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Original Research Article

Evaluation of anti-inflammatory and anti-arthritic activities of Benzimidazole derivative 2-((1H-benzo[d]imiadazol-2-yl) thio)-1-3, 5-diphenyl-1h-pyrazol-1-yl) ethanone

Muftia Arubah Basri[1], Aisha Mobashar[1.2], Komal Najam[1], Zeeshan Akbar[1], Kashif Barkat[1.2], Khalid Hussain[1] Humaira Nadeem [3], Hafiz Aamir Ali Kharl [4]

[1] Faculty of Pharmacy, The University of Lahore, Lahore, Pakistan | [2] Faculty of Health Sciences, Equator University of Science and Technology, Maska, Uganda | [3] Riphah institute of pharmaceutical sciences, Riphah International University, Islamabad, Pakistan | [4] HBS College of Pharmacy Park Road Islamabad, Pakistan

Correspondence should be addressed to Aisha Mobashar; aisha.mobashar@pharm.uol.edu.pk

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Arthritis, Immunomodulatory, Benzimidazole derivative, Cytokines

ABSTRACT

This study investigates the anti-inflammatory and immunomodulatory effects of a Benzimidazole derivative 2-((1H-benzo[d]imiadazol-2-yl) thio)-1-3, 5-diphenyl-1h-pyrazol-1-yl)ethanone (BZ), in both chronic and acute inflammation models. For induction of arthritis, Freund's Complete Adjuvant was used. After inducing arthritis in rats, three different doses of benzimidazole derivative (25,50 and 100 mg/kg) were given. The treatment started on the 7th day and continued for 3 weeks. Piroxicam was used as the standard drug. On the last day of treatment, the rats were sacrificed. Paw edema and arthritis were determined using digital water plethysmometry and arthritic score index. Various hematological parameters, including red blood cells (RBC), white blood cells (WBC) and hemoglobin (Hb), along with biochemical markers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine were also evaluated. The mRNA levels of IL-6 and TNF-α were determined using a quantitative polymerase chain reaction (qPCR). Prostaglandin E2 (PGE2) levels were measured via ELISA. Carrageenan induced paw edema was employed for determining anti-inflammatory activity. Treatment with different doses of Benzimidazole derivative decreased paw edema and decreased arthritic progression. All histopathological parameters were concluded to be decreased after the given treatment. The analysis of qPCR showed decreased levels of Tumour necrosis factor (TNF-α) and Interleukin-6 (IL-6). Enzyme-linked immunosorbent assay (ELISA) demonstrated a decreased level of PGE2 in all groups that were treated with the benzimidazole derivative. Results obtained from all groups treated with the benzimidazole derivative were significantly comparable to those of piroxicam. Treatment groups restored RBC and Hb levels. The Benzimidazole derivative exhibited significant anti-inflammatory and immunomodulatory effects by reducing paw edema, arthritic progression, and inflammatory markers (TNF-α, IL-6, and PGE2), demonstrating potential as a therapeutic agent for inflammatory conditions.

1. Introduction

Rheumatoid arthritis (RA) is a condition in which the immune system attacks one's bone cells, leading to the development of chronic inflammation owing to synovial hyperplasia followed by bone and cartilage destruction (Sattar et al., 2023). Globally, one percent of the population of adults is suffering in developed countries, with a yearly increase from 5 to 50 per 100,1000 (Sattar et al., 2023). Some other symptoms include loss of physical movement, stiffness, and disorders of the cardiovascular, pulmonary, physiological, and skeletal systems. A vital role for cytokines is played in the onset and course of rheumatoid arthritis (RA). Intracellular molecular signaling, initiated by the cytokines, IL-1β and TNF-α stimulates the mesenchymal cells, recruits innate and adaptive immune system cells, activates synoviocytes which further cause activation of many pro inflammatory markers such as interleukin-6 (IL-6), interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), causing an inflamed synovium, increased angiogenesis and decreased lymph angiogenesis. Treatment of RA aims at controlling pain and stopping or slowing further damage to the joints, as there is no cure for this condition. The mainstay of treatment for RA are non-steroidal anti-inflammatory drugs (NSAIDs). They limit the synthesis of prostaglandins, prostacyclin, and thromboxanes by inhibiting cyclo-oxygenase. Gastrointestinal bleeding, stomach discomfort, ulcers, and nausea are typical adverse effects. NSAIDs are less effective as anti-inflammatory drugs than corticosteroids, but corticosteroids have more adverse effects, such as immunosuppression, weight gain, diabetes, and bone weakening. Because of this, they should only be used briefly and at low doses. One of the most widely used medications for the management of rheumatoid arthritis is methotrexate. Similar to other disease-

modifying anti-rheumatic medications (DMARDs), methotrexate's potential side effects are a key drawback to its use. The new RA therapies have improved the disease course, and remission can be reached owing to an early diagnosis. The chances of remission increase due to early and rapid diagnosis and direct targeted approach. The recent and rapid increase in the identification of immune system targets like leukocyte surface antigens, cytokines, G-protein-coupled receptors (GPCRs), adhesion molecules, and co-stimulatory molecules have assured a steady supply of future anti-inflammatory therapeutic targets (Afnan et al., 2023).

