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Development of collagen-induced arthritis in male Sprague-Dawley rats using type ii chicken collagen and complete Freund’s adjuvant

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PHA413: Project & Dissertation

DEVELOPMENT OF COLLAGEN-INDUCED ARTHRITIS IN MALE SPRAGUE-DAWLEY RATS USING TYPE II CHICKEN COLLAGEN AND COMPLETE FREUND'S ADJUVANT

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Contents

Abstract....4
Chapter 1: Introduction....6
Chapter 2: Literature Review....9
2.1 Experimental Models of Rheumatoid Arthritis....9
2.2 Collagen-Induced Arthritis....10
2.3 Type II Collagen....10
2.4 Complete Freund's Adjuvant....11
2.5 Sprague-Dawley Rats....11
2.6 Assessment of Arthritis....12
Chapter 3: Aim and Objective....14
3.1 Aim....14
3.2 Objectives....14
Chapter 4: Reagent and Experimental Details....15
4.1 Experimental Animals....15
4.2 Chemicals and Reagents....15
4.3 Equipment....15
Chapter 5: Methodology....17
5.1 Experimental Design....17
5.2 Arthritis Induction....17
5.3 Observation Protocol....17
5.4 Paw Edema Measurement....17
5.5 Arthritis Scoring....18
5.6 Movement Assessment....18
5.7 Radiographic Examination....18
5.8 Blood Collection and Terminal Procedure....18

5.9 IL-6 and CRP Assay 20
5.10 Tissue Collection for Histopathology 20
Chapter 6: Method Development 22
6.1 Preparation of the Primary Collagen-CFA Emulsion 22
6.2 Booster Dose Calculation 22
Chapter 7: Results 23
7.1 Clinical Observations 23
7.2 Paw Edema (Plethysmometer Readings) 23
7.3 Arthritis Score 24
7.4 Movement Assessment 24
7.5 Radiographic Findings 24
7.6 IL-6 and CRP Results 26
7.7 Histopathology 26
7.8 Summary of Results 27
Chapter 8: Discussion 28
8.1 Limitations 30
Chapter 9: Conclusion 31
Chapter 10: Acknowledgment 32
Chapter 11: References 33

Abstract

Rheumatoid arthritis (RA) is a long-term autoimmune disease marked by ongoing inflammation of the synovium, joint swelling, cartilage breakdown, and bone erosion. Because the disease is difficult to study directly in humans, I turned to an animal model to understand its mechanisms and to test a candidate treatment. Collagen-induced arthritis (CIA) is one of the most widely used models for this purpose. Injecting type II collagen together with Freund's adjuvant reliably produces joint changes that mirror several hallmarks of human RA.

In this study, I set out to establish an experimental arthritis model in male Sprague-Dawley rats using chicken type II collagen combined with Complete Freund's Adjuvant (CFA). Eighteen rats weighing between roughly 187 and 247 g were split into five groups: a negative control (n = 4), a positive/arthritis control (n = 4), a standard-treatment group (n = 4), and two treatment-dose groups (n = 3 each). Chicken type II collagen (Sigma-Aldrich, Lot No. 0000536334) was dissolved in acetic acid and emulsified with CFA(Sigma-Aldrich,Lot No.000535580) I carried out the first induction on 16 July 2026 and then by a booster on 4 August 2026. I then tracked the animals for redness, swelling, weakness, reduced movement, paw edema, and an overall arthritis score. Paw volume was measured with a UGO Basile plethysmometer, radiographs were taken to check for structural joint damage, and blood was drawn to assess two inflammatory markers, IL-6 and CRP.

Redness and swelling occured in the arthritis-induced animals but not in the negative control group, and affected rats moved noticeably less than the negative control group. The positive-control group averaged about 6 movements per minute against roughly 14/min in the dose-2 group and 21/min in the negative control. Edema reached approximately 65.7%, and the highest arthritis score I recorded was 16. Radiographs results revealed visible bone erosion and structural joint changes. Laboratory analysis of three serum samples found IL-6 below 1.5 pg/mL and CRP below 6.00 mg/L in every sample tested.

Taken together, these findings show that the induction protocol successfully produced an inflammatory arthritis phenotype in this rat cohort, giving me a usable model for evaluating the anti-arthritic activity of the treatment under investigation.

Keywords: rheumatoid arthritis, collagen-induced arthritis, type II collagen, Complete Freund's Adjuvant, Sprague-Dawley rats, paw edema, arthritis score, bone erosion.

