World Journal of Pharmaceutical Science and Research

www.wjpsronline.com

Research Article

ISSN: 2583-6579
SJIF Impact Factor: 6.916
Year - 2026
Volume: 5; Issue: 7
Page: 136-148

# THERAPEUTIC EFFECT OF A SYNBIOTIC ON INFLAMMATORY SIGNALING AND GUT MICROBIOME MODULATION IN COLLAGEN-INDUCED ARTHRITIC C57BL/6 MICE

**Yara A. Nader[1], A. Helen*[2]**

[1]Research Scholar, Department of Biotechnology, University of Kerala, Kariavattom, Kerala 695581.

[2]Professor, Department of Biochemistry, University of Kerala, Kariavattom, Kerala 695581.

***Article Received: 04 May 2026 | | Article Revised: 24 May 2026 | | Article Accepted: 14 June 2026***

***Corresponding Author: A. Helen**
Professor, Department of Biochemistry, University of Kerala, Kariavattom, Kerala 695581.
**DOI:** https://doi.org/10.5281/zenodo.21033967

**How to cite this Article:** Yara A Nader, A. Helen (2026) THERAPEUTIC EFFECT OF A SYNBIOTIC ON INFLAMMATORY SIGNALING AND GUT MICROBIOME MODULATION IN COLLAGEN-INDUCED ARTHRITIC C57BL/6 MICE. World Journal of Pharmaceutical Science and Research, 5(7), 136-148.



## ABSTRACT

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic joint inflammation and gut microbiota dysbiosis. Modulating the gut microbiome may be a promising adjunctive strategy for RA management. This study evaluated the effects of combined probiotic and prebiotic (fructooligosaccharides, FOS) supplementation on inflammatory pathways and gut microbial composition in collagen-induced arthritic (CIA) C57BL/6 mice. Arthritis was induced using type II collagen with complete Freund's adjuvant (CFA). Mice were divided into three groups: control, disease, and Synbiotic-treated groups. The treated group received oral Synbiotics at fixed daily doses for 28 days. Inflammatory markers and signaling pathways were assessed. Colon histology was examined, and gut microbial diversity was evaluated using 16S rRNA gene sequencing. Treated mice exhibited reduced TLR2 expression decreased by approximately 10.2% compared with the disease group and decreased nuclear translocation of NF-κB ($p < 0.05$), decreased Level of TNF-α and increased level of IL-10 ($p < 0.05$). Microbiome analysis revealed descriptive compositional shifts, including increased relative abundance of Lactobacillus and partial normalization of the Firmicutes/Bacteroidetes ratio. Histological evaluation demonstrated improved colonic mucosal architecture in treated mice. In aggregate, these findings support the potential of synbiotic administration as a complementary strategy for modulating gut-immune interactions in RA, warranting further investigation in larger, controlled studies.

**KEYWORDS:** Collagen-induced arthritis; gut microbiome; dysbiosis; probiotics; prebiotics; rheumatoid arthritis.

## INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by persistent inflammation of the synovial joints, leading to cartilage degradation, progressive bone erosion, and systemic manifestations.[1] The development of RA is thought to result from a complex interplay of genetic predisposition, environmental factors, and immune dysregulation. Despite the availability of advanced therapeutic options, including biologic agents, Janus kinase inhibitors, and targeted synthetic disease-modifying anti-rheumatic drugs (DMARDs), many patients still experience disease flares and incomplete remission. This highlights the ongoing need for innovative and complementary therapeutic approaches.[2]

Recently, the gut microbiota has emerged as a critical regulator of immunological homeostasis throughout the body. The intestinal microbiota consists of trillions of microorganisms, including bacteria, archaea, fungi, and viruses.[3]

These microorganisms contribute to digestion and metabolism, support host development, and help maintain immune balance. The gut microbiota also plays an essential role in immune regulation by promoting the development and function of regulatory T cells. Several autoimmune diseases, including RA, have been linked to dysbiosis, defined as an imbalance in microbial community composition. Changes in gut flora structure can trigger immune dysregulation, cause systemic inflammation, and increase intestinal permeability.[4-6]

