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# Osteoarthritis: Trends and clinical implications in the standardization of a pathological induction protocol in rats using a single dose of monosodium iodoacetate. Model of induction of osteoarthritis in rats for preclinical study

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# Abstract

**Purpose:** Osteoarthritis (OA) is a disease that causes progressive erosion of articular cartilage. This study aimed to induce OA in rats with a single dose of MIA and monitor the temporal evolution of pathological progression, associating the changes with perimetry, behavioral tests, radiographic and histological images.

**Methods**: Rats were induced with OA with a single dose of MIA, monitored to analyze the progression of joint damage and associated with variables of joint edema, motor behavior, radiographic and histological images. At the end of the experiment, the animals were euthanized for removal of the joints for histopathological analysis. Statistical analyses employed analysis of variance with post hoc tests, ensuring methodological rigor with blinded evaluators and stratified comparisons between groups.

**Results**: Radiographic data showed classic characteristics of osteoarthritis (OA), with the presence of osteophytes, narrowing of joint spaces, subchondral bone sclerosis, cartilage erosion, increased synovial volume, and medial patellar displacement. Similarly, histological data showed excavation, loss of the superficial matrix layer, loss of the intermediate zone, and loss of the tidemark. These data were temporally associated with assessments of joint edema and motor behavior, showing greater joint damage between the 7th and 14th days of induction.

**Conclusion**: This experimental study provided a comprehensive temporal description of the progression phases of osteoarthritis (OA) using the MIA-induced rat model, combining data on joint edema, behavioral testing, and radiological and histological assessments, demonstrating a peak in pathology progression between the 7th and 14th days of induction.

**Trial registration number:** The research adhered to the ARRIVE standards and complied with the U.K. Animals (Scientific Procedures) Act of 1986 and the relevant EU Directive 2010/63/EU for animal experimentation. All experimental evaluation protocols were approved and authorized by the Animal Use Ethics Committee (CEUA) of the Federal University of Maranhão (UFMA), process n$^{o}$. 23115.031386/2019-28. The experiments were conducted in accordance with the Brazilian College of Animal Experimentation (COBEA) guidelines for the use of animals in research.


# Summary statement

We induced osteoarthritis (OA) in rats with a single dose of MIA and monitored the temporal evolution of the pathological progression, associating the changes with perimetry, behavioral tests, radiographic and histological images.

# BACKGROUND

Osteoarthritis (OA) is a chronic progressive disease that causes joint degeneration and loss of the articular cartilage surface, leading to an inflammatory process and localized pain, thus compromising

joint integrity (Carvalho, Costa and Silva, 2028). OA is a disease with a high incidence, ranking among the most common causes of chronic pain worldwide. It has an enormous psychosocial impact, affecting not only the patients, but also their families and professional careers (Carvalho, Costa and Silva, 2028). Experimental induction models play a key role in the development of new intervention strategies for OA, providing valuable insights into the mechanisms underlying the disease. One such model is monosodium iodoacetate **(**MIA), first described by Kalbhen and Blum in 1977 and subsequently standardized as a model for joint arthrosis **(**Kalbhem and Blum, 1977). MIA is a reagent that modifies cysteine residues of proteins. When administered into the joints of animals, MIA causes a change in proteoglycan concentration in the articular cartilage, disrupting glycolysis and breaking down the metabolism of chondrocytes, thus resulting in joint degradation. Once administered intra-articularly into the knee and present in the synovial fluid, MIA is absorbed by chondrocytes together with specialized cells of the joint capsule, causing chronic degradation of the cartilage, apparently offering a more predictive model for the study of OA in humans **(**Kalbhem and Blum, 1977).

Therefore, the objective of this study was to induce OA with a single dose of MIA and monitor the temporal evolution of joint damage progression over 28 days in 4 experimental groups, associating changes in perimetry and behavioral tests with radiographic and histological images in a control group.

# MATERIALS AND METHODS

### Animals and ethical aspects

Five 8-week-old female Wistar rats (*Rattus norvegicus*) weighing 250-300 g, obtained from the UFMA Central Animal Facility, were used. The rats were fed standard chow and received water *ad libitum* throughout the experimental period. They were kept under controlled conditions of temperature (23 ± 1º C) and humidity (40-60%) and under a 12-hour light-dark cycle. At the end of each experimental group, euthanasia was performed by deep anesthesia via the intraperitoneal route at a dosage of 0.3 ml of Xylazine (80mg/kg) + 0.3 ml of Ketamine (10mg/kg).

