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ORIGINAL ARTICLE

Goniothalamin Alleviates Inflammation and Arthritic Markers in Adjuvant-Induced Arthritis in Rats

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ABSTRACT

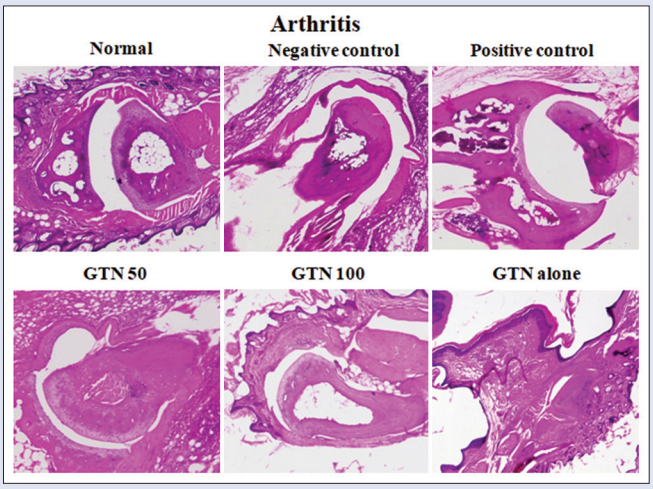
Background: Goniothalamin (GTN) is a well-known styryl lactone that possesses anti-inflammatory and anticancer properties. In this study, we evaluated the potential of GTN on experimental arthritis based on the complete Freund's adjuvant (CFA)-induced arthritis model in rats and the effect was compared with standard drug. **Materials and Methods:** The animals were divided into six groups: normal, CFA, CFA + indomethacin (10 mg/kg bw), CFA + GTN (50 mg/kg bw), CFA + GTN (100 mg/kg bw), and GTN (100 mg/kg bw) alone. The antiarthritic activity of GTN was measured by paw thickness, arthritic score, body weight, organ weight, biochemical markers, inflammatory mediators, and histopathologic analysis of ankle joints. **Results:** GTN significantly reduced the paw thickness, arthritic score, body weight changes, and organ weight. It reduced the levels of C-reactive protein, alkaline phosphatase, rheumatoid factor, and proinflammatory cytokines in a dose-dependent manner. It also minimized the histopathologic changes in ankle joints induced by CFA in a dose-dependent manner. **Conclusion:** These results demonstrate that GTN is effective against inflammation and is an alternative agent for the management of arthritis.

Key words: Antioxidant, arthritis, C-reactive protein, cytokines, goniothalamin, inflammation

SUMMARY

- GTN reduced body weight in CFA-induced arthritic rats.
- GTN diminished the levels of ALP, CRP, and RF in arthritic rats.
- GTN reduced the status of TNF-α, IL-6, and IL-1β in CFA-induced arthritic rats.

Abbreviations used: GTN = goniothalamin, CFA = complete Freund's adjuvant, RA = rheumatoid arthritis, FCA = Freund's complete adjuvant, CRP = C-reactive protein, ALP = alkaline phosphatase, RF = rheumatoid factor, NSAIDs = nonsteroidal anti-inflammatory drugs.



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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease that has impacted millions of people worldwide.[1] It causes restricted joint movement, synovial proliferation, cartilage destruction, and inflammation.[2] Anti-inflammatory and analgesic drugs are used to treat these symptoms, but prolonged use of these medications causes undesired effects.[3] Therefore, alternative and long-acting natural remedies are needed. Numerous models of experimental animals have been used to study the possible effect of combination drugs for treating RA. Among them, Freund's complete adjuvant (FCA)-induced arthritis model in rats shows similarity to RA in humans.[4,5] The administration of FCA instantly causes local inflammation that persists for a few days, which is followed by a chronic systemic reaction that continues for even several months.[6]

So far, the pathogenesis of RA remains unknown. According to the literature, cytokines are responsible for inflammatory reactions, initiation of RA, synovial inflammation, and destruction of joint tissue.[7] Several mechanisms contribute to synovitis, including T-cell activation, the persistence of cytokine networks such tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, and IL-6, antibodies to synovial tissue, proinflammatory small molecules, and angiogenesis.[8,9] Antiarthritic medicines can relieve joint pain and swelling. Several patients with RA commonly use herbal products for reducing pain.[10] Recent studies have focused their research on plant-based bioactive compounds as an alternative treatment of RA.[11,12]

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Goniothalamin (GTN) is a secondary metabolite of styryl lactone that is widely distributed in plants belonging to *Goniothalamus* genus and Annonaceae family. Previous research has revealed the cytotoxic, anti-inflammatory, apoptotic, and anti-proliferative properties of this compound,[13,14] but its antiarthritic mechanism of action remains poorly understood. Therefore, in this study, we aimed to assess the anti-inflammatory activity of GTN in CFA-induced arthritis in experimental rats.

