Ind. J. Pharm. Edu. Res., 2025; 59(2):727-737.
https://www.ijper.org

Original Article

Anti-Arthritic Effect of Traditional Chinese Medicinal Compound Bavachalcone on Freund's Complete Adjuvant-Induced Rheumatoid Arthritis in Rats

Xinglei Xiao*, Shanglun Liu, Guoqing Chen, Chen Cao

Department of Orthopedics, Huai'an Hospital of Traditional Chinese Medicine, Huai'an, CHINA.

ABSTRACT

Background: Oxidative stress and inflammation are suggested to be the major etiological factors for the autoimmune disease rheumatoid arthritis. Rheumatoid arthritis affects millions of individuals all over the world even though mortality rate is comparatively lower than the other autoimmune diseases it severely affects the quality life of the patients. Lack of timely and proper medical intervention could cause deformity bones leading to chronic pain, long term disability and also premature mortality. Traditional Chinese medicines are boon to treat these types of autoimmune diseases. For prolonged period these medicine were been trusted and preferred by Chinese and South Asian population. Bavachalcone is one such compound which possess diverse pharmacological properties and it proven to inhibit osteoclastogenesis activity. **Objectives:** In our study we evaluated the ameliorative potency bavachalcone in rats induced arthritis with Freund's complete adjuvant. **Materials and Methods:** The weight gain and relative index of thymus and spleen were measured to assess the inflammatory response in arthritis induced rats. Paw edema volume and arthritic index was calculated to confirm the induction of arthritis in test animals. The vasoregulatory effect and impact of bavachalcone on hepatic biomarkers enzymes were assessed. The antioxidant potency of bavachalcone was determined by estimating the levels of MDA and antioxidants in arthritis induced rats. The inflammatory cytokines and the inflammatory mediators iNOS, COX-2 and NF-κB were quantified to confirm the anti-inflammatory effect of bavachalcone in arthritis induced rats. Finally the anti-arthritic potency of bavachalcone was confirmed with synovial tissue histopathological analysis. **Results:** Bavachalcone significantly decreased the body weight, lymphatic organ relative index and paw edema volume in arthritis induced rats which was evidenced with decrease in arthritis index. It significantly decreased vasoregulators PGE2, NO and hepatic biomarker enzymes in arthritis induced rats. Bavachalcone increased the antioxidants and inhibited the synthesis of inflammatory response thereby prevented arthritis induction in rats. This was further confirmed with our histopathological results. **Conclusion:** All together our findings suggest bavachalcone possess anti-arthritic potency and it can be formulated as drug to treat arthritis.

Keywords: Rheumatoid Arthritis, Oxidative stress, Inflammation, Anti-arthritis drug, Traditional Chinese medicine, Bavachaclone.

Correspondence:
Dr. Xinglei Xiao
Department of Orthopedics, Huai'an Hospital of Traditional Chinese Medicine, Huai'an-223001, CHINA.
Email: xiaoxinglei123@outlook.com

Received: 22-05-2024;
Revised: 12-07-2024;
Accepted: 26-11-2024.

INTRODUCTION

An autoimmune disease which affects quality life of millions of individuals globally is rheumatoid arthritis.[1] Rheumatoid arthritis is presented as symmetrical polyarthritis caused due to chronic inflammation.[2] Most of the patients at initial stage suffers with mild discomforts such as pain, swelling and stiffness in small joints especially in interphalangeal and wrist joints. It further causes inflammation, hyperplasia in synovial join which eventually leads to neovascularization and cartilage damage.[3] In advanced stages rheumatoid arthritis causes joint deformation and affects vital internal organs such as lungs, kidney and heart.[4] Rheumatoid arthritis is a recurrent diseases which had affected about 17.6 million people worldwide by the year 2020. About 208 incidences were reported globally per one lakh population comparatively women are more prone to rheumatoid arthritis. It has been forecasted by the year 2050 about 31.7 million populations will be affected with rheumatoid arthritis.[5] Due to this patients experience tremendous psychological stress and it also imposes a significant financial burden on society.[6]

Diverse genetic and environmental factors triggers and worsens rheumatoid arthritis.[7] Molecular mimic and infectious agents induced immune response activation perpetuates inflammation

