ORIGINAL ARTICLE

Veterinary Research Forum. 2025; 16 (2): 117 - 124
doi: 10.30466/vrf.2024.2031860.4316
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Benefits of combining piperine with prednisolone in an experimental model of rheumatoid arthritis

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Article Info

Article history:

Received: 17 June 2024
Accepted: 14 August 2024
Available online: 15 February 2025

Keywords:

Autoimmunity
Piperine
Prednisolone
Rheumatoid arthritis

Abstract

This study evaluated the impact of combining piperine and prednisolone on clinical symptoms and immune responses in Wistar rats with rheumatoid arthritis (RA) induced by Freund's complete adjuvant due to piperine known anti-inflammatory and immunomodulatory properties. The RA rats were randomly divided into five groups (n = 10): The RA rats were treated with phosphate-buffered saline, RA rats treated with piperine (100 mg kg^{-1} orally), RA rats treated with prednisolone (10.00 mg kg^{-1} orally), and RA rats treated with a combination of piperine and prednisolone (half doses of each orally). Treatment started on day five post-induction when all rats had a clinical score of ≥ 1. Disease symptoms were monitored every other day until day 23 post-induction. Combining the two medications at half doses led to a more significant reduction in disease severity, weight improvement, and histopathological changes compared to using each drug alone at the full doses. The combined treatment group exhibited the most favorable response in C-reactive protein, myeloperoxidase, and nitric oxide biochemical tests compared to the other treatment groups. The combined treatment group showed decreased expression of *T-bet* and *RORɣt* genes. However, there was no statistically significant difference in the expression of *Foxp3* and *GATA3* genes compared to the group receiving prednisolone alone. Overall, combining piperine with prednisolone may prove to be a beneficial approach for managing RA.

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disorder characterized by a persistent inflammatory response that affects the joints and various organs outside the joints. These organs include the heart, kidney, lung, digestive system, eye, skin and nervous system.[1] The RA is among the most common chronic inflammatory joint diseases, affecting approximately 1.00% of the global population.[2] Various forms of arthritis have been extensively studied and categorized into non-inflammatory arthritis (such as osteoarthritis) and inflammatory arthritis resulting from crystal deposition (such as pseudogout, primary calcium phosphate disease, gout), bacterial and viral infections or autoimmune mechanisms.[1] Numerous biomolecular mechanisms have been suggested, however, the exact cause of RA remains unclear. One prevailing theory is that abnormal citrullination triggers the formation of anti-citrullinated protein antibodies.[3] Unpredictable flare-ups characterize the progression of RA and without effective treatment, symptoms worsen over time leading to irreversible joint damage and impacting both physical and psychological well-being.[4] Additionally, complications and associated conditions linked to RA can shorten patients life expectancy by a few years.[5]

Adjuvant-induced arthritis is an experimental arthritis model where arthritis is induced in a rat model by injecting Freund's complete adjuvant (FCA). This adjuvant consists of killed *Mycobacterium tuberculosis* bacteria suspended in sterile mineral oil. This method offers the advantage of sharing standard features with human arthritis. Histologically and immunologically, it involves swelling of the end organs, cartilage breakdown, loss of joint function, and an influx of lymphocytes to the affected area. Hence, this model of disease induction serves as a reliable approach to studying RA. It is also considered one of the most effective models for assessing the efficacy of

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various compounds as potential drugs for treating RAand other chronic inflammatory conditions.[6]

Numerous therapeutic medications, such as sulfasalazine, chloroquine, sodium aurothiomalate, corticosteroids and diclofenac sodium are accessible, however, these treatments have unwanted effects and have restricted effectiveness. Hence, alternative therapies are essential for RA. Identifying the bioactive components that show therapeutic advantages against RA is vital. Because of the limitations and side effects of traditional medications, patients and healthcare professionals are eagerly awaiting new treatments such as herbal bioactive substances to manage RA effectively.[7] Given that these patients need long-term medication, which raises the risk of side effects, a logical combination of current or new drugs could result in improved outcomes while decreasing the side effects of each drug.[8,9]

