Ind. J. Pharm. Edu. Res., 2025; 59(1):144-152.
https://www.ijper.org

Original Article

Protective Effects of Lysergol against Complete Freund Adjuvant-Induced Rheumatoid Arthritis in Rats

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ABSTRACT

Background: Rheumatoid Arthritis (RA) is a rapidly developing autoimmune disease caused by self-destruction of the immune system, resulting in deformed joints and erosion of the bones. In the current study, we explored the anti-arthritic impact of Lysergol against the Complete Freund Adjuvant (CFA)-stimulated arthritis in rat model. **Materials and Methods:** Administration of CFA was performed for inducing arthritis and the animals were divided into 4 distinct groups. The rats then received Lysergol at a dose of 20 mg/kg. The body weight, paw swelling, arthritic index score, inflammatory cytokine levels and other inflammatory parameters were assessed along with the histopathological examination. CFA-stimulated arthritic animals treated with Lysergol substantially increased the body weight and reduced the paw swelling, organ index and arthritic index. **Results:** Lysergol-treated rats further suppressed the levels of inflammatory cytokines including TNF-α, IL-6, IL-10 and IL-1β. Lysergol treatment also decreased the levels of Prostaglandin E2 (PGE2), Nitric oxide and Thromboxane B2 (TXB2), as well as C-Reactive Protein (CRP) and Cyclic Citrullinated Peptide (CCP). **Conclusion:** The present investigation thus found that Lysergol may be an appropriate substitute to the currently available treatments for RA, since it exhibits a remarkable anti-inflammatory effect against CFA-stimulated arthritis in rodent models.

Keywords: Arthritis, Lysergol, Complete Freund adjuvant, Anti-inflammatory, Inflammatory cytokines.

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Received: 05-06-2024;
Revised: 11-07-2024;
Accepted: 23-08-2024.

INTRODUCTION

A severe autoimmune, progressive and systemic inflammatory condition, Rheumatoid Arthritis (RA) is associated with irreparable joint destruction, musculoskeletal deficiencies, painful and stiff joints, as well as damaging bone erosions.[1,2] According to reports, 2% of people worldwide are estimated to be affected by RA.[3] Numerous attempts have been made to determine the cause of RA; nevertheless, the pathological mechanism of RA is still unclear and involves a number of crucial variables, including oxidative stress, chronic inflammation, the formation of self-antibodies and environmental and hereditary factors.[4]

Higher concentrations of numerous proinflammatory cytokines, including Interleukin-1β (IL-1β), Interleukin-6 (IL-6) and Tumor Necrosis Factor-α (TNF-α), have been linked to the advancement of inflammation in RA.[5,6] The rheumatoid synovium produces these cytokines that can diffuse into the blood stream and affect the function of several organs, particularly fat, liver, skeletal muscle and the endothelium of the vascular system. These functional changes may result in insulin resistance, elevated oxidative stress and lipid levels, dysfunctional endothelial cells and anemia.[7]

The adjuvant-stimulated arthritis animal model has been employed for years for studying the pathophysiology of arthritis, particularly RA, osteoarthritis and gout, as well as to assess the efficacy of specific anti-arthritic medications. Modeling of Complete Freund's Adjuvant (CFA)-stimulated arthritis in animals has since become prominent for studies aimed at developing therapeutic options for RA and other inflammatory arthropathies. This model is frequently employed to estimate the therapeutic efficacy of a medicine since it triggers analogous human arthritis.[8,9]

The current treatment options for managing the symptoms of RA target various mechanisms implicated in its progression. These treatments involve the intake of nonsteroidal anti-inflammatory medications, immune suppressors, disease-modifying anti-rheumatic medicines, biological substances and corticosteroids. These medicines have varied effectiveness and multiple side effects, including gastrointestinal discomfort, peptic

DOI: 10.5530/ijper.20250039

ulcers and adverse effects at both non-systemic and systemic levels.[10]

Alternative medicines, particularly herbal treatments/natural substances, have been adopted by RA patients owing to their apparent efficacy, safety and accessibility; much as they have been utilized for other serious illnesses.[11] The anti-rheumatic effects of several substances that exist naturally have been previously extensively studied via both *in vitro* as well as *in vivo* research. These substances are not only efficient in lowering the severe symptoms associated with RA, but have extremely few, if any, side-effects.[12,13] These bio-active compounds exhibit anti-oxidant and anti-inflammatory characteristics; thus, they can provide pain relief by reducing the formation of free radicals.[14] Lysergol (9, 10-Didehydro-6 methylergoline-8-α-methanol) is a bioenhancer alkaloid found in morning glory plants (Ipomoea spp.). It is efficient against a variety of microorganisms, including Gram-positive and Gram-negative bacteria.[15] Moreover, the anti-inflammatory activity of such clavine based alkaloids have been studied.[9] Hence, Lysergol was chosen in this particular study for investigation.

