

ERDOSTEINE: AN EFFECTIVE ANTIOXIDANT FOR PROTECTING COMPLETE FREUND'S ADJUVANT INDUCED ARTHRITIS IN RATS

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ABSTRACT

Objective: The objective of this study was to evaluate the protective effect of Erdosteine on complete Freund's adjuvant (CFA) induced arthritic rats.

Methods: Wistar Albino rats of 100–250 g were divided into five groups (n=6) and administered with 0.1 ml of CFA subcutaneously into the left hind paw except the negative control group. The standard group received methotrexate (MTX) 0.075 mg/kg body weight orally. Besides, the test groups received Erdosteine orally at a dose 10 mg/kg and 20 mg/kg bodyweight for 12 days. The changes in body weight, paw volume, hematological parameters, radiographical, and histological findings were the indicators to evaluate the efficacy of the test product.

Discussion: Significant change in the body weight, paw volume, radiographical, hematological, and histological parameters were observed which supports the remarkable reduction of the arthritic development in the standard and test groups compared to the untreated group. However, the test group (Erdosteine) with the dose 20 mg/kg shows to be more potent than the test group (Erdosteine) with a dose 10 mg/kg and the standard group (MTX) to reduce the arthritic effect.

Results: The test group with 20 mg/kg Erdosteine showed much better outcome than the standard group at significant (p<0.05). Therefore, Erdosteine acting as an anti-inflammatory and anti-oxidant is effective at a dose 20 mg/kg in treating the progression of rheumatoid arthritis in rats.

Keywords: Erdosteine, Arthritis, CFA, Methotrexate.

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects a large number of people throughout the world [1]. It is characterized by angiogenesis and micro-vascular lesions leading to the infiltration of a variety of inflammatory cells into the joint causing swelling, stiffness, and pain which finally leads to substantial loss of functioning and mobility in its advanced stages. The synovial membrane becomes highly vascularized, synovial fibroblasts proliferate, and inflammatory cells release numerous cytokines and growth factors into the joint [2]. The prevalence of RA varies between 0.3% and 1% worldwide and is more common in developed countries. In India, the prevalence of RA was reported to be 0.75% in the adult population [3]. It is rare in men under 30 years and prevalent in the geriatric population. In women, the incidence rises progressively from about 25 years of age to a broad peak during 45–75 years [4].

It is well established now that free radicals/reactive oxygen species play an important role in inflammation [5]. Control of inflammation is the key to slow down or prevent disease progression as well as manage symptoms of RA. Thus, it is an approach to treat RA by combating those reactive oxygen species with a compound having potent antioxidant activity along with traditional anti-arthritis effect [6]. The treatment is aimed to relieve pain, inflammation, and maintain joint function [7].

Methotrexate (MTX) is prescribed as monotherapy or in combination with other synthetic disease-modifying anti-rheumatic drugs (DMARDs) [8]. Hendawy *et al.* 2015, reported that MTX is considered by many rheumatologists to be the most important and useful DMARDs and is often considered as the first line of treatment [9]. It is most active in the developing adjuvant model where the opportunity exists

to dose it for a longer period (15 days). The ED50 doses are generally 0.06–0.075 mg/kg/day [10] Therefore, in this study MTX was used as a standard drug.

IUPAC name - 2-[2-oxo-2-[(2-oxothiolan-3-yl) amino]ethyl] sulfanylacetic acid

Erdosteine is a thiol derivative of the same class as acetylcysteine [11] developed for the treatment of chronic obstructive bronchitis. Erdosteine produces an active metabolite (Met 1) which was shown to produce antioxidant effects due to the presence of an SH group [12]. Its mechanism of action is its ability to inhibit some inflammatory mediators and some pro-inflammatory cytokines that are specifically involved in oxidative stress [12]. Based on its free radical scavenging activity, its protective effects against oxidant-induced tissue damage have been demonstrated in various inflammation models [13].

The study rationale is rooted on the fact that the test drug (Erdosteine) which produces low incidence of adverse events [14,15] and prevents the accumulation of free radicals [16] in the body can be used as a potential agent in RA.