Chapter 1: Introduction

Rheumatoid arthritis is a chronic, systemic autoimmune disease that targets the joints but is not limited to them. The defining feature of it is persistent inflammation of the synovial membrane, which brings swelling and pain and, over time, progressive destruction of cartilage and bone. Inflammatory activity may spread beyond the joints to other tissues, so Rheumatoid Arthritis is best understood as a systemic disorder rather than a purely local one.

Rheumatoid Arthritis is not a rare condition. Population studies generally put its prevalence at somewhere around 0.5 to 1 percent of adults worldwide, with women affected roughly two to three times as often as men, and onset most commonly falling between the ages of thirty and sixty. Due to its chronic and progressive nature rather than being self-limiting, the disease's burden builds up over time, and poorly managed cases are a major reason for work disability during the most economically productive years of an individual. The high prevalence, chronic nature, and potential for disability are reasons why I opted to focus on an animal model of the disease instead of a solely theoretical project.

A combination of genetic predisposition, environmental factors, and an erroneous immune response fuels the progression of the disease. Both innate and adaptive immune responses are involved: T cells, B cells, macrophages, neutrophils, and fibroblast-like synoviocytes all participate in the inflammatory processes occurring in the joint.

Under normal conditions the synovium is a thin lining around the joint cavity. In RA, it thickens and fills with inflammatory cells, eventually forming an abnormal tissue mass known as pannus. Left unchecked, pannus invades and erodes the surrounding cartilage and bone.

Multiple cytokines play a role in this damage. TNF-α, IL-1β, and IL-6 are among the most important. They activate synovial cells, draw more inflammatory cells to the joint, and accelerate the degeneration of cartilage and bone. IL-6 is particularly important because its effects reach beyond the joint: it encourages the liver to produce acute-phase

proteins such as CRP, which is why IL-6 and CRP are often used as laboratory markers of inflammatory activity.

Cartilage itself is rich in type II collagen, the protein that gives it structural strength and flexibility. Chronic inflammation drives up production of enzymes such as matrix metalloproteinases, which break down this collagen matrix over time. Bone erosion follows a related path, since sustained inflammatory signaling activates osteoclasts, and the resulting bone loss around the joint can eventually cause deformity and permanent loss of function.

Given how complicated the human disease is, researchers turn to animal models to work out its mechanisms and to test possible treatments. Several such models exist, including collagen-induced arthritis, adjuvant-induced arthritis, and antigen-induced arthritis, each capturing a different piece of the disease.

Collagen-induced arthritis is probably the most widely used of these. It grew out of early work showing that immunizing susceptible rats with type II collagen and Freund's adjuvant could reliably trigger inflammatory arthritis [1]. Later studies confirmed that type II collagen drives both antibody-mediated and cell-mediated immune responses tied to arthritis development [2–4]. How severe the disease becomes, and how many animals develop it, depends on a range of factors: the rat strain used, where the collagen comes from, its concentration, the adjuvant, the route of injection, and the immunization schedule.

CFA is typically added to boost the immune response to the collagen antigen. It contains mycobacterial components that strongly activate the immune system, so pairing it with collagen produces a robust inflammatory reaction in susceptible animals. It is worth noting, though, that collagen-induced and adjuvant-induced arthritis are not mechanistically identical, even though both involve inflammation. Comparative studies have shown real differences in how the two models develop [5,6].

The selection of strain is also important. Sprague-Dawley rats are important in pharmacological studies, yet their vulnerability to experimental arthritis varies among

strains. Previous research on induction in Sprague-Dawley rats has demonstrated that experimental conditions can significantly alter both incidence and severity [7,8].

With that background in mind, I set out in this project to establish experimental inflammatory arthritis in male Sprague-Dawley rats using chicken type II collagen combined with CFA, and to characterize the resulting model through clinical observation, movement, paw edema, arthritis scoring, radiography, and inflammatory biomarkers.

Chapter 2: Literature Review

2.1 Experimental Models of Rheumatoid Arthritis

A single animal model cannot have every feature of human Rheumatoid Arthritis, but different models can imitate different pieces of it, which is why animal work remains central to study inflammatory joint disease. The mostly used models include the following:

- Collagen-induced arthritis
- Adjuvant-induced arthritis
- Antigen-induced arthritis
- Pristane-induced arthritis
- Spontaneous autoimmune arthritis models

Of these, collagen-induced arthritis is especially well suited to studying autoimmune responses directed against type II collagen specifically.