Benzimidazole-based drugs have been developed as pain modulators and inflammation regulators with minimal adverse effects throughout the last decade. Benzimidazole is a significant heterocyclic pharmacophore that is made up of a benzene ring bonded with a 5-membered imidazole ring. It is sometimes referred to as 1H-benzimidazole and 1,3-benzodiazole. In heterocyclic chemistry, benzimidazole is recognized as a fortunate structure because of its connections to numerous biological processes. One research study has highlighted the role of the benzimidazole nucleus in specific biological activities, such as antitubercular, antitumor, pain reduction, anti-inflammatory, antibacterial, antiviral, ulcer healer, antihypertensive, and antidiabetic (Brishty et al., 2021). The majority of benzimidazoles have demonstrated anti-inflammatory activity against a range of target proteins (Veerasamy et al., 2021). Although a number of them have advanced to clinical trials, the majority of patents offer new treatment strategies for their intended illnesses. The substitutions seen in the majority of benzimidazole-based medications at positions 1 and 2 are probably significant for biological activity (Law and Yeong, 2021). Benzimidazole derivatives suppress inflammation by blocking the effect of cyclooxygenase (COX) enzyme, IL-6 and 5-lipoxygenase activating protein (Gaba et al., 2014). Benzimidazole derivatives disrupt fungal growth by targeting microtubules, preventing hyphal expansion and cell division. Beyond their antifungal role, they also aid in cancer treatment, regulate blood pressure, and function as tyrosine kinase inhibitors (Mohapatra and Ganguly, 2024). Benzimidazole derivatives offer a novel approach to arthritis treatment by targeting inflammation through unique pathways. Unlike traditional NSAIDs, they reduce arthritis symptoms by limiting lysosomal enzyme release from neutrophils instead of blocking COX enzymes (Bano, Nadeem et al. 2024). Some variants also inhibit IL-6, a key driver of rheumatoid arthritis, effectively easing symptoms in animal studies (Mishra et al., 2024).

In current study, benzimidazole derivative (BD) 2-((1H-benzo[d]imiadazol-2-yl) thio)-1-3, 5-diphenyl-1h-pyrazol-1-yl) ethenone was evaluated for its potential to reduce inflammation and arthritic inflammatory markers using Complete Freund′s Adjuvant (CFA) for induction of arthritic model and carrageenan induced paw edema model was used for evaluation of anti-inflammatory activity, respectively. Thus, our study sheds light on the possibility of benzimidazole derivatives as a new therapeutic strategy for this complex autoimmune disease.

2. Materials and Methods

2.1. Compound used

Benzimidazole derivative was prepared and obtained by Riphah University, Islamabad campus. Identification tests such as FTIR and mass spectroscopy were performed by the chemistry department of Riphah University, Islamabad Campus.

2.2. Experimental animal

The central animal house of the University of Lahore provided a total of 66 albino rats that weighed between 250-350 gm and were aged between 6-8 weeks. The male and female rats received standard feed and were kept in a controlled environment, such as humidity at 40-60%,12:12 light-dark cycle and a temperature range of between 24°C - 26°C in the Faculty of Pharmacy, University of Lahore. Thirty-six rats were used for the FCA-induced rat model, and 30 rats were used for the carrageenan-induced paw edema model. The guidelines that were followed by this experiment were permitted by the Institutional Research Ethics Committee (IREC-2022-09) for laboratory animals working in accordance with both national and international requirements at the Faculty of Pharmacy, University of Lahore. (Mashaal et al., 2023).

2.3. Determination of anti-arthritic potential of BD

2.3.1. Study Design

Thirty-six rats were divided into six groups, each group having six rats. At 0 day, CFA (0.15ml) was injected into the right hind paws of every rat for arthritis induction, excluding the vehicle control group. The treatment was started on day 7th and continued to day 28th. All treatments were given orally. On Day 28th, all rats were sacrificed using ketamine 87 mg/kg and 13 mg /kg xylazine for anesthesia (Tokimitsu et al., 2024). Vehicle control group I received two percent of Tween 80 dissolved in distilled water. Group II was the disease group that received no treatment. Group III was the reference group and received piroxicam (10 mg/kg b.w.) (Mobashar et al., 2022). Group-IV received 25 mg/kg; Group-V received 50 mg/kg and group-VI received 100 mg/kg of benzimidazole derivative (Ananta et al., 2024).

2.3.2. Evaluation of progression of arthritis

Parameters were visually observed on the 8th, 15th, 22nd and 28th days. Arthritic scoring was performed by observing inflammation of the paw, redness and swelling (macroscopic observation). Scores used to evaluate these characteristics were from 0 to 4 termed as normal, minimal, mild, moderate and severe, respectively. Score 0 = rat with normal walk and usual runs; 1 = rat shows difficulty in walking and running; 2 = rat limps with no withdrawal of hind paw; 3 = rat shows limping with retraction of hind paw (does not touch the hind paw on the floor) and 4 = rat only crawls or lies down.

Score 0: Rat walks and runs normally;
Score 1: Rat has difficulty walking and running;
Score 2: Rat limps but does not withdraw the hind paw;
Score 3: Rat limps and retracts the hind paw (hind paw does not touch the floor);
Score 4: Rat only crawls or remains lying down (Nazir, Ahmad et al. 2024).

2.3.3. Assessment of paw volume

After arthritic induction by CFA at day 0, paw edema was measured using a plethysmometer on every 7th day until sacrifice, i.e., days 7th, 14th, 21th, and 28th (Nazir et al., 2024).

2.3.4. Histopathological investigations

On the 28th day, the ankle joints of rats were cut and preserved in formalin. The joints were fixed in paraffin and then sliced into 5µm thin sections. These slides were assessed by a histopathologist for bone erosion, inflammation, and pannus formation (Mashaal et al., 2023).