METHODS

Drugs and chemicals

Complete Freund's adjuvant (CFA) and Erdosteine (test drug) were obtained from Sigma-Aldrich Chemicals, Mumbai. MTX was purchased from Ipca Laboratories Ltd, Mumbai, and used as a standard drug. Erdosteine is a white, microcrystalline powder slightly soluble in water, ethanol, methanol, and acetone. It is soluble in 20 mg/ml Dimethyl sulfoxide (DMSO) and since it is acidic, it is made neutral by adding NaHCO₃ Fig. 1. All other chemicals and solvents were of the highest grade commercially available.

Animals

Thirty adult Wistar albino rats, weighing 100–250 g were obtained from the Central Animal House, NSHM College of Pharmaceutical Technology, acclimatized for 7 days with a 12-h light/dark cycle. In each cage, five animals were housed with free access to commercial food pellet and water *ad libitum*. The protocol and the facility were maintained according to the guidelines provided by the Institutional Animal Ethical Committee, where all the animals under study were housed in an air-conditioned room 23±1°C and 50% humidity with a controlled. The animals were acclimatized in clean cage provided with husk bedding for 5 weeks before the study. The procedures throughout the study were carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals.

Acute oral toxicity study

An acute oral toxicity study was carried out using female Wistar rats according to the Organization for Economic Co-operation and Development guidelines 423. A total of 6 female rats were used, 3 for the control group (Group A) and 3 for the test group (Group B). Drug was administered orally to overnight fasted rat sat a volume of 1 ml/100 g body weight. Erdosteine was dissolved in DMSO and was administered at a single dose of 2000 mg/kg [17] in Group B and tap water was administered to (Fig. 2) Group A and food was withheld for another 3 h. They were observed continuously for 4 h for behavioral and autonomic profiles and regularly for the first critical 24 h and daily for a total of 14 days for any lethality. It was observed that none of the animals died at a dose of 2000 mg/kg body weight. Hence, one hundredth (1/100th) cut-off dose (i.e. 20 mg/kg) was selected for the subsequent study.

Experimental procedure

Thirty albino rats were randomly divided into five groups and were treated for 12 days and observed for 21 days. All the groups received 0.1 ml of CFA (10 mg/ml) in the left hind footpad except the negative control group of animals. Group 1, the negative control received only 5 ml/kg 0.9%w/v saline solution orally. Group 2, the positive control group received no test drug and group 3 animals were administered with standard MTX at a dose of 0.075 mg/kg body weight. Similarly, group 4 and group 5 rodents were administered with test drug Erdosteine at a dose of 10 mg/kg and 20 mg/kg body weight respectively. The time of adjuvant injection was referred to as day zero and treatment was initiated from day one and continued till the 12th day. The body weight and left hind paw volume of the animals were recorded consecutively on the 1st, 5th, 10th, 15th, and 20th day of experimentation [18,19]. The percentage inhibition of left paw edema was calculated by the following formula:

$$\% \text{ Inhibition} = (VC - VT) \times 100 / VC \quad (i) [20]$$

Where, VC = Paw volume of control group, VT = Paw edema volume of the test/standard group.

On the 19th day of experimentation, all the affected paws of the animals were photo radiographed to observe the soft tissue swelling and the bone space. On the 20th day of experimentation, the blood was collected from each group for hematological investigation and half of the population from each group were sacrificed, left leg was amputated at knee joints and were sent for histopathological evaluation [18,19].

The tissue samples of the tibiotarsal bone were stored in 10% Neutral Buffered Formalin. The bones were then decalcified by keeping in 5% Formic acid for 4–5 days. The histopathological tissue sections were sliced, stained with Hematoxylin-Eosin stain, and fixed on the slides and they were observed under a fluorescence microscope (OLYMPUS CX 21i TR) attached to a camera (OLYMPUS LC 30) [18].

RESULTS

Body weight

The graph Fig. 3 shows a quantitative change in the body weight of animals in each group. After induction of CFA there was a drastic decline

