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Evaluation of Anti-Arthritic Activity of Proton Pump Inhibitors in Freund's Adjuvant Induced Arthritic Rats

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Abstract

Background: Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by joint inflammation and systemic manifestations. Although non-steroidal anti-inflammatory drugs (NSAIDs) like diclofenac sodium are standard treatments, their adverse effects, particularly gastrointestinal toxicity, limit long-term use. Omeprazole, primarily used as a proton pump inhibitor, has been shown to exhibit anti-inflammatory effects in various preclinical models. Aim of the study was to evaluate the anti-arthritic activity of Omeprazole at different doses in comparison with Diclofenac sodium in a rat model of Complete Freund's Adjuvant (CFA)-induced arthritis. ***Materials and Methods:*** The study was conducted on 30 Wistar albino rats (150–250 g), divided into five groups (n=6 each). Arthritis was induced by intra-plantar injection of 0.1 ml CFA in the left hind paw. Treatment was given for 21 days as follows: Group I (control – distilled water), Group II (diclofenac sodium 10 mg/kg), Groups III-V (omeprazole 10, 20, and 30 mg/kg, respectively). Paw edema was measured using a digital plethysmometer on days 0, 7, 14, and 21. Hematological and serological parameters (Hb, WBC, RBC, SRF, and CRP) were assessed, and arthritis severity was evaluated by arthritis score and pain index. ***Results:*** Omeprazole reduced paw edema in a dose-dependent manner, with the 30 mg/kg group showing 54% inhibition by day 21, comparable to 60% in the diclofenac group. Hematological improvements, including increased Hb and RBC and reduced WBC levels, were noted at higher doses. Serological markers (SRF and CRP) significantly decreased in the 30 mg/kg Omeprazole group. Arthritis scores were also markedly reduced at higher Omeprazole doses. ***Conclusion:*** Omeprazole exhibited significant anti-arthritic activity in CFA-induced arthritis in rats, particularly at 30 mg/kg. These effects may be attributed to its anti-inflammatory and immunomodulatory properties. Omeprazole may represent a potential alternative or adjunct in arthritis management, especially where NSAID use is contraindicated.

Keywords: Rheumatoid arthritis, Omeprazole, Anti-arthritic activity, Complete Freund's Adjuvant (CFA), Diclofenac sodium, Hematological parameters, C-reactive protein, Rheumatoid factor, Wistar rats.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, progressive, and systemic inflammatory disease, characterized by synovial proliferation and joint erosions (1). Nonsteroidal anti-inflammatory drugs (NSAIDs) are used as an important part of therapeutic regime to suppress the pain and inflammation associated with RA (2). Although NSAIDs are very effective in minimizing RA-associated symptoms, its beneficial effect is strongly associated with severe side effects such as gastrointestinal complications, renal failure, and hepatic toxicity (3). Although the etiology underlying RA remains unknown, it is clear that inflammatory cytokine circuits are established in the synovial cells lining the joint in genetically susceptible individuals. The inflammatory process in RA initially affects the synovial membrane lining but can affect other organs also. The inflamed synovium leads to the aggressive cartilage destruction and progressive bony erosions. The disease is often progressive and results in pain, stiffness, and swelling of joints. In late stage

deformity and ankylosis develop (4). The modern drugs both steroidal and nonsteroidal anti-inflammatory drugs (NSAIDS) and disease modifying antirheumatic drugs (DMARDS) are used for the amelioration of the symptoms of the disease; however, they offer only temporary relief and also produce adverse effects (5). They are having important side effect i.e. gastric irritation and to avoidit proton pump inhibitors and other drugs like H2 blockers are addedin treatment.

Proton pump inhibitors (PPIs) are substituted benzimidazoles that block gastric acid secretion by inhibiting H+, K+ATPase the proton pump of the gastric parietal cell (6). They are the most effective anti-secretory agents available, and they are among the most commonly used medications in the world (7). They are widely regarded as the agents of choice for the treatment of acid-peptic disorders including gastric ulcer, duodenal ulcer, and gastroesophageal reflux disease (GERD). For patients with upper gastrointestinal symptoms of uncertain etiology, such as non-ulcer dyspepsia, improvement with PPI therapy is considered prima facie evidence of a pathogenetic role for acid-peptic disease.