Piperine (Pip) is a nutrient-rich compound in black and long pepper (*Piper nigrum* and *Piper longum*). It is commonly used as a seasoning in various cuisines worldwide and has also been utilized as a traditional medicine in Asia and the Pacific islands, particularly in India.[10,11] The current literature highlights a broad range of biological effects of Pip including its ability to boost pancreatic digestive enzymes, preventing oxidation reactions triggered by free radicals and improving the absorption of various therapeutic medications. Studies have confirmed its anti-inflammatory properties in different animal models.[12] Piper species have demonstrated blocking enzyme activity in producing leukotrienes and prostaglandins. Furthermore, Pip has been shown to suppress nitric oxide (NO), tumor necrosis factor-α and the expression of pro-inflammatory genes both *in vitro* and *in vivo*.[13] Research focusing on discovering secure and effective plant-based substances to reduce inflammation and oxidative stress could be crucial in treating RA. It is reported that Pip treatment in B16F-10 melanoma cells not only suppressed the nuclear translocation of p65, p50, c-Rel subunits of nuclear factor-kappa B, and other transcription factors like activating transcription factor 2, c-Fos and Cyclic-AMP response element binding but also hindered the production of matrix metalloproteinase.[14]

However, there is limited data on the role of combining Pip and prednisolone (Pred) in an inflammatory condition. Therefore, in this study, we assessed whether combining half doses of Pip and Pred could offer synergistic benefits in an animal model of RA.

Materials and Methods

Chemicals. Cell culture media and fetal calf serum were obtained from GIBCO/Life Technologies Inc. (Gaithersburg, USA). The enzyme-linked immunosorbent assay kits were purchased by PeproTech EC Ltd. (London, UK). The RNX-Plus solution for RNA isolation was procured from DENAzistAsia, Mashhad, Iran. SYBR Premix Ex TaqII and cDNA reverse transcription kits were purchased from TAKARA (Takara Biomedical Ltd., Beijing, China). Hematoxylin-Eosin staining kits were prepared by Sigma-Aldrich (St. Louis, USA). Piperine, methotrexate, MTT, and other reagents were procured from Sigma-Aldrich .

Animals. A group of 60 male Wistar rats, aged 8 weeks, were acquired from the Faculty of Veterinary Medicine at Urmia University in Iran. These rats had an average weight of 150 ± 7.00 g. They were housed in a controlled environment with a temperature of 23.00 ± 1.00 ˚C and a 12-hr light/dark cycle. The rats had unrestricted access to food and water. All experimental procedures were conducted following the ethical standards outlined in the laboratory laws published by the National Institute of Health Guide.

Induction of RA and animal groups. The RA was induced in rats through the intradermal injection of 0.10 mL of FCA into the hind paw. The adjuvant contained 10.00 mg mL^{-1} of killed Mycobacterium. To assess the severity of the disease, the volume of the non-injected hind paw was measured every other day using an electronic water plethysmograph. A scoring system was employed, where a score of four indicated complete swelling of the entire leg with an inability to bend it, a score of three represented swelling of the ankle, a score of two denoted erythema and swelling of the paws, a score of one indicated erythema of the toe, and a score of zero represented a normal paw. Evaluations were conducted every monday throughout the study, with three independent observers assessing each examination. The average of the measurements was reported. The maximum arthritis index possible was 12, and this index was evaluated solely for the non-injected paws. Additionally, the weight changes of each rat were recorded every other day following immunization.[15] The rats were placed in groups as follows: Group 1 consisted of 10 healthy rats that did not receive any treatment (Control group). Group 2 consisted of 10 rats in which RA was induced without any treatment (RA group). Group 3 comprised 10 rats in which RA was induced and 100 mg kg^{-1} of Pip was administered daily via oral gavage (RA + Pip). Group 4 comprised 10 rats in which RA was induced and Pred was administered orally at a rate of 10.00 mg kg^{-1} daily (RA + Pred group). Group 5 comprised 10 rats in which arthritis was induced and a combination of 50.00 mg kg^{-1} of Pip as well as 5.00 mg kg^{-1} of Pred were administered daily via oral gavage (RA + Com). The study continued until day 23 following RA induction. At that time, rats were deeply anesthetized with 100 mg kg^{-1} ketamine (Alfasan, Woerden, The Netherlands) and 10.00 mg kg^{-1} xylazine (Alfasan), and the samples were collected.