2.4. Assesment of mRNA expression levels of TNF-α and Interleukin-6

Blood was utilized for RNA extraction using the TRIzol reagent. The RNA that was extracted was then measured through a spectrophotometer. A reverse transcriptase kit (Thermo Scientific, Waltham, USA) was used for cDNA synthesis. The qPCR was performed with a SYBR Green maximum qPCR kit (Thermo Scientific, Waltham, USA). GAPDH was utilized as the reference

gene. The relative expression was calculated using the 2-ΔΔCT technique. The primers were constructed based on previously published data (Mobashar et al., 2020; Dar et al., 2023).

2.4. 1. Determination of serum PGE2 level through ELISA

For the measurement of serum PGE_2 levels, Elab Science's ELISA kit protocol (E-EL-0034 96T) was used (Mobashar, Shabbir et al. 2020).

2.5. Assessment of Hematological profile

Blood samples were collected after cardiac puncture. Hb, RBCs, platelets and WBC counts were performed using an automated hematology analyzer (Sysmex XT-1800i) (Mashaal, Shabbir et al. 2023)..

2.6. Assessment of Biochemical profile

Urea and creatinine were measured using serum for assessing renal function, while ALT and AST were determined using an automated chemical analyzer to assess liver function (Humalyzer3500) (Mashaal, Shabbir et al. 2023).

2.7. Measurement of anti-inflammatory potential using carrageenan-induced paw edema

Rats were allocated to five groups, with six rats in each. Vehicle and piroxicam were provided to groups 1 and 5, whereas BD was given orally to group 2 (25 mg/kg), group 3 (50 mg/kg), and group 4 (100 mg/kg). After one hour of BD administration, 0.1ml of carrageenan (1 percent solution) was injected into the subplantar tissue of the right hind paw to induce edema. After injecting carrageenan, paw edema was assessed every five hours using a water displacement plethysmometer. Percentage inhibition was evaluated (Mobashar et al., 2019).

2.8. Statistical analysis

For data interpretation, Graph Pad Prism (V 6.0) was used. Values were obtained as mean ± S.E.M (standard error of mean). One-way ANOVA (one-way analysis of variance), for comparison between groups Tukey's post-hoc test with significance $p < 0.05$ was applied.

3. Results

3. 1. Effect of BD on arthritic Score

Arthritis development, characterized by swelling and erythema, was observed macroscopically following sub-plantar induction of CFA. Inflammatory cell infiltration, pannus development, and bon treatment with BD commenced from the 8th day and continued until the 28th day. The arthritic control group exhibited a substantial (P<0.001) increase in arthritis development compared to the vehicle control group starting from the 8th day. In contrast, the BD-treated groups demonstrated a significant reduction in arthritis on the 14th, 21st, and 28th days, respectively, as compared to the disease control group. On the 21st and 28th days, visual examination revealed a noteworthy reduction in arthritis-related swelling and redness in the groups provided with BD, as indicated in Figure 1.

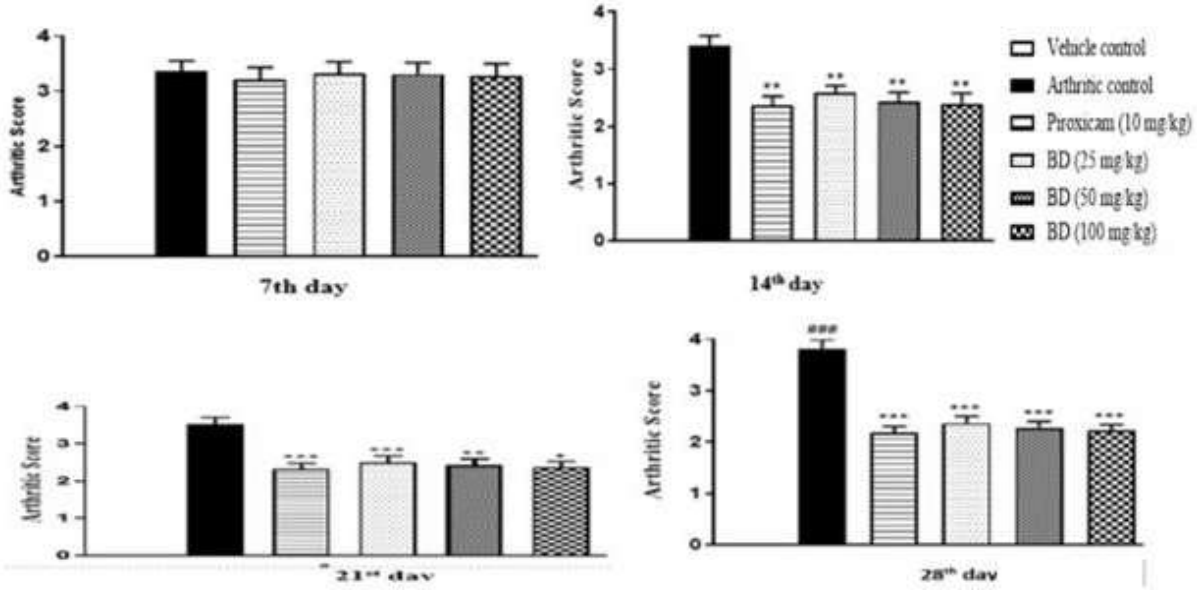

Figure 1. Arthritis scores were measured over 28 days in different treatment groups. The Benzimidazole derivative (BD) reduced arthritis severity in a dose-dependent manner, with effects comparable to piroxicam. Significant improvements were observed from Day 14 onward, with the greatest reduction by Day 28. Statistical significance is indicated ($p < 0.05$, $p < 0.01$, $p < 0.001$).