### Ethics declaration

The research adhered to the ARRIVE standards and complied with the U.K. Animals (Scientific Procedures) Act of 1986 and the relevant EU Directive 2010/63/EU for animal experimentation. All experimental evaluation protocols were approved and authorized by the Animal Use Ethics Committee (CEUA) of the Federal University of Maranhão (UFMA), process n$^{o}$. 23115.031386/2019-28. The experiments were conducted in accordance with the Brazilian College of Animal Experimentation (COBEA) guidelines for the use of animals in research. Euthanasia was performed by deep anesthesia via intraperitoneal injection with a dosage of 0.3 ml of Xylazine (80 mL/kg) + 0.3 ml of Ketamine (10 mg/kg).

### OA induction by MIA

The rats were previously sedated with 10% ketamine hydrochloride® (90 mg/kg) and then anesthetized using 2% xylazine hydrochloride® (5 mg/kg) intraperitoneally. Once anesthesia was confirmed, the right knee of all rats was shaved for injection and disinfected with 70% alcohol. 2 mg of MIA (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 50 µL of sterile saline solution and injected into the joint cavity of the right knee through the infrapatellar ligament with a 29G needle to induce knee OA Silva, Andersen and Tufik, 2008).

**Experimental design**

The experimental protocol resulted from a pilot project for preliminary implementation, whose objective was to analyze the feasibility, methodology, and pre-experimental instruments. Therefore, the experimental design was a preclinical and controlled trial. Five rats were randomly allocated into 5 groups (n = 1/group):

• Control Group (without OA induction and monitored for 28 days)

• Induced Group 1 (Induced with OA and monitored for 3 days of induction)

• Induced Group 2 (Induced with OA and monitored for 7 days of induction)

• Induced Group 3 (Induced with OA and monitored for 14 days of induction)

• Induced Group 4 (Induced with OA and monitored for 21 days of induction)

After induction, each group underwent assessments for joint circumference measurements (Joint Edema) and gait tests (Behavioral), followed by radiographic examinations. After reaching their respective time points (3, 7, 14, 21, or 28 days), the animals were euthanized for histological analysis. The tibiofemoral joints were excised and fixed in 10% formaldehyde in phosphate-buffered saline (PBS) for slide preparation and subsequent analysis.

**Perimetry - Joint edema**

Joint edema was measured using a stainless steel universal digital caliper (MTX®) for measurements up to 150 mm, quantifying joint thickness resulting from the inflammatory response in MIA-induced OA. Following the experimental schedule, the animals were evaluated at 0, 3, 7, 14, 21, and 28 days after induction. Knee joint thickness was measured three times, with the animals under anesthesia during preparation for euthanasia.

**Behavioral Test - Motor activity assessment - forced walking (*Rotarod test*)**

The animals were placed on a Rotarod (IITC Life Science, CA, USA), a rotor-like device, at a speed of 4 to 40 rpm for 300s. Two preliminary training tests were carried out followed by two recorded evaluations. After induction, three evaluations were performed and the average latency was recorded. The animals rested for 15 min between assessments. The data were expressed as walk values and were coded by a

single examiner. Observational analysis was conducted using a 5-point numerical scale, where 1 indicated normal limb use; 2, slight limping; 3, severe limping; 4, intermittent disuse of the affected limb; and 5, complete disuse of the limb (Tonussi and Ferreira, 1992).

### Radiographic imaging

Radiographic images were acquired in anteroposterior and laterolateral planes using a portable digital X-ray machine equipped with an image capture sensor (Diox-602®, DigiMed Co., Ltd., South Korea). Radiographs were obtained at the end of each time point/group, i.e., on days 3, 7, 14, 21, and 28, immediately after euthanasia. All images were analyzed by a veterinary imaging specialist who was unaware of the experimental protocol. After evaluation, each knee was classified according to the Kellgren-Lawrence (KL) system for osteoarthritis (OA) (0 - normal, 1 - doubtful, 2 - minimal, 3 - moderate, and 4 - severe). The average score was then calculated for comparison (Keyes et al, 1992).