The main objective of the current study is to elucidate the anti-arthritic potentials of Lysergol against CFA-stimulated rheumatoid arthritis in rats. The rats exposed to CFA administration were then subjected to Lysergol treatment. Their hind paw volume, arthritis index score, inflammatory cytokines, prostaglandin E2, nitric oxide, thromboxane B2, Osteoprotegerin, receptor activator of nuclear factor-kB ligand, C-Reactive Protein (CRP) and Cyclic Citrullinated Peptide (CCP) and histopathological analysis was carried out to examine the anti-arthritic property of Lysergol.

MATERIALS AND METHODS

Experimental animals

Wistar rats weighing 170-220 g were housed in metal enclosures with wire mesh bottoms. They were subjected to a 12 hr dark/light cycle at 25±2°C and 55±5% humidity. They were fed a pellet chow meal and had unlimited access to water. The Institutional Animal Ethical Committee (IAEC) approved the studies.

Experimental protocol

The rats were divided into four groups of with six rats in each (n=6). Group I was considered as a normal control group, whereas Group II rats were RA-induced by injecting 0.1 mL of Complete Freund's Adjuvant (CFA) subcutaneously at the tail base to induce RA. The rats in group III were RA-stimulated and subsequently administered with Lysergol at a dose of 20 mg/kg for 25 days. The group IV rats were RA-induced and administered with indomethacin (3 mg/kg) for 25 days. Both Lysergol and indomethacin were dispersed in distilled water before administration.

Group I: Normal control.

Group II: RA-induced by injecting 0.1 mL of Complete Freund's adjuvant.

Group III: RA-induced with Lysergol at a dose of 20 mg/kg.

Group IV: RA-induced with Indomethacin (3 mg/kg).

Estimation of body weight and organ index in the experimental rats

The body weight of the experimental rats was measured at equal time intervals and recorded in grams. On the 29th day of the experiment, rodents were starved overnight and euthanized with chloral hydrate. Blood samples were collected via the abdominal aorta and subjected to centrifugation to collect serum for biochemical examination. The thymus and spleen were quickly removed and weighed. The thymus and spleen indexes were computed.

Estimation of hind paw volume and arthritis index score in the experimental rats

The hind paw volumes of all experimental animals were recorded at regular points (on 5, 10, 15, 20 and 25 days) to determine visible arthritic modes. A plethysmometer was utilized to assess the changes in paw volume in all groups of experiments. A plethysmometer is a tool used to evaluate the swollen hind paw of an inflammatory rat.

From the start of the trial to the end, the observer recorded visible arthritic modifications every 5 days. The severity of arthritis in the rat paw was measured and graded from 0 to 4, where grade 0 (no swelling), grade 1 (light redness or swelling on paw finger), grade 2 (evident swelling in one or more swelling in paw finger), grade 3 (persistent swelling in ankle or joint), grade 4 (severe arthritic edema in the wrist and fingers) was assigned. The maximum arthritic score determined for rats induced by CFA is an 8.

Assessment of inflammatory cytokines in the experimental rats

Proinflammatory cytokines, including TNF-α, IL-10, IL-1β and IL-6, were measured in the rat serum by employing standard ELISA kits (Abcam, USA).

Estimation of prostaglandin E2 (PGE2), Nitric oxide and Thromboxane B2 (TXB2)

Blood serum was obtained from rodents on day 22 and maintained at -80°C before analysis. PGE2, Nitric oxide and thromboxane B2 concentrations in serum were measured using ELISA kits according to the company's specifications (Abcam, USA). The levels of PGE2, Nitric oxide and thromboxane B2 were expressed in terms of ng/mL, μM and Pg/mL, respectively.

Assessment of Osteoprotegerin (OPG) and Receptor Activator of Nuclear factor-kB Ligand (RANKL) in serum by ELISA

A commercial Enzyme-Linked Immunosorbent (ELISA) test kit (Abcam, USA) was employed to measure the amounts of RANKL and OPG in the serum samples. The levels of RANKL and OPG were measured in terms of pmol/L and ng/L, respectively.

Determination of C-Reactive Protein (CRP) and Cyclic Citrullinated Peptide (CCP) in serum by ELISA

A commercial ELISA test kit (Abcam, USA) was utilized to analyze the levels of CRP and CCP in the serum samples. The levels were measured in terms of Pg/mL.

Histopathological analysis of rat organ tissues

On the 29th day, anaesthetized rodents were sacrificed via cervical dislocation. The abdomen was sliced apart to extract the thymus and the spleen was rinsed with distilled water and measured. Ankle joints were separated, cleaned and fixed in formalin (10%) for 48 hr before being decalcified with EDTA (10%) for 1 month. The tissues were subsequently treated by embedding in paraffin wax. Joint slices of 5 µm thickness were obtained with a microtome, placed on a glass slide and stained with hematoxylin and eosin dye before being examined under a microscope for inflammation, bone erosion, as well as pannus development. A semi-quantitative evaluation technique was used, with scores that ranged from 0 to 4.

Statistical analysis

Results were represented as mean±standard deviation and analyzed using one-way and two-way ANOVA with Tukey's post hoc test. Additionally, a regression analysis was conducted to determine the dose-response association.

