.7 ± 0.01*	0.7 ± 0.1ø	0.6 ± 0.03	0.4 ± 0.1*ø	0.6 ± 0.07
LDL	3.6 ± 0.6	1.6 ± 1.6	5.5 ± 3.5	6.4 ± 4.6	5.0 ± 3.6
Weight A	255.8 ± 4.0	260.8 ± 1.3	260.6 ± 1.3	260 ± 1.2	261 ± 1.0

Means in a same line, followed by the same symbol differ significantly from each other by Kruskal Wallis (P > 0,05) test.

compared to GI, GIV and GV [x^2 (4) = 18,24; p > 0,05]. The other groups presented no differences when the mean values between the groups were compared (Images from "A" to "E" of Figure 1), as it is possible to evidence on the graph with mean and standard deviation for quantification in micrometers (Image "F" of Figure 1).

From histochemical analysis, the staining for detection of deposits of calcium ions presented positive marking for all experimental groups (Images from "A" to "E" of Figure 2). Nevertheless, there was no difference between groups, which is statistically confirmed by image "F" of Figure 2, on the measure bars in pixels [x^2 (4) = 5,102; p > 0,05] (Figure 2).

The hyaline cartilage in which covers the articular surfaces of movable joints, named articular cartilage, does not have perichondrium and come into contact with the subchondral bone. The articular cartilage is avascular and it enables the diffusion of substances such as the calcium among the chondrocytes inside its extracellular matrix. The articular cartilage, different from the hyaline cartilage, is organized into layers in which have intense calcification near to its watermark line, also named the TideMarck, both in the deep zone and in the calcified zone, mainly in adults.

The histological analysis of the slides produced from livers of experimental groups enabled to identify resemblances on the tissue when