Histopathology assay. The right ankle of each rat was amputated at the end of the study. The joints were fixed in 4.00% paraformaldehyde, decalcified in 10.00 % ethylene diamine tetraacetic acid for up to 30 days at 4.00 ˚C. Subsequently, the tissues were dehydrated, processed and embedded in paraffin. Serial paraffin sections (5.00 µm) were stained with Hematoxylin and Eosin, and examined under a light microscope (Olympus, Tokyo, Japan). The severity of arthritis was evaluated based on pathological changes such as inflammatory cell infiltration, synovial hyperplasia, joint swelling and inflammation.[16]

Biochemical assays. On 28th day post-induction and under deep anesthesia, blood was drawn from the hearts of the rats to obtain the serum needed for subsequent tests. One of the tests conducted was the myeloperoxidase (MPO) activity test. For this test, a serum sample of 10.00 µL was mixed with 80.00 µL of 0.75 mM H_2O_2 and 110 µL of the reaction solution containing 2.90 mmol of 3,3′,5,5′-Tetramethylbenzidine (TMB) in 14.50% dimethyl sulfoxide along with 150 mM of sodium phosphate buffer (pH = 4.50). The samples were then incubated at 37.00 ˚C for 15 min. Afterward, 50.00 µL of sulfuric acid (2.00 M) was added to halt the reaction. The absorbance of light was measured at 450 nm and the reference point at 620 nm using a microplate reader (Dynatech, Denkendorf, Germany). The 10.00 µL of horse-radish peroxidase was used at 2.50 and 25.00 mU mL^{-1} concentrations to establish a positive control. Finally, the MPO activity was determined by comparing the difference in absorbance to the horseradish peroxidase standard curve and the results were reported in mIU mL^{-1}.[17,18] The 0.10% sulfanilamide was combined with 100 µL of the serum sample. This mixture was then placed in a dark environment at 25.00 ˚C for 10 min. Subsequently, the absorbance of light at 540 nm was measured. The NO level was determined by comparing it to the standard curve obtained through Griess method.[15] The total antioxidant capacity (TAC) was determined in serum samples using the ferric reducing antioxidant power assay which evaluates the ability of an antioxidant to reduce a ferric tripyridyltriazine complex to a colored ferrous tripyridyltriazine. In this experiment, 20.00 µL of serum sample was mixed with 1.00 mL of working solution and vortexed. The optical absorption of the sample was measured at 593 nm initially and after 4 min compared to that of the control sample (Blank). The absorbance value was plugged into the TAC formula to determine the antioxidant capacity. Finally, the TAC value was calculated using a standard curve.[19,20]

Real-time polymerase chain reaction. To assess GATA3, T-bet, ROR-C and FOXP3 levels, total mRNA was isolated from rat joints using RNX-Plus solution as per the manufacturer's instructions. The isolated RNA was then used to generate complementary DNA. Polymerase chain reaction amplification was carried out in triplicate using a SYBR Green kit following the manufacturer's protocols. The *GAPDH* gene, serving as a housekeeping gene, was used as a control. Forward and reverse primers for mRNA amplification in the case of GATA3 were 5′-CAA AGC CAG AGT CCT TCA GA-3' and 5′-GAT GGT CTT GGT CCT TAG CC-3', respectively. The forward and reverse sequences for T-bet were 5'-CGG CTG CAT ATC GTT GAG GT-3' and 5'-GTC CCC ATT GGC ATT CCT C-3', for GATA3 were 5'-TCA TTA A GC CCA AGC GAA GG-3' and 5'-GTC CCC ATT GGC ATT CCT C-3', for ROR-ɣT were 5'-GCA GCG CTC CAA CAT CTT CT-3' and 5'-ACG TAC TGA ATG GCC TCG GT-3', and for FOXP3 were 5'-CAC CTG GCT GGG AAA ATG G-3' and 5'-GGA GCC CTT GTC GGA TGA-3', respectively.The outcomes were reported as $2^{-\Delta\Delta Ct}$ (mean fold change).[21-23]

Statistical analysis. The data underwent assessment for normal distribution by utilizing the Shapiro-Wilk test. The Kaplan-Meier test was employed to analyze the disease activity index and survival probability. Then, a one-way analysis of variance and Tukey's post hoc test were conducted to investigate the findings further. The means ± SD was utilized to express the results with a significance level set at $p < 0.05$.