3.2. Effects of BD on paw edema

The data in Table 1 depicts a notable reduction ($p < 0.001$) in paw edema with standard piroxicam and BD treated groups as compared to the disease control group. The maximum reduction in paw edema was achieved on the 28th day of treatment. The value of vehicle control is considered zero as a normal paw has no swelling, inflammation or redness (Table 1).

3.3. Analysis of Histopathological Findings

Animals treated with CFA to induce arthritis showed maximum intrusion of inflammatory cells, along with pronounced pannus formation and bone erosion, in contrast to the vehicle control group, which exhibited no sign of inflammation. Rats treated with the standard drug piroxicam had a marked decrease in degradation (Table 2)

3.4. Effect of BD on TNF-α & IL-6 mRNA expression

Arthritic control group expressed a significant ($p < 0.001$) increase of TNF-α level to an average of 49.20 ±1.979 with respect to vehicle group (32.42±1.785) indicating arthritic control group exacerbated maximum inflammation. However, when treated with Piroxicam, a

well-known anti-inflammatory drug, the TNF-α levels were markedly reduced to 32.82 ± 1.044 ($p < 0.001$). Similarly, treatment with BD at different doses also significantly decreased TNF-α levels: BD 25 mg/kg reduced the levels to 34.96 ± 1.739, BD 50 mg/kg to 36.88 ± 0.782, and BD 100 mg/kg to 36.38 ± 0.571, all with a significance of $p < 0.001$ (Figure 2A). In addition to TNF-α, IL-6 values were also obtained. The arthritic control group showed an elevated IL-6 level of 39.19 ± 1.236. Treatment with Piroxicam significantly lowered IL-6 levels to 26.59 ± 0.8532 ($p < 0.001$). Similarly, BD treatment led to a notable reduction in IL-6 levels: BD 25 mg/kg reduced it to 35.61 ± 0.5859 ($p < 0.01$), BD 50 mg/kg to 31.70 ± 0.6461 ($p < 0.01$), and BD 100 mg/kg to 28.95 ± 0.7713 ($p < 0.01$) (Figure 2B).

Table 1. Paw edema increased in the Arthritic Control group over time, while treatment with Benzimidazole derivative (BD) and Piroxicam significantly reduced swelling from Day 14 onward.

Days	Vehicle Control	Arthritic Control	Piroxicam 10 mg/kg	BD 25 mg/kg	BD 50 mg/kg	BD 100 mg/kg
	Mean ± SEM (ml)					
Day 7	0	0.940 ± 0.010	0.959±0.012	0.950 ± 0.008	0.943 ±0.007	0.937 ± 0.008
Day 14	0	1.142 ± 0.018	0.780 ± 0.007***	0.778 ± 0.020***	0.775 ± 0.006***	0.770 ± 0.013***
Day 21	0	1.241 ± 0.014	0.717 ± 0.008***	0.752 ± 0.013***	0.748 ± 0.013***	0.740 ± 0.009***
Day 28	0	1.375 ± 0.008	0.626 ± 0.007***	0.678 ± 0.011***	0.660 ± 0.017***	0.640 ± 0.016***

The highest BD dose (100 mg/kg) showed the greatest effect, comparable to Piroxicam ($p < 0.001$).

Table 2. Histopathological parameters attenuated by BD.

Parameters	Disease control	Piroxicam (Standard control)	BD (25 mg/kg)	BD (50 mg/kg)	BD (100 mg/kg)
	Mean ± SEM				
Infiltration of inflammatory cells	2.584± 0.084	1.573 ± 0.084***	2.232 ± 0.084***	2.083 ± 0.084*	1.917 ± 0.154*
Pannus formation	3.416 ± 0.084	2.344 ± 0.105***	2.672 ± 0.084***	2.656 ± 0.105***	2.572 ± 0.084***
Bone erosion	2.580 ± 0.084	2.093 ± 0.084	2.5 ± 0.084	2.333 ± 0.116**	2.166 ± 0.084*

Data presented as Mean± SEM ***$P < 0.001$ indicates significance when compared with disease control group.

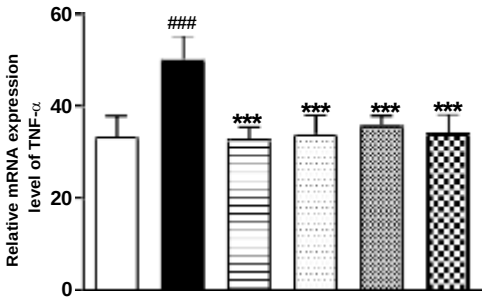

Figure 2A. Treatment with piroxicam, BD 25 mg/kg, BD 50 mg/kg, and BD 100 mg/kg decreased TNF-α and IL-6 levels. **$p < 0.01$ and ***$p < 0.001$, in contrast with the arthritic control group. ### shows the difference between two control groups.

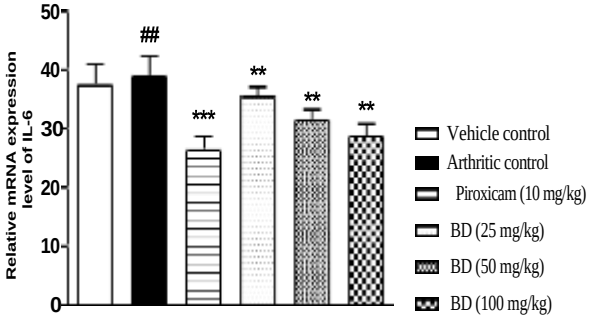

Figure 2B. BD significantly attenuated Levels of IL-6 levels in treatment groups. **$p < 0.01$ and ***$p < 0.001$, in contrast with the arthritic control group. ### shows the difference between the two control groups.