DOI: 10.5530/ijper.20257346

in rheumatoid arthritis. Individuals exposed to occupational pollutants are more prone to arthritis.[8] The pathology of rheumatoid arthritis harbors exaggerated immune response, deregulated cytokine signaling and increased activation of chondrocyte and osteoclast.[9] Oxidative stress plays a crucial role in the pathogenesis of rheumatoid arthritis. Reactive oxygen species activates NF-κB signaling thereby triggers inflammatory response.[10,11] Suppressing antioxidant system also reported to elicits inflammatory response in arthritis patients.[12] At present arthritis is treated with glucocorticoids, non-steroidal anti-inflammatory drugs and other pain relieving drugs which improves pain, stiffness and fatigue in patients but the complete cure is not achieved.[13] Side effects also hinders the usage of these drugs for longer period.[14,15] Antagonizing inflammatory response for treating rheumatoid arthritis is the new strategy focused by the researchers.

Traditional Chinese Medicine (TCM) may be potent and most trusted alternative for treating various autoimmune disorders. These medicines are not only effective but also safe and economical.[16] In TCM rheumatoid arthritis is categorized as "Bi Zheng" or arthralgia it focus on strengthening the body's vital energy (qi) and improving the quality and circulation of blood which not only relieves pain but also treats the root cause of the disease.[17] It eliminates the pathogenic factors and strengthens the body resistance against it. TCM therapy are multi targeted which regulates multiple pathways therefore they render minimal side effects and they are cost effective.[18,19] Decoction and extracts of herbs like *Tripterygium wilfordii* Hook, F, Wu-Tou Tang, Guizhi-Shaoyao-Zhimu were prescribed and proven beneficial to ameliorate rheumatoid arthritis.[20-22]

One such promising TCM possess diverse pharmacological properties such as antioxidant, anti-inflammatory, bacteriostatic, antiviral and anti-tumor activities is bavachalcone. Bavachalcone is chalcone compound segregated from Fructus Psoraleae.[23,24] This compound possess osteoclastogenesis, angiogenesis and tissue repairing property[25] hence in this present we hypothesized to evaluate the ameliorative potency of bavachalcone in arthritis induced rats.

MATERIALS AND METHODS

Animals

Twenty four healthy male Wistar albino male rats weighing about 210±20 g were procured after obtaining ethical clearance from institutional animal ethical clearance board. The animals were fed with standard laboratory rodent pellet diet and water *ad libitum*. The animals were acclimatized in laboratory condition with 23±2°C temperature, 50±5% humidity and 12 hr light dark cycle for a week. Sterile condition was strictly maintained in the laboratory the cages and the beddings were periodically changed as per the guidelines. The animals were treated with utmost care and concern.

Experimental grouping

The acclimatized rats were segregated into four each group with 6 healthy rats. Group I are categorized as control rats which were treated with olive oil. Group II are arthritis stimulated rats administered with 0.1 mL of FCA through sub-cutaneously in the tail base. Group III are arthritis stimulated rats treated with 20 mg/kg of Bavachalcone for 25 days. Group IV are drug control rats which are arthritis stimulated rats treated with 3 mg/kg of indomethacin for 25 days. Both the drugs bavachalcone and indomethacin were orally treated 30 min before the FCA induction and continued till end of the 25th day treatment. The occurrence and development of arthritis was assessing with paw thickness. On completion of 25 days treatment the rats were sacrificed by euthanasia and the blood was collected for biochemical and molecular analysis. The thymus, spleen and bone were immediately dissected and stored at -80°C until further analysis.

Determination of relative organ weight

The final weight gain in arthritis induced rats untreated and bavachalcone rats were measured and the relative organ weight were calculated. Thymus and spleen were excised rinsed with PBS dried with tissue paper and weighed using digital weighing machine. The relative organ weight of thymus and spleen were calculated using the formula

$$\text{Relative organ weight} = (\text{Organ weight}/\text{Total body weight}) \times 100 \ (\text{mg/g})$$

Paw edema analysis

The paw volume of arthritis induced rats untreated and bavachalcone rats were measured using YLS-7B plethysmometer (China).The paw swelling was analyzed using the formula,

$$\text{Paw swelling} = \text{Final Paw swelling volume} - \text{Initial Paw swelling volume}$$[26]