Results

Histopathological changes. Figure 1 illustrates the histopathological examination of joints affected by RA. The control rats (Fig. 1A) displayed normal articular cartilage and no mononuclear cell infiltration in the synovium. On the other hand, the knee joints of arthritic rats (RA group; Fig. 1B) exhibited cartilage erosion, synovial hyperplasia and mononuclear cell infiltration in the synovial membrane. Additionally, there was a more pronounced extent of bone damage and a significant infiltration of inflammatory cells consisting of lymphocytes, macrophages and congested vessels. Rats that received Pred treatment (RA + Pred; Fig. 1C) displayed a structure similar to that of the normal group. The inflammatory response was decreased in the group treated with Pip (RA + Pip; Fig. 1D), although not as effectively as with Pred. Notably, a significant reduction in joint inflammation and lymphocyte accumulation was observed in the group that received a combination of Pip and Pred (RA + Com; Fig. 1E). Overall, the results indicated that the combination treatment of Pip and Pred yielded favorable outcomes in terms of joint pathology.

Clinical evaluations. Inflammatory responses within the joint environment are a crucial clinical indicator in RA and its animal counterparts. Therapeutic regimens commenced on the fifth day following immunization upon observation of an arthritis index of ≥ 1 in each rat.[24] The maximum level of paw swelling was documented every alternate day following adjuvant immunization (Fig. 2A and Table 1). On the 23rd day after the administration of the adjuvant (precisely 18 days after the manifestation of disease symptoms), the rats were euthanized humanely.

The absence of a statistically significant correlation between the RA + Pip and RA + Pred groups in terms of average RA index can be observed in Figure 2 and Table 1. The group receiving simultaneous treatment with Pip and Pred (RA + Com) exhibited the lowest average RA index, indicating a notable decrease in value compared to other groups. It is worth noting that the treatment and control groups demonstrated a significant impact ($p < 0.05$).

The arthritis index findings on the final day showed slight variations. The RA + Pred, RA + Pip, and RA + Com groups exhibited the lowest values, followed by the control group. There were statistically significant differences among all groups ($p < 0.05$).

Notably, the RA + Com group showed a minor weight difference and no weight difference was observed between the Pip and Pred groups from the beginning to the end of the study. These findings suggested that Pip treatment alone might not effectively manage the pain and discomfort symptoms of the disease. Pred, on the other hand, seemed to have a better effect. However, the combined treatment of Pip and Pred (RA + Com group) effectively reduced the inflammation, pain, and discomfort.

