



Research Article

Investigation of Antiarthritic Potential of Spironolactone in CFA Induced Model of Rheumatoid Arthritis in Rats

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ABSTRACT

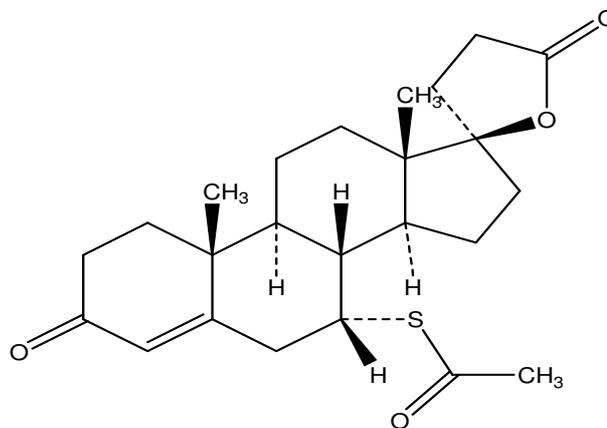
Rheumatoid arthritis includes inflammation of joints and classified as autoimmune disorder. The complete Freund's Adjuvant induce Arthritis is the most widely used animal model of arthritis. Spironolactone have immunomodulatory, antioxidant and anti-inflammatory properties, so might be it can use as an antiarthritic drug. The present work is to investigate the antiarthritic effect of spironolactone on CFA induced arthritis. The female rats were divided into five group containing six animal in each group and CFA induce arthritic rat were treated with spironolactone (56 mg/kg and 28 mg/kg), methotrexate and vehicle till 21st day. The results of treatment is evaluate by paw volume, body weight, ESR, arthritis index, TNF- α and IL-6. The female rats were immunized with CFA to induce rheumatoid arthritis. Spironolactone shows significant changes in all the parameters. The level of cytokines is reduced by oral administration of spironolactone. The results are nearly related as compare to methotrexate. Improvement in paw volume, body weight, ESR, arthritic score, TNF- α and IL-6 indicates that spironolactone might be used as the novel treatment of arthritis.

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INTRODUCTION

Arthritis is a collective term which include over 100 types of rheumatic diseases, which are described by inflammation, inelasticity and pain in muscular and skeleton system, categorized as systemic and autoimmune disorder [1]. The age which is generally affected by arthritis is between 35-40 [2]. About 1% population is influenced by RA, in which women are more frequently influenced as compare to men in the ratio of 3:1 [3]. The joint damage in RA is because of disproportion in pro and anti-inflammatory cytokines [4]. The synovial tissue is damaged by generation of inflammatory cell in synovium [5,6]. These inflammatory cells such as macrophages and Fibroblast derived cytokines produce cytokines and chemokines [7]. TNF- α and IL-6 play an important role in the pathophysiology of RA [8].

Spironolactone (Fig. 1) is a steroid, used as a diuretic it also possess anti-inflammatory, immune modifying, antioxidant and antiproliferative properties [9].

**Figure 1:** Spironolactone

Few studies describe its effect in Rheumatoid Arthritis also [10]. Spironolactone (SPIR) acts by blocking the binding of aldosterone to mineralocorticoid receptor and exert anti-inflammatory effect [11]. Aldosterone enhances the level of cytokines by acting on pro inflammatory cells [12]. The elevated level of aldosterone in synovium leads to pannus formation and cartilage destruction [13]. Only few works were accessible related to spironolactone in Rheumatoid Arthritis [14]. Spironolactone also suppress the production of TNF- α , Granulocyte

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macrophages colony stimulating factor and IL-6 [15]. Few studies report that spironolactone exert anti-inflammatory effect in severe cardiovascular disorders [16]. Bradstreet describe the immune modifying and anti-inflammatory effect of Spironolactone in autism. Spironolactones exert anti-inflammatory effect in ocular disease [17].