RESULTS

Impact of Lysergol on the bodyweight and organ index in the experimental rats

Figure 1A displays the body weight of all the experimental groups. The suppression of body weight during the arthritic condition was noticed, which was due to the increase of joint swelling and a reduction in intestinal glucose absorption. Lysergol treatment successfully increased the body weight of the RA-stimulated rats. The thymus and spleen index score has been depicted in Figure 1B. The RA-stimulated rats exhibited an elevated organ index, which was suppressed by Lysergol treatment.

Impact of Lysergol on the hind paw volume and arthritis index score in the experimental rats

The effect of Lysergol on the hind paw volume and arthritis index score of CFA-triggered rats is depicted in Figure 2A and 2B, respectively. Contrary to normal control rats, arthritis control rats showed a substantial linear increase in paw volume over the course of the analysis. Lysergol, however, significantly decreased the inflammatory hind paw volume on days 5, 10, 15, 20 and 25 compared to the arthritis control group.

The arthritic improvement pattern was evaluated using the scoring system illustrated in Figure 2B. During arthritis, the arthritic score improves dramatically, indicating that the condition is progressing. The CFA-stimulated arthritic animals demonstrated a similar outcome. Arthritis control rats exhibited a considerably higher arthritic index score on day 25 than did the usual control group. The arthritic score was drastically reduced after Lysergol treatment.

Impact of Lysergol on the levels of inflammatory cytokines in the experimental rats

The levels of proinflammatory cytokines, including TNF-a, IL-10, IL-1b and IL-6, in experimental rats are depicted in Figure 3. There was no discernible substantial difference between the rats in the lysergol treatment group and the control group. Rats with arthritic stimulation exhibited a marked increase in proinflammatory cytokines when compared to normal control rats. However, the rats treated with Lysergol exhibited a significant decrease in these proinflammatory cytokines, revealing that Lysergol possesses anti-inflammatory properties.

Effect of Lysergol on the levels of Prostaglandin E2 (PGE2), Nitric oxide and Thromboxane B2 (TXB2)

As demonstrated in Figure 4A, the serum PGE2 levels of arthritis-stimulated rats were higher than those of the normal control rats. Lysergol administration markedly decreased PGE2 concentrations in comparison to the arthritic control animals. The PGE2 concentrations of Indomethacin treated rat group were further lowered than that of the lysergol treated group. The outcome of nitric oxide and thromboxane B2 concentrations in the experimental rat groups was also similar. These findings further strengthen the anti-inflammatory nature of Lysergol against arthritic-induced rats.

Effects of Lysergol on the levels of Osteoprotegerin (OPG) and Receptor Activator of Nuclear factor-κβ Ligand (RANKL)

The effect of Lysergol on the OPG and RANKL levels in the experimental rat groups was examined using ELISA. Upon arthritic induction, rats expressed higher levels of RANKL and lower levels of OPG. Treatment with Lysergol suppressed the levels of RANKL and elevated the OPG levels and treating the arthritic-stimulated rats with indomethacin further reduced the levels of RANKL and increased the OPG levels (Figure 5).

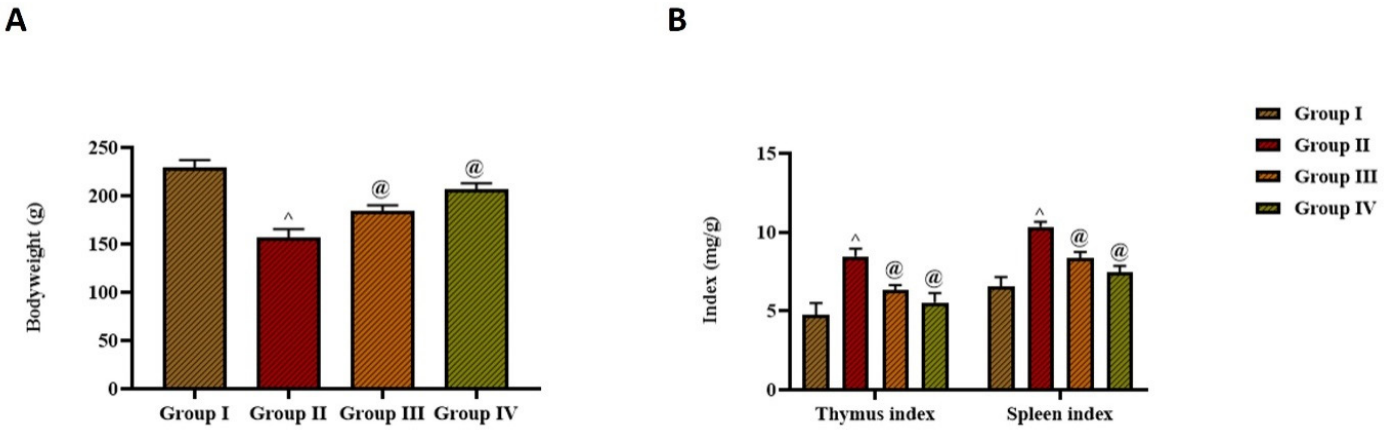

Figure 1: Effects of Lysergol on the body weight and organ index of CFA-induced arthritic rats. Values are expressed as mean±SD for three experiments. ^p<0.05 compared to the control group; @p<0.05 compared to the CFA group.

