

# A Comparative Study of CFA and MIA Induction Models in Rat Knee Arthritis

Rena Normasari<sup>1</sup>, Bambang Purwanto<sup>2,\*</sup>, Damayanti Tinduh<sup>3</sup>

Rena Normasari<sup>1</sup>, Bambang Purwanto<sup>2,\*</sup>, Damayanti Tinduh<sup>3</sup>

<sup>1</sup>Doctoral Program, Medical Faculty, Airlangga University, Surabaya, INDONESIA.

<sup>2</sup>Physiology Department, Medical Faculty, Airlangga University, Surabaya, INDONESIA.

<sup>3</sup>Physical Medicine and Rehabilitation Department, Medical Faculty, Airlangga University, Surabaya, INDONESIA.

## Correspondence

Bambang Purwanto

Bambang Purwanto

E-mail: bambang-purwanto@fk.unair.ac.id

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## ABSTRACT

This study presents another comparative review of knee joint arthritis induction in rats using two distinct methods: Complete Freund's Adjuvant (CFA) and monosodium iodoacetate (MIA). Different variables are assessed, including bodyweight changes, knee bend scores, and knee diameter measurements, as well as the quantification of interleukin-1 $\beta$  (IL-1 $\beta$ ) and C-telopeptide of type II collagen (CTX-II) levels. CFA or MIA induction was used on rats, and 14 days were observed. Our data show that the impact of arthritis induction varies significantly across the two models. Both the CFA and MIA groups showed different changes in terms of bodyweight changes, knee bend scores, and knee diameter variations. Furthermore, the levels of IL-1 $\beta$  and CTX-II, both known indicators of inflammation and cartilage degeneration, were measured. Notably, IL-1 $\beta$  levels in the CFA group were considerably higher than in the MIA-induced rats, although CTX-II concentrations showed a contrary pattern. These findings highlight the need to carefully consider the induction approach when performing arthritis investigations in rats since the model used has a major impact on the reported physiological alterations. This study's comparative analysis provides useful information for researchers looking to use rat knee joint arthritis models, laying the groundwork for a better-informed selection of the best induction strategy depending on desired outcome metrics.

**Key words:** Osteoarthritis, MIA, CFA, IL-1  $\beta$ , CTX-II.

## INTRODUCTION

Arthritis, a chronic inflammatory illness that affects joint integrity and function, continues to be a major healthcare concern across the world. Animal models have emerged as crucial research tools, with the aim of understanding their complicated genesis and devising effective treatment approaches. Rat models have received a lot of interest because of their physiological similarities to humans and their ability to manipulate genetic and environmental variables. The selection of an effective induction technique becomes critical as researchers strive to construct increasingly accurate and trustworthy models.<sup>1-3</sup>

Complete Freund's adjuvant (CFA) and monosodium iodoacetate (MIA) are two extensively used methods for causing knee joint arthritis in rats. These techniques were chosen for their ability to imitate important features of arthritis etiology, such as inflammation, cartilage deterioration, and pain. However, because of the different processes behind CFA and MIA induction, a comprehensive comparison is required to determine their relative impacts on arthritis development and severity.<sup>4-6</sup>

This report describes a complete comparative analysis of knee joint arthritis induction in rats using CFA and MIA. The evaluation includes a variety of metrics such as bodyweight changes, knee bend scores, and knee diameter measurements, which provide insight into the overall influence of each induction technique on the rats' physiological condition and joint function. The study also analyzes molecular aspects of arthritis by assessing levels of interleukin-1 $\beta$  (IL-1 $\beta$ ) and type II collagen C-telopeptide (CTX-II) in joint tissues. IL-1 $\beta$  is a potent inflammatory mediator in arthritic joints, and CTX-II is a validated marker

for the degradation of type II collagen, a significant component of articular cartilage.