MATERIAL AND METHODS

Animals

Adult female Sprague dawley rats 6-8 week old weighing about 200-250gms were purchased from NIB, Noida. Animals were accommodated in a constant room temperature (24-26°C) and humidity (40-60%). Food (Pellet, Golden feed, Mehrauli) and drinking water available ad libitum. The protocol was approved by IAEC and caring of animals were performed as per the CPCSEA guidelines (Registration Number: **1149/PO/ERe/S/07/CPCSEA**).

Chemicals and Drugs

The chemicals were used CFA injection, Spironolactone and Methotrexate obtained from Sigma Adrich company USA, RPG life sciences Mumbai and IPCA labs Mumbai, respectively. All the chemicals, reagents and drugs used in the study were of analytical grade.

Complete Freund's Adjuvant Induce Arthritis

After the 7 days period of acclimatization to laboratory condition, the female Sprague dawley rats were divided in five groups (six animals in each group), i.e control, arthritic control, Spironolactone 28 mg/kg, Spironolactone 56 mg/kg and standard. Each group is treated as follows:

| | |
|------------|--|
| Group I: | control group which receive 0.5% Carboxymethylcellulose p.o |
| Group II: | arthritic control group which receive 0.5% Carboxymethylcellulose p.o + 0.1 ml CFA s.c |
| Group III: | 0.1 ml CFA p.o + 28 mg/kg Spironolactone p.o |
| Group IV: | 0.1 ml CFA s.c + 56 mg/kg Spironolactone p.o |
| Group V: | 0.1 ml CFA s.c + 1 mg/kg Methotrexate (MTX) p.o |

Arthritis was induced by injected 0.1ml of CFA (10 mg/kg of Mycobacterium tuberculosis in heated killed form which is suspended in 1 ml paraffin oil) on day 0 in right hind paw. The body weight and paw volume were measured at initial

day by using plethysmometer and weighing balance respectively. Drugs treatment was started at day 0 and continued till day 21. Paw volume and body weight were again measure at 7th, 14th and 21st day. At the end of experiment animals were anaesthetized and blood sample is withdrawn by retro orbital plexus and collected in plain and EDTA Tubes for haematological and biochemical analysis. The level of ESR, TNF- α and IL-6 were determined.

Evaluation of Arthritis

Arthritis Score

The arthritic scale was measured by 4 point scale as follows:

- 0 = no swelling
- 1= erythema of fingers
- 2= erythema and swelling of ankle
- 3= inflammation of whole paw
- 4= deformity of paw

At the end of experiment arthritic score of all rats were recorded and for control group it is describe as 0 represent the normal appearance and no swelling.

Measurement of TNF- α and IL-6

TNF- α and IL-6 are measured by ELISA method from the rat serum. TNF- α and IL-6 is estimated by ELISA kit.

Measurement of ESR

ESR was measured in wintrobe tube, which is 100mm long. The blood sample was collected in tube containing anticoagulant. EDTA tube is generally used for this procedure. After mixing the blood sample was withdrawn in wintrobe tube .1hour later, the fall is RBCs was measured in millimeters.

Statistical Analysis

The data were expressed as mean \pm S.D (n=6). The statistical significant analysis was done by one way ANOVA followed by tukey test by using statistical Graph Pad Prism software, version 8.1.1. $P < 0.05$, $P < 0.01$ and $P < 0.001$ are contemplating as statistically significant.

RESULTS

Effect on Body Weight

The rheumatoid arthritis is usually achieved with reduction in body weight. Thus altered body weight functions as a parameter during rheumatoid arthritis. The effect of body weight is shown in the table and figure in which body weight is gradually reduced in CFA induced group as compare to control group. In treatment

groups altered body weight is compared with CFA induced arthritis.

Fig. 2, Represent a comparison of body weight in the control, arthritic control, SPIR 28 mg/kg, SPIR 56 mg/kg and MTX group on day 0, 7, 14 and 21. Control, SPIR and standard groups have show minute changes in weight. Group –II is arthritic group, which show decrease in body weight as compare to day 0 ($P < 0.001$).

Effect on Paw Volume

The antiarthritic effect of Spironolactone was determined by measuring paw volume. It is the crucial parameter to assess the Rheumatoid Arthritis. The effect of paw volume is shown in the table and figure in which paw volume is gradually increased in CFA induced group as

compare to control group. In treatment groups altered paw volume is compared with CFA induced arthritis.