### Histological analysis

The animals were euthanized according to the schedule, i.e., at 3, 7, 14, 21, and 28 days after the completion of joint edema assessments, motor behavior, and radiographic examinations. Each group (n=1), after the induction period, was euthanized for joint removal. For histological analysis, the tibiofemoral joint was excised and fixed in 10% formaldehyde in PBS. The samples were decalcified by immersion in 20% ethylenediaminetetraacetic acid (EDTA) for 20 days. Subsequently, the samples were embedded in paraffin blocks, cut into 5 µm sections, and the organic cartilage matrices were stained with hematoxylin-eosin (H&E). The analysis was performed blindly by a veterinary histopathologist. The histopathological evaluation of the cartilage followed protocols adapted from the Osteoarthritis Research Society International (OARSI) guidelines (Pritzker, et al, 2006). The parameters correspond to the stages of osteoarthritis (OA), focusing on the extent, surface area, or volume of articular cartilage involved in the local OA. The degree of osteoarthritis was determined according to the depth of the pathology in the cartilage, with points assigned on a scale ranging from 0 (intact surface) to 6 (total cartilage loss and bone deformation).

### Statistical analysis

The data were analyzed using GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA). One-way ANOVA followed by Tukey's post-hoc test was applied for comparisons between multiple groups. Two-way ANOVA was used for variables involving two independent factors (Induced vs. Non-Induced). The normality of the data was assessed using the Shapiro-Wilk test. A p-value $< 0.05$ was considered statistically significant.

## RESULTS

### Perimetry - Joint edema

Joint edema assessment was performed using calipers, which evaluated the bone diameter measurements of the induced joint at the end of each group during the experimental protocol (**Figure 1A**). The Control Group obtained an average score of 6.07 ± 0.14 and served as a comparative basis between the groups to analyze the level of joint damage through local edema. Thus, we observed that Group 3, which was induced and monitored for 14 days, presented greater joint involvement on the last day of induction when compared to the Control Group, with statistically significant values (20.32 ± 3.62; *p = 0.001). Group 2, which was induced and monitored for 7 days, presented slight joint edema on the last day of induction when compared to the Control Group (16.02 ± 3.62), but without significant values, demonstrating a possible sudden increase in joint edema. The graphic trends indicated a slight increase in joint edema on the 7th day of induction; however, on the 14th day of induction, as presented by group 3, there was a sudden peak in joint edema caused by the inflammatory process, altering the morphology of the joint.

**Behavioral Test - Motor activity assessment - forced walking (*Rotarod test*)**

Motor behavior was assessed using the rotarod test at the end of each group throughout the experimental protocol (**Figure 1B**). In the Control Group, the animals presented preserved gait patterns (mean 5.0 ± 1.82). However, when comparing the Control Group with Group 2 (induced and monitored for 7 days), the latter showed a marked impairment in gait performance on the last day of induction, with statistically significant values (1.0 ± 0.47; *p=0.001), and Group 3 (induced and monitored for 14 days) also showed a marked impairment in gait from the 7th day of induction until the last day of induction, corresponding to the 14th day (mean 1.0 ± 0.62; *p=0.001). Group 4 showed a peak in marked motor impairment between the 7th and 14th day of induction, but on the last day of induction, showed a slight improvement in gait pattern compared to days 7 and 14 (mean 2.0 ± 1.56). The graphic trends indicated a possible peak in motor impairment between the 7th and 14th day of induction.

**Clinical course and pathophysiological changes in radiographic and histological images of MIA-induced OA**

Radiographic and histological examinations of the rats' knees were performed at the end of each time point for the experimental groups.

For quantitative classification using the Kellgren-Lawrence (KL) system for radiological images, grade 0 was observed for the Control Group, characterized by smooth and regular articular surfaces with normal opacity. Radiographs also showed a mineralized structure in the intra-articular space of the knee in the cranial and caudal portions of the meniscus, with the patella overlapping its respective trochlear groove, and without signs of inflammatory changes or open fissures, consistent with the animal's age. This pattern served for comparison with the other experimental groups (**Figure 2: 2A**). In groups 1 and 2 (3 and 7 days of induction, respectively), the joints presented a grade 3 score, with slight narrowing of the intra-articular space and slight sclerosis of the subchondral bone, demonstrating a slight progression of osteoarthritis (**Figure 2: 2B-2C**). In group 3 (14 days of induction), we observed clear characteristics of osteoarthritis reaching its peak, with extensive articular destruction (grade 4 score). Notable findings

included prominent marginal osteophytes, narrowing of intra-articular spaces, subchondral bone sclerosis, cartilage erosion, partial loss of the normal appearance of structures, increased synovial volume, and medial displacement of the patella (**Figure 2: 2D**). This pattern of osteoarthritis lesion was maintained in group 4 (**Figure 2: 2E**).

