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Original Research Article

Arglabin as a potential drug in the treatment of Freund's complete adjuvant-induced arthritis in rats

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Abstract

Purpose: *To investigate the anti-arthritic activity of arglabin in Freund's complete adjuvant- induced arthritis in rats, and the likely underlying mechanism.*
Methods: *A total of 40 male albino Wistar rats weighing between 120 and 150 g were used for this study. The rats were divided into four groups of ten rats each: control group, arthritis group, arglabin-treated group, and standard (STD) group. Chronic arthritis was induced by injecting Freund's complete adjuvant in the plantar region of the rats. Rats in the arglabin-treated group received 5 ng/g arglabin intraperitoneally (i.p.), while those in the STD group received 1.5 mg/kg indomethacin, p.o. for 4 weeks. The development of arthritis was assessed at 0, 7, 14, 21 and 28th day of protocol by measuring thermal hyperalgesia, mechanical nociceptive threshold, arthritic score and paw volume. Activities of liver alkaline phosphatase (ALP), alanine amino-transaminase (ALT) and aspartate amino-transaminase (AST), and the levels of inflammatory cytokines -tumor necrosis factor-α (TNF-α), interferon gamma (IFN-γ), interleukin (IL)-6 and interleukin (IL)-1β were measured in the synovial fluid, while those of inflammatory mediators - thromboxane B_2 (TXB_2), prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4) were determined in serum. The expressions of mRNAs of nuclear factor kappa-light-chain-enhancer of activated B cells (NF_kB), cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) were also determined in rat synovial tissues.*
Results: *Arglabin significantly decreased the paw swelling and arthritic scores, but significantly increased the paw withdrawal latency, when compared to the arthritis group ($p < 0.05$). It also attenuated the altered levels of inflammatory cytokines in arthritic rats, and significantly reduced the levels of inflammatory mediators, when compared to the arthritis group ($p < 0.05$). The expressions of mRNAs of NF_kB, COX-2 and iNOS also significantly decreased in arglabin-treated group, relative to the arthritis group ($p < 0.05$).*
Conclusion: *The anti-arthritic activity of arglabin is due to its effect on inflammatory pathway via decreases in the levels of inflammatory mediators and cytokines, and decrease in the expressions of NF_kB, COX-2 and iNOS in the synovial tissues of arthritic rats.*

Keywords: *Arglabin, Arthritis, Freund's complete adjuvant, Inflammatory cytokines*

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INTRODUCTION

Arthritis is an inflammatory disorder of the joints which affects approximately 10 % of the world's population [1]. Osteoarthritis (OA) and rheumatoid arthritis (RA) are the two major types of arthritis. The latter is an autoimmune disorder associated with chronic inflammation that results in the loss of body movement [2]. There are about 10 million people afflicted with RA in China [3]. The disease leads to destruction of joints, damage to cartilage and bones, thus affecting the quality of life of sufferers [4]. Arthritis mainly affects the large joints, and causes swelling and pain due to alteration in the morphology of the joints. Osteoarthritis affects approximately 3.6 % of the world's population [5], and it is an inflammatory disorder of joints characterised by joint pain, cartilage damage and development of osteophytes. Studies have revealed that inflammatory cytokines play important role in the pathogenesis of RA and OA [6], which are conventionally managed with non- steroidal anti-inflammatory drugs (NSAIDs) and analgesics. However, apart from proffering only symptomatic relief, frequent use of NSAIDs causes gastric ulcer. Recently, the use of alternative medicine has been shown to elicit some beneficial effects in the management of arthritis. Arglabin, a sesquiterpene gamma-lactone isolated from different plant species such as *Artemisia glabella,* has tremendous pharmacological potential [7,8]. It possesses antimicrobial, anti-inflammatory, neuroprotective and anticancer activities [9-11]. The anti-inflammatory property of arglabin is due to its capacity to alter the level of inflammatory cytokines, which confers on it a potential for the management of lung, ovarian, colon and breast cancers [12]. The aim of this study was to investigate the anti-arthritic activity of arglabin in Freund's complete adjuvant-induced arthritis in rats, and to identify the likely mechanism involved.