Assessment of arthritic index

Severity of arthritis in rats was evaluated using five point scaling method. The scoring was given based on the observation by three independent observers. 4-edema and erythema from ankle joints to entire paw, 3-moderal edema and erythema observed from ankle to tarsal bone, 2-edema in toes and toe joints, 1-slight edema and erythema in toe joints, 0- no edema and erythema. The arthritis index score of individual rat was the sum of four limbs.[27]

Evaluation of Vasodilation

The vascular tone modulation and immune cell function in arthritis induced untreated and drugs treated animals were assessed by quantifying the levels of prostaglandin E2 and nitric oxide. Prostaglandin E2 (PGE2) was estimated with the

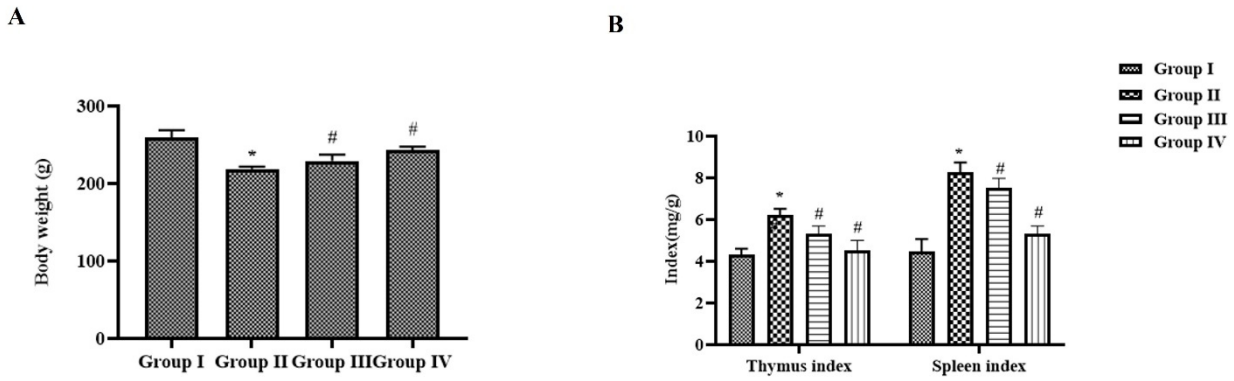

Figure 1: Impact of bavachalcone on body weight and lymphatic organs in arthritis induced rats. Body weight B) Relative organ index of thymus and spleen. Analysis was conducted in triplicates using samples randomly collected from test groups, each containing six rats. Statistical assessment was performed using GraphPad Prism version 6.02 software and results were presented as means±SD. *p*-value<0.05 was considered statistically significant.