Beyond CIA, adjuvant-induced arthritis is produced simply by injecting CFA alone, usually at the base of the tail, and tends to run a more acute, mono- or oligo-articular course driven largely by innate immune activation rather than by a defined autoantigen. Antigen-induced arthritis instead sensitizes an animal systemically to a protein such as methylated bovine serum albumin and then challenges the joint directly, producing a localized, reproducible synovitis useful for testing local anti-inflammatory strategies. Pristane-induced arthritis, seen mainly in certain rat strains, follows a single injection of the hydrocarbon oil pristane and develops more slowly, giving a chronic, relapsing course that some investigators find closer to the waxing-and-waning pattern seen in human RA. Spontaneous models, such as the K/BxN or hTNF-transgenic mouse lines, remove the need for external immunization altogether since the animals develop joint disease on their own as a consequence of a defined genetic lesion, though they are used far less often in rat-based pharmacology work. Choosing these models among themselves is ultimately a trade-off between how closely the induced pathology resembles human Rheumatoid Arthritis, how reproducible the onset and severity are, and how practical the protocol is to

run at the scale a pharmacology study needs. That trade-off is part of why CIA remains the default choice for screening candidate anti-arthritic compounds.

2.2 Collagen-Induced Arthritis

The model originates from Trentham et al., who initially demonstrated that type II collagen could induce experimental arthritis in vulnerable animals [1]. Morgan et al. continued this research by examining native type II collagen-induced arthritis in rats and associating the progression of the disease with the humoral immune response targeting collagen [2]. Later research expanded on this by demonstrating that cell-mediated immunity to type II collagen also plays a role in the disease [3]. Collectively, these results confirmed CIA as a truly immune-mediated model instead of merely a response to mechanical damage.

Mechanistically, the disease follows a fairly predictable timeline in susceptible rats. An initial sensitization phase after the primary immunization is typically followed by a clinically silent interval of one to two weeks, and then a fairly abrupt onset of joint swelling once anti-collagen antibodies reach a sufficient titre and begin depositing as immune complexes within the joint. The activation of complement and engagement of Fc-receptors by these deposited complexes attract neutrophils and macrophages into the synovium, and it is this cellular influx, rather than the antibody response alone, that causes the observable redness, swelling, and tenderness used for clinical scoring of the animals.

Susceptibility to this cascade is strongly influenced by the major histocompatibility complex haplotype an animal carries, which is why not every strain, and not even every individual within a susceptible strain, develops disease of the same severity after an identical immunization protocol.

2.3 Type II Collagen

Type II collagen makes up much of articular cartilage's structure, so an immune response directed at it naturally tends to land on joint tissue. Chicken type II collagen in particular has a track record in this kind of work; several studies have used it successfully in rats to study arthritis development and to screen anti-arthritic treatments.

The immunodominant epitopes recognized during CIA sit within the triple-helical region of the collagen molecule, and because this region is relatively conserved across species, collagen purified from one species can still trigger a cross-reactive response against an animal's own cartilage collagen even though the injected protein is foreign. Collagen sourced from different species and tissues, whether bovine, porcine, or chicken, differs somewhat in how arthritogenic it is and in how consistently it induces disease. The chicken-derived preparations used in rat studies have generally been reported to give a robust and fairly reproducible incidence when paired with an appropriate adjuvant.

2.4 Complete Freund's Adjuvant

CFA is used to amplify the immune response to whatever antigen it is paired with. It is an oil-based emulsion containing killed mycobacterial components, and on its own it can produce arthritis-like inflammation even without collagen. A collagen-CFA protocol is therefore really combining two things at once: an antigen-specific response to collagen and a strong, nonspecific adjuvant effect.

This adjuvant effect comes largely from the mycobacterial cell-wall components in CFA, which are recognized by pattern-recognition receptors such as TLR2 and TLR4 on innate immune cells and drive a strongly Th1-skewed response, the kind of response known to favor both delayed-type hypersensitivity and the pathogenic antibody responses seen in CIA. Because the whole-mycobacteria formulation used in CFA can cause substantial local granuloma formation and discomfort, it is normally reserved for the priming dose. The milder Incomplete Freund's Adjuvant, essentially the same oil-based emulsion without the mycobacterial component, is used for any booster, where a second, gentler stimulus is all that is needed to sustain the response.

2.5 Sprague-Dawley Rats

Sprague-Dawley rats are popular in pharmacology because they are readily available, grow predictably, and tolerate repeated clinical and laboratory measurements well. That said, arthritis does not develop the same way in every strain. Comparisons between Sprague-Dawley and Wistar rats have turned up real differences in susceptibility to

collagen-induced arthritis [7,8], which is exactly why protocols developed specifically for Sprague-Dawley rats matter.