MATERIALS AND METHODS

Chemicals

CFA, GTN, and all other chemicals were bought from E. Merck (Darmstadt, Germany).

Experimental Protocols

Male Wistar albino rats (150–180 g) were obtained from the animal house of the Central Hospital Affiliated to Shandong First Medical University, and the animals were provided with feed and water as per the guidelines. This research was approved by The Third Hospital of Jinan animal ethical committee, approval no. 2020JN335.

Arthritis Induction

Arthritis was induced following the method of Pearson.[15] For the induction, 0.1 mL of 10 mg/mL of *Mycobacterium tuberculosis* (heat-killed) was inoculated into the rear paw of the right side in Wistar albino rats. The animals were divided into six sets of six rats in each set as follows: normal control, CFA-induced group, CFA + indomethacin (10 mg/kg bw), CFA + GTN (50 mg/kg bw), CFA + GTN (100 mg/kg bw), and GTN alone (100 mg/kg bw), respectively.

The experimental period was 28 days.[3,16] Indomethacin was used as the standard drug.[16] GTN and indomethacin were administered through intragastric tube. The measurements of paw joint and body weight were taken at a fixed interval. After the experimental period, the rats were anesthetized, puncturing the retro-orbital plexus, and blood samples were collected. The excised spleen and thymus were weighed after cleaning. The ankle joints were dissected out and used for the histopathologic examination.

Serum Preparations

The blood sample was allowed to clot, and the serum was separated by centrifuging it at 1000–2000 × *g* for 10 min in a cold centrifuge. The supernatant was separated and used for biochemical analysis.

Paw Thickness and Arthritic Score

The diameter of the paw edema was measured using a digital micrometer screw gauge at regular intervals from day 0 to 28.[17] Arthritis was scored by a previously reported procedure.[18]

Biochemical Analysis

C-reactive protein (CRP), alkaline phosphatase (ALP), and rheumatoid factor (RF) in serum were measured by using the kit provided by SPINREACT Company (Girona, Spain).

Measurement of Cytokines

In serum, cytokines such as TNF-α, IL-6, and IL-1β were analyzed by using the enzyme-linked immunosorbent assay (ELISA) kit (Cayman Chemicals, Michigan, USA). These readings were read on an ELISA plate Reader (Lisa Plus, Germany), and the concentration was expressed as pg/mL.

Histopathologic Examination

The ankle joints were dissected instantly after the completion of the experimental period and stored in 10% formalin for 1 day. Then, 5% formic acid was added for decalcification. The tissues were stained with hematoxylin and eosin and observed through a light microscope.

Statistical Analysis

Results are presented as mean ± standard error of mean (SEM). Statistical Package for the Social Sciences (SPSS) software (version 17.0) was used to analyze the data. Significant values were those with *P* values less than 0.05.

RESULTS

Effect of GTN on Changes in Body Weight

Body weight was reduced significantly in CFA animals ($P < 0.05$) when compared with control rats. Animals in GTN + indomethacin group exhibited substantial improvement in body weight. Indomethacin-treated rats exhibited better body weight after the experimental duration. There was no significant change in body weight in control and GTN alone supplemented rats [Figure 1].

Effect of GTN on Arthritis

The results of this study showed significant increase in the thickness of paw of CFA-induced rats. The paw edema increased on day 7 and gradually increased till day 25 and remained unchanged. GTN reduced paw thickness and swelling in a dose-dependent manner [Figure 2]. The arthritic score increased significantly ($P < 0.05$) in CFA-induced arthritic rats, whereas GTN successfully reduced the arthritic score in a dose-dependent manner. Indomethacin showed similar effect. Normal and GTN alone treated animals did not show any arthritic symptoms.