Studies in RA patients have revealed that gut microbiota composition differs significantly from that of healthy individuals, with reduced abundance of beneficial commensal genera, such as *Bacteroides*, and increased abundance of pro-inflammatory species, such as *Prevotella copri*. Furthermore, RA patients often exhibit impaired intestinal barrier function, which facilitates translocation of microbial antigens into the systemic circulation and contributes to immune activation.[7] This microbial imbalance is thought to contribute to sustained joint inflammation and dysregulated immune responses. Accordingly, microbiome-targeted strategies aimed at restoring gut microbiome homeostasis—such as probiotic and prebiotic supplements—are increasingly being explored as complementary approaches for RA management.[8] Due to its well-characterized genetic background and the availability of genetically manipulated lines, C57BL/6 mice are widely used in immunological research. C57BL/6 mice are considered relatively resistant to RA because of their H-$2^b$ major histocompatibility complex (MHC) haplotype. Consequently, strong immunization strategies, including booster injections of type II collagen (CII) emulsified with complete Freund's adjuvant (CFA), are required to induce arthritis in this strain. Despite all the challenges, C57BL/6 mice provide a valuable model for studying immunological and microbiome dynamics in arthritis under well-controlled experimental environments.[9-11]

In the current study, a multi-strain probiotic formulation was chosen to address various facets of rheumatoid arthritis (RA) pathophysiology via the gut–immune axis. The formulation comprised a range of species from the genera *Lactobacillus* and *Bifidobacterium*, as well as *Bacillus coagulans*, *Streptococcus thermophilus*, and the probiotic yeast *Saccharomyces boulardii*. These strains were selected for their synergistic functions in modulating gut microbiota composition, enhancing intestinal barrier integrity, and regulating host immune responses. Members of the *Lactobacillus* and *Bifidobacterium* genera have been documented to diminish pro-inflammatory cytokines such as TNF-α and IL-6, stimulate regulatory T cell responses, and facilitate the production of short-chain fatty acids, which are essential for sustaining immune homeostasis. Furthermore, *Saccharomyces boulardii* contributes to mucosal protection and inhibits pathogenic microbial overgrowth, whereas *Bacillus coagulans* provides enhanced gastrointestinal stability and has been associated with reduced inflammation in arthritis models. The use of a multi-

strain formulation was therefore intended to exert synergistic effects on microbial balance and immune modulation, which are key components in RA progression.[12-15]

## MATERIALS AND METHODS

### Animals

A total of 18 Female C57BL/6 mice, aged 3 months and possessing a fully developed immune system, were used in this study. The mice weighed 22.0 ± 1.0 g and were bred and housed in the department's animal facility. Mice were kept in an environment with controlled temperature of 25-26°C, humidity of 39%, and a 12:12 h light-dark cycle. A standard commercial diet (Amrut Laboratory Animal Feeds, Maharashtra, India) and tap water were provided ad libitum. All experimental procedures were conducted with the guidelines of the Animal Ethics Committee (CPCSEA), Government of India (Reg No.218/GO/ReBiBt/2000/CPCSEA), and were approved by the Institutional Animal Ethics Committee (IAEC-1-KU-4/2024-BCH-AH (54)).

### Probiotics and Prebiotics

Commercially available probiotics and prebiotics supplements were used in this study. The probiotic supplement was supplied as tablets with a maximum strength of 50 billion CFU per capsule, including 20 probiotic strains (*Lactiplantibacillus plantarum, Lactobacillus acidophilus, Lacticaseibacillus rhamnosus, Lactobacillus salivarius, Lactobacillus gasseri. Lactobacillus fermentum. Limosilactobacillus casei, Lacticaseibacillus reuteri, Lacticaseibacillus paracasei, Lacticaseibacillus lactis, Lactobacillus bulgaricus, Bifidobacterium lactis, Bifidobacterium longum, Bifidobacterium breve, Lactobacillus helveticus, Bifidobacterium infantis, Bifidobacterium bifidum, Bacillus coagulans, Streptococcus thermophilus, Saccharomyces boulardii*) and prebiotic fructooligosaccharides (FOS; 150 mg per capsule). The product was obtained from Carbamide-Forte (India). In addition, Prebiotics fructooligosaccharides (FOS) powder (300 g) was purchased from Sharrets, India.

### Chemicals

The high-grade analytical reagents used in this study were obtained from various national and international corporations. Type II collagen derived from chicken sternal cartilage (C9301; 5mg) and complete Freund's adjuvant (CFA) (F5881; 10 mg/ml) were purchased from Sigma-Aldrich. Primary antibodies were procured from Sigma-Aldrich (St Louis, MO, USA), Imperial Life Science, and Abcam (Cambridge, UK). Antibodies used in the analysis included IL-10, TNF-α, NF-κB, TLR4, TLR2, and MyD88. 16s rRNA gene sequencing was performed by Nucleome Informatics Private Limited, Hyderabad, India.

### Experimental Design

C57BL/6 mice were randomly divided into three groups of six each (n=6 per group): control, disease, and treatment.