Gender is another factor that merits attention alongside strain. In various rodent CIA protocols, males exhibit a slightly different susceptibility pattern compared to females, which is one reason studies often limit the group to just one sex, as I have done here. Age and body weight during immunization are important factors, as younger, growing animals may react differently to the adjuvant load compared to older, weight-stable ones. Maintaining a narrow initial weight range, such as the 187–247 g range applied in this research, aids in reducing that variability source among animals.

2.6 Assessment of Arthritis

Various supplementary techniques are used to monitor the advancement of experimental arthritis.

Clinical evaluation: indicators usually observed include redness, swelling, paw swelling, joint inflammation, limited movement, and overall weakness.

Paw-volume measurement: a plethysmometer gives an objective read on paw volume, with larger volumes indicating more inflammatory edema.

Arthritis score: animals can be assigned a numerical score reflecting how severe and how widespread the joint involvement is.

Radiography: X-rays pick up structural changes such as bone erosion, altered joint spacing, and other skeletal abnormalities.

Biochemical assessment: markers such as IL-6, TNF-α, IL-1β, and CRP add a systemic-inflammation dimension to the clinical picture.

Histopathology: tissue examination can reveal synovial hyperplasia, inflammatory infiltration, cartilage breakdown, bone erosion, and pannus formation.

No single measurement fully captures disease activity on its own, which is why CIA studies typically combine several of these readouts. Clinical scoring is fast and needs no specialized equipment, but it is somewhat subjective and depends on the scorer's

consistency, so many labs pair it with the more objective plethysmometer reading. Radiography provides a structural aspect that clinical scoring lacks, although traditional X-rays have limited sensitivity for detecting early or subtle bone loss compared to micro-computed tomography where this technology is accessible. Biochemical markers provide a systemic readout that can pick up inflammatory activity even before it is visible at the joint, while histopathology remains the most detailed, if also the most invasive and terminal, method of confirming what is actually happening within the synovium and cartilage. I tried to use several of these measures together in this project, since that gives a more complete and more convincing picture of disease development than any single method would on its own.

Chapter 3: Aim and Objective

3.1 Aim

My aim was to establish an experimental collagen-induced arthritis model in male Sprague-Dawley rats using chicken type II collagen and Complete Freund's Adjuvant, and to characterize its development through clinical, functional, radiological, and biochemical measures.

3.2 Objectives

- 1. Induce experimental arthritis in male Sprague-Dawley rats using chicken type II collagen and CFA.
- 2. Record clinical signs of arthritis, including redness and swelling.
- 3. Measure paw edema with a plethysmometer.
- 4. Grade arthritis severity using an arthritis scoring system.
- 5. Track changes in spontaneous movement.
- 6. Assess structural joint damage by X-ray.
- 7. Measure the inflammatory biomarkers IL-6 and CRP.
- 8. Compare arthritis-induced animals against negative controls.
- 9. Evaluate two doses of the investigational treatment.
- 10. Compare the investigational treatment against a standard treatment.

Chapter 4: Reagent and Experimental Details

4.1 Experimental Animals

Eighteen male Sprague-Dawley rats, weighing roughly 187–247 g, were split into five groups as follows.

Group	Treatment	Number of rats
Group I	Negative control	4
Group II	Positive/arthritis control	4
Group III	Standard treatment	4
Group IV	Dose 1	3
Group V	Dose 2	3
Total		18

4.2 Chemicals and Reagents

- Chicken type II collagen
- Complete Freund's Adjuvant
- Acetic acid
- Distilled water
- Syringes and needles
- Ketamine Hydrochloride Injection
- Blood collection materials
- IL-6 assay reagents
- CRP assay reagents

Collagen supplier: Sigma-Aldrich. Product: chicken type II collagen. Lot number: 0000536334. Ketamine Hydrochloride Injection was used for the terminal procedure to draw blood for biochemical assay.

4.3 Equipment

Equipment	Specification

Plethysmometer	UGO Basile, Model No. 37140
Centrifuge	800 Centrifuge — Max speed 4000 r/min, Max. RCF 1790×g, capacity 20 mL × 6, AC 220V 50Hz, power 50W
Radiographic equipment	X-ray unit for structural joint imaging

Chapter 5: Methodology

5.1 Experimental Design

The eighteen male Sprague-Dawley rats was divided across five groups. The negative-control group served as a baseline, showing what normal, uninduced animals look like. The positive/arthritis-control group demonstrated the arthritis phenotype produced by the induction protocol on its own, without any treatment to counter it.