EXPERIMENTAL

Animals

Male albino Wistar rats (120-150 g) were procured from Jing Mei Company, China, and were housed under standard condition based on the guidelines of Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC) for experimentation and animal use [13].The rats were acclimatized to laboratory conditions for a period of seven days, during which they had free access to normal standard chow and tap water. The protocol for this study was approved by the Institutional Animal Ethical Committee of Dongzhimen Hospital, Beijing University of Chinese Medicine, China (DHBUCM/IAEC/02-2017).

Induction of arthritis

The rats were divided into four groups of ten rats each: control group, arthritis group, arglabin-treated group and standard (STD) group. Rats in the arglabin-treated group received 5 ng/g arglabin, i.p., while those in the STD group received 1.5 mg/kg indomethacin, p.o., for a period of four weeks. Inactivated *Mycobacterium tuberculosis* (10 mg) was dried in 1 ml of paraffin oil for the preparation of complete adjuvant. Freund's complete adjuvant (0.1 ml) was injected on the first day of protocol in the sub-plantar region of the rats, and on the 29th day of protocol, blood was collected from the retro-orbital plexus of the rats under mild anesthesia and centrifuged at 3000 rpm for 10 min to obtain serum which was used for biochemical analysis. The rats were sacrificed by cervical dislocation and their ankle joints were isolated for biochemical, histological and gene expression studies.

Assessment of the development of arthritis

Development of arthritis was assessed in the rats by measuring thermal hyperalgesia, mechanical nociceptive threshold, arthritic score and paw volume on 0, 7, 14, 21 and 28 days of protocol. The severity of arthritis was estimated on the five-point scale: score 0 was considered as no swelling, 1 for limited erythema and edema; 2 for erythema from the tarsal bone to ankle with slight edema, 3 for erythema from the tarsal bone to ankle associated with moderate edema, and 4 for erythema of entire leg associated with edema.

Determination of the activities of liver enzymes in serum

The activities of alkaline phosphatase (ALP), alanine amino-transaminase (ALT) and amino transaminase (AST) were estimated in the sera using their respective kits.

Determination of the levels of inflammatory cytokines and mediators

Enzyme-linked immunosorbent assay (ELISA) was used for the determination of concentrations of TNF-α, IFN-γ, IL-6 andIL-1β in synovial fluid, and serum levels of TXB_2, PGE_2 and LTB_4.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA extraction reagent (RNAiso Plus) was used for the extraction of total RNA from the

synovial tissues, while cDNA synthesis kit was used to perform the cDNA synthesis reaction according to the instructions of the manufacturer. Light Cycler 1536 Real-time PCR Detection System was used for the estimation of the expressions of mRNAs of NF_kB-p65, COX-2 and iNOS by quantitative RT-PCR. Variation in the cDNA content was normalized using glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The PCR medium (20 µl) which consisted of 6.4 µl of dH_2O, 1.6 µl of gene-specific primer (10 µM), 2 µl of synthesized cDNA and 10 µl of SYBR Premix Ex Taq™ II, was used to carry out the PCR reaction. Data was expressed as the mRNA expression of gene of the arthritic group relative to that of the normal or treated group.

Statistical analysis

Data are expressed as mean ± SEM, and analyzed using Graph pad prism (6.1), while post-hoc comparison of the means was carried out using Dunnett's post-hoc test. The level of statistical significance was set at $p < 0.05$.

RESULTS

Effect of arglabin on paw swelling, arthritic score and paw withdrawal latency

Effect of arglabin on paw swelling, arthritic score and percentage of paw withdrawal latency in arthritic rats was shown in Figure 1. There were significant increases ($p < 0.01$) in the paw swelling and arthritic score, and significant decrease in the paw withdrawal latency in the arthritis group, compared to the control group. However, in the arglabin-treated group, there were significant decreases ($p < 0.01$) in the paw swelling and arthritic score, and significant increase in the paw withdrawal latency in RA rat model, relative to the arthritis group ($p < 0.01$).