3.5. Effect of BD on PGE levels

The disease control group had an enhanced PGE2 level (0.987 ± 0.028) compared to the vehicle group. Considerably decreased PGE2 was seen in the standard (0.638 ± 0.032), BD 25 mg/kg (0.769 ± 0.055), 50 mg/kg (0.790 ± 0.045), and BD 100 mg/kg (0.847± 0.018) treated groups (Figure 3). Comparing the disease control group to the vehicle group, the study revealed a significant decline in RBC, hemoglobin (Hb), WBC (white blood cells), and platelet counts. Afterwards, the treatment with BD significantly increased blood cell counts and Hb counts to approaching normal levels. In a similar vein, BD treatment

assisted in returning WBC counts to normal and decreased the elevated platelet count observed in the disease control group (Figure 4).

3.6. BD effects on biochemical parameters

No significant differences were observed in the measured levels of urea, creatinine, AST, and ALT.

3.7. Effects of Pre-treatment with BD on carrageenan-induced paw edema

BD significantly reduced the inflammation in carrageenan-induced paw edema. The maximum inhibition was demonstrated by BD 100 mg/kg at the 5th hour (0.164 ± 0.004). Percentage inhibition was calculated (Table 3).

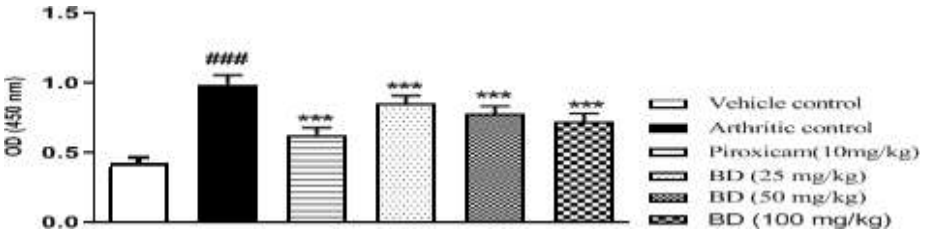

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Figure 3. Piroxicam, BD 25 mg/kg, BD 50 mg/kg and BD 100 mg/kg decreased prostaglandin E2 levels. ***p<0.001, as compared to arthritic control group. ### indicates a significant difference among control groups. OD is optical density, which shows the concentration of PGE2 in the sample's hematological parameters.

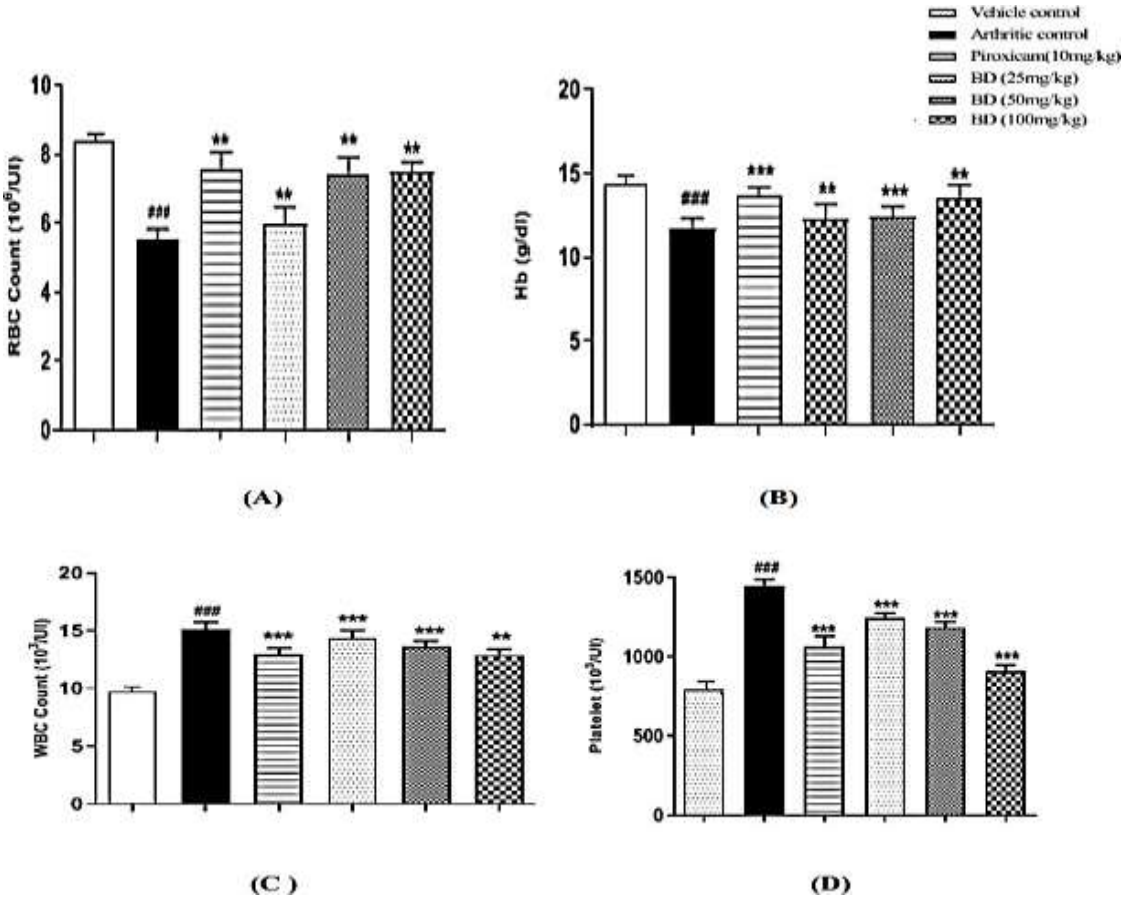

Figure 4. A. Shows that treated groups with BD significantly normalized RBC count in comparison to arthritic control. B. It shows that the BD-treated group significantly normalized Hb content compared to the arthritic control group. C. BD significantly decreased WBC count in comparison to arthritic control. D. shows BD significantly decreased Platelet count in comparison to arthritic control. **p < 0.01 and ***p < 0.001 as compared with disease control group. ### indicates a difference between the disease control group and the vehicle control group

Table 3. Significant reduction in edema was observed with BD Data presented as Mean ± SEM $^{***}P < 0.001$ indicates significance when compared with the disease control group.