5.2 Arthritis Induction

The first induction was carried out on 16 July 2026, weighing every rat before the induction. The collagen-CFA emulsion was administered as per the approved protocol, at the knee joints, back, and base of the tail. A booster dose was given on 4 August 2026, after the primary and secondary observation stages, to help sustain and develop the arthritic response.

5.3 Observation Protocol

I recorded a primary observation on 20 July 2026, covering movement, inflammation, redness, swelling, weakness, and overall condition. A secondary observation followed on 23 July 2026. At that stage the negative-control group still showed no visible redness or swelling, while the arthritis-induced groups had clearly developed inflammatory changes. The booster dose described above was administered after these two observation points.

5.4 Paw Edema Measurement

I tracked paw volume with a UGO Basile plethysmometer (Model No. 37140) as a measure of inflammatory swelling, taking readings before induction and again once swelling had developed. Percentage edema was calculated as:

$$\textit{Edema (\%)} = [(\textit{Final paw volume} - \textit{Initial paw volume}) / \textit{Initial paw volume}] \times 100$$

5.5 Arthritis Scoring

Each animal was scored using an arthritis scoring system that accounts for redness, swelling, inflammation, reduced movement, and joint abnormalities, with higher scores reflecting more severe and more widespread joint involvement.

5.6 Movement Assessment

I recorded each animal's spontaneous movement over a one-minute period, counting discrete movements to give a simple functional readout alongside the clinical scoring.

5.7 Radiographic Examination

Radiographs were taken of the hind limbs to check for structural changes associated with the induced arthritis, including bone erosion, altered joint spacing, and other skeletal abnormalities.

5.8 Blood Collection and Terminal Procedure

At the end of the study, I used Ketamine Hydrochloride Injection for the terminal procedure so that blood could be drawn for biochemical assay. Blood was collected following the approved sampling procedure and then centrifuged using an 800 Centrifuge (max. speed 4000 r/min, max. RCF 1790×g, capacity 20 mL × 6) to separate serum from the cellular fraction before analysis.



Figure 1. Preparing a micropipette for sample handling during blood processing.



Figure 2. Transferring a collected blood sample into a labeled tube for centrifugation and later assay.

5.9 IL-6 and CRP Assay

Serum samples were analyzed at Neuron Healthcare Center (report dated 29 August 2026, referred by Md. Jahir Alam) using a Gentin-1100/PA-50 Random Access Multibatch chemiluminescence immunoassay (CLIA) analyzer, which was used to quantify both IL-6 and CRP from the same serum samples.

5.10 Tissue Collection for Histopathology

Following the terminal procedure, I opened the abdominal cavity to recover tissue for histopathological assessment of the joints and to check the general condition of the internal organs. The dissection was carried out promptly after euthanasia to keep the tissue as close to its in-life state as possible.



Figure 3. Gross dissection following the terminal procedure, showing the abdominal organs of three animals recovered for further analysis.

Chapter 6: Method Development

6.1 Preparation of the Primary Collagen-CFA Emulsion

For the primary induction, I dissolved 12.6 mg of type II collagen in 2.1 mL of acetic acid and left the solution at roughly 4°C overnight. The next day, I mixed in 2.1 mL of CFA, giving a collagen solution of 2.1 mL, a CFA volume of 2.1 mL, and a total emulsion of 4.2 mL.

The intended dosing volume was 300 µL per rat. For 14 rats, that works out to 300 µL × 14 = 4200 µL = 4.2 mL, so the emulsion I prepared was exactly enough for 14 rats at 300 µL each.

6.2 Booster Dose Calculation

The booster was prepared to deliver 600 µL per rat, giving a final emulsion volume of 8.4 mL for the cohort. Using a reference concentration of 200 µg collagen in 200 µL, I worked out the required stock strength as 0.0168 (mg collagen per µL of emulsion). Applying the dilution relationship $V_1S_1 = V_2S_2$, with V_1 as the volume of stock collagen solution needed:

$$V_1 = (30 \times 0.1) / 7.4 = 0.172$$

On this basis, the total collagen required to prepare the booster emulsion came to 16.8 mg.

Chapter 7: Results

7.1 Clinical Observations

The animals that underwent the induction procedure developed visible redness and swelling in the paws and joints, while the negative-control group showed neither redness nor swelling at any stage. Figure 4 shows the swelling and redness that developed in an arthritis-induced animal's paw following induction, compared with the smooth, unswollen appearance expected in an unaffected limb.