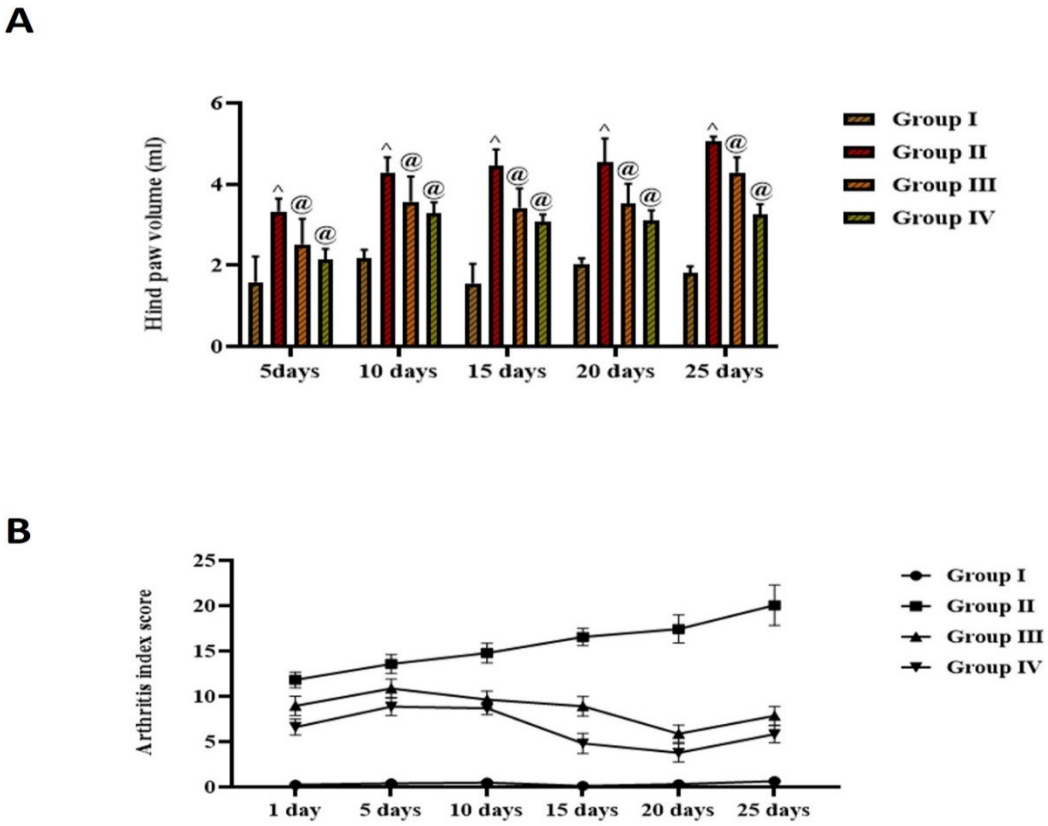

Figure 2: Effects of Lysergol on the hind paw volume and arthritis index score of CFA-induced arthritic rats. Values are expressed as mean±SD for three experiments^p<0.05 compared to the control group; @p<0.05 compared to the CFA group.

Effects of Lysergol on the levels of C-Reactive Protein (CRP) and Cyclic Citrullinated Peptide (CCP)

Figure 6 depicts the impact of Lysergol on the levels of CRP and CCP in experimental rats upon arthritis induction. Both CRP and CCP levels were elevated in RA-induced rats whereas these levels were lowered when Lysergol was administered. With the administration of indomethacin, these levels were reduced even further.

Histopathological examination of rat organ tissues

As illustrated in Figure 7, control group rats exhibited whole synovial membrane in their ankle joints and no signs of pannus development, bone erosion, or inflammation. Increased infiltration, synovial hyperplasia and pannus development were observed in the rats with polyarthritis. Rats administered with Lysergol demonstrated decreased pannus development and bone

degradation in their ankle joints compared to arthritic control group.

DISCUSSION

Rheumatoid arthritis is a common autoimmune disease characterized by joint inflammation and significant discomfort. Drugs such as NSAIDs, methotrexate and glucocorticoids are currently the only effective therapeutic choices for RA.[10] However, the possible future uses of these medications for RA treatment are not promising considering their major side effects as well as the significant financial burden linked to their usage, leaving a large opportunity for the development of novel therapies from natural medicines.[16,17]