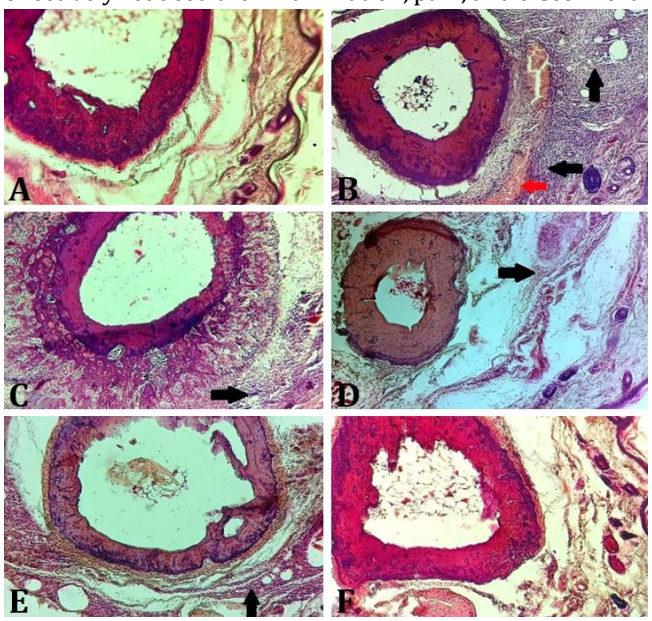

Fig. 1. The histopathology of joints affected by rheumatoid arthritis (RA). Histological sections of hind paws from animals with adjuvant-induced arthritis. **A)** Normal group with only phosphate-buffered saline administration (Control). **B** and **C)** No-treatment group (RA group). In this group, the extent of bone damage was more pronounced accompanied by significant infiltration of inflammatory cells as well as congested vessels. **D)** Rats treated with prednisolone (Pred) exhibited a structure similar to that of the normal group (RA + Pred). **E)** Piperine (Pip) -treated group. The inflammatory reaction was decreased in this group, however, was not as effective as Pred (RA + Pip). **F)** A significant reduction in joint inflammation and leukocyte infiltration was observed in the Pred and Pip group. (RA + Com). Black arrows: Inflammatory cells, and red arrow: Congested vessels**.** (Hematoxylin and Eosin staining; 100 ×).

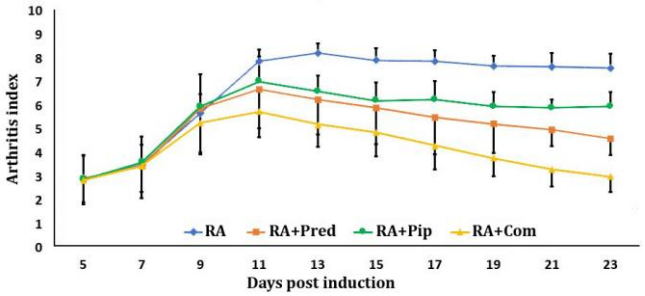

Fig. 2. The clinical features in rheumatoid arthritis (RA) rats. The RA rats were treated by piperine (Pip) and prednisolone (Pred) alone and in combination as described in the Materials and Methods section. The combined treatment with both Pip and Pred showed a more significant reduction in the clinical features of RA compared to treatment with either medication alone using optimal doses.

Table 1. Results of arthritis index, arthritis index and mean body weight on the last day. Data are presented as mean ± SD.

Groups	Average arthritis index	Arthritis index on the last day	Weight loss (g)
RA	6.61 ± 0.73^{a}	7.51 ± 0.72^{a}	8.94 ± 0.85^{a}
RA + Pip	5.57 ± 0.60^{b}	5.90 ± 0.59^{b}	4.45 ± 0.73^{b}
RA + Pred	5.06 ± 0.72^{cb}	4.53 ± 0.61^{c}	4.02 ± 0.61^{b}
RA + Com	4.10 ± 0.54^{dc}	2.92 ± 0.67^{d}	3.18 ± 0.57^{c}

RA: Rheumatoid arthritis; Pip: Piperine; Pred: Prednisolone; Com: Combination-treated RA group.
$^{a\text{-}d}$ Different letters indicate statistical significance at a $p < 0.05$.

Changes in biochemical factors. Figure 3 illustrates the levels of biochemical factors including C-reactive protein (CRP), MPO, and NO in the serum of rats. The control groupexhibited the lowest levels of these factors and the RA groupshowed the highest levels. Among the treated groups, the RA + Com, RA + Pred and RA + Pip groups demonstrated the highest response to treatment for all measured factors. It is worth noting that there were statistically significant differences among all groups in terms of the measured factors. The group treated with Pred exhibited a more effective reduction in the inflammation-related factors than those treated with Pip alone. However, when these two treatments were combined, a synergistic effect was observed.

Effect of treatment on the mRNA expression of master regulator of T helper (Th) cells. Based on the results, the expression of transcription factors T-bet, GATA3, RORγT, and FOXP3 in the joints of RA rats showed a significant increase compared to normal rats (Fig. 4). The expression of T-bet and RORγt were significantly decreased in treatment groups compared to RA rats without treatment. Here, the combined treatment was significantly more effective in reducing the expression of T-bet and RORγt compared to the groups received monotherapy (Fig. 4). The expression of GATA3 was significantly increased in treatment groups compared to RA rats without treatment. Here, the treatment with Pred and combined treatment was significantly more effective

in reducing the expression of T-bet compared to the RA + Pip group. There was no significant difference between RA + Pred and RA + Com groups in terms of the expression of this factor (Fig. 4). Statistically, treatment with Pip was not effective in changing the expression level of FOXP3. The treatment with Pred and combined treatment was significantly effective in reducing the expression of T-bet compared to the RA + Pip group. There was no significant difference between RA + Pred and RA + Com groups in terms of the expression of FOXP3.