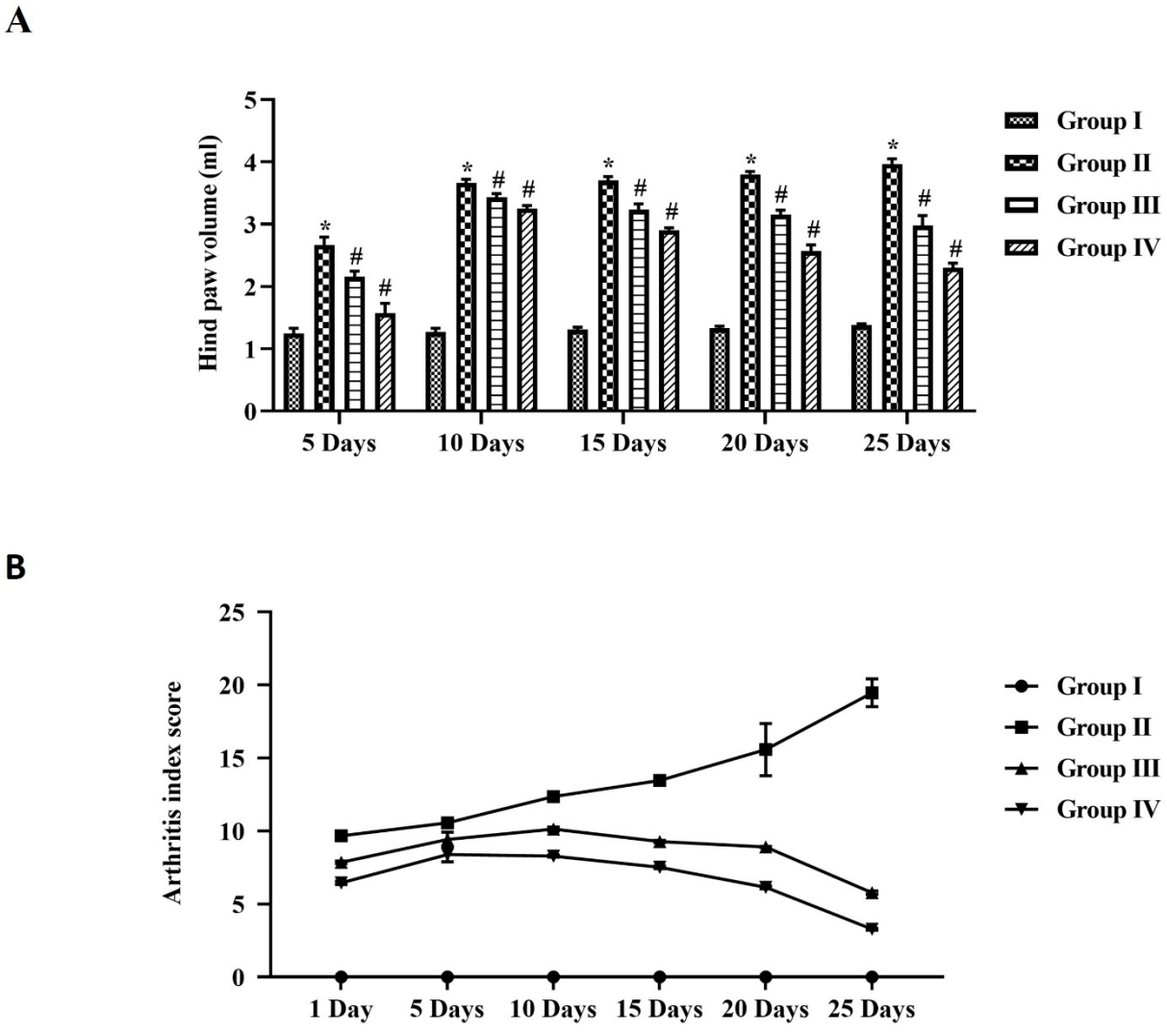

Figure 2: Role of bavachalcone on preventing Paw edema and arthritic index in arthritis induced rats. Paw edema volume B) Arthritic index assessed throughout the period with regular intervals. Analysis was conducted in triplicates using samples randomly collected from test groups, each containing six rats. Statistical assessment was performed using GraphPad Prism version 6.02 software and results were presented as means±SD. *p*-value<0.05 was considered statistically significant.

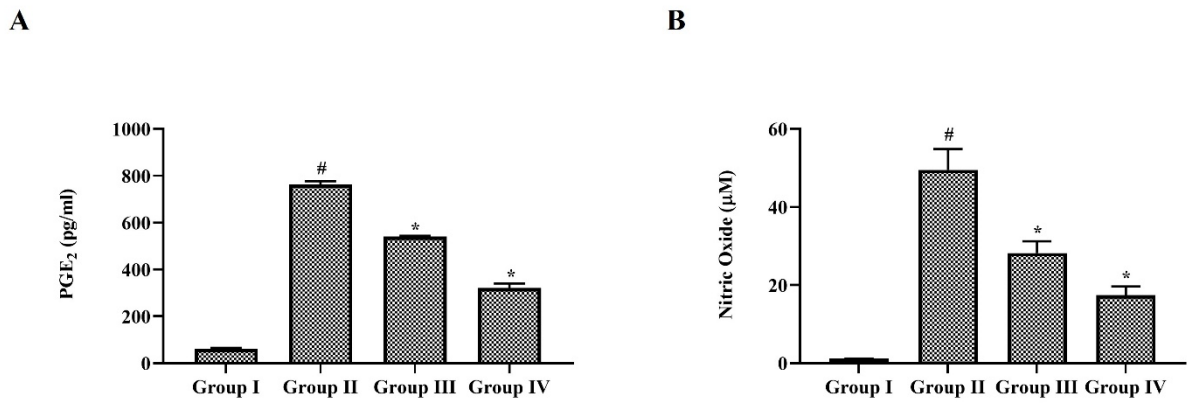

Figure 3: Vasoregulatory effect of bavachalcone in arthritis induced rats. Prostaglandin E2 B) Nitric oxide. Analysis was conducted in triplicates using samples randomly collected from test groups, each containing six rats. Statistical assessment was performed using GraphPad Prism version 6.02 software and results were presented as means±SD. *p*-value<0.05 was considered statistically significant.