Effect of arglabin on the activity of liver enzyme

Effect of arglabin on the activities of liver enzymes in the sera of arthritic rats was shown in Figure 2. There were significant increases ($p < 0.05$) in the activities ALT, AST and ALP in the arthritis group, when compared to control group. However, the arglabin and indomethacine-treated groups had significant reductions ($p < 0.05$) in the activities of these enzymes, when compared to arthritis group.

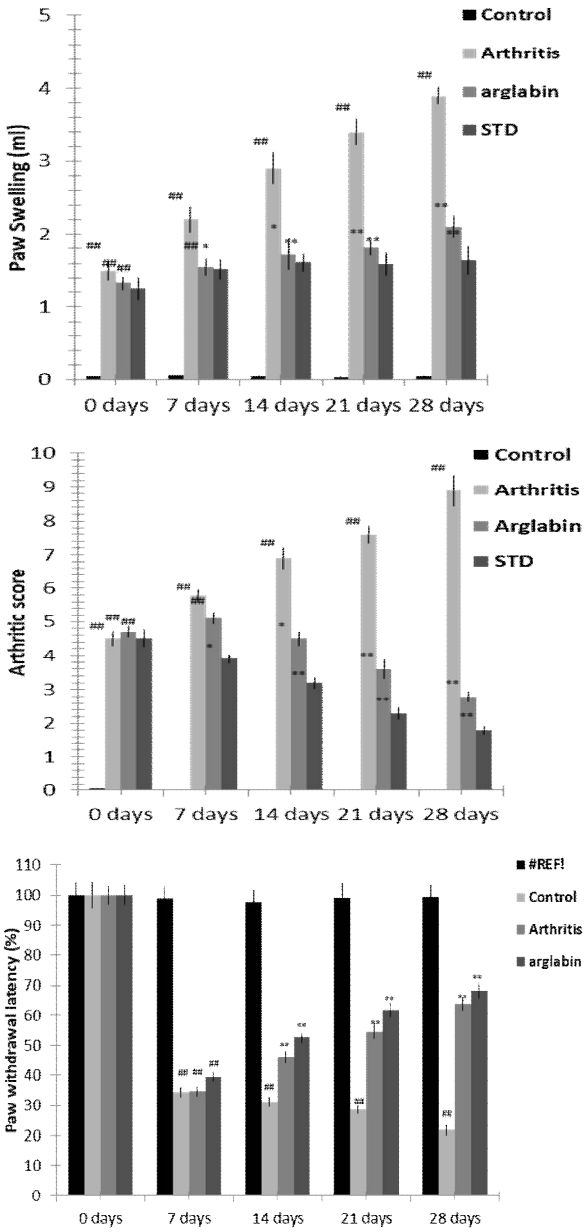

Figure 1: Effect of arglabin on paw swelling, arthritic score and paw withdrawal latency in arthritic rats. Mean ± SEM (n=6); $^{\#\#}p<0.01$, compared to control; $^{**}p < 0.01$, compared to arthritis group

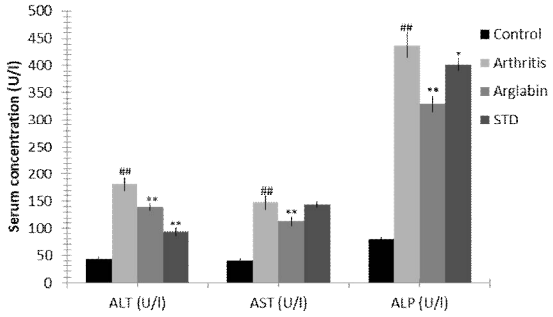

Figure 2: Effect of arglabin on the activities of liver enzymes in the sera of arthritic rats. Mean ± SEM (n = 6); $^{\#\#}p < 0.01$, compared to control; $^{**}p < 0.01$, compared to arthritis group

Effect of arglabin on the levels of circulating inflammatory cytokines

The effect of arglabin on the concentrations of inflammatory cytokines in the synovial fluid of arthritic rats are shown in Table 1. The levels of inflammatory cytokines (IL-1β, IL-6, TNF-α, and INF-γ) were significantly enhanced up to 4 to 5 folds in the arthritis group, compared to control group ($p < 0.05$).