The CFA contained 10 mg/ml of Mycobacterium tuberculosis and type II collagen, obtained from chicken sternal cartilage, which was dissolved in 0.05 M acetic acid. Mice in the disease group and treatment group received an intradermal injection of 0.1 ml of type II collagen emulsified with CFA (1:1), yielding a final concentration of 0.5 mg/ml of M. tuberculosis, administered at the base of the tail on day 0. A booster injection of 0.1 ml of CII + CFA was administered on day 21 at a different site at the base of the tail. The treatment phase targeted early immune activation and initial gut microbiome disruption following disease onset. Beginning on day 46 post-immunization, treated mice received ($1x10^9$ CFU) of probiotics and (FOS; 13.6 mg/kg body weight) via oral gavage five days per week for 28 days

(4 weeks). Dosages of probiotics and prebiotics were selected based on previous studies showing enhanced anti-inflammatory and immunomodulatory effects in a mouse model of autoimmune disease, including RA. Furthermore, FOS promotes the growth of beneficial bacteria. Both of these dose ranges have been reported to be sufficient to induce measurable changes in gut microbial composition and host immune responses without causing adverse gastrointestinal effects. The selected dose also reflects commonly used concentrations in preclinical probiotic intervention studies. [16-19]

The synbiotic approach, which combines probiotics and prebiotics, aims to improve the survival, colonization, and functional activity of gut microbes. The chosen doses aimed to elicit a biologically significant, synergistic effect on gut microbiota modulation and immune response regulation. Because probiotics and prebiotics were coadministered, the experimental design does not allow discrimination of their individual effects; therefore, all conclusions are restricted to combined (synbiotic) outcomes. Dry stool samples were collected to study gut microbiome composition, and then mice were sacrificed on day 73, and different tissues were collected to assess Inflammatory markers and signaling pathways readouts. (Fig. 1)

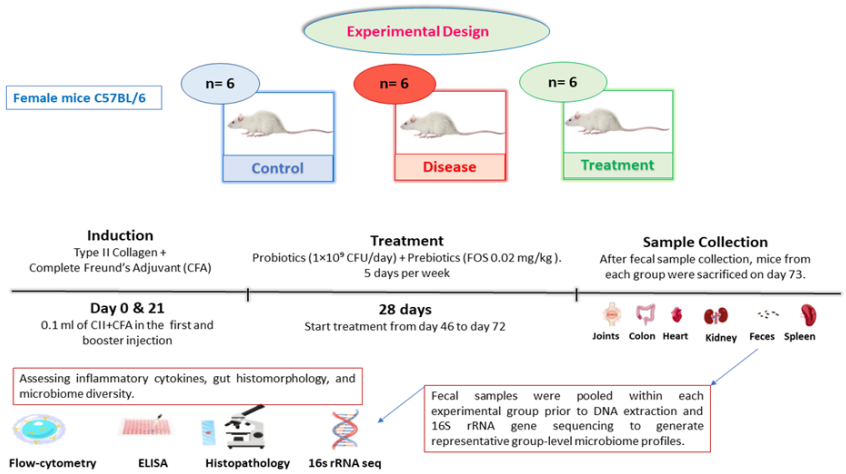


**Figure 1: Schematic representation of the experimental design and methodology.**

**Measurement of inflammatory mediators by ELISA (Enzyme-Linked Immunosorbent Assay)**

An enzyme-linked immunosorbent assay (ELISA) was performed to quantify cytokine levels in different tissues, including the heart, kidney, spleen, colon, and joints, according to the method described by Engvall and Perlman (1971). [20] Pro-inflammatory cytokines assessed included TNF-α, while the anti-inflammatory cytokine IL-10 and components of inflammatory signaling pathways (NF-κB and TLR4-MyD88) were also evaluated. Total protein concentrations were determined after grinding tissues in lysis buffer. Extracted proteins were used as antigens and coated on ELISA plates. Plates were incubated overnight at room temperature and subsequently washed with PBS containing Tween-20. Non-specific binding sites were blocked using blocking buffer, after which wells were incubated with primary antibodies for 2 hrs at room temperature. Following thorough washing with PBS-Tween, the secondary antibody was added and incubated for 1 hour. The colorimetric reaction was developed using an appropriate substrate, and absorbance was measured at 490 nm using an automated microplate reader (Thermo Multiskan Spectrum).

**Estimation of protein**

Total protein concentration in tissue samples was determined using the Lowry method (1951).[21]

**Analysis of TLR-2 by Flow cytometry**

Flow cytometry was performed to assess TLR2 expression. Spleen tissue was mechanically dissociated in phosphate-buffered saline (PBS), and the resulting cell suspension was passed through a 70 µm nylon mesh strainer to obtain single-cell suspensions. Cells were incubated with a primary antibody against TLR2 for 1 h at room temperature, then washed with FACS buffer. Subsequently, a FITC-conjugated secondary antibody was added, and samples were incubated for 45 min. After washing, live cells were gated and analyzed using a FACSAria™ II flow cytometer (BD Biosciences), and data were processed using FlowJo software.