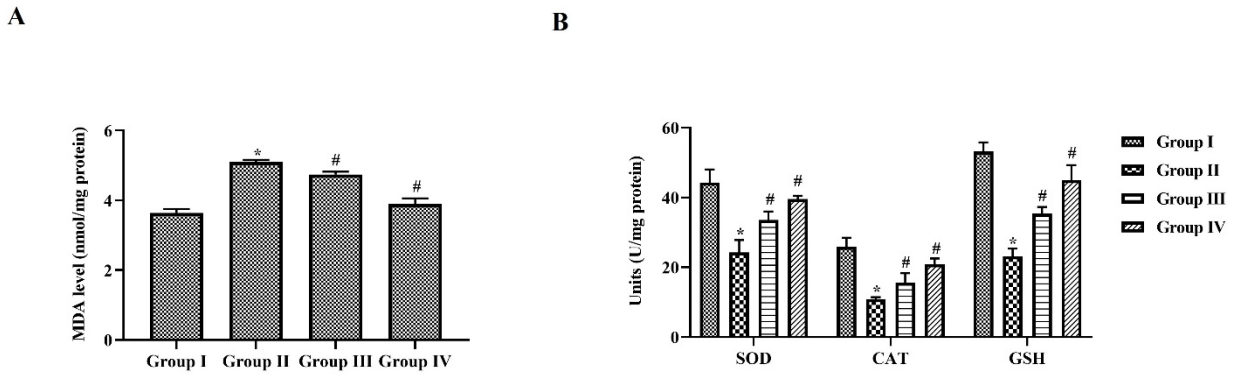

Figure 4: Ameliorative effect of bavachalcone against oxidative stress in arthritis induced rats. Malondialdehyde content B) Superoxide dismutase, Catalase and Reduced glutathione. Analysis was conducted in triplicates using samples randomly collected from test groups, each containing six rats. Statistical assessment was performed using GraphPad Prism version 6.02 software and results were presented as means±SD. *p*-value<0.05 was considered statistically significant.

ELISA kit procured from LS BIO. The reagents were brought to room temperature before initiation of experiment. As per the instructions provided in the kit the reagents were prepared and the standard, samples were diluted. 50 µL of standards and sample were added to each wells and the assay was performed according to kit manual. Finally 50 µL of stop solution was added to all the wells and the yellow coloration formed was read at 450 nm using ELISA microplate reader. The assay was performed in triplicates and the concentration of PGE2 in samples were calculated using the standard curve plot.

Nitric oxide levels in the experimental animals were quantified using colorimetric assay kit obtained from ThermoFisher Scientific. The total nitric oxide content in the samples were measured by incubating the sample with nitrate reductase and NADH. The nitrate reductase and NADH reduces nitrate to nitrite. The final color absorbance was measured at 540 nm and the concentration of nitric oxide was calculated as per the kit manual instructions.

Assessment of oxidative stress

Oxidative stress in arthritis induced untreated and drugs treated rats were analyzed by estimating the extent of lipid peroxidation and the levels of antioxidants in the samples. Lipid peroxidation was estimated using the Lipid peroxidation (MDA) Assay kit obtained from Abcam. The samples, standards were prepared as per the protocol provided in the manual before initiation of experiment. The reagents prepared were equilibrated to room temperature and gently agitated prior usage. The assay was performed in triplicates in microplate and the final absorbance was read at 532 nm.

Antioxidants superoxide dismutase, catalase and reduced glutathione were quantified using the commercially available colorimetric assay kits procured from Cayman Chemicals. The assays were performed as per the instructions and the concentrations were determined using the standard curve plot drawn with known standard concentration OD values.