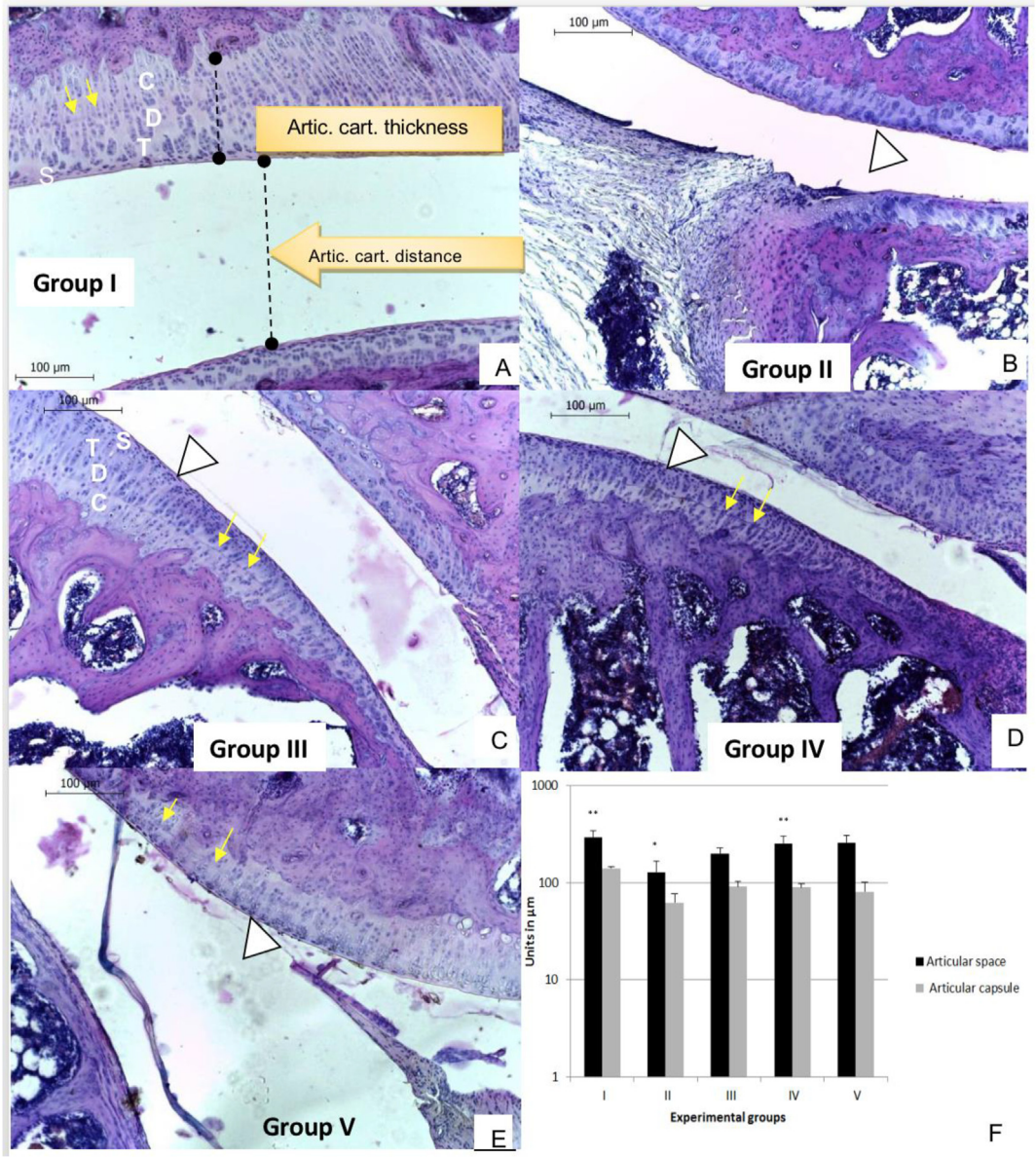

Figure 1. Image "A" of the articular cartilage of rats from group I, control, without induction to OA. Observe the preservation of the tissue, chondrocytes (black arrow) in its gaps; zones: superficial (S), transitional (T) and deep (D) of the articular cartilage and calcified zone (C). Deposit of collagen fibers "TideMarck" (yellow arrow). "A" to "F" Thickness of the articular cartilage and distance between the articulation and the head of the femur of rats induced to osteoarthritis. A) Rat from group I, control, without induction to OA; B) Group II, placebo, induced to OA and without drug treatments, observe the reduction statistically significant when compared to groups I (control) and IV (treatment with melatonin after the induction to OA). Notice, also, the difficulty to identify the characteristics of the articular capsule; C) Group III, induced to OA and treated with strontium ranelate; D) Group IV, induced to OA and treated with melatonin; E) Group V, induced to OA and treated with the association of the drugs; F) Graph showing the mean values of the measures of the articular space and the articular capsule in micrometers and their deviations in all experimental groups. The tip of the arrow (Δ) indicates the head of the femur – articular capsule. Routine staining (H&E). Means followed by the same symbol in the parameters analyzed did not differ significantly, according to the Kruskal-Wallis test of independent samples (P > 0.05).

compared to GI. When reading the slides from all groups, it was possible to perceive salutary characteristics, not considered pathological for the liver of the animals. It was identified the integrity of the hepatic lobule and the periportal space, hepatic veins well defined, and hepatocytes forming confluent cords for the centrilobular vein. Among the cords, it was observed hepatic sinusoids with rounded and voluminous nuclei, normally euchromatic; intact sinusoidal cords and with some red cells. The hepatic cells presented intact nuclei, generally centralized, nucleoli well evidenced and cytoplasm with basophilic and eosinophilic areas, within normality (Figure 3).

In the kidneys, it was perceived similar histological characteristics between the experimental groups when compared to GI, in which were non-pathological, with integrity of the renal corpuscles and contorted proximal and distal tubules; glomeruli formed by capillaries; presence of podocytes, endothelium and mesangial cells without alteration; integrity of Bowman's capsule and space and histological pattern preserved in the medulla and renal pelvis (Figure 4).

4. Discussion

It is known that OA is one of the most frequent causes of musculoskeletal pain and functional incapacity [23]. In our study, the daily monitoring demonstrated that the induction to OA obtained success and did not lead to any inflammatory damage, since the tissue was presented well preserved and with similar quantification of calcium between the experimental groups.