In animal studies on certain non-peptic disorders however, PPIs have been found to have beneficial effects that cannot be explained by a reduction in gastric acid secretion. In rats, for example, lansoprazole has been found to protect against ischemia-reperfusion injury of the bowel and against indomethacin-induced injury of the distal small intestine (8). A beneficial effect of PPIs has even been described for the colon of a patient with ulcerative colitis (9).

Recent studies have elucidated a number of mechanisms whereby PPIs can exert anti-inflammatory effects unrelated to the inhibition of gastric acid production (10). Those anti-inflammatory properties of the PPIs, which have nothing to do with their effects on parietal cells, may contribute to the beneficial clinical actions of the PPIs, even in acid-peptic disorders. But their anti-inflammatory and anti-arthritic effect in rheumatoid arthritis was not yet reported.

The present study was aim to evaluate the potential of omeprazole in producing anti-arthritic effects using the Complete Freund's Adjuvant (CFA) model of rheumatoid arthritis in albino rats. Specifically, the study is designed to assess the anti-arthritic activity of omeprazole in CFA-induced arthritis and to compare its anti-inflammatory and anti-arthritic effects with the standard drug diclofenac sodium.

MATERIALS AND METHODS

The study was carried out in the post graduate research laboratory, Department of Pharmacology, Navodaya medical college, Raichur. Study is conducted as per CPCSEA guidelines. In the present study omeprazole was screened primarily for its anti-arthritic activity. The results obtained were compared with control and also with known standard agent, Diclofenac sodium. The materials used and the methods adopted during the present investigation are being described briefly.

Animals

Animals used in the present experiment were wistar albino rats weighing 150 – 250 g of either sex, obtained from National Institute of Nutrition, Hyderabad, maintained at central animal house, Navodaya Medical College, Raichur under suitable conditions of housing, temperature, ventilation and nutrition. Ethical clearance from the Institutional Animal Ethical Committee was obtained. All the drugs were administered orally with the help of a sterile, nontoxic tube made up of polyvinyl chloride.

Chemicals

Complete freund's agent (CFA) :

1) CFA, most frequently used arthritic agents as 1% suspension in normal saline was used to induce arthritis in left hind paw. Not more than 0.1 ml was administered by intra plantar route.CFA was obtained from SIGMA – ALDRICH.
2) Drugs: Omeprazole and Diclofenac Sodium were obtained frompharmaceutical company.
3) Distilled water: used as vehicle.

Induction of Arthritis:

Arthritis will be induced in rats by the intra plantar injection of 0.1 ml of CFA in the left hind paw. The adjuvant contained heat killed Mycobacterium tuberculosis in sterile paraffin oil (10 mg/ml).

To induce arthritis 30 rats will be injected with 0.1 ml of complete freund's adjuvant on their left hind paw. These rats then will be divided into 5 groups.

Duration of treatment is 21 days. Each group will receive different treatment as follows:

Group 1: Control - Receive 2ml of distilled water daily

Group 2: Standard - Receive diclofenac sodium 10 mg/kg/day orally

Group 3: Omeprazole 10 mg/kg orally

Group 4: Omeprazole 20 mg/kg orally

Group 5: Omeprazole 30 mg/kg orally

Edema formation in the injected hind paw peaked at 3-5 days after injection of the CFA and is measured by calculating percent inhibition of the edema volume of the injected paw .

$$\text{Percentage inhibition} = \frac{Vc - Vt}{Vc} \times 100.$$

Where

Vc = Volume of paw edema in control animals.

Vt = Volume of paw edema in treated animals.

Secondary lesions are immunologically mediated changes characterized by inflammation of the non injected sites. Changes in Primary (lesions in adjuvant injected paw) and secondary (non injected paw) lesions are assessed by using digital Plethysmometer (Marsap) before and on 7th, 15th and 21st day post adjuvant injection.