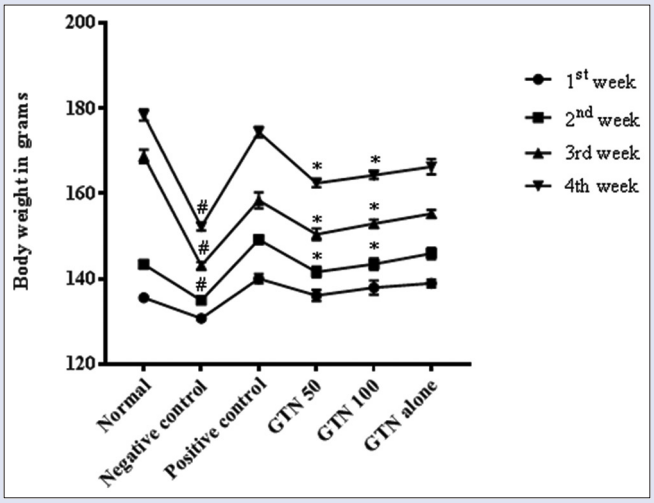

Figure 1: Effects of GTN on body weight in control and experimental rats. Results were conveyed as mean ± SD of six observations. #,*$P<0.05$ indicate the level of significance compared with arthritic control rats

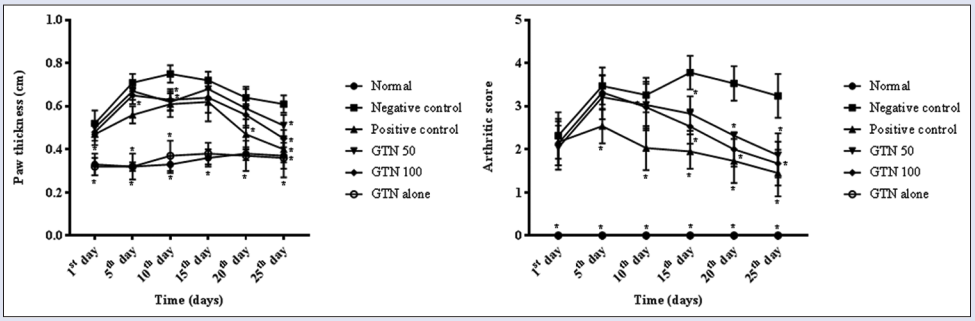

Figure 2: Influence of GTN on paw thickness and arthritic score changes in control and experimental rats. Results were conveyed as mean ± SD of six observations. *P<0.05 indicate the level of significance compared with arthritic control rats

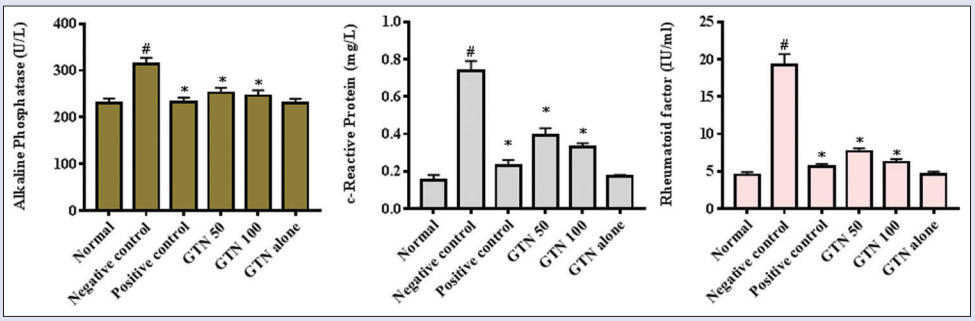

Figure 3: Effects of GTN on biochemical markers in control and experimental rats. Results were conveyed as mean ± SD of six observations. #,*P<0.05 indicate the level of significance compared with arthritic control rats

Effect of GTN on Biochemical Markers

CFA-induced animals showed increased levels of ALP, CRP, and RF. GTN significantly (P < 0.05) reduced the levels of aforementioned biochemical markers in a dose-dependent manner, when compared with CFA-induced arthritic rats. Indomethacin also showed similar results. There was no significant difference between normal and GTN alone treated rats [Figure 3].