The morphometric analysis revealed that the measures of the articular capsule and space from groups in which received some treatment

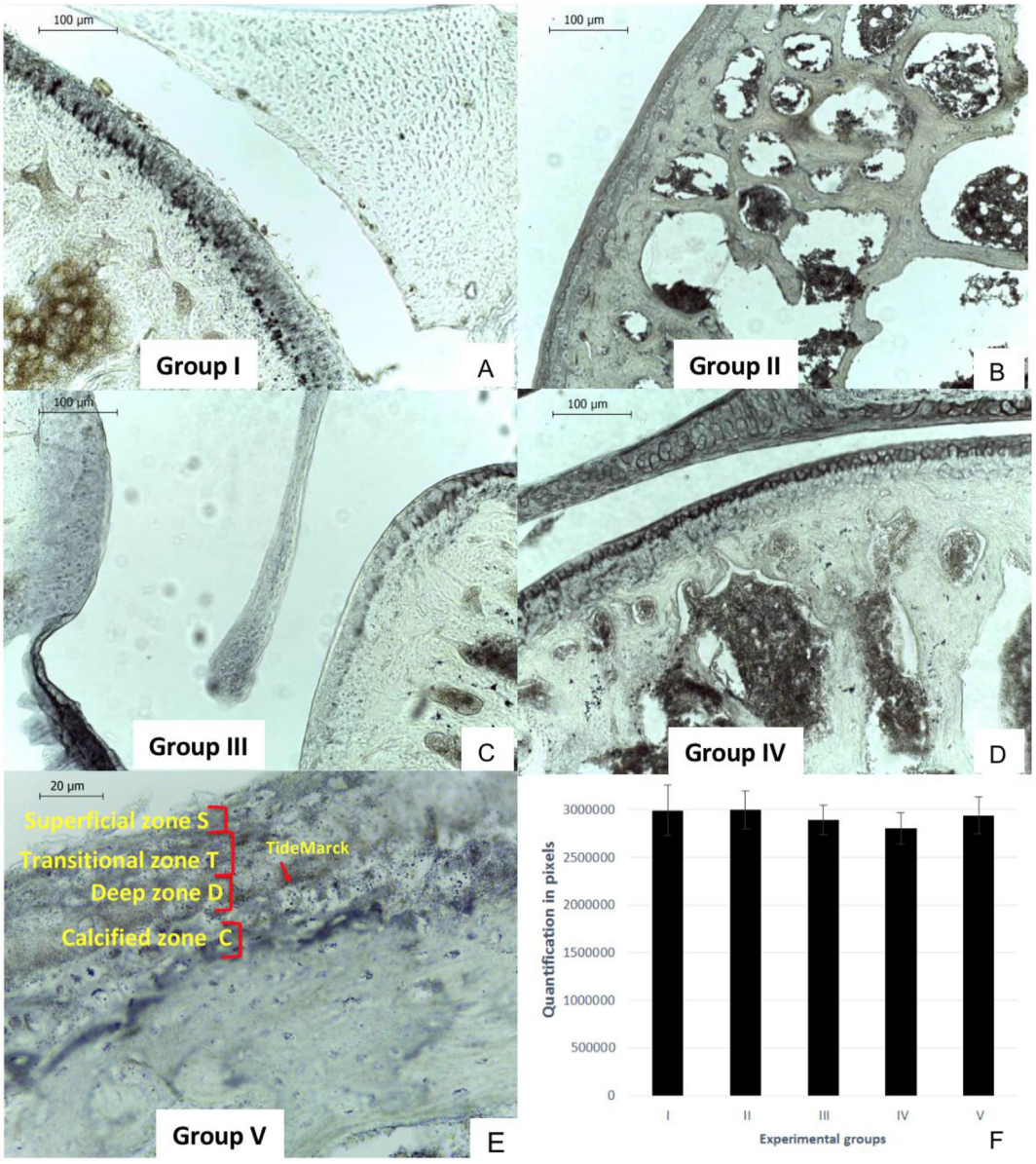

Figure 2. Images of bone structures of the head of the femur and the knee of rats from all experimental groups induced to OA. Histochemical for Calcium (Von Kossa). A) Rat from group I, control, without induction to OA; B) Group II, placebo, induced to OA and without drug treatments; C) Group III, induced to OA and treated with strontium ranelate; D) Group IV, induced to OA and treated with melatonin; E) Group V, induced to OA and treated with the association of the drugs; F) Graph with mean and standard deviation for the quantification in pixels of the stain in the area of the articular capsule, statistically presenting similarity between the data. Von Kossa staining.