This study attempts to comprehend the subtle variations in illness presentation and development between the two models by evaluating the responses of rats treated with CFA and MIA induction. Such insights are critical for researchers and clinicians who want to choose the best arthritis induction strategy based on their individual research aims and treatment goals. Finally, this study adds to our knowledge of arthritis pathogenesis and the optimization of animal models for preclinical research, which has implications for developing arthritis treatment methods and improving patient outcomes.

## MATERIALS AND METHODS

Eighteen adult male rats weighing 178.28  $\pm$  2.97 grams were used in this study. Rats were placed in cages with mineral water and food ad libitum and acclimatized for 2 weeks before the study. The animals induced osteoarthritis and rheumatoid arthritis respectively using MIA 3 mg in 50  $\mu$ l saline and 100  $\mu$ l CFA into the left knee joint. The rats were weighed and the diameter of the left knee joint was measured on days 1, 3, 5, 7, 11, and 14 using a calibrated digital caliper. The knee-bend test was done on days 1, 3, 5, 7, 11, and 14 to evaluate the movement-induced pain caused by CFA and MIA (Table 1). On day 14, synovial fluid was collected by infusing 100  $\mu$ l of saline intra-articularly, and then the fluid was aspirated. The liquid was immediately centrifuged the supernatant was taken and the levels of IL-1 $\beta$  and CTX-II were measured using ELISA. The study protocol was approved by the Faculty of Medicine's ethics committee at Jember University in Indonesia.

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# Statistical analysis

Values are expressed as the mean  $\pm$  SD. The bodyweight, knee diameter, knee-bend score, IL-1 $\beta$ , and CTX-II, levels were tabulated and analyzed statistically by one-way ANOVA, followed by a student t-test to compare variables between different groups. p values of less than 0.05 were considered significant.

# RESULTS AND DISCUSSION

Complete Freund's Adjuvant (CFA) and Monosodium Iodoacetate (MIA) were used in the current investigation to examine two alternative approaches for producing knee joint arthritis in rats. The study aimed to shed light on the differential effects of these induction techniques on the progression and severity of arthritis by analyzing multiple parameters such as bodyweight changes, knee bend scores, knee diameter measurements, and quantification of interleukin-1 $\beta$  (IL-1 $\beta$ ) and C-telopeptide of type II collagen (CTX-II) levels. The findings shed light on the feasibility and implications of each technique for arthritis research.

## Rat bodyweight

The rat bodyweight in the control group increased by about 27 grams during the fourteen-day study. The CFA and MIA groups had a similar decrease in fourteen days ( $p = 0.5041$ ) (Figure 1A).

**Table 1. Knee-bend score, an indication of the animal's movement-induced nociception. The sum of the animal's reactions gives a maximal value of 20.<sup>7</sup>**

0	no responses
0.5	struggle to maximal flexion/extension
1	struggle to moderate flexion/extension or vocalizations to maximal flexion/extension
2	vocalizations to moderate flexion/extensions