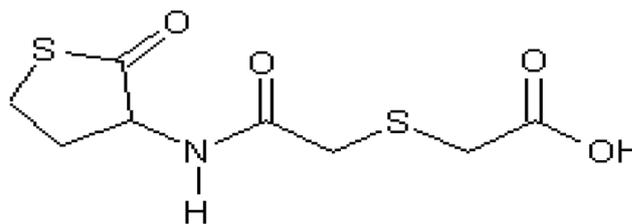


Fig. 1: Chemical structure of erdosteine

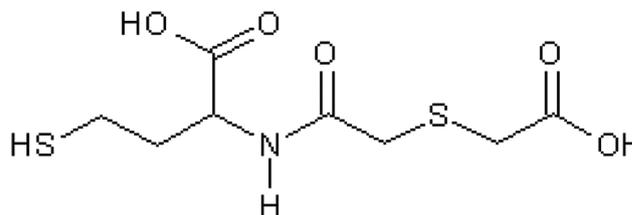


Fig. 2: Erdosteine- metabolite 1

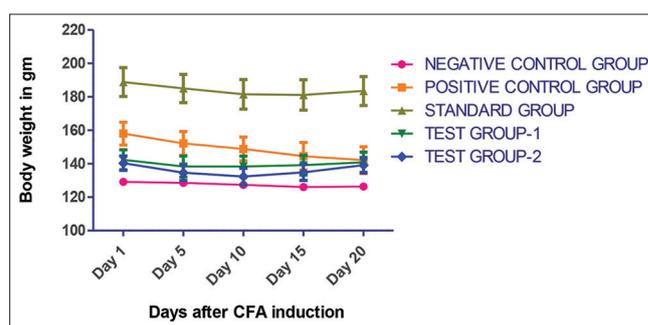


Fig. 3: Change in the body weight. Significant change in the body weight of Methotrexate and Erdosteine treated group. n=6, values are expressed as mean±SEM, Tukey. Comparison of all parameters of the control group with standard and test group were done, Non-significant (p>0.05) and significant (p<0.05)

in the body weight on day 10, which elevated due to the administration of the drugs (standard and test drug). However, no significant difference was found in the body weight between the two test groups administered with 10 mg/kg and 20 mg/kg of test drug. However, a significant difference between standard and group 5 (test drug 20 mg/kg) was found (p<0.05).

Paw volume

Fig. 4 clearly indicates maximum swelling in the paw during 5–10 days post-administration of CFA. However, reduction in the paw volume was seen during days 10–20 which indicated decrease in the inflammation. There was a significant decrease in the hind paw volume of all the drug-treated groups compared to the positive control group. Furthermore, substantial design in paw volume was observe in test group 2 compared to the standard group and test group 1. Table 1 represents mean and percent inhibition of paw volume. Percent Inhibition of arthritis increased in the standard and in both the test groups. However, better outcome was observed in test group 2 (Fig. 5). Tukey's comparison of the data shows significant difference between the groups.

Haematological and biochemical estimation

The hemoglobin and RBC levels decreased in the positive control group which indicates that the induced arthritic rats were anemic. However, when compared with the standard and test groups their levels were normal post-induction. WBC drastically multiplied in number in positive control group indicating elevated inflammation in the hind paws. However, in the standard and test group, WBC count become normal

on day 21. Serum albumin is also one that can act as an inflammatory marker. Previous studies showed that decrease in the albumin level may be correlated with alteration in the RA condition related to the augmented level of pro-inflammatory cytokines [21]. Lower levels of serum albumin correspond to higher levels of inflammatory activity, as the albumin accumulates in the inflamed joint. The ESR value of the inflamed paws of arthritis rats in the positive control group was very high compared to the groups being treated with MTX and Erdosteine (Table 2). Besides, there is a significant change in the RF in all the drug-treated groups and in positive control. It was observed that the untreated group had a high level of RF than the groups treated with MTX and Erdosteine. Eventually, the test group being treated with

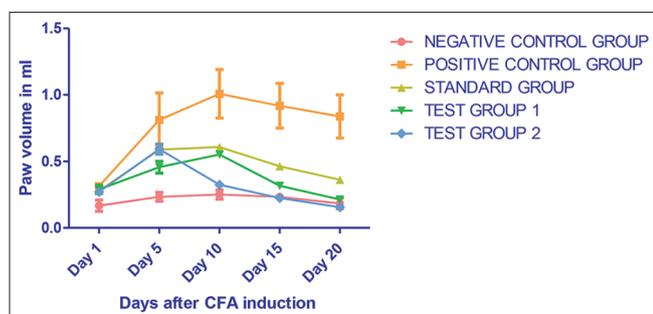


Fig. 4: Change in Paw volume. Reduction of the paw volume after drug treatment on the arthritis-induced Albino rats. n=6 rats in each group, values are expressed as mean±SEM, Tukey. Comparison of all parameters of the control group with standard and test group, Non-significant ($p>0.05$), Significant ($p<0.05$)