Table 1: Effect of arglabin on inflammatory cytokines in the synovial fluid of arthritic rats

Group	IL-1β (ng/l)	IL-6 (ng/l)	TNF-α (ng/l)	INF-γ (ng/l)
Control	43.72 ± 3.16	41.48 ± 2.17	79.23 ± 3.61	3.16 ± 0.21
Arthritis	181.40 ± 11.83$^{\#\#}$	147.33 ± 12.29$^{\#\#}$	437.30 ± 23.10$^{\#\#}$	7.59 ± 0.37$^{\#\#}$
Arglabin	98.60 ± 6.26**	93.27 ± 9.12**	219.10 ± 14.70**	6.28 ± 0.27**
STD	93.71 ± 7.29**	89.38 ± 4.91**	186.40 ± 11.30**	4.49 ± 0.23**

Mean ± SEM (n = 6); $^{\#\#}p < 0.01$, compared to control; $^{**}p < 0.01$, compared to the arthritis group

Effect of arglabin on levels of inflammatory mediators in the sera of arthritic rats

Effect of arglabin on the levels of inflammatory mediators in the sera of arthritic rats was shown in Table 2. The concentrations of inflammatory mediators (TXB_2, PGE_2, and TLB_4) were significantly enhanced in the arthritis group, relative to the control group ($p < 0.05$). However, treatment with arglabin and indomethacin significantly reduced the levels of these inflammatory mediators in arthritic rats ($p < 0.01$).

Table 2: Effect of arglabin on levels of inflammatory mediators

Group	TXB_2 (ng/l)	PGE_2 (µg/l)	LTB_4 (ng/l)
Control	39.81 ± 3.14	1.09 ± 0.08	10.28 ± 1.01
Arthritis	79.26 ± 5.38$^{\#\#}$	4.18 ± 0.39$^{\#\#}$	16.37 ± 1.35$^{\#\#}$
Arglabin	42.71 ± 2.93**	1.23 ± 0.31**	12.69 ± 1.13**
STD	34.29 ± 1.73**	1.85 ± 0.23**	15.27 ± 1.28**

Mean ± SEM (n=6); $^{\#\#}p < 0.01$, compared to control; $^{**}p < 0.01$, compared to arthritis group

Effect of arglabin on the expression of mRNAs of NF_kB, COX2 and iNOS

Effect of arglabin on the expression of mRNAs of NF_kB, COX2 and iNOS was shown in Figure 3. The expressions of mRNAs of NF_kB, COX2 and iNOS were significantly enhanced ($p < 0.05$) in the synovial tissues of arthritis group, compared to control, whereas they were significantly reduced ($p < 0.05$) in arglabin and indomethacin-treated groups, when compared to the arthritis group.