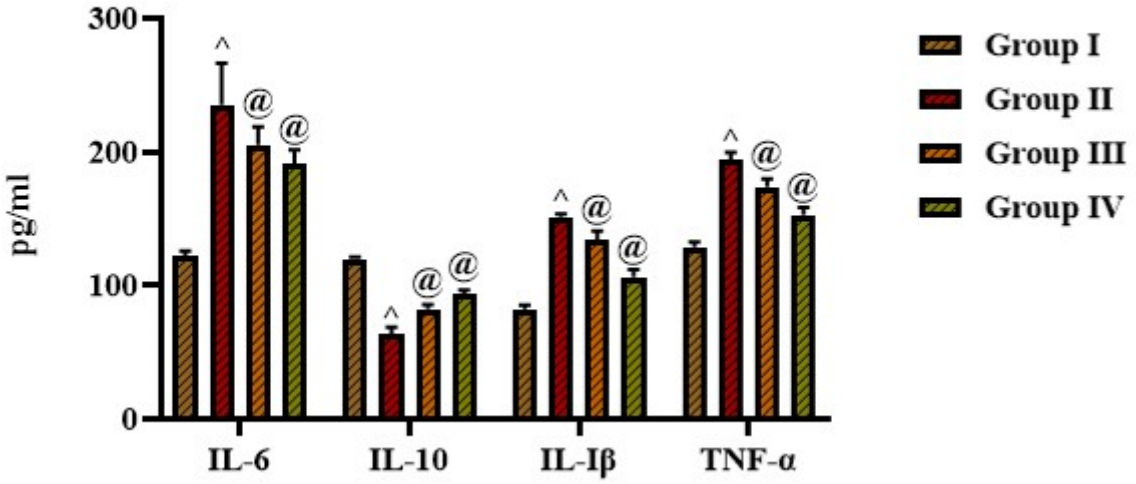

Figure 3: Effect of Lysergol on inflammatory cytokine levels in CFA-induced arthritic rats. Values are expressed as mean±SD for three experiments. ^p<0.05 compared to the control group; @p<0.05 compared to the CFA group.

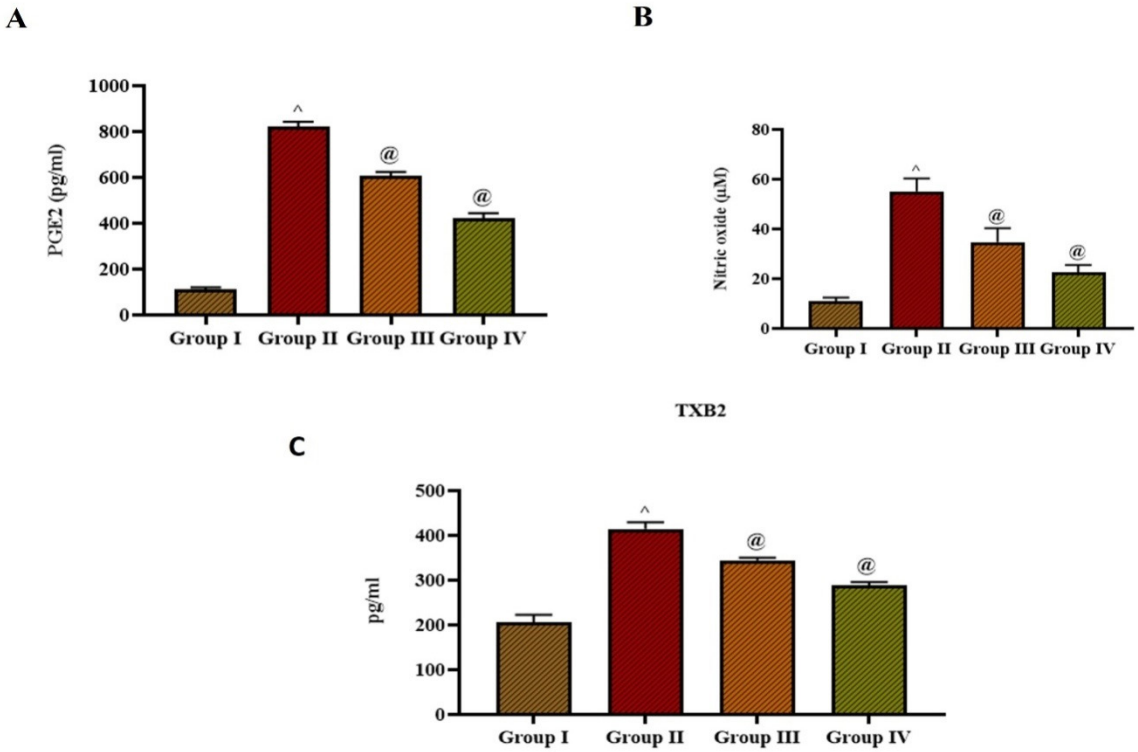

Figure 4: Effects of Lysergol on Prostaglandin E2 (PGE2), Nitric oxide and Thromboxane B2 (TXB2) levels in CFA-induced arthritic rats. Values are expressed as mean±SD for three experiments. ^p<0.05 compared to the control group; @p<0.05 compared to the CFA group.