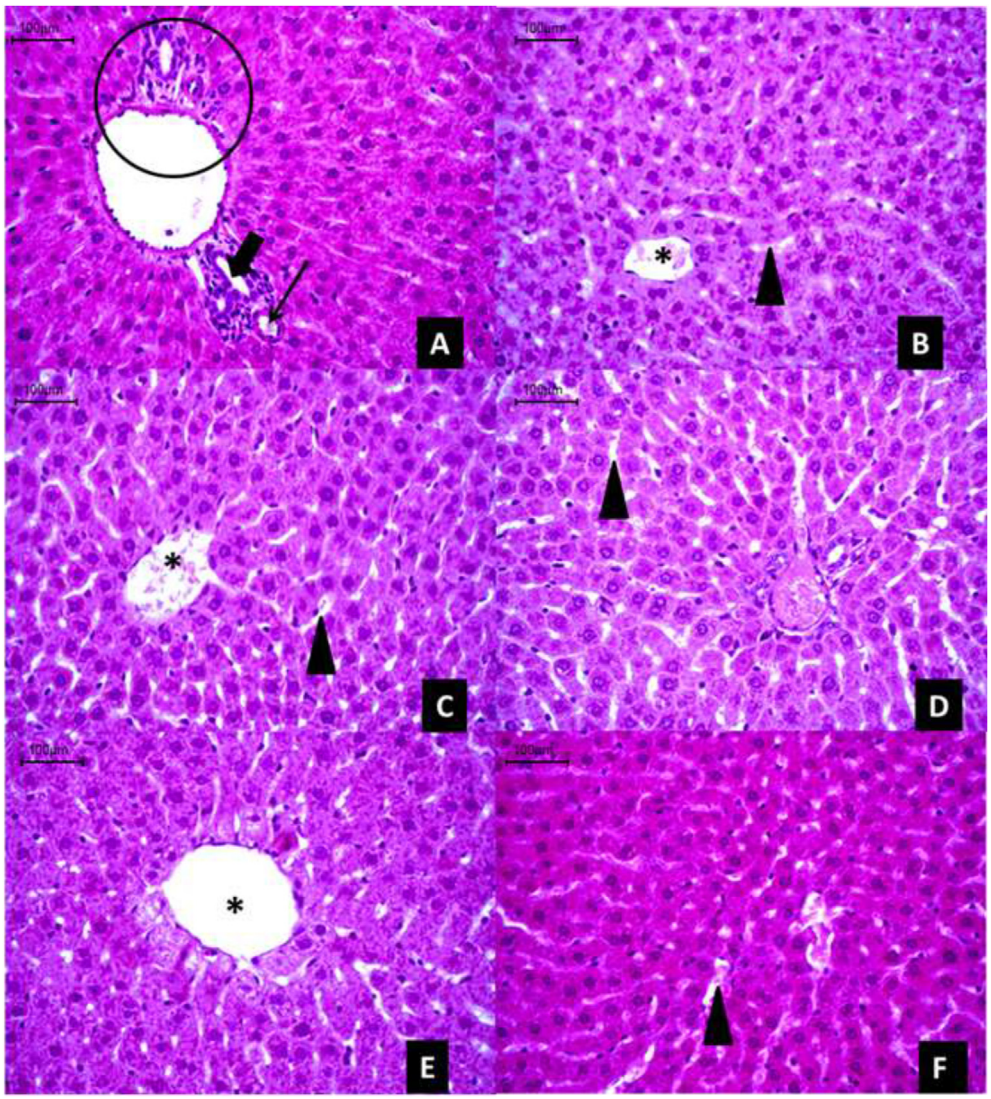

Figure 3. Histopathology of the liver from all experimental groups with histopathology preserved and healthy (H&E staining) [A-F] with increase of ±428X; A) Observe the periportal space delimited by the circle, bile duct (large arrow) and arteriole (thin arrow) of group I, control, without induction to OA; B) sinusoidal cords (tip of the arrow) intact and converging to the centrilobular vein (asterisk) from group I, control, without induction to OA; C) Group II, placebo, induced to OA and without drug treatments; D) Group III, induced to OA and treated with strontium ranelate; E) Group IV, induced to OA and treated with melatonin; F) Group V, induced to OA and treated with the association of the drugs.

were similar to the control group. It demonstrates the protective effect of the melatonin and the SR, and for the first time it was observed that such benefit is maintained when these drugs are associated.