Arthritis evaluation:

1. Primary and Secondary Lesions :
   Primary lesion refers to the edema formation in the injected hind paw that peaked 3-5 days after injection of the phlogistic agent and was measured on day 5 by calculating percent inhibition of the edema volume of the injected paw . Secondary lesions are immunologically mediated changes characterized by inflammation of the non injected sites (hind leg, forepaws, ears, nose and tail) Changes in Primary (lesions in adjuvant injected paw) and secondary (non injected paw) lesions were assessed by using digital Plethysmometer (Marsap) before and on 7th ,15th and 21st day post adjuvant injection. Body weight was taken every 3rd day after adjuvant injection till 21st day.
2. Hematological parameters :
   Changes in blood parameters which have been included in this study are associated with inflammatory conditions. The blood samples were collected by retro-orbital puncture of anaesthetized rats. Hematological parameters (RBC, WBC, and Hb) were determined using automated cell counter. Blood was centrifuged at 3500 rpm for 20 minutes and serum was separated for estimation of serological parameters like serum rheumatoid factor (SRF) and C- reactive protein (CRP) estimated.205
3. Severity inflammation and pain in arthritis:
   Severity of inflammation was assessed by arthritis index given by Colpert.
   Pain associated with arthritis was assessed by pain scoring system mentioned by Kalpesh .Patil et. al. 207

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using SPSS 11.0 software. The results of anti-arthritic activity were expressed as "mean ±SD" of paw volume and haematological parameters. Analysis of variance (one way ANOVA) was followed by Dunnett s t-test for control, standard and test group comparisons were used for statistical evaluation. P values <0.05 were considered as significant.

RESULTS

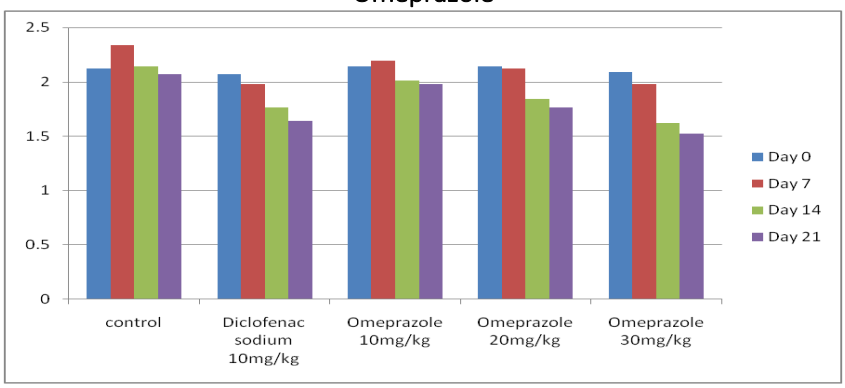
Table 1: Effect of Omeprazole on Paw Edema in Complete Freund's Adjuvant-Induced Arthritis in Rats Comparison with Diclofenac Sodium

Treatment Group (Dose)	Day 0	Day 7	Day 14	Day 21	P value	F value
Control (D/W 2 ml/kg)	2.12±0.34	2.34±0.35	2.14±0.33	2.07±0.37	0.97	0.11
Diclofenac sodium (10 mg/kg)	2.07±0.19	1.98±0.19	1.76±0.16	1.64±0.18	0.05	2.74
Omeprazole (10 mg/kg)	2.1Â±0.2	2.19±0.19	2.01±0.21	1.98±0.21	0.003	5.097
Omeprazole (20 mg/kg)	2.14±0.19	2.12±0.17	1.84±0.19	1.76±0.09	0.0008	6.75
Omeprazole (30 mg/kg)	2.09±0.17	1.98±0.17	1.62±0.18	1.52±0.11		

The table 1 presents the mean paw volume (edema) measurements in milliliters (± standard deviation) recorded on Days 0, 7, 14, and 21 in rats with Complete Freund's Adjuvant (CFA)-induced arthritis. Five groups were studied: a control group receiving distilled water, a standard group treated with diclofenac sodium (10 mg/kg), and three test groups receiving omeprazole at doses of 10, 20, and 30 mg/kg. Initially, all groups showed similar paw volumes. Over time, the diclofenac and higher-dose omeprazole groups (especially 30 mg/kg)

demonstrated a significant reduction in paw edema, indicating anti-inflammatory and anti-arthritic effects. This is supported by one-way ANOVA analysis, which showed statistically significant differences among groups on Days 14 ($p = 0.003$) and 21 ($p = 0.0008$), suggesting that omeprazole, particularly at higher doses, may have potential in reducing inflammation associated with arthritis.