Groups	Time (hour)				
	1	**2**	**3**	**4**	**5**
Disease control	0.277 ± 0.003 (11.45%)	0.295 ± 0.004 (28.55 %)	0.320 ± 0.008 (33.96 %)	0.339 ± 0.008 (46.17 %)	0.356 ± 0.009 (53.91 %)
Piroxicam (10 mg/kg)	0.207 ± 0.008*** (27.77%)	0.216 ± 0.003*** (32.74 %)	0.212 ± 0.004*** (11.94 %)	0.193 ± 0.003*** (23.52 %)	0.124 ± 0.004*** (34.07 %)
BD (25 mg/kg)	0.231 ± 0.006*** (21.39 %)	0.236 ± 0.005*** (13.89 %)	0.231 ± 0.006*** (27.35 %)	0.211 ± 0.007*** (38.23 %)	0.196 ± 0.009*** (45.53 %)
BD (50 mg/kg)	0.240 ± 0.003** (18.40 %)	0.220 ± 0.004*** (18.76 %)	0.207 ± 0.003*** (35.22 %)	0.194 ± 0.004*** (43.23 %)	0.174 ± 0.004*** (51.11 %)
BD (100 mg/kg)	0.211 ±0.004*** (27.08 %)	0.195 ± 0.002*** (24.72 %)	0.169 ± 0.004*** (45.91 %)	0.160 ± 0.004*** (54.11 %)	0.164 ± 0.004*** (65.08 %)

4. Discussion

The anti-inflammatory and immunomodulatory role of medicinal compounds in the treatment of chronic inflammatory conditions is well established (Veerasamy et al., 2021). Rheumatoid arthritis is characterized by the increased production of pro-inflammatory cytokines such as TNF-α, IL-6, and PGE2. Substances that decrease the release of pro-inflammatory cytokines have been pronounced important for the treatment of inflammatory disorders (Shabbir et al., 2016; Dar et al., 2023).

The benzimidazoles are a large chemical family used to treat nematode and trematode infections. They are biologically active and of significant importance in medicinal chemistry (32) and pharmacology due to their wide range of therapeutic effects, such as antiparasitic, antiulcer, antihypertensive, antihistaminic, anti-cancer, and antiemetic/antipsychotic (Brishty et al., 2021).

The FCA-induced rheumatoid arthritis model is most commonly utilized because it has a profile comparable to the actual arthritic condition, which is characterized by synovial hyperplasia, vascular formation, cartilage destruction, and severe bone erosion. Pannus development, which consists of macrophages and fibroblasts such as synoviocytes, is a significant feature of RA. TNF- and IL-6 are thought to be responsible for the erosion seen in rheumatic illnesses. BD significantly reduced bone degradation and pannus development in joints by downregulating pro-inflammatory.

In the therapy of RA, the primary and most important goal is to minimize inflammation induced by pro-inflammatory cytokines. TNF-α is the main cytokine involved in inflammatory diseases, and it promotes IL-6 in both a paracrine and autocrine manner. TNF-α stimulates and activates fibroblasts, which then release matrix degrading enzymes after bone resorption, destroying cartilage (Shabbir et al., 2016). These processes result in the establishment of the Th1 response, which is a activates additional cytokines such as IL-6 and TNF-α, as well as neutrophil activation. This study discovered that BD reduced TNF-α and IL-6 mRNA expression levels, which could have resulted in less bone degradation, inflammation, and pannus development (Ashiq et al., 2023).

Prostaglandin (PGE2) levels in the diseased control group were found to be high as compared to treatment groups. The interaction of PGE2 to prostaglandin receptors causes juxta-articular cartilage to erode (Shabbir et al., 2016). PGE2 suppression is associated with bone remodeling and RA inflammation suppression (Uttra et al., 2018). The substantial suppression of TNF-α, IL-6 and PGE2 in BD treated groups confirms its immunomodulatory role (Mobashar et al., 2020; Dar et al., 2024).

In the arthritic control group, Hb content and RBC counts were lower, indicating anemia in rats. Low plasma iron levels, which are inversely related to significantly higher IL-6 levels, might be associated with anemia (Song et al., 2013; Avau et al., 2014). In anemia, the bone marrow did not produce enough cells, and abnormal iron deposits in the synovial tissues were discovered (Shabbir et al., 2018). These altered hematological indicators were normalized by BD, which is consistent with the findings of the previous study (Akhtar and Shabbir, 2019). WBC and platelet count, on the other hand, were found to be higher, which could be due to IL-6. White blood cell levels and platelet counts were restored after treatment with BD. Elevated levels of ALP may lead to bone deterioration and mineralization (Shabbir et al., 2016). In the treatment groups, both BD and piroxicam dramatically lowered ALP levels. We measured numerous biochemical markers such as urea, creatinine, ALT, and AST levels to determine the safety profile of BD. The findings revealed that there was no significant difference between the groups, supporting BD's safety in terms of hepatic and kidney parameters.