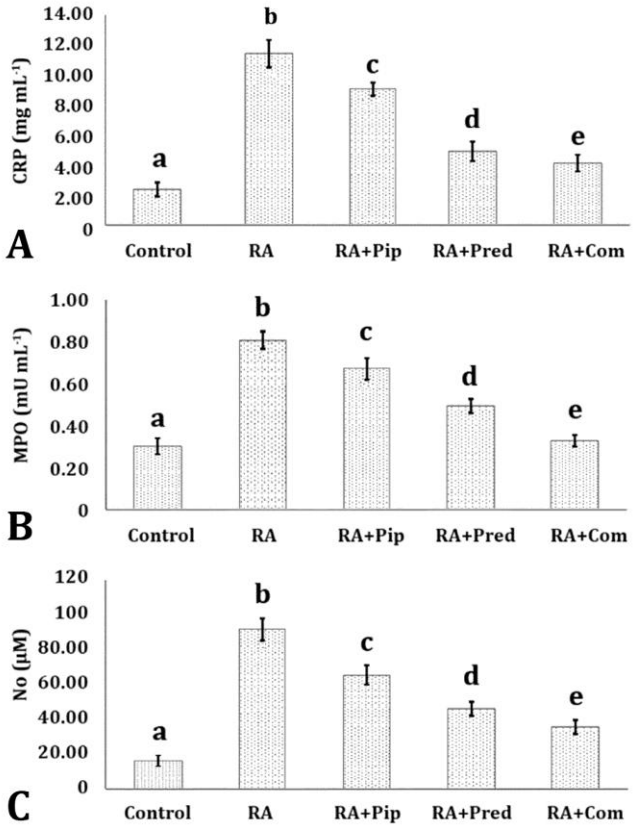

Fig. 3. Biochemical modifications in the sera of rats with rheumatoid arthritis. The outcomes revealed that the combined treatment of piperine (Pip) and prednisolone (Pred) resulted in a more significant decrease in the levels of **A)** C-reactive protein (CRP), **B)** myeloperoxidase activity (MPO), and **C)** nitric oxide enzyme activity (NO) compared to each drug used alone. The results were presented as mean ± SD, and different letters indicated statistical significance at a $p < 0.05$. RA: Rheumatoid arthritis, and Com: Combination-treated RA group.

Discussion

Long-term management of chronic inflammatory diseases like RA often involves the prescription of anti-inflammatory drugs to regulate the dysfunctional immune system. Consequently, there is a pressing requirement to create secure and efficient medications suitable for extended periods of use. Numerous research teams have investigated non-steroidal anti-inflammatory compounds