Fig. 3.1 & 3.2, Represent a comparison of paw volume in the control, arthritic control, SPIR 28 mg/kg, SPIR 56 mg/kg and MTX group. The graph show the comparison of paw volume in group I, II, III and IV on day 0, 7, 14 and 21. Maximum paw volume was observed on day 7 in arthritic control group, and then there is gradual reduction in all the groups except control. SPIR 28 mg/kg, SPIR 56 mg/kg and MTX group produced a significant reduction in paw swelling on day 21, as compare to control ($\dagger = P \text{ value} < 0.001$). Further the antiarthritic activity of SPIR 56 mg/kg was comparable that of Methotrexate throughout the observation period.

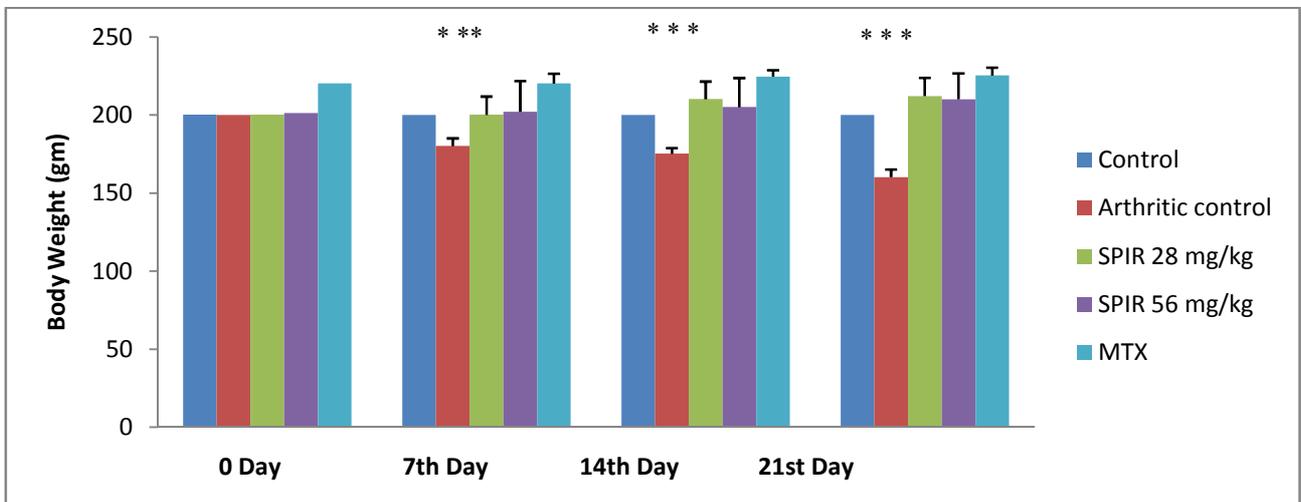


Figure 2: Showing effect CFA, SPIR 28 mg/kg, SPIR 56 mg/kg and MTX on body weight, n = 6 in each group. The data represent the mean ± S.D of all groups. Statistical analysis was done using one way ANOVA and Tukey test. *** = p – value < 0. 001 vs. control groups.