Figure 1: Percentage Inhibition of Paw Edema in Rats Treated with Diclofenac and Different Doses of Omeprazole



The Figure 1 presents the percentage inhibition of paw volume edema in arthritic rats treated with Diclofenac sodium and varying doses of Omeprazole (10, 20, and 30 mg/kg) over a 21-day period. Diclofenac, a standard anti-inflammatory drug, showed the highest inhibition across all time points, peaking at 60% by day 21. Omeprazole demonstrated a dose-dependent response, with higher doses yielding greater anti-edematous effects. By day 21, Omeprazole at 30 mg/kg achieved 54% inhibition, closely approaching the standard drug, indicating its potential anti-arthritic effect at higher doses.

Table 2: Evaluation of Hematological Parameters in CFA-Induced Arthritic Rats Treated with Omeprazole

Groups	Evaluation of Hb levels µg/ml (Mean ± SEM)	Evaluation of WBC levels×10^6/mm^3 (Mean ± SEM)	Evaluation of RBC levels×10^6/mm^3 (Mean ± SEM)
Control (n=6)	9.23±0.23	11.98±0.23	3.85±0.33
Diclofenacsodium 10mg/kg (n=6)	12.22±0.12	6.56±0.28	6.06±0.43
Omeprazole 10mg/kg (n=6)	9.89±0.28	11.58±0.32	4.01±0.26
Omeprazole 20mg/kg (n=6)	11.23±0.52	7.86±0.26	5.46±0.32
Omeprazole 30mg/kg (n=6)	11.56±0.19	8.98±0.3	6.12±0.26

This table 2 summarizes the effect of Omeprazole at varying doses (10, 20, and 30 mg/kg) on hematological parameters in rats with Complete Freund's Adjuvant (CFA)-induced arthritis. Compared to the control group, arthritic rats treated with Diclofenac sodium showed a marked improvement in hemoglobin (Hb), white blood cell (WBC), and red blood cell (RBC) levels. Omeprazole-treated groups exhibited a dose-dependent improvement in all three parameters. Notably, the 30 mg/kg Omeprazole group showed hemoglobin and RBC levels close to the Diclofenac group, indicating potential hematological protective effects in arthritis.

Table 3: Evaluation of Serum Rheumatoid Factor (SRF) and C-Reactive Protein (CRP) Levels in CFA-Induced Arthritic Rats Treated with Omeprazole

Groups	Evaluation of SRF levels IU/ml (Mean±SEM)	Evaluation of CRP levels in µg/ml (Mean±SEM)
Control (n=6)	41.97±1.26	427.4±2.21
Diclofenacsodium10mg/kg (n=6)	29.33±2.14	178.62±1.72
Omeprazole10mg/kg (n=6)	40.34±1.34	410.34±2.02
Omeprazole20mg/kg (n=6)	37.24±2.2	388.56±2.02
Omeprazole30mg/kg (n=6)	32.43±1.2	247.86±1.93

The table 3 presents the serum levels of SRF and CRP, which are key inflammatory markers, in CFA-induced arthritic rats treated with Diclofenac and different doses of Omeprazole. Diclofenac significantly reduced both SRF and CRP levels compared to the control group. Omeprazole showed a dose-dependent decrease in these inflammatory markers, with the 30 mg/kg dose showing the most notable reduction (SRF: 32.43 IU/ml, CRP: 247.86 µg/ml), suggesting that higher doses of Omeprazole may exert moderate anti-inflammatory effects similar to standard treatment.