Effect of GTN on Organ Weights

The weights of spleen and thymus were significantly (P < 0.05) increased in CFA-induced arthritic rats than in normal animals. GTN significantly (P < 0.05) reduced the weight of spleen and thymus in a dose-dependent manner, when compared with the CFA group. Indomethacin significantly (P < 0.05) reduced the weight of these, when compared with GTN-treated rats. There was no significant difference between control and GTN alone group [Figure 4].

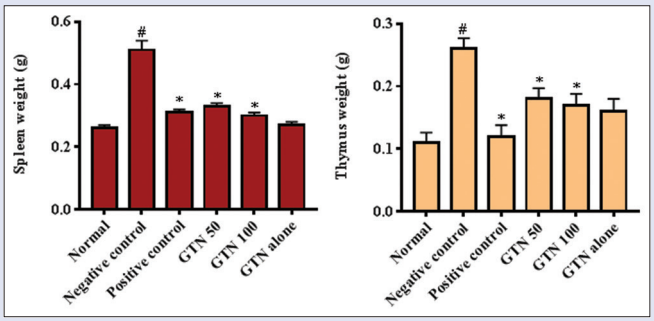

Figure 4: Effects of GTN on organ weights spleen and thymus in control and experimental rats. Results were conveyed as mean ± SD of six observations. #,*P<0.05 indicate the significance compared with arthritic control rats

Influence of GTN on Proinflammatory Cytokines

CFA-induced arthritic rats showed increased levels of cytokines in serum. GTN significantly (P < 0.05) reduced the levels of TNF-α, IL-6, and IL-1β, when compared with arthritic rats. Indomethacin significantly reduced the levels of these proinflammatory cytokines. GTN alone treated rats showed similar levels of cytokines, when compared with normal control [Figure 5].

Effect of GTN on Histopathology of Ankle Joints

The histopathologic analysis of specimens of ankle joint of normal and GTN alone treated animals showed no inflammatory signs and cartilage destruction. CFA-induced arthritic animals showed moderate hyperplasia, synovitis, cartilage destruction, and inflammation. Indomethacin showed mild synovial hyperplasia and inflammation. GTN significantly reduced synovial hyperplasia and swelling in a dose-dependent manner [Figure 6].

DISCUSSION

CFA-induced arthritis in rats is a well-established model that closely resembles the immunological, pathological, and clinical manifestations of human RA. Therefore, it is widely used to screen antiarthritic agents.[19,20] Nonsteroidal anti-inflammatory drugs (NSAIDs) are generally used in the medical treatment of RA to reduce pain and swelling in joints. Prolonged usage of NSAIDs leads to undesired side effects.[21] NSAIDs cause reoccurrence of osteoarthritis due to the degeneration of cartilage. Therefore, natural antioxidants with anti-inflammatory properties and

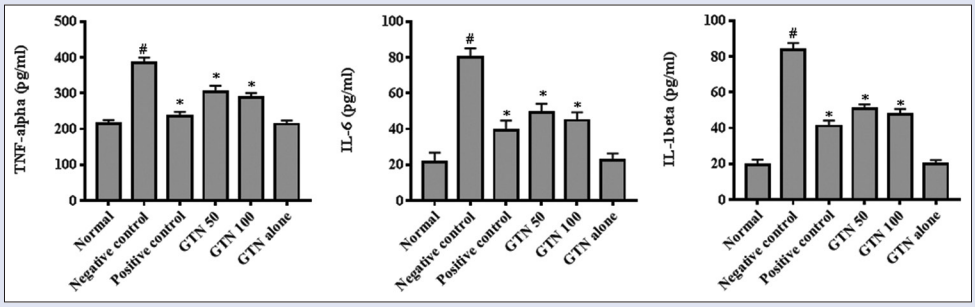

Figure 5: Effects of GTN on serum cytokines TNF-α, IL-6, and IL-1β in control and experimental rats. Results were conveyed as mean ± SD of six observations. #,*$P<0.05$ indicate the level of significance compared with arthritic control rats