Furthermore, it was demonstrated that the group submitted to OA and without pharmacological treatments reduced such measurements when compared to GI (control) and GIV (treated with melatonin), suggest superior pharmacological effect of the melatonin, in which is mentioned as a chondroprotector on experimental models of OA in rabbits, which was overserved the reduction of the extension and the severity of the degradation of the articular cartilage [24, 25].

In vivo experiments demonstrate that the intra-articular injection of melatonin relieved the progression of the induced OA in mice [26]. Also, the melatonin can revert the destruction of the cartilage by the inhibition of proinflammatory cytokines and the activation of chondrogenic marker genes [27].

These facts indicate that melatonin may be beneficial for the cartilaginous tissue, since with advancing age it occurs a reduction of the secretion of melatonin and concomitant increase of the prevalence of OA. Therefore, the administration of melatonin could prevent from the development of OA.

The treatment with melatonin reduced the levels of creatinine, suggesting renoprotective effect, as mentioned by other researches with melatonin [28, 29]. Furthermore, the melatonin operates as an antioxidant of broad spectrum for the elimination of free radicals [30, 31, 32].

The plasmatic data suggest that did not happen any hepatic injury in any of the groups, since there was no difference on the dosages of glycose and GPT. However, the levels of GOT in GIII and GV were increased and, in such cases, it emphasizes possible harmful effects of SR in the cardiac muscle tissue. Similar results were demonstrated previously, when the treatment with SR caused ischemic damages, alterations in the vascular smooth muscle and heart damages [33, 34, 35].

Despite of that, in similar experimental model of OA in which the SR was used, it was observed the significant reduction of lesions on the cartilage and subchondral bone, also the reduction on the expression of the genes related to the destruction of articular cartilage [21]. Preclinical studies *in vitro* indicate that the SR inhibits the resorption of subchondral bone and it stimulates the formation of cartilage matrix in normal and osteoarthritic chondrocytes [36, 37].

The increase of levels of cholesterol from group IV was observed, pertinent with the higher values of HDL identified at the same group, and this effect was maintained when associating the drugs. The remaining plasmatic dosages demonstrated no significant differences, in corroboration with the data from Atteritano *e col.* [38]. Regarding the effects of