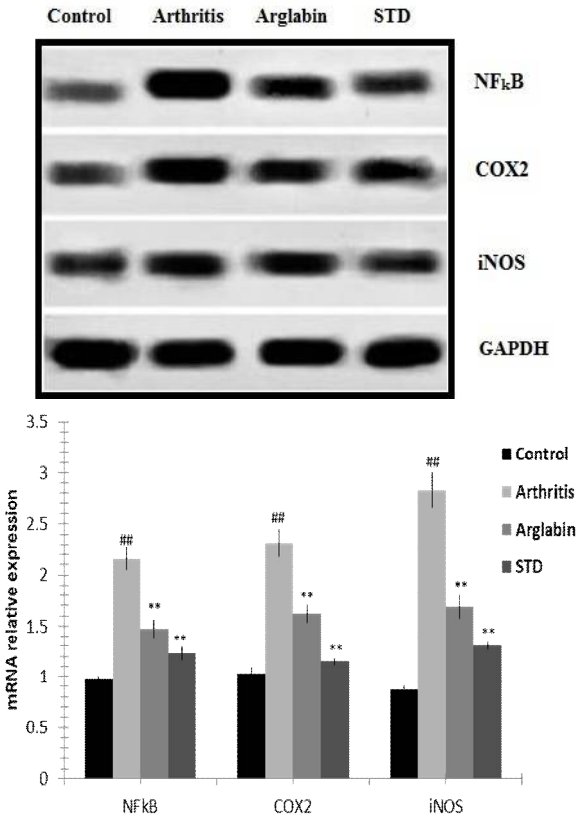

Figure 3: Effect of arglabin on the expressions of mRNAs of NF_kB, COX-2 and iNOS in the synovial tissues of arthritic rats. Mean ± SEM (n=6); $^{\#\#}p < 0.01$, compared to control; $^{**}p < 0.01$, compared to arthritis group

DISCUSSION

The present study investigated the anti-arthritic activity of arglabin and attempted to postulate its possible mechanism of action. Arthritis was induced in the rats by a single injection of Freund's complete adjuvant and they were treated with arglabin for a period of four weeks. The results showed that paw swelling and arthritic score were significantly reduced, while paw withdrawal latency was significantly enhanced in arglabin treated-group compared to the arthritis group. Arthritis is mainly characterized by swelling of the synovium due to the proliferation of synovial cells [14], and studies have shown that the serum activities of ALP, ALT and AST increase due to hepatic lesions [15]. In the present study, activities of these hepatic enzymes were significantly increased in the sera of arthritic rats, which is in agreement with reports of previous studies that reported suggested an activation of enzymes and

inflammatory mediators in arthritis [16]. However, the arglabin and indomethacine-treated groups showed a significant reduction in the activities of these enzymes, when compared to the arthritis group.

In arthritis, the concentrations of inflammatory mediators like LTB_4, PGE_2 and TXB_2 are enhanced by lipoxygenase (LOX) and cyclooxygenase (COX) pathways. These mediators together enhance the vasodilatation, vascular permeability and blood flow in arthritis [17]. However, in arthritis, they cause resorption of bone by stimulating the production of matrix metalloproteinases (MMPs) [18]. Pro-inflammatory cytokines like IL-1β, IL-6, TNF-α and INF-γ play important roles in the pathogenesis of arthritis [19]. This is so because they increase erosion of bone, degradation and destruction of articular cartilage, and inflammation. Anti-inflammatory drugs used in the management of arthritis attenuate the altered levels of these inflammatory mediators and cytokines. The results of the present study revealed that the concentrations of the measured inflammatory cytokines increased by 4 to 5 folds in arthritis group, when compared to control. However, treatment with arglabin significantly decreased the levels of inflammatory mediators, relative to the arthritis group.

Parameters of inflammatory cascade such as NF_kB, COX-2 and iNOS play substantial roles in the pathogenesis of arthritis. The expressions of adhesion molecules and inflammation mediators depend on the activation of NF_kB, while COX-2 catalyzes the synthesis of PGE_2 whose concentration increases in arthritis [20]. However, activation of iNOS enhances the synthesis of nitric oxide (NO) which causes tissue damage and inflammation of joints [21]. In the present study, the expressions of mRNAs of NF_kB, COX-2 and iNOS were significantly enhanced in the synovial tissues of rats in the arthritis group, whereas they were significantly reduced in arglabin and indomethacin-treated groups, when compared to the arthritis group. However, treatment with arglabin attenuated the altered expressions of NF_kB, COX-2 and iNOS in the synovial tissues of the arthritic rats.

CONCLUSION

These results indicate that the anti-arthritic activity of arglabin is due to its potential effect on inflammatory pathway through decrease in the levels of inflammatory mediators and cytokines, and reduction in the expressions of NF_kB, COX-2 and iNOS in the synovial tissues of arthritic rats.

DECLARATIONS

Acknowledgement

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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