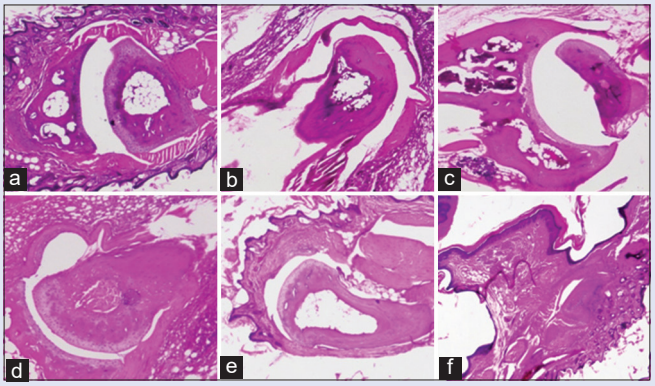

Figure 6: Influence of GTN on the histopathology of ankle joint. (a) Control rats showing normal ankle histology. (b) CFA rats showing moderate synovial hyperplasia, cartilage destruction, and inflammation in joints. (c) CFA + indomethacin-treated ankle joint showing mild inflammation. (d) CFA + GTN (50 mg/kg bw)-treated rats displaying slight synovial hyperplasia and inflammation. (e) CFA + GTN (100 mg/kg bw) rats showing only mild inflammation in the joint. (f) GTN alone treated ankle joint showing normal histology (stained with hematoxylin and eosin, magnification 40×). CFA = complete Freund's adjuvant, GTN = goniothalamin

less toxicity in humans are highly warranted.[22] GTN has proven to possess anti-inflammatory activities without side effects.[23,24]

Our results showed that GTN reduced the symptoms of CFA-induced arthritis in rats. CFA-induced rats exhibited resemblance to human RA in the pathological, immunological, and clinical features. Their body weight decreases owing to inflammation and severity.[25] GTN treatment gradually improved the body weight in CFA-induced arthritic rats. Regaining body weight is a common phenomenon found after treatment with herbal remedies.[26] These results revealed a link among body weight reduction, arthritis severity, and metabolic activity variations in CFA-induced arthritic rats. Furthermore, GTN reduced the paw thickness and arthritic score in rats in a dose-dependent manner, which demonstrates the efficiency of GTN in inhibiting arthritis. The paw thickness and the arthritic score are the visual tools for the estimation of severity of RA.[27] The degree of paw thickness and arthritic scores are the most objectives to measure the severity of arthritis.[28] Therefore, the primary symptoms of arthritis, such as redness, swelling, and destruction of joints, were reduced in GTN-induced and indomethacin groups, when compared with control arthritic rats.

Next, biochemical makers such as ALP, CRP, and RF were routinely analyzed in this study to measure the effect of GTN.[29] According to the results, the aforementioned biomarkers were significantly increased in CFA-induced rats. GTN reduced the levels of these biomarkers, indicating that it is effective in treating CFA-induced arthritis. Inflammatory disorders, especially RA, show elevated levels of CRP.[30] GTN effectively reduced the level of CRP in CFA-induced arthritic rats. RF is an autoantibody against self, which generates immune complexes activating complement that affects tissue destruction.[31] Several researchers have documented that the discharge of inflammatory mediators triggers an immune response in arthritis.[32,33] The spleen and thymus are the vital organs that control the immune response. They produce immune cells and remove dead cells from the circulatory system.[34] The organ weight of spleen and thymus in CFA-induced arthritic rats was increased, which shows the infiltration of immune cells. In this study, we found that the organ weight of spleen and thymus was reduced by GTN in a dose-dependent manner.

A previous study has suggested that proinflammatory cytokines play an important role in the pathogenesis of CFA, such as IL-1β, TNF-α, and IL-6.[35] In this study, we obtained similar results. The levels of the aforementioned proinflammatory cytokines were elevated in the CFA-induced rats when compared with the control animals. Administration of indomethacin and GTN (50 and 100 mg/kg bw) could reduce the cytokine levels, when compared with CFA-induced arthritic rats. These results point to the antiarthritic activity of GTN. Histopathologic analysis of ankle joints of CFA-induced rats showed articular damage, revealing inflammation, synovial hyperplasia, cartilage, and joint destruction. Indomethacin and GTN reduced the inflammation and joint destruction. The antiarthritic effect of GTN is demonstrated by the underlying mechanism associated with diminished production of cytokines and regulation of immunologic function.

CONCLUSION

In conclusion, GTN demonstrates a protective effect in RA in CFA-induced rats by decreasing the paw thickness, inflammation, arthritic score, biochemical markers, organ weight, and histopathologic damage. It might be a potent anti-inflammatory and antiarthritic candidate. Further studies are required to analyze the molecular mechanisms of its anti-arthritic potential.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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