Figure 4. Swelling and redness in the hind paws of an arthritis-induced rat following collagen-CFA immunization.

7.2 Paw Edema (Plethysmometer Readings)

Paw volume was recorded with the UGO Basile plethysmometer (Model No. 37140) for the negative-control and standard-treatment groups. The individual readings and group averages are given in Table 1.

Group	Reading 1	Reading 2	Reading 3	Reading 4	Average
Negative	1	1.1	0.9	0.9925	0.9925

control					
Standard treatment	1.4	1.7	3	1.5	1.475

Across the cohort as a whole, percentage edema reached approximately 65.7%, calculated as described in Section 5.4.

7.3 Arthritis Score

The negative-control group showed no redness or swelling at any point, while arthritis-induced animals displayed redness, swelling, inflammation, reduced movement, and joint abnormalities consistent with disease. The highest arthritis score I recorded across the cohort was 16.

7.4 Movement Assessment

Table 2 gives the movement recorded for each group over a one-minute observation period.

Group	**Movement/minute**
Negative control	21
Positive/arthritis control	6
Standard	N/A
Dose 1	N/A
Dose 2	14

The arthritis-control group moved at roughly 6 times per minute against 21 per minute in the healthy negative controls and 14 per minute in the dose-2 group, a pattern consistent with the swelling and joint discomfort seen in the untreated arthritic animals.

7.5 Radiographic Findings

Radiographs of the hind limbs showed bone erosion, joint structural abnormalities, and changes consistent with cartilage and joint destruction, adding independent structural evidence alongside the clinical findings also soft tissue swellings.

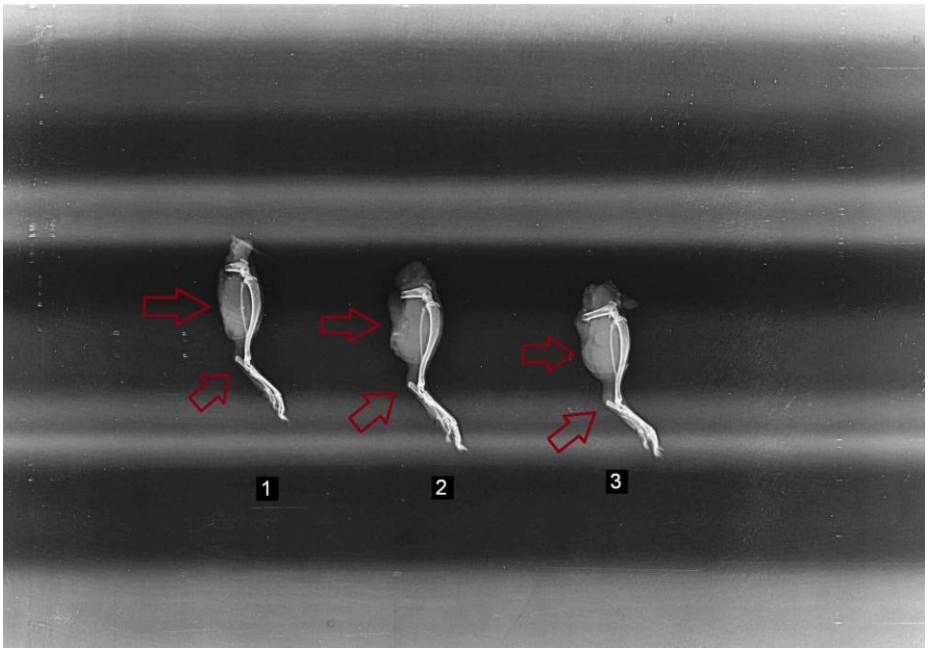


Figure 5. Hind-limb radiographs of three representative animals (labeled 1–3 on the film).