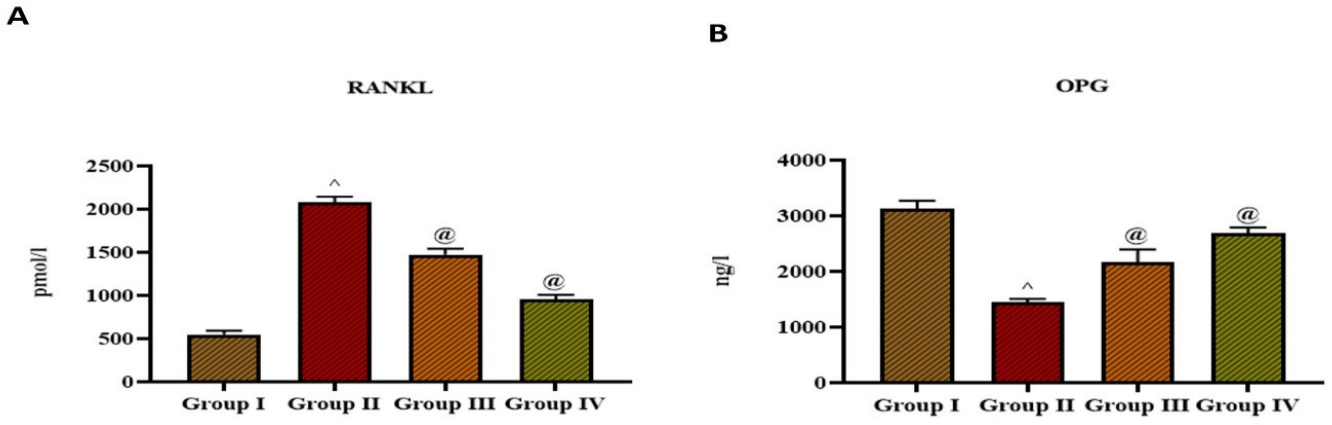

Figure 5: Effects of Lysergol on Osteoprotegerin (OPG) and Receptor Activator of Nuclear factor-kB Ligand (RANKL) levels in CFA-induced arthritic rats. Values are expressed as mean±SD for three experiments. ^p<0.05 compared to the control group; @p<0.05 compared to the CFA group.

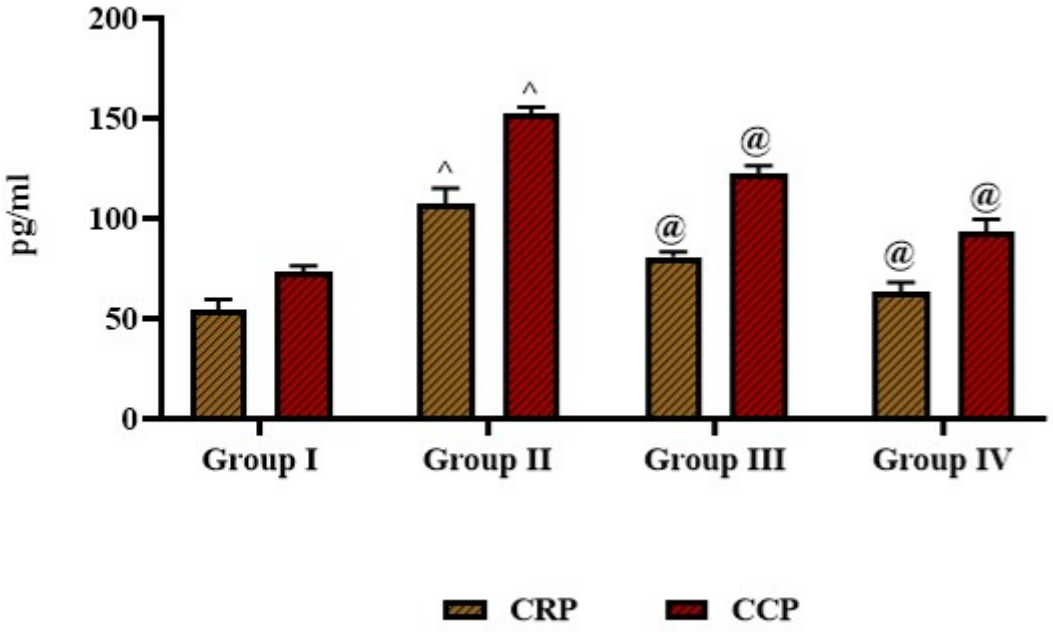

Figure 6: Effects of Lysergol on analysis of C-Reactive Protein (CRP) and Cyclic Citrullinated Peptide (CCP) levels in serum of CFA-induced arthritic rats. Values are expressed as mean±SD for three experiments. ^p<0.05 compared to the control group; @p<0.05 compared to the CFA group.

Adjuvant-stimulated arthritis has been observed to be the most effective approach for studying anti-arthritic medications. CFA-stimulated chronic polyarthritis exhibits symptoms that are comparable to RA. As a result, the CFA-triggered arthritis model has been utilized extensively for pre-clinical evaluation of various medications to manage the symptoms of RA.[18,19] In this investigation, we explored Lysergol's anti-arthritic activity in CFA-induced arthritic rats.