**Histopathology of colon tissue**

Large intestines were fixed in neutral buffered formalin. Colonic samples were embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin (H&E). Stained sections were examined using a light microscope at 40X magnification.

**16s rRNA sequencing of gut microbiome**

Fecal samples were pooled within each experimental group prior to DNA extraction and 16S rRNA gene sequencing to generate representative group-level microbiome profiles. Due to sample pooling, microbiome analyses were conducted as descriptive and exploratory comparisons rather than definitive hypothesis-driven statistical tests. Taxonomic composition was visualized using relative abundance bar plots, and alpha diversity indices were calculated to summarize within-group microbial diversity. Genomic DNA was isolated from pooled fecal samples using the NucleoSpin® Stool Kit (Cat. No. 740472.50) according to the manufacturer's instructions. DNA concentration and purity were assessed using a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific, MA, USA), and DNA integrity was evaluated by 1% agarose gel electrophoresis (Lonza, Belgium). Libraries targeting the V3–V4 hypervariable region of the 16S rRNA gene (~600–650 bp) were prepared using the QIAseq® 16S/ITS Region Panel (Qiagen; Cat. No. 333845). Library quality and integrity were confirmed by 2% agarose gel electrophoresis. Amplified libraries were quantified using the Qubit™ dsDNA High Sensitivity Assay Kit (Invitrogen; Part No. 2491434) on a Qubit fluorometer and further validated for size distribution by gel electrophoresis (BioPrint). Sequencing was performed on the Illumina NextSeq platform using NextSeq 1000/2000 P1 reagents (Illumina), following the manufacturer's guidelines. Sequencing output was used for downstream analysis of microbial taxonomic composition and diversity metrics. Because 16S rRNA gene sequencing provides only relative abundance data, absolute microbial abundance and functional activity could not be determined.

**Statistical analysis**

Statistical analyses were performed using GraphPad Prism version 5.01. Group differences were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons post hoc test to identify differences between individual groups. A P value of $< 0.05$ was considered statistically significant.

**RESULTS**

**Effect of probiotics and prebiotics on TLR-2**

Toll-like receptors (TLRs) are transmembrane receptors that play an essential role in innate immunity by recognizing and binding to harmful pathogens, thereby eliciting an immune response. As the TLR pathway is activated, the transcription factor NF-κB is translocated from the cytoplasm to the nucleus, leading to the transcription of genes involved in the immune response, such as cytokines and inflammatory enzymes.

The disease group showed increased TLR2 expression in splenic tissue compared with the control group (up to 25.2%). In contrast, the treatment group showed a reduction in TLR2 expression (10.2% lower than in the disease group), indicating partial modulation of the inflammatory response (Fig. 2).

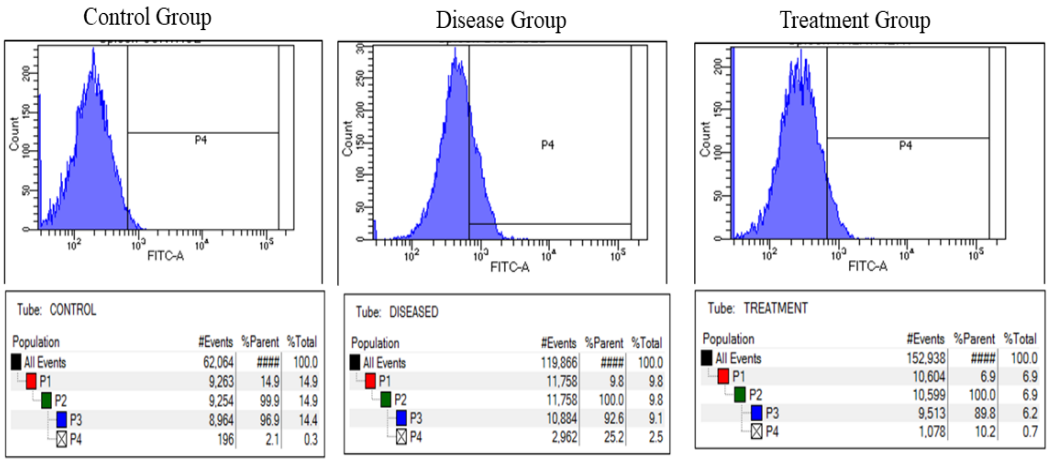


**Figure 2: Flow cytometry analysis of TLR2 in spleen.**

**Effect of Probiotics and prebiotics on TLR4, MyD88 and NF-κB in joints**

Toll-like receptor 4 (TLR4) and myeloid differentiation primary response 88 (MyD88) are key signalling molecules involved in inflammatory responses. In the experimental arthritis model, RA mice displayed significantly higher levels of TLR4 and MyD88 in joint tissues compared with controls. In contrast, treated mice with probiotics and prebiotics showed a significant reduction in the expression of both markers ($p < 0.05$) relative to the disease group (Fig. 3A).