In the histological analysis, using the OARSI scale, structural integrity of the joint was observed (score 0), presenting a regular surface, preserved marking lines, and viable chondrocytes with the presence of nuclei (**Figure 2: 1A**). In group 2 (7 days of induction), the joint lesion showed marked pathological progression, classified as grade 4 according to the OARSI scale, in addition to erosion and excavation with loss of the superficial matrix layer and the intermediate zone, loss of the tidemark, and further progression in the two days following induction (**Figure 2: 1C**). This peak of joint lesion was maintained in the following groups (**Figure 2: 1D-1E**).

These combined radiographic and histological findings provide us with a broader dimension of the onset of osteoarthritis progression and suggest a possible peak of osteoarthritis between the 7th and 14th day of induction.

# DISCUSSION

Guzman et al, 2003, report that OA induction was first described by Kalbhen and Blum in 1977. In line with this, the results of this experimental OA induction study provide a temporal analysis of the progression of the pathology. The relationship between the variables analyzed, such as radiological and histological parameters, joint edema, and behavioral assessment, offers a clear and objective perspective, aligning with the existing literature and highlighting joint damage at various points in the experimental induction model. Thus, with the results of this pilot study, we were able to provide a descriptive analysis of the temporal evolution of joint damage, providing useful data for the combined assessment of structural and functional changes in OA.

In this way, one of the characteristics caused by OA is joint edema, due to joint degradation, with an initial and local inflammatory process occurring, resulting in a degenerative and painful process, common in the stages of disease progression. These events lead to excessive fluid accumulation in the joint (Minji, Dongyeon, and Junesun, 2023; Chu et al., 2010). Calipers are widely used instruments in clinical and experimental assessment, serving to accurately measure joints and bone structures (Chu et al., 2010). Thus, in the results of joint edema using calipers, a peak of joint edema was observed on the 14th day of induction ($p = 0.001$), suggesting greater joint damage promoted by the peak of the inflammatory process in advanced stages of OA. This is in agreement with Chu et al., 2010, who highlighted an increase in joint edema promoted by MIA around the 16th day of induction, reinforcing that induction promotes synovial inflammation based on chondrocyte hypercellularity, collapse of joint integrity, and blood vessels (Chu et al., 2010).

The motor behavior test was used to assess joint damage through the observed limping pattern. This behavioral pattern appears to be directly related to bone contact due to joint wear and tear and,

consequently, the mechanical activation of nociceptors, as well as their sensitization by cytokines derived from the necrotic process present, being useful in the direct and objective assessment of joint functionality. Thus, the tests evaluated confirmed that there was a peak in the behavioral gait pattern between the 7th (1.0 ± 0.47; *p = 0.001) and the 14th (mean 1.0 ± 0.62; *p = 0.001) day of induction. This replicates the degree of joint damage associated with OA, as observed by Silva et al, 2025, who highlighted a peak in the rotarod test between the 7th and 14th day of induction when compared to the healthy group.

Radiographic data, despite being from a pilot study with a small sample, demonstrate that a single dose of MIA administered to rats caused greater joint damage, consistent with osteoarthritis, between the 7th and 14th day of induction. The presence of prominent marginal osteophytes, narrowing of intra-articular spaces, subchondral bone sclerosis, cartilage erosion, partial loss of the normal appearance of the structures, increased synovial volume, and medial displacement of the patella observed on the 14th day of induction are compatible with those of Silva et al, 2025.

In the histological analysis, in our findings, we observed grade 4 erosion and excavation with loss of the superficial matrix layer and the intermediate zone, and loss of the tidal line. These results were consistent with those found in the study by Santiago et al, 2023.

The radiographic and histological findings, combined with assessments of joint edema and gait behavior, provide us with a broader dimension of the onset of osteoarthritis progression and suggest a possible peak of joint damage consistent with osteoarthritis between the 7th and 14th day of induction.

# CONCLUSION

This experimental study provided a comprehensive temporal description of the progression phases of osteoarthritis (OA) using the MIA-induced rat model, combined with validated perimetry, behavioral tests, radiological and histological assessments. As this was a pilot study with a small sample size, our study was able to present positive results, demonstrating a peak in joint damage between the 7th and 14th day of induction, as shown in the assessments performed. Thus, the practical applicability of these findings presents a temporal peak phase of joint damage. This integrated approach more closely reflects possible clinical scenarios in which structural and symptomatic outcomes should be considered together. The strengths of this study include a descriptive analysis of the temporal evolution of a single-dose MIA induction model, which may be valuable for future research evaluating the therapeutic efficacy of potential agents for the treatment of osteoarthritis.