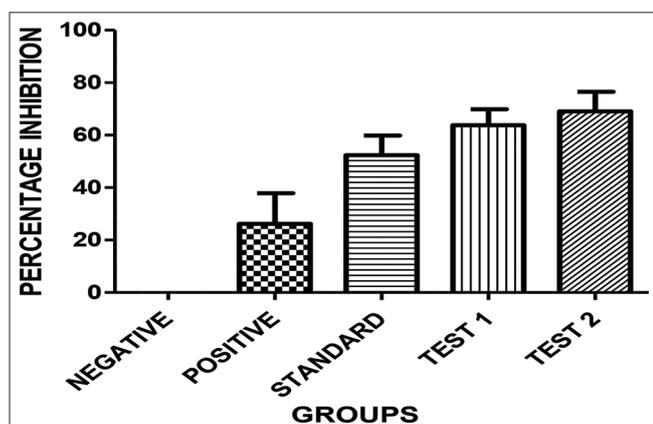


Fig. 5: % inhibition of paw volume using one way ANOVA, n=6 rats in each group, values are expressed as mean±SEM

Table 1: Average paw volume and %inhibition of paw volume of the adjuvant-induced arthritis in Wistar Albino rats

Groups	Day 1	Day 5	Day 10	Day 15	Day 20
Negative control					
Average	0.12±0.11	0.18±0.06	0.21±0.05	0.22±0.06	0.17±0.06
%Inhibition	0	0	0	0	0
Positive control					
Average	0.33±0.05	0.64±0.1	1.01±0.04	0.88±0.06	0.85±0.12
%Inhibition	67	36	1	12	15
Standard group					
Average	0.27±0.08	0.68±0.05	0.60±0.09	0.46±0.05	0.37±0.12
%Inhibition	73	32	40	54	63
Test group 1					
Average	0.29±0.06	0.45±0.15	0.55±0.05	0.31±0.09	0.21±0.07
%Inhibition	71	55	45	69	79
Test group 2					
Average	0.27±0.09	0.59±0.09	0.32±0.08	0.22±0.07	0.15±0.05
%Inhibition	73	41	68	78	85

20 mg/kg Erdosteine shows better result than the standard and test group.

Radiographical evaluation

Radiographs were evaluated to study the clinical features of the adjuvant-induced arthritis in rats. The soft skin thickening and the bone erosion were looked for. Pathological changes were compared between the control and the treatment groups. X-ray of normal rats (Fig. 6a) shows no pathological features, however, that of non-treated arthritic rats (Fig. 6b) shows severe soft tissue swelling (arrow), severe joint swelling, and very narrow joint space. X-ray of arthritic rats treated with MTX (Fig. 6c) at a dose of 0.075 mg/kg showed moderate soft tissue edema (arrow), moderate joint swelling, and very narrow joint space. Consequential changes were seen in x-rays of Erdosteine treated arthritic rats at a dose of 10 mg/kg (Fig. 6d) manifesting soft-tissue edema, mild joint swelling, and moderate narrow joint space and at a dose of 20 mg/kg (Fig. 6e) showing no soft tissue edema or joint swelling and improved narrow joint space.

From the clinical radiographic features, it was observed that there was an improved prognosis in the groups treated with MTX and Erdosteine compared to the untreated group. Besides, improved treatment outcomes were observed in a dose-dependent manner.

Histopathological evaluation

Different slides from different groups were observed on the computer screen at a magnification of 10× and 40× and the photomicrographs were snapshot.

Photomicrograph of a tissue section of a rat from the Negative Control group showed no sign of inflammation, bone destruction, or inflammatory cells (Fig. 7a). Section of the tissue from the rat paw of the positive control group i.e., non-treated arthritic group, showed massive numbers of inflammatory cells infiltration, pannus formation, bone destruction, and narrow synovial space (Fig. 7b). Similarly, paw section of standard group demonstrates reduced inflammation and improved synovial space. In addition, lesser macrophages and neutrophils infiltration were observed in standard treated group. However, pannus formation and bone destruction were evident in this section (Fig. 7c). Bone destruction and inflammatory cells infiltration were much lesser in this section in test treated group 2 compared to group 1. From the result, it is evident that Erdosteine at a dose of 20 mg/kg is potent enough to decrease inflammation in RA.