With TLR2/4 and MyD88 activation confirmed, we next examined the downstream effect on NF-κB. RA mice exhibited increased nuclear translocation of NF-κB, consistent with enhanced inflammatory signaling, while synbiotic-treated mice showed a statistically significant reduction in NF-κB nuclear translocation ($p < 0.05$) compared with the disease group. These findings suggest an association between synbiotic administration and attenuation of joint inflammatory signalling (Fig. 3B).

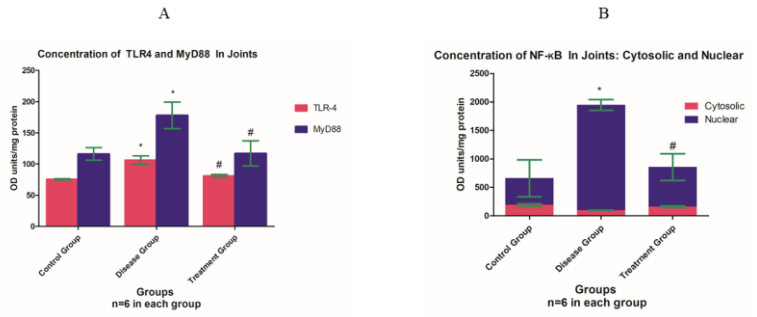


**Figure 3: Effect of Probiotics and prebiotics on MyD88 and TLR4 (A) and NF-κB (B) in joints. Values expressed as the average of 6 values ± SEM in each group of each stage of treatment. *: p< 0.05 vs. control group. #: p< 0.05 vs. disease group.**

**Effect of Probiotics and prebiotics on IL-10 and TNF-α in Heart and kidney tissue**

Compared with normal mice, RA mice exhibited significantly higher TNF-α levels ($p < 0.05$), confirming a pronounced inflammatory response associated with RA development. TNF-α levels were significantly lower in treated mice than in RA mice ($p < 0.05$), indicating an association between treatment and reduced inflammatory burden. In contrast, the anti-inflammatory cytokine IL-10 was significantly reduced in RA mice compared with normal controls, suggesting an imbalance between pro- and anti-inflammatory responses. Treated mice showed a significant increase in IL-10 expression relative to RA mice ($p < 0.05$), indicating partial modulation of anti-inflammatory pathways (Fig. 4).

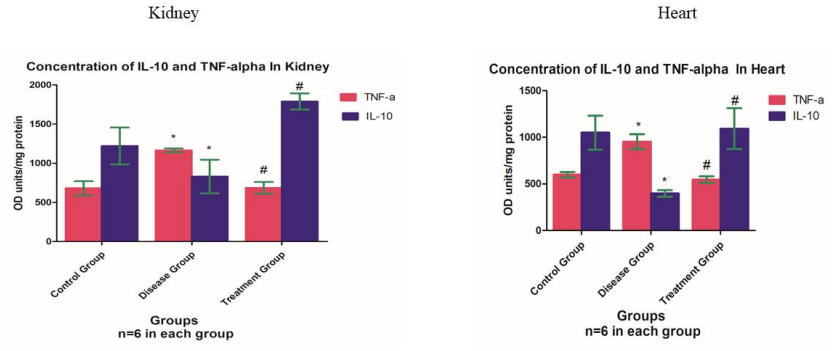


**Figure 4: Effect of Probiotics and Prebiotics on IL-10 and TNF-α in Heart and Kidney Tissue. Values expressed as the average of 6 values ± SEM in each group of each stage of treatment. *: p< 0.05 vs. control group. #: p< 0.05 vs. disease group.**

**Histopathological analysis of the colon**

Reflecting the influence of gut microbiota status on intestinal tissue integrity, distinct histological differences were observed among the control, disease, and treated groups during treatment. Colonic tissue from the control group exhibited normal architecture, with intact mucosa and submucosa, consistent with a healthy, non-inflamed colon. In contrast, the disease group showed mild mucosal injury and inflammation, characterized by surface erosion and inflammatory cell infiltration, suggesting compromised intestinal barrier integrity and early tissue alterations, often associated with dysbiosis. The treated group demonstrated noticeable histological improvement, with reduced epithelial denudation and a near-normal mucosal appearance. These findings indicate that synbiotic treatment was associated with partial preservation of colonic mucosal integrity and attenuation of inflammatory damage, rather than complete restoration. (Fig. 5)