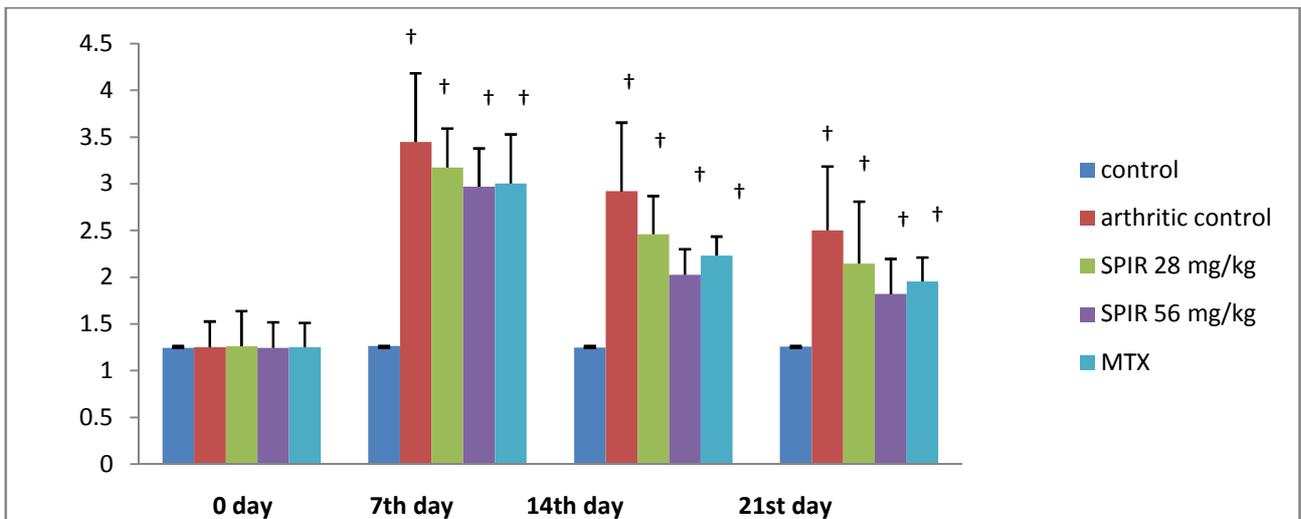


Figure 3.1: Showing effect CFA, SPIR 28 mg/kg, SPIR 56 mg/kg and MTX on paw volume, n = 6 in each group. The data represent the mean ± S.D of all groups. Statistical analysis was done using one way ANOVA and Tukey test. † = p – value < 0.001 vs. Control group.

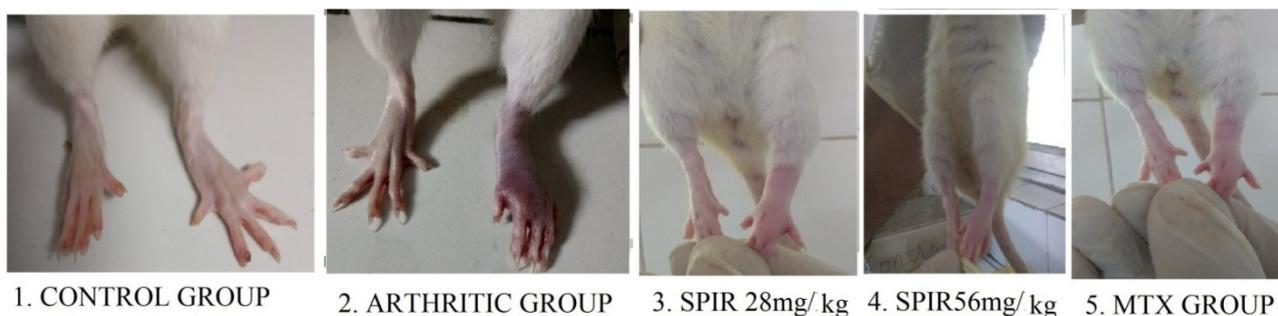


Figure 3.2: Showing effect of Spironolactone on paw swelling in CFA induced arthritis in rats.

Effect of Spironolactone on Arthritic Scale

Fig. 4 Represent a comparison of arthritic scale in the control, arthritic control, SPIR 28 mg/kg, SPIR 56 mg/kg and MTX group. Control group shows normal appearance although arthritic group show increase in arthritic score. Group - III (SPIR 28 mg/kg) show decrease in arthritic group as compare to arthritic group (P < 0.001). Group - IV (SPIR 56 mg/kg) show decrease in arthritic score as compare to group - III (P < 0.001). Standard group show decrease in arthritic index, as compared to group - III (P value < 0.001).