## Knee diameter

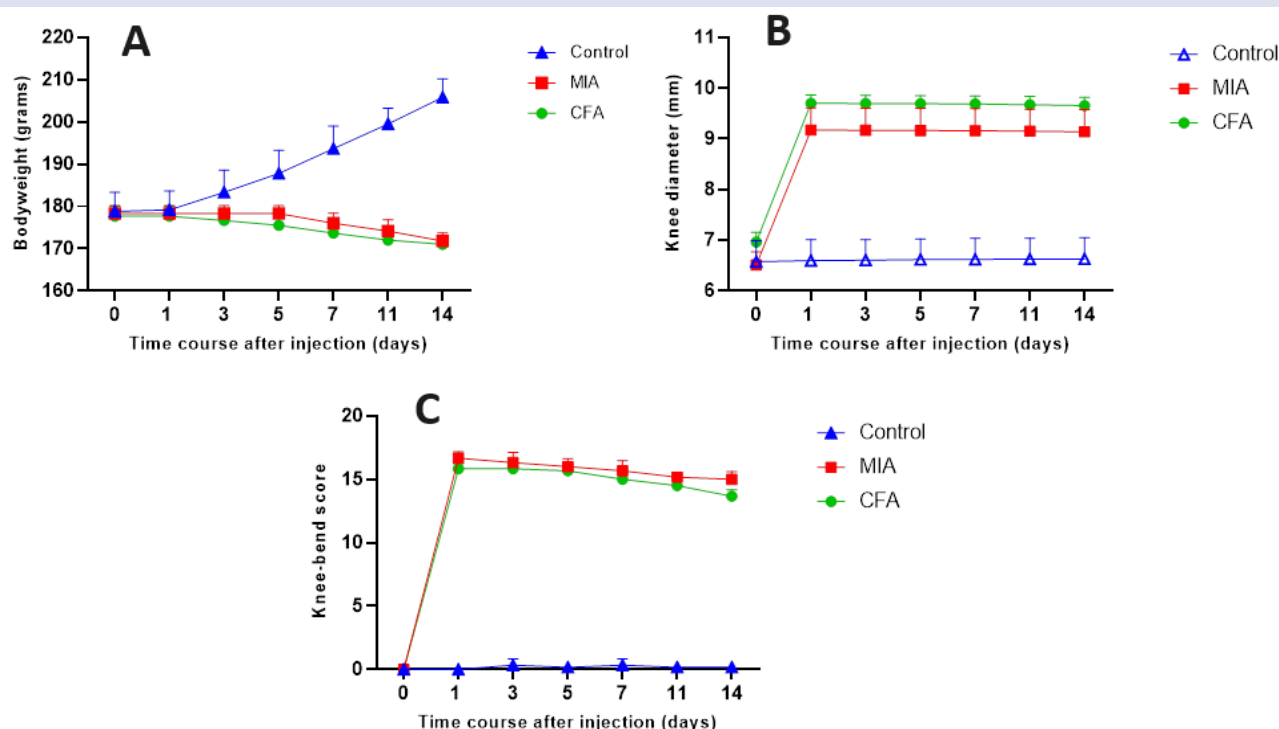
There was a significant increase in left knee joint diameter in the MIA and CFA groups compared to the control group ( $p < 0.05$ ). Left knee joint diameter was measured on days 0, 3, 5, 7, 11, and 14 post-injection. The MIA and CFA groups had a similar increase in left knee joint diameter ( $p = 0.3616$ ) (Figure 1B).

## Knee-bends score

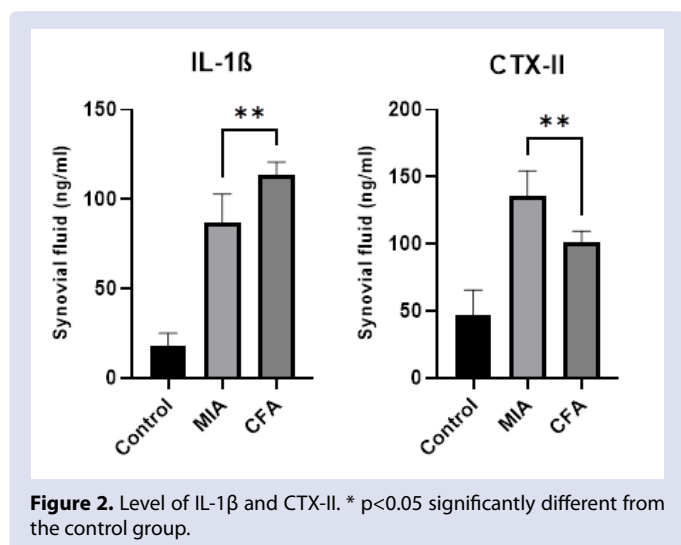
No signs of spontaneous nociceptive behavior were found prior to injection. Meanwhile, there was a significant increase in knee-bend scores in the MIA and CFA groups compared to the control group ( $p < 0.05$ ). There was no significant difference between the MIA and CFA groups, though the MIA group had a slightly higher knee-bend score than the CFA group ( $p < 0.05$ ) (Figure 1C).