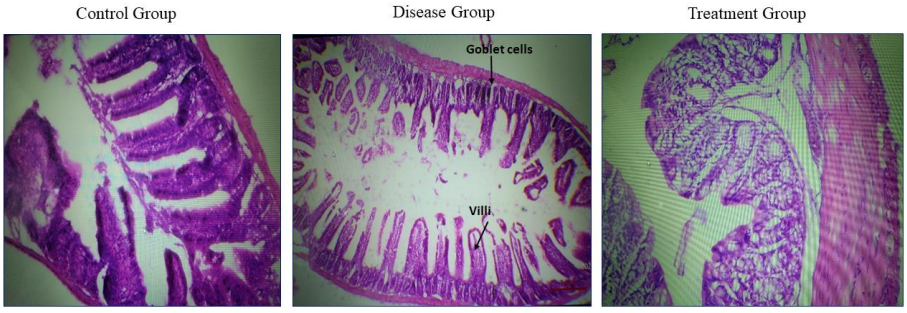


**Figure 5: Effect of Probiotics and Prebiotics in the colon. Macroscopy: colon measuring 24 cm, Magnification: 40x. The normal group shows normal colonic mucosa, submucosa, and muscle. The disease group shows mild denudation/unclear mucosa / mild inflammation. The treatment group shows less denudation. Almost normal.**

**Studying Microbiome by 16s rRNA sequencing**

Gut microbiome profiling revealed observable compositional differences between control, disease, and treated groups.

As fecal samples were pooled within each group prior to sequencing, microbiome findings are presented descriptively and interpreted qualitatively. Taxonomic analysis identified 6 phyla, 7 classes, 10 families, and 11 genera across the experimental groups. In Alpha diversity analysis, descriptive comparison of pooled samples suggested reduced microbial diversity in CIA mice relative to controls; however, statistical inference is not possible given the pooled sampling design, consistent with previous reports of arthritis-associated dysbiosis. The treated group showed a partial increase in alpha diversity relative to the disease group, suggesting a trend toward diversification of the microbial community rather than complete normalization (Fig. 6).

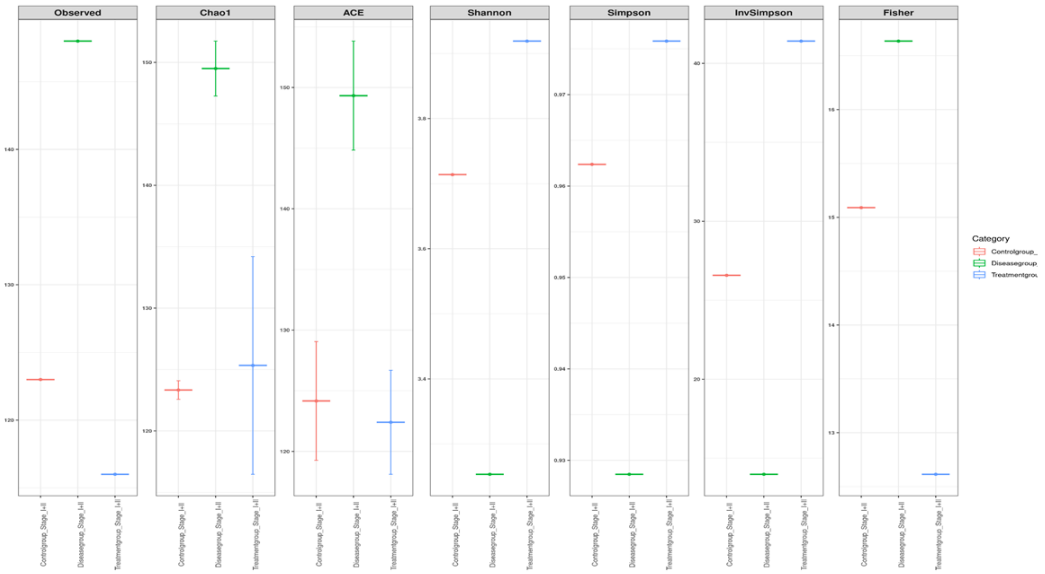


**Figure 6: Alpha diversity based on different metrics summarize the structure of an ecological community with respect to its richness (number of taxonomic units) across experimental groups using 16S rRNA gene sequencing.**