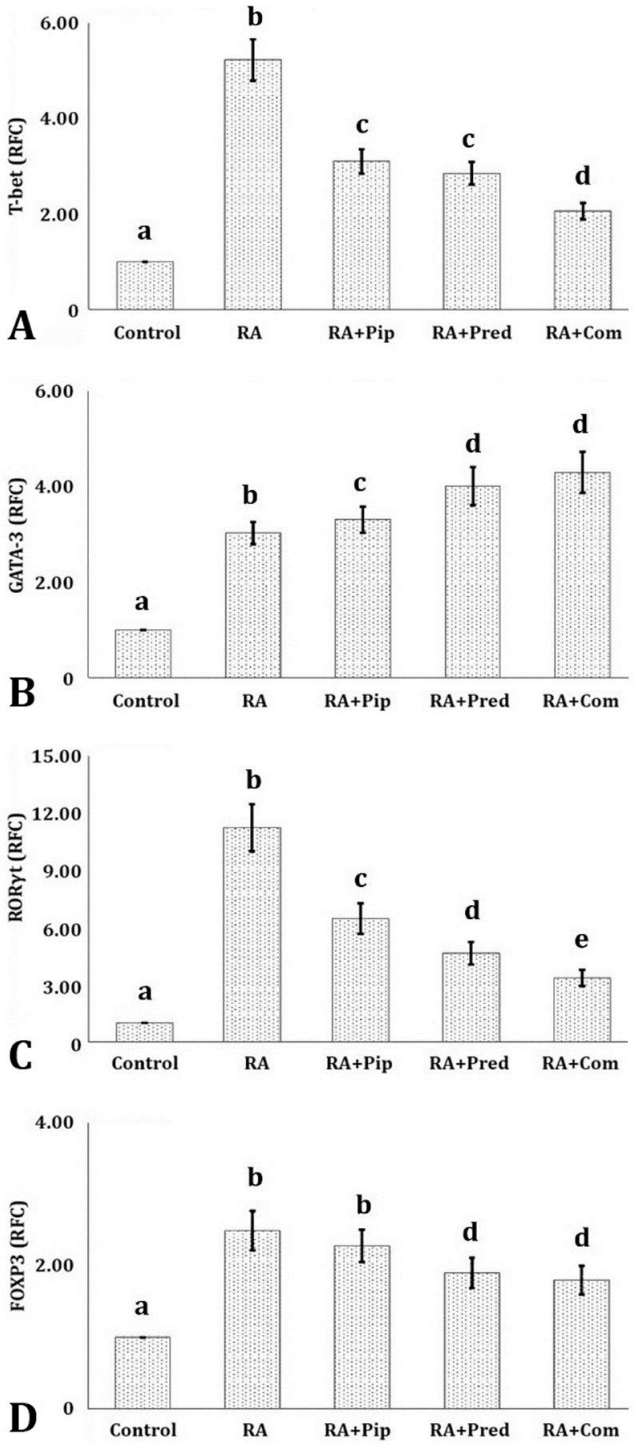

Fig. 4. Effect of treatment on the mRNA expression of master regulator of T helper (Th) cells. Gene expression of various transcription factors each responsible for directing the differentiation of Th0 cells into distinct subsets including **A)** T-bet, **B)** GATA-3, **C)** RORγt and **D)** FOXP3 are shown. The transcription factor gene expression was quantified using the real-time polymerase chain reaction technique and reported as the mRNA fold change in $\log_2$. RFC: Realative fold change. The results were presented as mean ± SD.
[a-e] Different letters indicated statistical significance at a $p < 0.05$.

derived from natural sources to discover novel therapeutic options for clinical application.[25] The components and extracts derived from plants are the subject of increasing research resulting in a rise in their consumption globally for their positive impact on health.[12]

However, Pred is considered the most effective chemical treatment for RA. It is recommended to administer a low dosage of Pred as it has been proven to be safe and efficient in managing RA. Low doses of Pred have shown significant efficacy in reducing inflammation associated with RA. Furthermore, substantial evidence indicates that low doses of Pred can slow down the progression of bone erosion in RA. By taking preventive measures and implementing appropriate management, potential side effects of low-dose glucocorticoids can be anticipated and avoided.[26] Prolonged and high-dose corticosteroid usage poses significant risks such as skin issues, electrolyte imbalances, high blood pressure, elevated blood sugar levels, pancreatitis, and various effects on the blood, immune system and brain. While rare, there is a chance of experiencing clinically significant side effects. Extended corticosteroid use could lead to more severe consequences like osteoporosis, joint damage without infection, adrenal insufficiency, gastrointestinal problems, liver issues, eye complications, high cholesterol levels, stunted growth and potential congenital disabilities.[27] Given these explanations, it appears imperative to discover organic substances that can replace the reliance on Pred or, at the very least, reduce the required dosage.

Piperine is noted for its strong antioxidant properties and its ability to reduce paw inflammation in treated animals by eliminating free radicals, which are believed to cause cellular damage in cartilage.[28,29] Free radicals are believed to trigger cellular damage in cartilage in experimental animals. Nitric oxide (NO) is a crucial signaling molecule that activated cells and macrophages generate during the inflammatory response.[30-32] Elevated NO levels have been observed in arthritic rat models, mirroring findings in synovial fluids of individuals with RA.[33] It is believed that during sterile inflammation, MPO and the oxidants produced by MPO contribute to the escalation of inflammation and tissue injury. Elevated levels of MPO and the inflammatory process are commonly seen in various autoimmune conditions. The MPO is known to enhance vascular permeability and trigger inflammatory immune reactions.[34]

The CRP is commonly evaluated as an indicator of systemic inflammation in RA. Nevertheless, it also functions as an immune modulator that is crucial in the inflammatory processes linked to RA and encourages atherogenic impacts.[35] Atained data in this study indicated that the combination of half-doses of Pip and Pred showed the most favorable response in MPO and NO levels compared to the other treatment groups that received each agent at full doses.