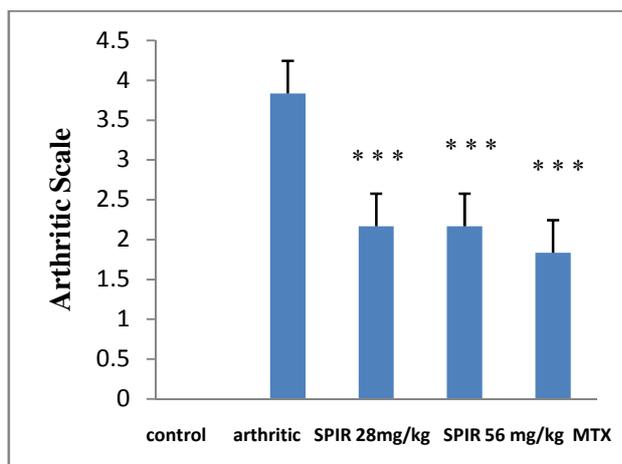


Figure 4: comparison of the effect of Spironolactone on arthritic score of CFA induced arthritis with methotrexate treatment on rat. Statistical analysis was done using one way ANOVA and tukey test. *** p value < 0.001 vs arthritic group.

Effect on ESR

Fig. 5 represents the effect of spironolactone on ESR. As compare to arthritic control group the spironolactone and standard group show decrease in the value of ESR. Group I which is control group show normal level of ESR. Group II which is arthritic group show increased level of ESR. Group III (SPIR 28 mg/kg) has show decrease level of ESR as compare to group II (P <

0.001). Group IV (SPIR 56 mg/kg) has show decrease level of ESR as compare to group III (P < 0.001). Group V which is Methotrexate group (Standard group) has show decrease level of ESR as compare to group IV.

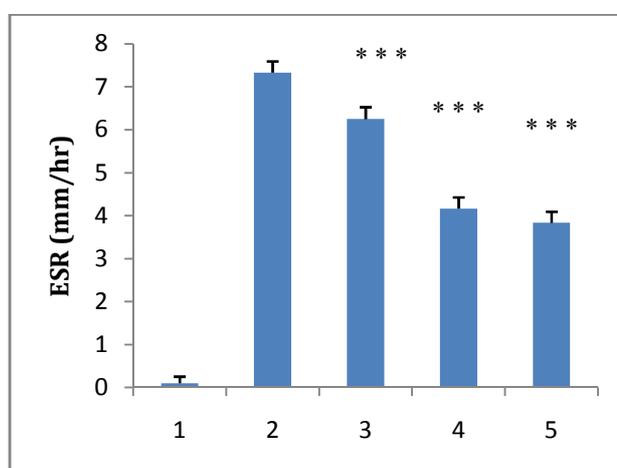


Figure 5: Showing effect CFA, SPIR 28 mg/kg, SPIR 56 mg/kg and MTX on ESR, n = 6 in each group. The data represent the mean ± S.D of all groups. Statistical analysis was done using one way ANOVA and Tukey test. *** p value < 0.001 vs. arthritic group.

Effect of SPIR on TNF-α and IL-6 level

Fig 6.1 & 6.2, represent the effect of spironolactone on TNF-α and IL-6. Injecting CFA produced an elevation in the level of TNF-α and IL-6 in all tested animal. As compare to arthritic group the spironolactone and standard group show decrease in the value of TNF-α and IL-6. Group I which is control group show normal level of TNF-α and IL-6. Group II which is arthritic group show increased level of TNF-α and IL-6. Group III (SPIR 28 mg/kg) has show decrease level of TNF-α as compare to group II (P < 0.05). Group IV (SPIR 56 mg/kg) has show decrease level of TNF-α and IL-6 as compare to group III (P < 0.001). Group V which is Methotrexate group (Standard group) has show

decrease level of TNF- α and IL-6 as compare to group IV.