At the phylum level, control mice displayed a microbial profile dominated by *Firmicutes* (64.75%) and *Bacteroidetes* (24.48%). CIA mice showed a compositional shift characterized by increased *Firmicutes* (84.17%) and reduced *Bacteroidetes* (6.7%). In synbiotic-treated mice, *Firmicutes* decreased (53.35%), and *Bacteroidetes* increased (39.64%), indicating a partial compositional shift toward the control profile rather than full restoration (Fig. 7A). Class-, family-, and genus-level analyses revealed parallel trends. CIA mice exhibited increased relative abundance of taxa previously reported to be associated with inflammatory conditions, including *Bacilli*, *Clostridia*, *Campylobacteria*, *Staphylococcaceae*, and *Helicobacteraceae*, alongside reductions in *Bacteroidia* and *Bacteroidaceae*. Following probiotics and prebiotic treatment, relative abundances of *Lactobacillaceae*, *Bacteroidaceae*, *Muribaculaceae*, and *Prevotellaceae* increased, while *Staphylococcaceae* and *Helicobacteraceae* were markedly reduced or not detected in pooled samples. These changes suggest selective modulation of community composition during early treatment, without evidence of complete microbiome normalization (Fig. 7B–D).

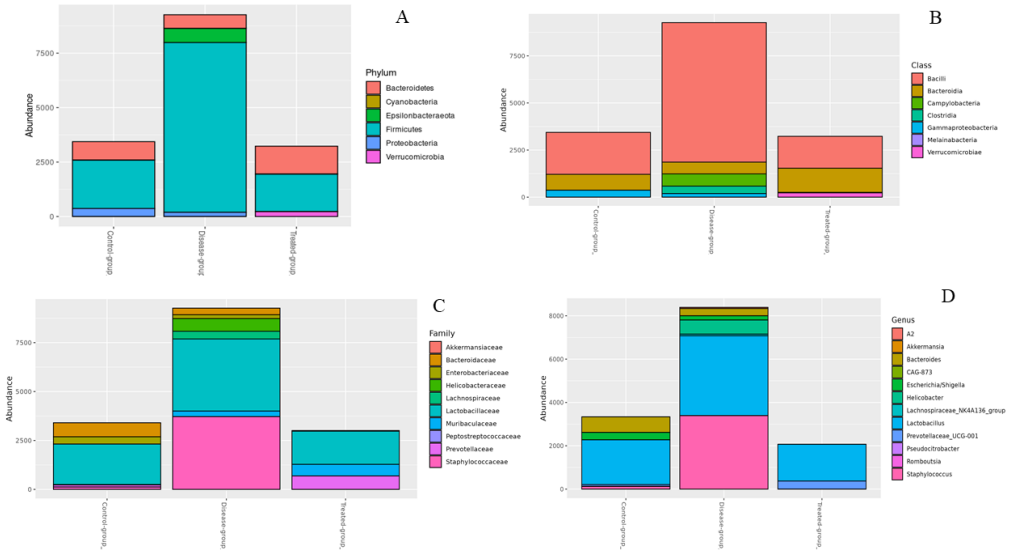


**Figure 7: Bar plots depict the relative abundance (%) of dominant taxa across control, disease, and treatment groups using 16S rRNA gene sequencing. A: Relative taxonomic abundance at the phylum level; B: Relative taxonomic abundance at the class level; C: Relative taxonomic abundance at the family level; D: Relative taxonomic abundance at the Genus level.**

## DISCUSSION

The present study examined the modulatory effects of synbiotic administration on inflammatory signaling and gut microbiome composition in the CIA mouse model of RA. Its pathogenesis is associated with activation of innate immune sensors such as Toll-like receptors (TLRs), which trigger adaptor proteins like MyD88 and downstream transcription factors, including NF-κB, leading to the expression of inflammatory cytokines such as TNF-α.[23-26]

In parallel, the gut–joint axis has emerged as an important regulatory component in RA pathophysiology. Alterations in gut microbial composition have been associated with systemic immune dysregulation, potentially mediated by microbial metabolites and impaired intestinal barrier function. Accordingly, modulation of this axis has shown therapeutic potential in preclinical and clinical studies.[22]

Synbiotic-treated mice exhibited reduced TLR2 expression in splenic tissue compared to RA mice, suggesting an association with lower innate immune activation. Reduced nuclear localization of NF-κB was also observed in joint tissues of treated mice, consistent with decreased inflammatory signaling activity. [27-29] These changes were associated with reduced inflammatory burden and joint pathology rather than direct evidence of pathway inhibition or structural protection. Similarly, decreased MyD88 expression was observed in treated mice, indicating altered engagement of TLR/IL-1R–related and T-cell–associated inflammatory signaling pathways. Collectively, these findings are consistent with previous studies that have highlighted the involvement of these signaling pathways in rheumatoid arthritis pathogenesis. In addition, Cardiac and renal tissues were included, given the established extra-articular systemic manifestations of RA, including pericarditis and renal amyloidosis, which reflect systemic inflammatory burden.