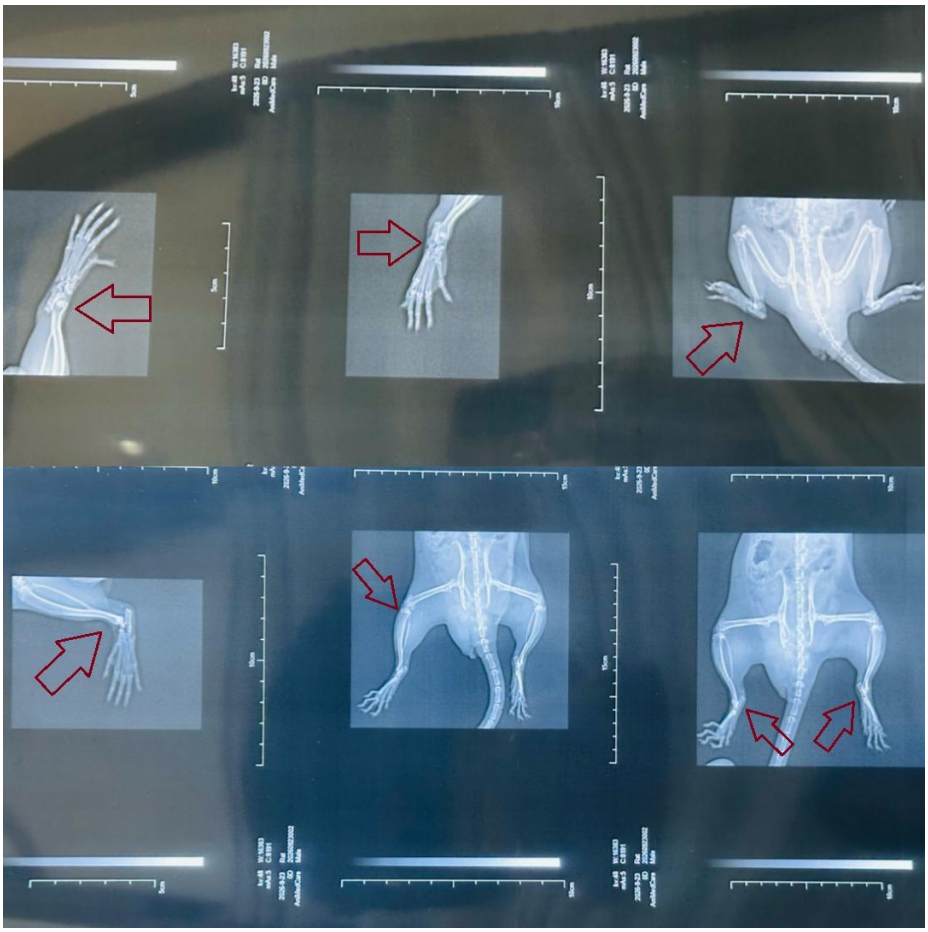


Figure 6. Composite radiographic scan showing additional limb regions across the sampled animals.

7.6 IL-6 and CRP Results

IL-6 came back below the assay's detection threshold in every sample tested (Table 3), and CRP came back below the assay's reported limit in the same three samples (Table 4).

Sample	IL-6
01	<1.5 pg/mL
02	<1.5 pg/mL
03	<1.5 pg/mL

Sample	CRP
01	<6.00 mg/L
02	<6.00 mg/L
03	<6.00 mg/L

7.7 Histopathology

Table 5 summarizes the histopathological picture across groups.

Group	Synovial inflammation	Cartilage damage	Bone erosion
Negative control	N/A	N/A	N/A
Positive control	Present	Present	Present
Standard	Present	Present	Present
Dose 1	Present	Present	Present
Dose 2	Present	Present	Present

7.8 Summary of Results

Altogether, the clinical inflammation, edema, movement changes, arthritis scores, and radiographic findings converge on the same conclusion: the induction protocol that was used successfully produced experimental inflammatory arthritis in this cohort.

Chapter 8: Discussion

The aim was to establish experimental inflammatory arthritis in male Sprague-Dawley rats using chicken type II collagen and CFA, and the results broadly support that this was achieved. The animals developed redness, swelling, and reduced movement, a clinical picture consistent with what is expected from a working CIA model.

The model itself traces back to work showing that type II collagen given with Freund's adjuvant can induce arthritis in susceptible animals [1], with Morgan et al. later tying arthritis development to the humoral immune response against collagen [2] and further work implicating cell-mediated immunity as well [3]. I used chicken-derived collagen specifically, which has precedent in prior Sprague-Dawley work.

Strain choice is relevant here too, since susceptibility to CIA is known to vary between rat strains, and the same induction protocol does not necessarily produce identical results across strains [7,8]. Pairing collagen with CFA adds a layer of nonspecific immune stimulation on top of the antigen-specific response, since CFA's mycobacterial components activate innate immune pathways in their own right, though it is worth restating that collagen-induced and adjuvant-induced arthritis remain mechanistically distinct models, as comparative work has shown [5,6].