DISCUSSION

As the etiology behind RA prognosis is not well understood confirmed curative measures are not discovered till date [14]. Current treatments include non-steroidal anti-inflammatory drugs (NSAIDs), DMARDs, and biologics, but none are curative and there is a significant "non-responder" rate [22]. NSAIDs and DMARDs are associated with adverse

Table 2: Change in haematological and biochemical evaluation

S. No.	Parameters	Negative control group	Positive control group	Standard group	Test group 1 (10 mg/kg)	Test group 2 (20 mg/kg)
1.	Haemoglobin (g/dL)	16.1±5.5	9.7±2.4	12.8±4.7	12.5±4.2	13.8±3.3
2.	Total WBC count (mm ³)	7.3×10 ³ ±5.4	10.3×10 ³ ±4.4	11.6×10 ³ ±4.3	8.4×10 ³ ±4.6	6.6×10 ³ ±5.1
3.	Neutrophils (mm ³)	2.6×10 ³ ±2.6	3.6×10 ³ ±2.5	4.6×10 ³ ±1.9	1.3×10 ³ ±3.2	1.9×10 ³ ±2.6
4.	Lymphocytes (mm ³)	6.8×10 ³ ±3.4	6.1×10 ³ ±5.6	5.6×10 ³ ±4.0	3.7×10 ³ ±4.7	4.1×10 ³ ±4.6
5.	Monocytes (mm ³)	0.02×10 ³ ±0.02	0.03×10 ³ ±0.02	0.03×10 ³ ±0.01	0.01×10 ³ ±0.01	0.01×10 ³ ±0.01
6.	Eosinophils (mm ³)	0.05×10 ³ ±0.03	0.07×10 ³ ±0.02	0.04×10 ³ ±0.01	0.04×10 ³ ±0.02	0.03×10 ³ ±0.01
7.	Basophils (mm ³)	0.01×10 ³	0.0×10 ³	0.0×10 ³	0.0×10 ³	0.0×10 ³
8.	RBC (mm ³)	7.76×10 ⁶ ±2.1	5.80×10 ⁶ ±1.6	6.1×10 ⁶ ±2.5	6.50×10 ⁶ ±3.2	7.40×10 ⁶ ±2.6
9.	ESR (mm/h)	19.3±1.6	40±5.5	26.16±3.2	28±4.1	23.16±2.3
10.	RA factor (mIU/ml)	3.9±1.6	5.2±2.6	3.8±2.2	3.2±1.5	2.9±0.7
11.	Albumin (g/dL)	3.8±1.8	3.2±0.9	3.6±0.5	3.7±1.2	3.8±1.4

n=6, values were expressed as mean±SEM, Tukey. Comparison of all parameters of control group with standard and test group, Non significant (p>0.05), *Significant (p<0.05). RA: Rheumatoid arthritis.

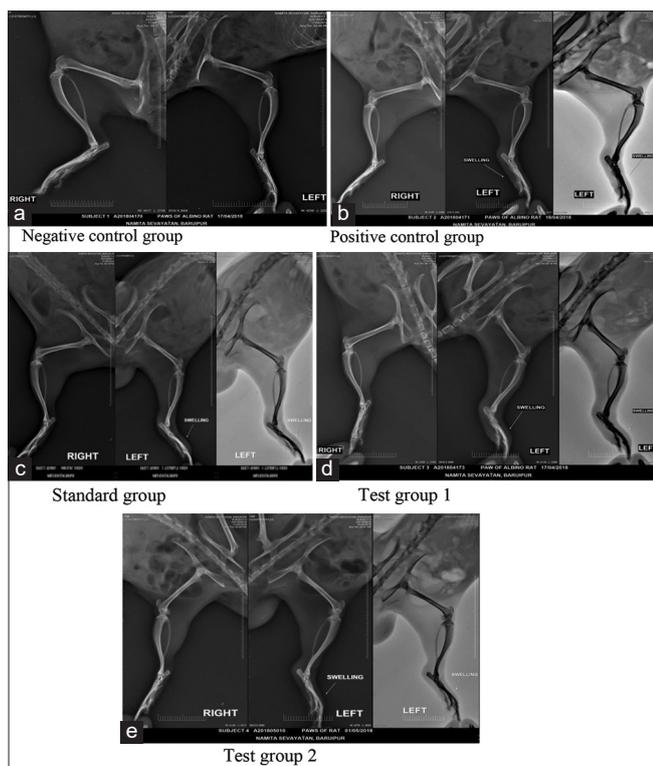


Fig. 6: Radiographical images of paws of the rats under study. (a) X-ray image of a rat without CFA induced, treated with saline; (b) X-ray figure of a rat induced with CFA treated with saline; (c) X-ray of rat induced with CFA and treated with methotrexate; (d) X-ray image of rat induced with CFA treated with 10 mg/kg Erdosteine; (e) X-ray of rat induced with CFA treated with 20 mg/kg Erdosteine. CFA: Complete Freund's adjuvant