The changes in bodyweight noticed in both the CFA and MIA groups indicate the systemic impact of arthritis induction on the animals' overall health. The CFA group's consistent loss of bodyweight might be attributed to CFA's chronic immunological reaction, which could potentially alter the animals' metabolic rate. On the other hand, the sudden and severe loss of bodyweight in the MIA group is most likely due to the acute and localized inflammation caused by cartilage injury. The different trajectories of bodyweight changes imply that while organizing experiments and evaluating findings, researchers should consider the temporal dynamics of these models. Similarly, knee bend scores indicated joint function and discomfort, emphasizing the possible differences in pain sensations between the two techniques, with the MIA group demonstrating more limits in joint movement. These data imply that MIA induction may result in more severe joint dysfunction, which is consistent with its method of causing localized cartilage destruction.<sup>8-11</sup>

Increased knee diameter in both induction groups indicates joint inflammation and edema. The magnitude of knee diameter alterations



**Figure 1.** Time course of (A) bodyweight increase, (B) knee joint diameter, (C) ipsilateral knee-bend score in control group rats, before and after injection of MIA and CFA into the left knee joint.



differed between the CFA and MIA groups, highlighting the different processes of both induction approaches. The broader immune response elicited by CFA may result in more extensive joint inflammation, whereas MIA induction, due to its direct impact on cartilage, may result in a more localized yet severe inflammatory response. This information is useful for researchers who want to understand the inflammatory reactions linked to each approach.<sup>12-14</sup>

### Biochemical markers

IL-1 levels in the MIA group were significantly higher than in the control group ( $87.18 \pm 15.8$  ng/mL,  $18.35 \pm 6.79$  ng/mL, respectively,  $p < 0.05$ ). IL-1 was observed to be higher in the CFA group compared to the control group ( $113.75 \pm 7.03$  ng/mL,  $p < 0.05$ ). The CFA group's IL-1 levels were also found to be significantly higher than those of the MIA groups ( $p < 0.05$ ) (Figure 2).

CTX-II levels were considerably higher in the MIA group compared to the control group ( $135.69 \pm 18.89$  ng/mL,  $46.94 \pm 18.83$  ng/mL respectively,  $p < 0.05$ ). CTX-II levels were found to be higher in the CFA group than in the control group ( $100.96 \pm 8.74$  ng/mL,  $p < 0.05$ ). CTX-II levels in the MIA group were similarly observed to be significantly higher than those in the CFA groups ( $p = 0.0037$ ) (Figure 2).

The analysis of IL-1β and CTX-II levels presented molecular insights into the processes behind the observed physiological changes. Elevated IL-1β levels in both induction groups demonstrated the inflammatory nature of arthritis. The substantially higher levels of IL-1β in the CFA group imply a possibly more severe inflammatory response, most likely as a result of CFA's direct cartilage injury. On the other hand, greater CTX-II concentrations in the MIA group may imply greater cartilage disintegration. These discrepancies in molecular markers correspond to the processes of CFA and MIA induction and contribute to a better understanding of their varied impacts on joint health.<sup>15-20</sup>

The measurement of IL-1β and CTX-II levels revealed important information on the molecular pathways underlying arthritis induction.<sup>21,22</sup> Elevated IL-1β levels in both induction groups highlight the disease's inflammatory character. Surprisingly, the CFA group had much higher IL-1β levels than the MIA group.<sup>23,24</sup> This study supports the idea that inducing MIA by directly injuring cartilage might result in a powerful inflammatory response defined by increased IL-1β production<sup>25</sup>. CTX-II levels, on the other hand, showed opposing tendencies. While both induction procedures resulted in greater CTX-II levels than controls, the MIA group had higher levels. This finding implies that MIA-induced arthritis may result in more widespread

cartilage deterioration<sup>26</sup>, possibly as a result of a larger immune response impacting joint tissues.<sup>13,27</sup>

The observed variations in bodyweight changes, knee bend scores, knee diameter measures, and molecular markers highlight the necessity of selecting the best arthritis induction approach for the research aims. Researchers interested in researching systemic immune responses, inflammation, and joint-related consequences may choose the CFA model, whereas those interested in studying localized cartilage damage and molecular mechanisms associated with cartilage degradation may prefer the MIA model. These findings highlight the significance of matching study aims to the strengths and limits of each approach.