The CRP is commonly evaluated as an indicator of systemic inflammation in RA. Nevertheless, it also functions as an immune modulator that is crucial in the inflammatory processes linked to RA and encourages atherogenic impacts.[35]

Greater levels of CRP have been linked to increased disease activity in RA as determined by the core components of the 28-joint disease activity score.[35,36] Additionally, specific indicators of disease activity such as the count of swollen joints and patient-reported measures like functional status, morning stiffness, fatigue and pain have also shown associations with CRP.[35] In our research, the group treated with Pip and Pred in the RA+Com group exhibited the lowest levels of CRP. The activity of MPO and NO enzymes indicates inflammation and disease severity, while CRP is an acute phase factor. Therefore, it can be inferred that RA was effectively managed. Additionally, the lower Pred dose minimizes side effects and elicits a more efficient response than higher doses. The combined treatment reduces the reliance on Pred by half making it the optimal treatment option due to the numerous advantages of Pip.

It is anticipated that T-bet will play a significant role in guiding Th1 differentiation leading to the expectation of T-bet's involvement in autoimmunity mediated by the adaptive immune system. Surprisingly, it was discovered that the central location for T-bet's influence on inflammation regulation was within the DC population.[37] Furthermore, the phenotype and function of regulatory T cells (Tregs) residing in the bone marrow (BM) of patients with RA and animal models of RA have been successfully characterized by the researchers. The investigations into the *ex vivo* function of Tregs, specifically their suppressive activity on effector T cells, have yielded valuable insights. The findings indicated that the reduced number and impaired functional properties of $CD4^{+}FOXP3^{+}$ T cells found in the BM of RA patients might contribute to the inflammatory process observed in RA BM. This discovery highlights the significance of the FOXP3 factor in immune modulation and inflammation, thus, offering significant implications for our understanding of RA pathogenesis.[22,37]

The primary cytokines found in the joints of individuals with RA are mainly Th1, suggesting a Th1-driven condition. This is due to an imbalance between Th1 and Th2 cells with inadequate Th2 cells to reduce inflammation. Although there is strong evidence supporting RA as a Th1-driven disease, there have also been reports of defective Th1 polarization in RA. The association between T-bet mRNA expression and interferon-γ levels provides additional confirmation of the involvement of Th1 cells in the development of RA.[38,39] The RORγ isoform specific to T cells, known as RORγt, has also been demonstrated as the crucial transcription factor that defines the lineage and initiates the differentiation process of Th17 cells.[40] The Th17 cells produce the inflammatory

cytokine interleukin (IL) 17, a protein identified in synovial fluid associated with RA. The IL17 works in collaboration with IL1β and tumor necrosis factor-α to promote inflammatory conditions in joints and other tissues.[41] In this study, the highest polarization from Th0 cells to Treg and Th2 cells was observed in the groups that received RA + Pred and the RA + Com group. Interestingly, there was no significant difference between these two groups. This finding suggested the establishment of an anti-inflammatory state and immune modulation. Considering the considerable side effects of Pred, the combination of Pred and Pip to reduce the dosage proves to be highly beneficial. On the contrary, the polarization towards Th1 and Th17, which are involved in inflammation propagation and the release of pro-inflammatory cytokines, was the lowest in the group that received the combined treatment. The results obtained from the analysis of transcription factors and other findings demonstrated the effectiveness of the combined therapy in controlling arthritis. Nevertheless, further studies on both human and animal models are recommended to gain more comprehensive insights.

In conclusion, this study showed that Pip effectively alleviated symptoms in an animal model of RA. Moreover, combining a lower dose of Pred with Pip led to a more significant improvement in both clinical and laboratory markers of RA compared to using either treatment alone. This indicated that co-administration of these compounds could be a promising strategy for managing RA. However, further research is needed to understand the precise mechanism of action and the synergistic effects of Pred, a commonly used immunosuppressant, and Pip.

Conflicts of interest

The authors declare no conflicts of interest.

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