For future research, better strategies for assessing joint damage can be implemented, with a larger sample size, placebo control groups, and more modern and advanced imaging and behavioral tests.

Finally, this study reinforces the value of the MIA-induced rat model as a relevant tool for experimental research in OA.

# Abbreviations

Hematoxylin-Eosin (H&E); Kellgren-Lawrence (KL); Monosodium Iodoacetate**,** MIA; Osteoarthritis, OA; Osteoarthritis Research Society International, OARSI.

# Declarations

**Availability of data and materials:** The data is in Excel documents and stored in a Google Drive affiliated with the Federal University of Maranhão and can be made available to anyone upon request for access.

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**Author's contributions** All authors were involved with the design of the study, interpretation of the data, critical review of the manuscript and approval of the final version for submission. **LÂMS, LSD and EM de S**: Conceptualization, Methodology, Software. **LÂMS, LSD and EM de S**: Data curation, Writing - Preparation of the original draft. **LÂM S and L S D** Visualization. **LÂMS,** (luis.angelo@ufma.br), **LSD** (laercio.dias@ufma.br) and **EM de S** (edmsousa@hotmail.com) take full responsibility for the integrity of the work from the beginning to the finished manuscript.

**Ethics declaration** The research adhered to the ARRIVE standards and complied with the U.K. Animals (Scientific Procedures) Act of 1986 and the relevant EU Directive 2010/63/EU for animal experimentation. All experimental evaluation protocols were approved and authorized by the Animal Use Ethics Committee (CEUA) of the Federal University of Maranhão (UFMA), process n$^{o}$. 23115.031386/2019-28. The experiments were conducted in accordance with the Brazilian College of Animal Experimentation (COBEA) guidelines for the use of animals in research. Euthanasia was performed by deep anesthesia via intraperitoneal injection with a dosage of 0.3 ml of Xylazine (80 mL/kg) + 0.3 ml of Ketamine (10 mg/kg).

**Data declaration** Data is available upon request.

**Conflicts of interest** The authors declare that there are no conflicts of interest in the collection, analysis or interpretation of the data; in the writing of the manuscript, or in the decision to publish the results.

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# Figures

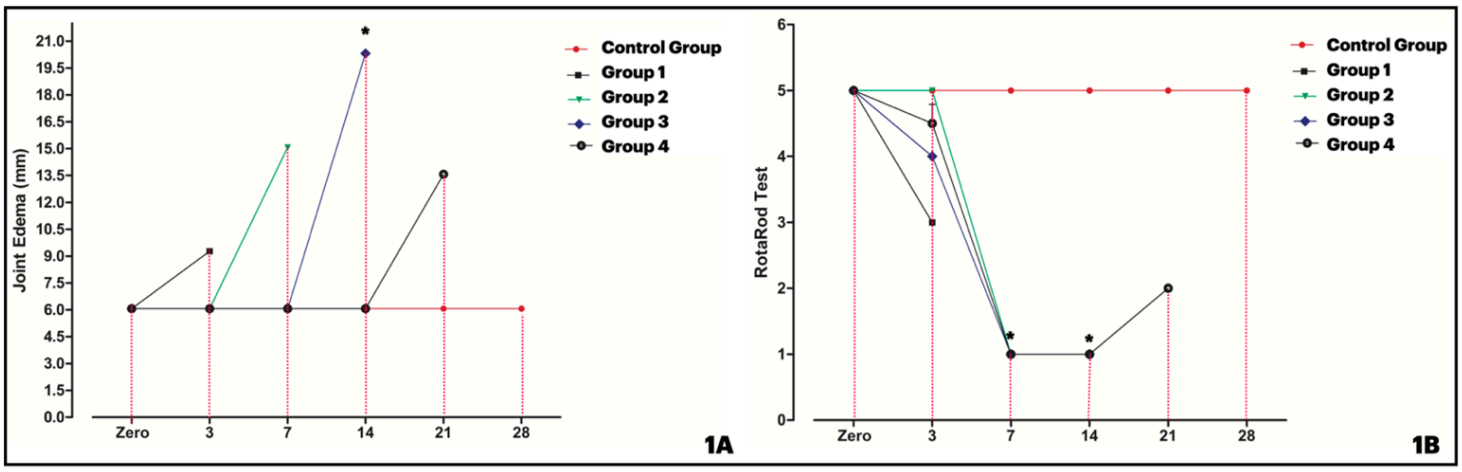



**Figure 1**

**A and B - Evaluation of joint edema and gait in rats subjected to the experimental osteoarthritis protocol induced by sodium monoiodacetate.**

**A:** Assessment of joint edema using a digital caliper in rats subjected to the OA induction protocol.

**B**: Behavioral assessment using the rotarod test in rats subjected to the OA induction protocol. The analysis was performed at the experimental time points of each group: 0 (pre-induction), 3, 7, 14, 21, and 28 days post-induction, respectively. Data are presented as mean values for each group. $p < 0.001$ – Comparison between animals induced and not induced to the experimental protocol.