The absence of redness and swelling in the negative-control group, set against their clear presence in the induced groups, is a reasonably direct confirmation that an inflammatory response was triggered. Paw edema, measured objectively via plethysmometer, is one of the more commonly used quantitative readouts in this kind of study. The negative-control group averaged 0.9925 on the plethysmometer against 1.475 in the standard-treatment group, and percentage edema across the cohort reached roughly 65.7%, pointing to substantial swelling having occurred in the induced animals.

Movement dropped accordingly. The arthritis-control group averaged about 6 movements per minute against 21 in the negative controls and 14 in the dose-2 group, plausibly reflecting the discomfort and mechanical limitation that comes with joint inflammation. Radiography added a structural dimension to this picture, showing apparent bone erosion and joint abnormalities, an important finding since sustained inflammatory signaling

driving osteoclast activity is what actually causes this kind of bone loss in chronic arthritis. Other CIA studies have used radiography alongside clinical scoring and histopathology in much the same way [7,9], and finding bone erosion here suggests the disease process went beyond surface-level inflammation into genuine structural damage.

The biomarker side of the picture is less conclusive. IL-6, an important driver of inflammatory signaling and acute-phase responses, came back below 1.5 pg/mL in all three samples analyzed, meaning this dataset does not currently show elevated circulating IL-6. CRP came back below 6.00 mg/L in the same samples. Since only three of the eighteen animals have been tested so far, and it is not yet confirmed which groups those three samples represent, these figures should be read as a preliminary signal rather than a group-wise comparison, and alongside the clinical and radiographic findings rather than in isolation.

Cytokines including TNF-α, IL-1β, and IL-6, along with pathways such as NF-κB, COX-2, and JAK/STAT signaling, have all been studied extensively in prior CIA work [10–19], and a number of studies using total glucosides of paeony, Periploca forrestii extracts, and cyclosporine in rats have shown that anti-arthritic treatments can reduce paw swelling, arthritis scores, inflammatory cytokines, and histopathological damage [9,10,13,15–19]. That body of work supports using the model that was established here to evaluate the treatment under investigation, though its actual effectiveness cannot be judged from the summary data alone. The individual results from the standard, dose-1, and dose-2 groups still need to be statistically compared against the arthritis-control group before any efficacy claims can be made.

Taken as a whole, the model that was established here sits comfortably within the range of outcomes reported for chicken collagen-CFA protocols in Sprague-Dawley rats elsewhere in the literature, and it reproduces the clinical, functional, and radiographic hallmarks that define a usable CIA model even before the full biochemical and histopathological picture is in hand. That gives me reasonable confidence that the differences expected between the arthritis-control group and the treated groups, once the complete dataset is analyzed, will reflect genuine treatment effects rather than an unreliable disease model.

8.1 Limitations

A few limitations are worth noting. The exact collagen dose used in the primary induction needs verification, since 12.6 mg prepared for 14 rats works out to 0.9 mg/rat rather than the 9 mg/rat figure recorded elsewhere in my notes. Plethysmometer readings are so far available for the negative-control and standard-treatment groups only; the corresponding readings for the positive-control, dose-1, and dose-2 groups still need to be added. IL-6 and CRP results are so far only available for three of the eighteen animals, and the correspondence between those three samples and the five experimental groups has not been confirmed. Histopathology was graded qualitatively as present or absent rather than scored on a quantitative scale, and the radiographs obtained so far are not yet matched to specific groups or animals. The dose groups were also relatively small in size. Finally, as with any animal model, collagen-induced arthritis does not reproduce every feature of human rheumatoid arthritis, and results should be interpreted with that in mind.

Chapter 9: Conclusion

Experimental Inflammatory Arthritis was successfully established in male Sprague-Dawley rats using chicken type II collagen combined with Complete Freund's Adjuvant. The induced animals showed redness, swelling, paw edema, reduced movement, an elevated arthritis score, and radiographic evidence of joint damage including apparent bone erosion, together indicating that the collagen-CFA protocol produced a usable inflammatory arthritis phenotype for testing anti-arthritic treatments. Statistical analysis of the complete individual-animal dataset is still needed to determine how effective the investigated treatment actually is, and whether the two doses differ in their response.

Chapter 10: Acknowledgment

I sincerely appreciate the Department of Pharmacy, School of Pharmacy and Public Health, Independent University, Bangladesh, for their academic assistance and resources throughout the study.

I would especially like to express my gratitude to Mossammat Rima Akter, Lecturer B, Department of Pharmacy, School of Pharmacy and Public Health, Independent University, Bangladesh, for her important academic advice, oversight, and assistance during the research process.

I would also like to acknowledge my classmate Naaz Rahman for her assistance and cooperation during the experimental work.

Chapter 11: References

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