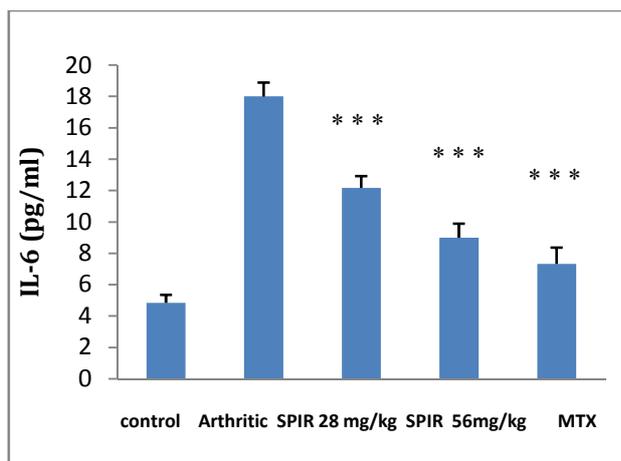


Figure 6.1: Showing effect CFA, SPIR 28 mg/kg, SPIR 56 mg/kg and MTX on IL-6, n = 6 in each group. The data represent the mean \pm S.D of all groups. Statistical analysis was done using one way ANOVA and Tukey test. *** p value < 0.001 vs arthritic group.

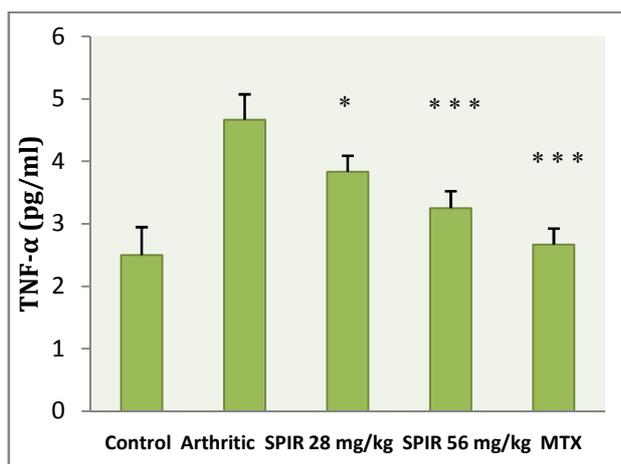


Figure 6.2: Showing effect CFA, SPIR 28 mg/kg, SPIR 56 mg/kg and MTX on TNF- α , n = 6 in each group. The data represent the mean \pm S.D of all groups. Statistical analysis was done using one way ANOVA and Tukey test. *** p value < 0.001 and *p value < 0.05 vs arthritic group.

DISCUSSION

RA is a very annihilating condition, which deficient in proficient treatment option. A synthetic DMARDs are recently used to treat arthritis, still low coasting and fewer side effect drug treatment is deficit. The present research work investigates the effect of spironolactone in CFA induced arthritis. CFA induced arthritis is the most widely used antiarthritic model and it possess nearly similar pathological and biochemical changes as compare to human RA. In

the present study there was a dose dependent reduction in paw volume in the SPIR treated group as compare to control.

In present work, the level of cytokines (TNF- α and IL-6) are also determined, that are overexpressed in Rheumatoid Arthritis. SPIR produce inhibition of cytokines as compare to control group. Improvement in the ESR level in the SPIR 28 mg/kg indicates its positive effects in the recovery of arthritis. In the present work, the arthritic index is slightly higher in spironolactone 28 mg/kg dose and slightly lower than in spironolactone 56 mg/kg as compare to Methotrexate group. This work suggests that the spironolactone possess antiarthritic activity as compare to methotrexate. Methotrexate is a potent immunomodulatory, antiarthritic synthetic DMARD. NSAIDs used in the treatment of rheumatoid arthritis give only symptomatic relief and they do not completely cure the disease. DMARDs used in the treatment of arthritis act on inflammatory mediators and inhibit the proinflammatory cytokines. The serum cytokines TNF- α and IL-6 are responsible in the progression of RA. The present work describe that spironolactone reduce the level of cytokines as compare to arthritic group. Spironolactone nearly reduces the level of serum cytokines as compare to methotrexate.

CONCLUSION

The current study investigates that dose of 56 mg/kg of spironolactone showed the antiarthritic activity in CFA induced arthritic model. It can nearly prevent the joint, cartilage and bone destruction. The therapeutic effect of spironolactone is nearly similar to the antiarthritic effect of spironolactone. The present study suggested that spironolactone may be used in the treatment of RA, as potent alternative DMARDs.

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