An investigation of BD's anti-inflammatory activities was performed using carrageenan as a phlogistic agent in a carrageenan-induced paw edema model. The role of autacoids as an anti-inflammatory mediator was studied using a well-known animal model to clarify the multifaceted events such as carrageenan-induced edema. The release of histamine, serotonin, and kinin was observed in the initial phase (1-2 hours). In contrast, the release of prostaglandin and bradykinins was observed in the second phase (Mobashar et al., 2022). BD inhibited paw edema at the first stage and prevented till 5th hour, indicating its

effectiveness in the second stage. The findings of the reported literature were used to validate the results (Mobashar et al., 2019).

The toxicity of benzimidazole derivatives varies based on their chemical structure. Research indicates that RU-891, a benzimidazole compound, has a minimum toxic dose (TDmin) ranging from 230 mg/kg to 460 mg/kg in mice when administered orally (Spasov et al., 2021). Another derivative, labeled as 8d, showed an LD50 of 500 mg/kg in mice, highlighting the need for individual toxicity assessments (Lee et al., 2023). These studies emphasize the careful evaluation of each compound's safety profile.

Furthermore, its favorable safety profile at different doses in this study, as indicated by the restoration of RBC and Hb levels and stable biochemical markers (AST, ALT, urea, and creatinine), suggests reduced systemic toxicity. The combination of benzimidazole and pyrazole moieties contributes to its unique mechanism of action, potentially offering a safer and more effective alternative for managing chronic inflammatory diseases. Further exploration of its molecular interactions could establish it as a novel therapeutic candidate with enhanced efficacy and safety.

5. Conclusion

This study establishes the benzimidazole derivative's efficacy in inflammatory and autoimmune diseases. The ability of BD to reduce joint inflammation in both acutr and late chronic models demonstrated that it had considerable immunomodulatory and anti-inflammatory potential. The reduction in RA symptoms could be attributable to a decline in pro-inflammatory markers such as TNF-α and IL-6. Furthermore, after treatment with BD, PGE2 levels were found to be lower. Among the possible reasons for the anti-inflammatory effect is autacoid suppression, as evident by the carrageenan model of acute inflammation. Further studies are needed to explore BD's long-term safety, optimal dosage, and bioavailability in clinical settings.

Author's contributions

Conceptualization and original draft writing, Muftiah Arubah, Aisha Mobashar, and Khalid Hussain; Editing and formatting Komal Najam, Zeeshan Akbar, and Kashif Barkat; Reviewing and data validation: Humaira Nadeem, Hafiz Aamir Ali Kharl

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References

Afnan, A., Saleem, A., & Akhtar, M. F. (2023). Chrysin, a 5,7-dihydroxyflavone, restrains inflammatory arthritis in rats via subsiding oxidative stress biomarkers and inflammatory cytokines. *Inflammopharmacology* 31, 1863–1878. doi: 10.1007/s10787-023-01229-6

Akhtar, G., & Shabbir, A. (2019). Urginea indica attenuated rheumatoid arthritis and inflammatory paw edema in diverse animal models of acute and chronic inflammation. *J. Ethnopharmacol* 238:111864. doi: 10.1016/j.jep.2019.111864

Ananta, M. F., Saha, P., Spriha, S. E., Rahman S. A., and Chowdhury A. A. (2024). Analgesic, Anti-inflammatory and Antidiarrheal Properties of Synthesized Benzimidazole Derivatives via In vivo and In silico Methods. *Dhaka Univ. J. Pharm. Sci.* 23, 157-169. doi: 10.3329/dujps.v23i2.78573

Ashiq, K., Ashiq, S., Mobashar, A., Abid, F., Yasmeen, A., Shehzadi, N., el al. (2023). An updated review on rheumatoid arthritis (RA): Epidemiology, pathophysiology, diagnosis, and the current approaches for its treatment. *Sudan J. Med. Sci.* 18, 539–551. doi:10.18502/sjms.v18i4.14742

Avau, A., Mitera, T., Put, S., Put, K., Brisse, E., Filtjens, J., et al. (2014). Systemic juvenile idiopathic arthritis–like syndrome in mice following stimulation of the immune system with Freund's complete adjuvant: regulation by interferon-γ. *Arthritis Rheumatolo* 66, 1340-1351.doi: 10.1002/art.38359

Bano, S., Nadeem, H., Zulfiqar, I., Shahzadi, T., Anwar, T., Bukhari, A., et al. (2024). Synthesis and anti-inflammatory activity of benzimidazole derivatives; an in vitro, in vivo and in silico approach. *Heliyon* 10, e30102. doi: 10.18502/sjms.v18i4.14742

Brishty, S. R., Hossain, M. J., Khandaker, M. U., Faruque, M. R. I., Osman, H., and Rahman, S. A. (2021). A comprehensive account on recent progress in pharmacological activities of benzimidazole derivatives. *Front. Pharmacol.* 12, 762807. doi: 10.3389/fphar.2021.762807

Dar, E., Mobashar, A., Shabbir, A., Mushtaq, M. N., Anjum, I., Gaafar, A. R., et al. (2023). Mechanistic evaluation of antiarthritic effects of citronellol in CFA-induced arthritic rats. *ACS omega* 8, 44955-44963. doi: 10.1021/acsomega.3c06374

Gaba, M., Singh, S., and Mohan, C. (2014). Benzimidazole: an emerging scaffold for analgesic and anti-inflammatory agents. *Eur. J. Med. Chem.* 76, 494–505. doi: 10.1016/j.ejmech.2014.01.030.