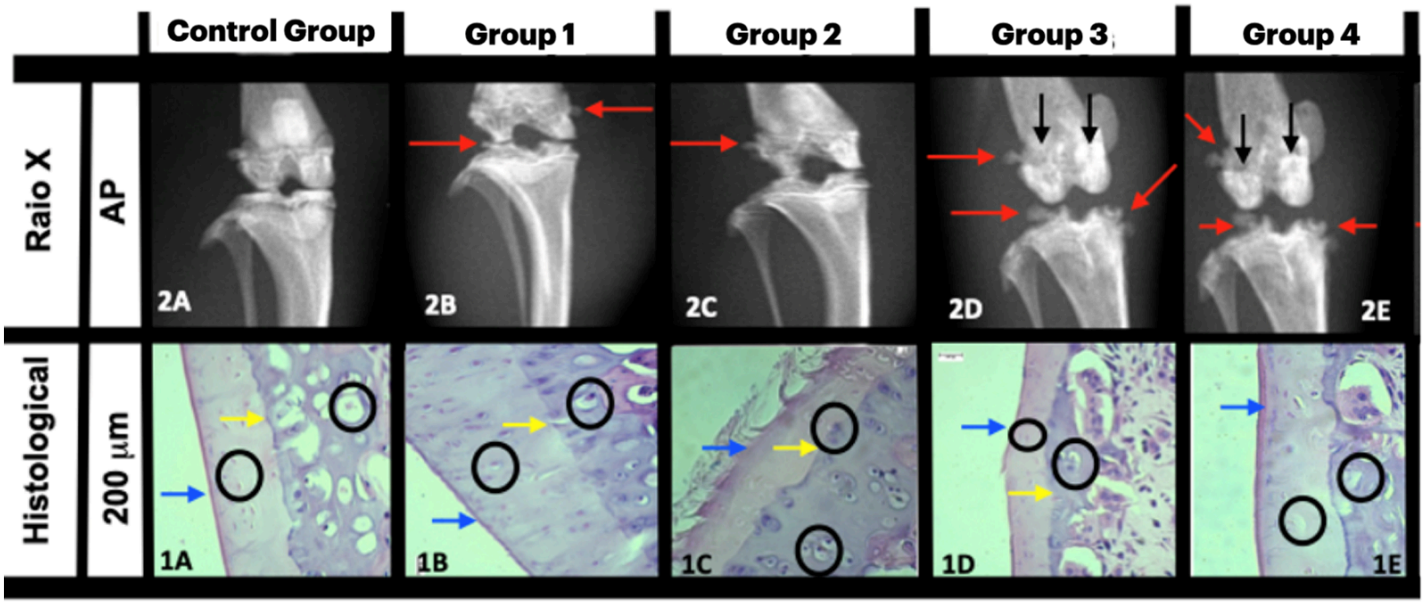



**Figure 2**

**Radiographic and histopathological changes in the articular cartilage of the right knee of *Rattus norvegicus* up to 28 days after MIA-induced OA.**

**Fig. 2** Anteroposterior radiographic images of the right tibiofemoral joint (**2A-2B-2C-2D-2E-**). Histological section of the medial portion of the knee joint at 4x magnification, stained with H&E (**1A-1B-1C-1D-1E**). Radiographic images: **Red Arrow** - Early marginal osteophyte formation (**2B-2C**); **Red Arrow** - Prominent marginal osteophytes (**2D-2E)**; **Black Arrow** - Subchondral bone sclerosis (**2D- 2E**). Histological images: **Blue arrow** - superficial zone of the cartilage. **Yellow arrow** - Mark line. **Black circles** - chondrocyte cells before and after the tidemark. Degenerative changes in osteoarthritis (OA). Radiographic images classified according to the KL method. Histopathological images classified in accordance with OARSI guidelines. Abbreviations: OA (Osteoarthritis); MIA (Monosodium iodoacetate); H&E, (hematoxylin-eosin), KL (Kellgren-Lawrence), (OARSI (Osteoarthritis Research Society International).

## Supplementary Files

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