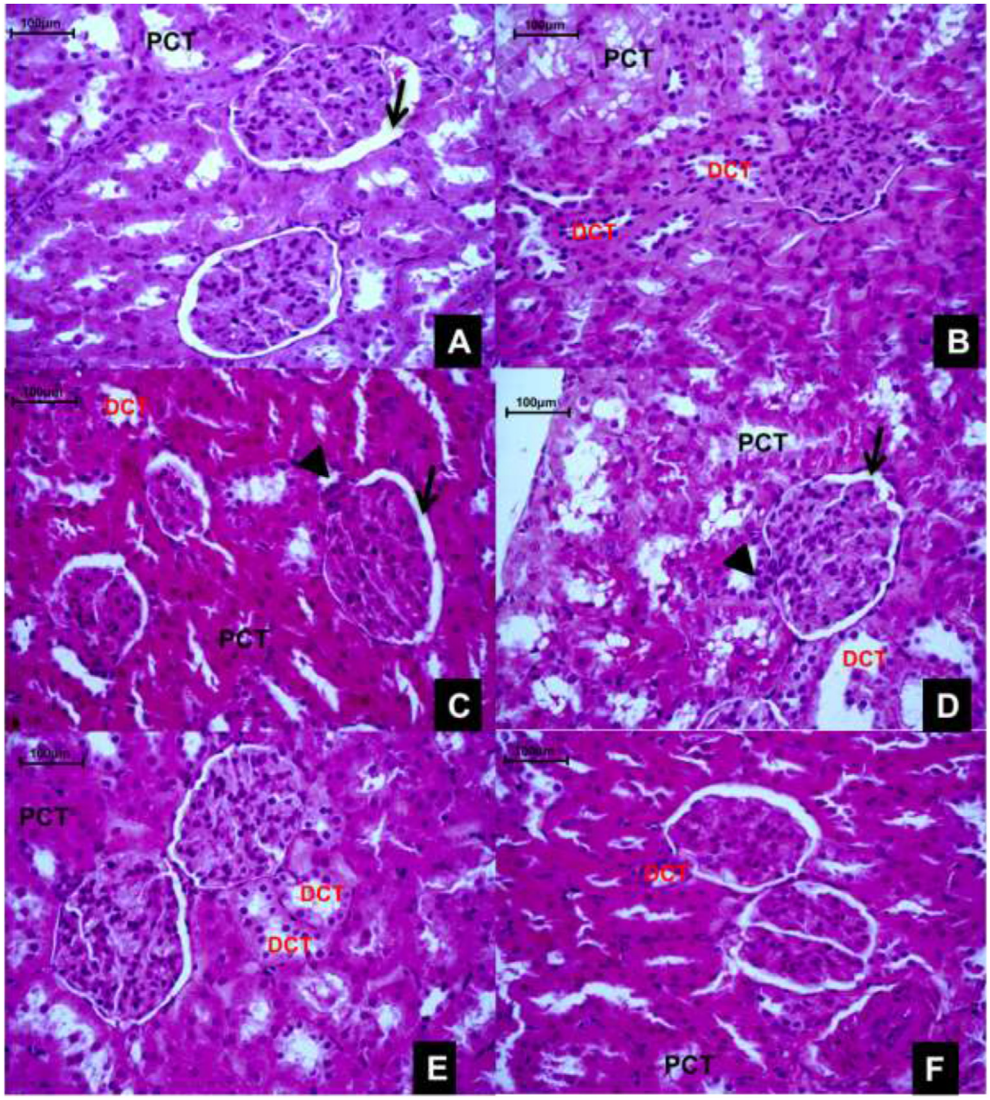

Figure 4. Histopathology of the kidney from all experimental groups with histopathology preserved and healthy (H&E staining) [A-F] with increase of ±428X; A) Observe the Bowman's space, intact renal corpuscles and glomeruli (arrow) from group I, control, without induction to OA; B) proximal contorted tubule (PCT) and distal contorted tubule (DCT) from group I, control, without induction to OA; C) Group II, placebo, induced to OA and without drug treatments; it was possible to identify excellent image from macula densa (tip of the arrow), Bowman's space well preserved, similar to GI, as well as the other experimental groups; D) Group III, induced to OA and treated with strontium ranelate; E) Group IV, induced to OA and treated with melatonin; F) Group V, induced to OA and treated with the association of the drugs.

melatonin, other studies were efficient for most of the rates of biochemical lipids and the increase of HDL [2, 39, 40].

Studies show that the tissue manifestations of OA are mediated by inflammatory factors, not being restricted to articular structures [41]. An cross-sectional study demonstrated that the majority of the patients presents associated comorbidities, including liver and kidneys problems [42]. The histopathological analysis demonstrated that the isolated use of SR or associated to melatonin was not capable to cause perturbation to the structure of liver and kidneys, in our study.

Due to its several properties and the virtual absence of toxicity, the melatonin has been utilized in a long term [43]. It was observed in the current study that its use in isolation or in association with the SR was not capable of modifying the cytoarchitecture of the hepatic and the renal tissues in experimental model of OA, which makes its utilization secure. The administration of melatonin presents several beneficial effects facing hepatic disorders, which are not limited to antioxidant effects [44].

5. Conclusions

There are solid indications that the association of melatonin and SR is a significant achievement, the administration of these drugs in association or not, presented chondroprotective effect and prevented from the aggravation of articular damages as hypothesized, in addition to not damaging other filtering tissues, such as liver and kidneys. However, it is necessary to be aware of possible cardiovascular damages in which may be caused by SR and, besides the benefits presented by the use of melatonin, additional studies are necessary to comprehend the roles of this hormone and of the circadian rhythms in OA. It is suggested that further researches are developed in order to standardize the utilization of the drugs proposed in this study facing the treatment of patients with OA.

Declarations

Author contribution statement

Kássya Mycaela Paulino Silva; Francisco Lucas de Sousa: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Ana Carolina Barreto Alves; Pollyana Alves Rocha; Hildegard Naara Alves Furtado da Costa; Waldilene Rodrigues Ferreira; Taianara Sampaio Reis; Tharcia Kiara Beserra de Oliveira; Sandra Rejane Cabral Batista; Clovis José Cavalcanti Lapa Neto; Anne Gabrielle Oliveira: Performed the experiments; Analyzed and interpreted the data.

Ana Janaina Jeanine M. de Lemos Jordão: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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