effects [9]. Because of this reason, many patients and practitioners are seeking alternative approaches for providing an effective cure for the treatment of disease and to overcome the serious drawbacks such as gastrointestinal bleeding and bone loss. Hence, there is an urgent need to find safer compounds for the management of RA [11].

Rat adjuvant arthritis is an experimental model of polyarthritis which has been widely used for preclinical testing of numerous anti-arthritis agents which are either under preclinical or clinical investigation or are currently used as therapeutics in this disease [10]. It has been experimentally proved that adjuvant-induced arthritis in rat mimics the pathological, immunological, and clinical significance of human RA [20].

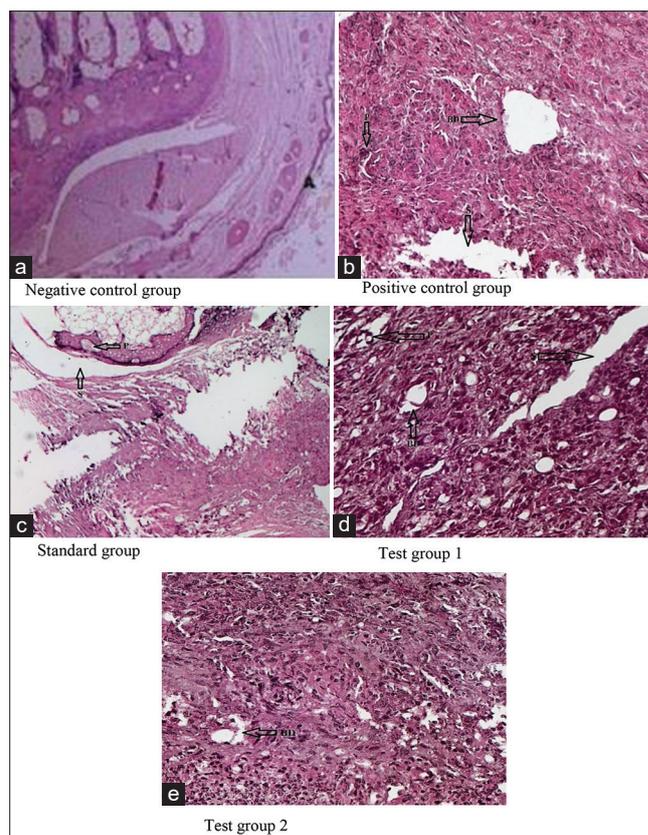


Fig. 7: Sections of tibiotarsal tissue sample of the rats for histological evaluation at 10× and 40×. (a) Negative control group (b) Positive control group without treatment; Sections of tibiotarsal tissue sample of the rats for histological evaluation at 10× and 40×. (c) Standard group treated with 0.075 mg/kg Methotrexate; (d) Test group 1 treated with 10 mg/kg Erdosteine; (e) Test group 2 treated with 20 mg/kg Erdosteine

There was a significant difference in the result between the control and treatment groups in this study. It was observed that the body weight of the animals being treated started to improve on Day 10 along the course of the treatment but no improvement was seen in the untreated group of rats. Patil *et al.* suggest that the decrease in body weight during inflammation is due to deficient absorption of nutrients through the intestine [23]. In this study, standard group treated with MTX and the group treated with Erdosteine 20 mg/kg had a significant change p<0.05 proving the decrease in inflammation.

CONCLUSION

In this study, treatment with MTX and Erdosteine as standard and test drug, respectively, in the arthritis induced groups recede the inflammation significantly. Furthermore, Erdosteine given at a dose of 20 mg/kg was more effective compared to the standard drug. The results, therefore, supports that the test drug, Erdosteine can be a potential anti-inflammatory and anti-oxidant lead for the treatment of RA.

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CONFLICT OF INTEREST

There was no conflict of interest among the authors.

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