It is essential to recognize that this study has limitations. The response of the animal model may differ depending on parameters such as strain, age, dose, and CFA and MIA delivery procedures. Furthermore, the duration and endpoint of the research may have an impact on the observed outcomes. Future research might look into these aspects to gain a better understanding of the model's dynamics and implications.

## CONCLUSION

This comparison analysis of CFA and MIA induction approaches provides a thorough evaluation of rat knee joint arthritis models. The various profiles of bodyweight changes, knee bend scores, knee diameter measures, IL-1β, and CTX-II levels show each model's distinctive features. Researchers may use these insights to make educated judgments on the best induction approach for their study aims. Researchers can improve the relevance and translatability of data by improving the choice of induction method, enhancing our understanding of arthritis pathophysiology, and developing viable therapy methods.

## DISCLOSURE STATEMENT

The authors have no conflicts of interest regarding this study.

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## AUTHORS' CONTRIBUTIONS

All authors participated in data analysis, article preparation, and paper revision and agreed to accept responsibility for all parts of this study.

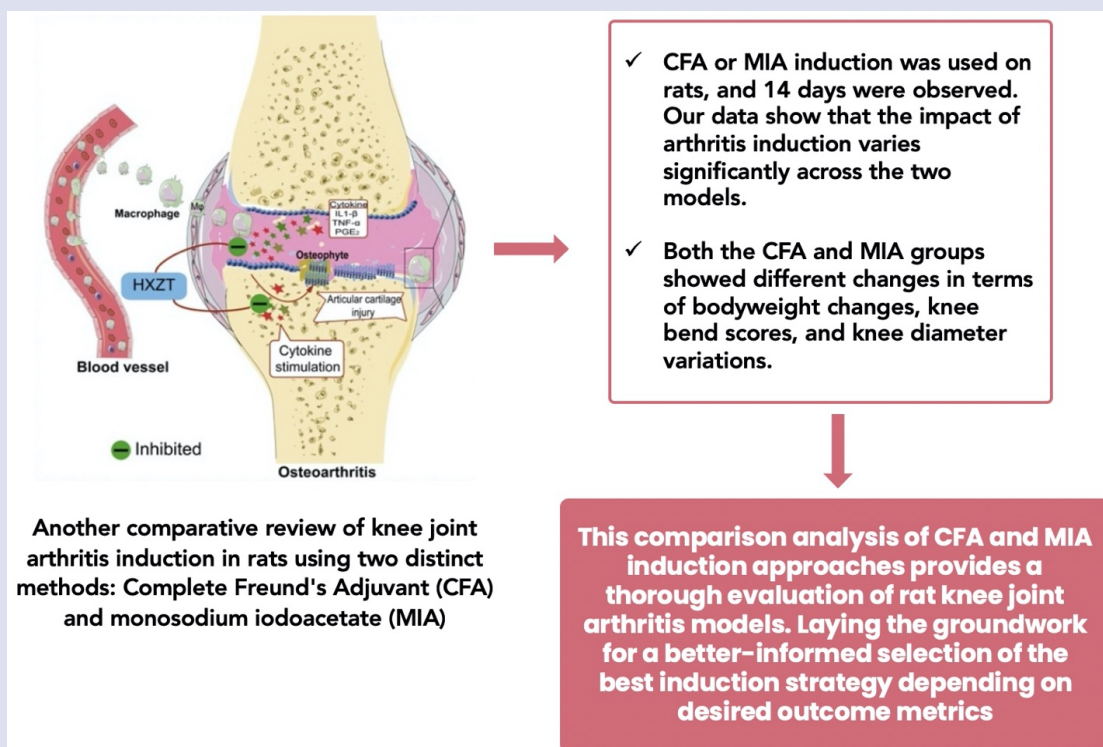
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## GRAPHICAL ABSTRACT



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