Title 4: Arthritis Score Evaluation in CFA-Induced Arthritic Rats Treated with Omeprazole

Day of study	Control (n=6) (Mean±SEM)	Diclofenac sodium 10mg/kg (n=6) (Mean±SEM)	Omeprazole 10mg/kg (n=6) (Mean±SEM)	Omeprazole 20mg/kg (n=6) (Mean±SEM)	Omeprazole 30mg/kg (n=6) (Mean±SEM)
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
14	6.67±0.33	6.83±0.17	7.43±0.19	7.12±0.26	6.98±0.17
21	8.50±0.22	2.67±0.33	5.6±00.17	4.2±0.19	3.2±0.27

The table 4 shows arthritis scores recorded on days 0, 14, and 21 in rats with CFA-induced arthritis treated with Diclofenac sodium and different doses of Omeprazole. On day 14, all groups except control showed elevated scores due to arthritis induction. By day 21, Diclofenac significantly reduced the score to 2.67, reflecting strong anti-arthritic activity. Omeprazole also reduced arthritis severity in a dose-dependent manner, with the 30 mg/kg dose lowering the score to 3.2, suggesting a beneficial effect, though less potent than Diclofenac. The control group continued to show worsening of symptoms, reaching a score of 8.5.

DISCUSSION

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic joint inflammation, synovial hyperplasia, and progressive cartilage and bone destruction. Among several experimental models, Complete Freund's Adjuvant (CFA)-induced arthritis in rats is widely accepted as a standard model due to its close resemblance to human RA, both in immunopathogenesis and clinical features (11).

In this study, CFA administration resulted in the development of pronounced paw edema, arthritis scores, and histological changes resembling chronic polyarthritis. These were associated with significant alterations in hematological and serological markers such as increased white blood cell (WBC) count, decreased hemoglobin (Hb) and red blood cell (RBC) levels, and elevated serum rheumatoid factor (SRF) and C-reactive protein (CRP) levels. These findings reflect systemic inflammation and immune activation, consistent with reports in arthritic models (12).

Omeprazole, commonly known as a proton pump inhibitor, was evaluated for its potential anti-arthritic effects at doses of 10, 20, and 30 mg/kg. The results demonstrated a dose-dependent reduction in paw edema, with the 30 mg/kg group showing a 54% inhibition on day 21 comparable to the 60% observed with Diclofenac sodium (10 mg/kg), a known NSAID standard. The arthritis scores also showed significant improvement in the Omeprazole 30 mg/kg group (score reduced to 3.2 on day 21), suggesting a notable anti-inflammatory effect.

Hematological evaluation showed that higher doses of Omeprazole helped reverse CFA-induced anemia and leukocytosis. Improved Hb and RBC levels, along with reduced WBC count in the 20 and 30 mg/kg groups, suggest a restoration of hematological balance, potentially due to Omeprazole's anti-inflammatory or immunomodulatory properties.

Serological analysis revealed that Omeprazole significantly reduced CRP and SRF levels in a dose-dependent manner, with the 30 mg/kg group showing values closer to those treated with Diclofenac sodium. These reductions suggest that Omeprazole can suppress systemic inflammatory mediators, further supporting its anti-arthritic action.

The exact mechanism underlying the anti-arthritic activity of Omeprazole is not fully defined, but several preclinical studies suggest that Omeprazole can modulate immune responses and reduce inflammation by suppressing pro-inflammatory

cytokines such as TNF-α, IL-1β, and IL-6, and by inducing the cytoprotective enzyme heme oxygenase-1 (HO-1) (13, 14). These effects have been shown to reduce oxidative stress and cytokine-induced joint damage in various inflammatory conditions.

Although Omeprazole is primarily used as a gastroprotective agent, accumulating evidence supports its role in modulating inflammatory pathways, which may account for the observed therapeutic effects in this study (15). Compared to Diclofenac, Omeprazole did not demonstrate superior efficacy but showed comparable anti-arthritic potential at higher doses, with possibly fewer gastrointestinal side effects.

CONCLUSION

In conclusion, Omeprazole exhibited significant anti-arthritic activity in CFA-induced arthritis in rats, particularly at 30 mg/kg. Its ability to reduce paw edema, improve arthritis score, correct hematological alterations, and suppress inflammatory markers suggests a promising therapeutic potential, warranting further mechanistic and clinical investigations. Future studies should include molecular pathway analysis, longer treatment durations, and comparative toxicity evaluations to confirm its safety and efficacy in arthritis management.

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