In line with earlier research, the CFA-stimulated arthritic rodents exhibited a substantial reduction in body weight gain, drastically elevated paw redness and swelling, joint distortion and limp and paw edema, indicating the onset of uncontrolled inflammation.[20] Administration with Lysergol substantially lowered the increased arthritic score, paw edema and swelling in RA-stimulated rats.

During the course of the RA disease, body weight is consistently lowered owing to poor leucine and glucose absorption through the intestinal tract.[21] In this investigation, we noticed a loss in the body weight of RA rodents, while Lysergol treatment significantly augmented the body weight, indicating a protective action against glucose and leucine absorption. Previous study reveals that the thymus and spleen index serve an important role by acting as a repository for antibody storage. The weight of these organs is

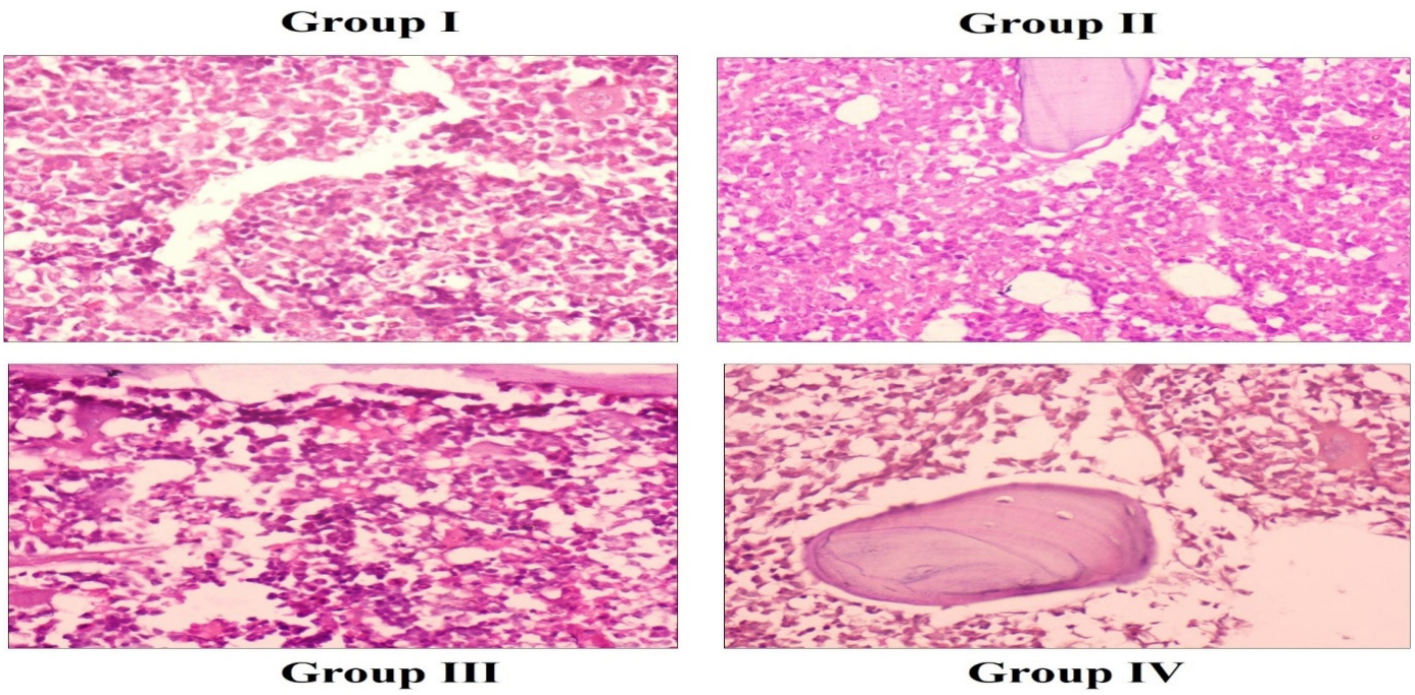

Figure 7: Histopathological changes in rat organ tissues. Group I is normal control, Group II is CFA Induced, Group III is CFA+Lysergol treated and Group IV is CFA+Indomethacin treated.

frequently employed to assess the immunomodulatory effects of test drugs.[22] The organ index elevated during the RA state and a similar effect was discovered in our study. Lysergol treatment in rats dramatically reduced the organ index, indicating an immunostimulant action. C-Reactive Protein (CRP) is an acute phase protein of liver source that increases due to IL-6 release from T cells and macrophages in inflammatory processes, resulting in elevated levels in the blood of RA patients.[23] As a result, CRP levels were elevated upon arthritis induction, whereas Lysergol treatment led to a reduction in these levels, suggesting its anti-inflammatory action.