Law, C. S., and Yeong, K. Y. (2021). Benzimidazoles in drug discovery: a patent review. *ChemMedChem* 16, 1861-1877. doi: 10.1002/cmdc.202100004

Lee, Y. T., Tan, Y. J., and Oon, C. E. (2023). Benzimidazole and its derivatives as cancer therapeutics: The potential role from traditional to precision medicine. *Acta Pharm. Sin. B.* 13, 478–497. doi: 10.1016/j.apsb.2022.09.010

Mashaal, K., Shabbir, A., Khan, M. A., Hameed, H., Shahzad, M., Irfan, A., et al. (2023). Anti-arthritic and immunomodulatory potential of methanolic, n-hexane, and ethyl acetate fractions of bark of *Acacia modesta* on complete Freund's adjuvant-induced arthritis in rats. *Pharm.* 15, 2228. doi: 10.3390/pharmaceutics15092228

Mishra, S., Gupta, S., Kaur, S., Bansal, Y., and Bansal, G. (2024). Design, Synthesis and Evaluation of Benzimidazole Derivatives as IL-6 Inhibitors and Their Role in Rheumatoid Arthritis. *Chem. Biol. Drug Des.* 104, e70008. doi: 10.1111/cbdd.70008

Mobashar, A., Shabbir, A., and Hassan, S. U. (2019). Anti-inflammatory effects of *Trigonella gharuensis*: Comparative analysis in BALB/C mouse model. *Pak. J. Pharm. Sci.* 32, 2287–2293. doi: 142.54.178.187:9060/xmlui/handle/123456789/14646

Mobashar, A., Shabbir, A., and Shahzad, M. (2020). Evaluation of immunomodulatory and antiarthritic potential of *Trigonella gharuensis* extracts. *Evid.-Based Complement. Altern. Med.* 2020, 8836080. doi: 10.1155/2020/8836080

Mobashar, A., Shabbir, A., Shahzad M., and Gobe, G. (2022). Preclinical Rodent Models of Arthritis and Acute Inflammation Indicate Immunomodulatory and Anti-Inflammatory Properties of *Juglans regia* Extracts. *Evid.-Based Complement. Altern. Med.* 2022, 1695701. doi: 10.1155/2022/1695701

Mohapatra, T. R. and Ganguly, S. (2024). The recent development of benzimidazole derivative as a promising pharmacological scaffold. J. Indian Chem. Soc. 101, 101237. doi: 10.1016/j.rechem.2024.101911

Nazir, S., Ahmad, I., Mobashar, A., Sharif, A., Shabbir, A., and Chaudhary, W. A. (2024). Mechanistic evaluation of antiarthritic and anti-inflammatory effect of campesterol ester derivatives in complete Freund's adjuvant-induced arthritic rats. *Front. Pharmacol.* 14, 1346054. doi: 10.3389/fphar.2023.1346054

Sattar, S., A. Shabbir, M. Shahzad, T. Akhtar, A. Ahmad, S. M. Alnasser, B. et al. (2023). *Eichhornia crassipes* Ameliorated Rheumatoid Arthritis by Modulating Inflammatory Cytokines and

Metalloproteinase Enzymes in a Rat Model. *Medicina* 59, 1594. doi: 10.3390/medicina59091594.

Shabbir, A., Batool, S. A., Basheer, M. I., Shahzad, M., Sultana, K., Tareen, R. B., et al. (2018). *Ziziphora clinopodioides* ameliorated rheumatoid arthritis and inflammatory paw edema in different models of acute and chronic inflammation. *Biomed. pharmacother.* 97, 1710-1721. doi: 10.1016/j.biopha.2017.11.118

Shabbir, A., Shahzad, M., Ali A., and Zia-ur-Rehman, M. (2016). Discovery of new benzothiazine derivative as modulator of pro- and anti-inflammatory cytokines in rheumatoid arthritis. *Inflammation* 39, 1918-1929. doi: 10.1007/s10753-016-0427-y

Song, S. N. J., Iwahashi, M., Tomosugi, N., Uno, K., Yamana, J., Yamana, S., et al. (2013). Comparative evaluation of the effects of treatment with tocilizumab and TNF-α inhibitors on serum hepcidin, anemia response and disease activity in rheumatoid arthritis patients. *Arthritis Res. Ther.* 15, R141. doi: 10.1186/ar4323.

Spasov, A. A., Kucheryavenko, A. F., Maltsev, D., Miroshnikov, M.V., Sirotenko, V. S., Sultanova, K. T., et al. (2021). Acute toxicity of a new benzimidazole derivative with antithrombogenic properties. *Toxicol. Rev.* 1, 52–57. doi: 10.36946/0869-7922-2021-1-52-57

Tokimitsu, K., Yachida, M., Miki, W., Kuramoto, T., Mochizuki, N., Yoshida, S., et al. (2024). Clinical Consideration in Total Intravenous Anesthesia Using Xylazine and Propofol in Calves. *Thai J. Vet. Med.* 54, 1-5.

Uttra, A. M., Shahzad, M., Shabbir, A., and Jahan, S. (2018). Ephedra gerardiana aqueous ethanolic extract and fractions attenuate Freund Complete Adjuvant induced arthritis in Sprague Dawley rats by downregulating PGE2, COX2, IL-1β, IL-6, TNF-α, NF-kB and upregulating IL-4 and IL-10. *J. Ethnopharmacol.* 224, 482–496. doi: 10.1007/s10787-023-01229-6.

Veerasamy, R., Roy, A., Karunakaran, R., Rajak, H (2021). Structure–activity relationship analysis of benzimidazoles as emerging anti-inflammatory agents: An overview. *Pharmaceuticals* 14, 663. doi: 10.3390/ph14070663.