Across the treatment phase, synbiotic administration was associated with a consistent reduction in pro-inflammatory marker expression, most notably TNF-α, alongside with increased IL-10 levels in kidney and heart tissues following treatment, suggesting an association with enhanced systemic anti-inflammatory regulation.[30-32]

Histopathological analysis revealed that CIA induction was associated with modest colonic mucosal alterations, characterized by mild villus thinning and limited inflammatory infiltration. Treatment with prebiotics and probiotics was associated with partial preservation of colonic architecture. Taken together, these findings suggest that synbiotic administration was associated with attenuation of arthritis-related colonic alterations. However, the extent and durability of these effects remain exploratory, and further studies with extended treatment duration, optimized dosing strategies, and direct mechanistic assessments are required to determine whether more complete recovery of gut structure and function can be achieved.

Microbiome analysis indicated marked compositional alterations in the disease group, characterized by an elevated *Firmicutes*/*Bacteroidetes* (F/B) ratio, increased relative abundance of taxa such as *Staphylococcus* and *Helicobacter*, and reduced representation of genera including *Lactobacillus* and *Bacteroides*. Following synbiotic treatment, partial compositional shifts were observed, including relative increases in several taxa commonly reported as beneficial and reductions in taxa frequently associated with inflammatory states. Given the pooled sampling strategy and limited sample size, these findings should be interpreted descriptively rather than as definitive evidence of the absence of microbial change. Although improvements in inflammatory markers were observed, functional microbial outputs (such as short-chain fatty acid production) were not directly measured in this study. Similar observations—where clinical or immunological improvements occur without large shifts in relative bacterial abundance—have been reported in previous RA microbiome studies. [34] Consistent with these observations, alpha diversity analysis demonstrated reduced microbial diversity in RA mice, with partial recovery trends in the synbiotic-treated group. These findings highlight the complexity of microbiome–host interactions in RA and suggest that microbiome modulation may involve subtle compositional changes rather than complete reversal of disease-associated alterations.[18,34]

In aggregate, these results indicate that combined probiotic–prebiotic (synbiotic) administration was associated with reduced expression of pro-inflammatory cytokines, altered readouts of inflammatory signaling pathways (TLR/MyD88/NF-κB), partial preservation of joint and gut tissue morphology, and descriptive shifts in gut microbial composition. Overall, these data support the concept that modulation of the gut–immune axis through synbiotic intervention may represent a promising adjunctive and hypothesis-generating approach in rheumatoid arthritis research, consistent with emerging evidence in this field.[15,22,33,35]

**Study Limitations and Future Perspectives**

The present study has several limitations that should be considered when interpreting the findings. Future investigations should incorporate Larger animal numbers and extended follow-up periods to evaluate probiotics and prebiotics (synbiotics) administration results in enhanced or sustained effects. In addition, targeted microbial functional analyses (e.g., metabolomics) and immune cell profiling would be valuable to further elucidate the biological processes potentially underlying the observed anti-inflammatory associations. Future studies may also explore the use of tailored prebiotic formulations designed to support specific microbial taxa in combination with probiotics, as such strategies may enhance synbiotic efficacy; however, this remains to be experimentally validated.

## CONCLUSION

This study demonstrates that combined probiotic–prebiotic (synbiotic) administration was associated with modulation of inflammatory readouts, partial preservation of gut histological features, and descriptive compositional shifts in the intestinal microbiome in CIA-induced arthritis in C57BL/6 mice. The treatment phase was associated with more

apparent microbiome compositional changes. Overall, these findings are exploratory and hypothesis-generating and support the concept that microbiome-targeted interventions may represent a potential complementary approach in the study of autoimmune conditions such as rheumatoid arthritis.

**Statements & Declarations**

**ACKNOWLEDGEMENTS**

We extend our sincere gratitude to the Indian Council for Cultural Relations (ICCR) and the Government of India for their generous funding. Special thanks also go to the University of Kerala for providing the facilities necessary for this research.

**Funding**

This work was supported by the Indian Council for Cultural Relations (Grant WC1746326071346 to YAN) under the Government of India's initiative for foreign students.

**Conflict of Interest**

The authors declare that they have no conflicts of interest.

**Author Contributions**

Study concept and drafting of the manuscript (YAN), administrative support and study supervision (AH). All authors read and approved the final manuscript.

**Data availability**

The datasets generated and/or analysed during the current study are included in this article and its supplementary information files. For further inquiries, please contact the corresponding author.

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