Employing a plethysmometer to evaluate paw thickness in CFA-stimulated arthritis is a well-known and established technique.[24] In the current investigation, an inflammatory rat's edematous hind paw was estimated with a plethysmometer. Severe swelling, joint pain and edema in hind paws of animals following CFA administration could be the result of continuous inflammatory responses. Joint inflammation can predict the progression of RA in rats.[25] Synovial edema arises in experimental rats treated with CFA as a result of increased synovial fluid generation and vascular involvement into the inflammation area.[26] Our findings demonstrated enhanced edema and cell penetration in rats induced with RA, both of which indicate chronic inflammation. Lysergol administration led to a reduction in arthritis score and paw volume in animals exposed to CFA. Lysergol treatment lowered paw thickness, potentially by inhibiting inflammatory mediators, suggesting that it possesses anti-inflammatory capabilities against CFA-stimulated arthritis.

Inflammation is clearly involved with RA and cytokines are thought to serve significant roles in the progression of the condition. Existing evidence suggests that these cytokines may lead to immune cell infiltration, resulting in the production of matrix metalloproteinases, which have been linked to cartilage breakdown in arthritis as well as osteoarthritis.[27] Furthermore, they may stimulate the NF-κB pathway, increasing proinflammatory cytokine levels and exacerbating the inflammatory cascade.[17] In RA, macrophages, immunological cells and T cells can increase the expression of TNF-α and IL-6, accelerating inflammation.[28] The current research found that Lysergol lowered the cytokine levels in RA-stimulated rats, indicating that its anti-inflammatory abilities could help treat RA.

TNF-α buildup has been demonstrated to elevate the levels of PGE2, IL-6 and IL-1, leading to synovial joint hyperplasia, greater accumulation of enzymes that cause destruction and collagenase activation, all of which are associated with arthritic decay.[29] Elevated PGE-2 levels in CFA-stimulated rats have been shown to be strongly associated with joint edema, redness, discomfort, blood vessel dilation and cartilage erosion. Similarly, Lysergol's antiarthritic properties must suppress the levels of cytokines in order to lower arthritis symptoms. Therefore, the present investigation concluded that Lysergol, when provided to CFA-stimulated arthritic rodents, led to an apparent decline in PGE2 anti-inflammatory regulators. TNF-α promotes inflammation by increasing the migration of leukocytes, synovial fibroblasts and cellular adhesion molecules to painful joints.[30] IL-1 stimulates cartilage deterioration, bone resorption and

alters PGE2 and Nitric Oxide (NO) production. IL-6 induces inflammation by expanding blood vessels. PGE2 stimulates pain receptors and triggers fever.[31] As a result, an imbalance between pro-inflammatory and anti-inflammatory condition has led to joint damage and the development of synovial membrane inflammation.[32] Overall, Lysergol was demonstrated to exhibit a protective role against CFA-stimulated RA by lowering paw volume, arthritic score, body weight, PGE2, NO and TXB2 levels, as well as CRP and CCP levels. It further exhibited an anti-inflammatory effect by successfully suppressing the inflammation in RA-stimulated rodent models.

CONCLUSION

In conclusion, Lysergol treatment effectively suppressed arthritic disease progression by increasing the body weight and by lowering the paw swelling and arthritic score. Lysergol administration in rats further reduced the levels of inflammatory cytokines and inflammatory markers. It has been shown to protect against RA by reducing inflammation, suppressing PGE2, NO and TXB2 levels and lowering CRP and CCP levels. The present investigation found that Lysergol may be an appropriate substitute to the currently available treatments for RA. In addition, the further research in this context can improve Lysergol as a supplementary or combination medicine with existing therapies.

ACKNOWLEDGEMENT

This work was supported by Children's Hospital of Capital Institute of Paediatrics, Beijing, 100020, China.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

Clinical study of ultrasound-guide dintra-articular injection of triamcino lone acetonide in the treatment of juvenile idiopathic arthritis (2023BEG03004).

ETHICAL APPROVAL

This work has approved by the institutional animal ethical committee by Children's Hospital of Capital Institute of Paediatrics, Beijing, 100020, China.

ABBREVIATIONS

CFA: Complete Freund adjuvant; **RA:** Rheumatoid Arthritis; **ELISA:** Enzyme-linked immunosorbent assay; **IL-1:** Interleukin-1; **TNF-α:** Tumor Necrosis Factor-alpha; **IL-6:** Interleukin-6; **NO:** Nitric oxide**; PGE2:** Prostaglandin E2; **NO:** Nitric oxide; **TXB2:** Thromboxane B2**; OPG:** Osteoprotegerin; **RANKL:** Receptor activator of nuclear factor-kB ligand; **CRP:** C-reactive protein; **CCP:** Cyclic citrullinated peptide.

SUMMARY

Our investigation proved that Lysergol exhibited protective effect against CFA induced arthritis in animal models. It increased the body weight and reduced the paw swelling and arthritic score. Their ability to reduce the levels of PGE2, NO, TXB2, CRP and CCP renders them a potentially valuable strategy for the treatment of arthritis.

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Cite this article: Wang L, Ma LY, Yue YT, Xu LL, Lai JM. Protective Effects of Lysergol against Complete Freund Adjuvant-Induced Rheumatoid Arthritis in Rats. Indian J of Pharmaceutical Education and Research. 2025;59(1):144-52.