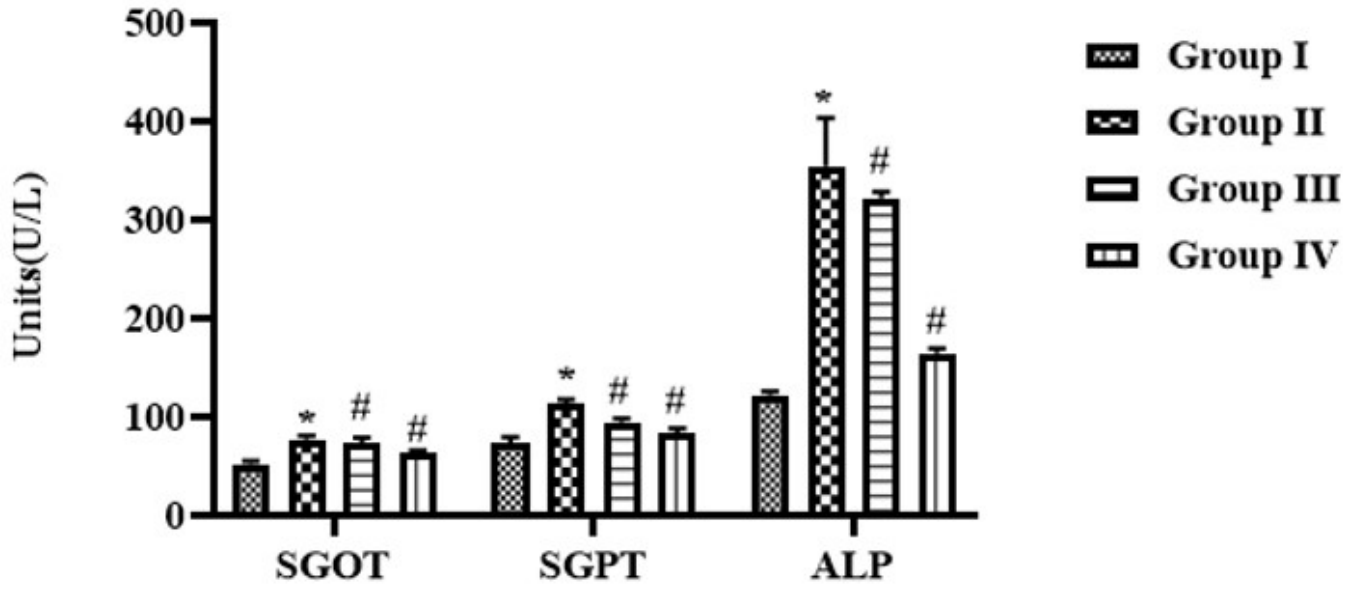

Figure 5: Effect of bavachalcone on hepatic biomarker enzymes in arthritis induced rats. Level of hepatic biomarkers Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT) and Alkaline Phosphatase (ALP). Analysis was conducted in triplicates using samples randomly collected from test groups, each containing six rats. Statistical assessment was performed using GraphPad Prism version 6.02 software and results were presented as means±SD. *p*-value<0.05 was considered statistically significant.

Evaluation of hepatic marker enzymes

The hepatic function in arthritis induced untreated and drugs treated rats were assessed by quantifying the hepatic biomarker enzymes SGOT, SGPT and ALP. Standards were prepared with known concentration of enzymes and the reagents were prepared prior to experiment. The blank, standard and samples were dispensed in the microplates in triplicates. The enzyme-substrate and the colorimetric reagents were added according to the manual and finally the reaction was stopped with stop solution. The absorbance was read and the enzymes activities in the samples were determined by interpolating with the standard curve.

Analysis of inflammatory cytokines

Inflammatory cytokines interleukins 1β, 6, 10 and tumor necrosis factor alpha were quantified in the arthritis induced untreated and drugs treated rats. The inflammatory cytokines were estimated using the ELISA assay kits from R and D Systems. The standard and the samples were prepared according to the instruction and dispensed in the antibodies coated microplates. The assay was performed according to kit protocol and the final absorbance was measured. The cytokines concentration was determined using the standard curve.

Assessment of inflammation mediators

The key inflammation mediators of rheumatoid arthritis Cyclooxygenase-2 (COX-2), inducible Nitric Oxide Synthase (iNOS) and Nuclear Factor-kappa B (NF-κB) were quantified in the experimental animals. The levels of COX-2, iNOS and NF-κB were estimated using the ELISA kits procured from Abcam. The assay was performed according to the kit manual and the final absorbance was measured using ELISA microplate reader. The concentration of inflammatory mediators was ascertained with standard curve plot.

Histology of Synovial tissue

The rats were sacrificed at the end of experiment and the right hind limb was dissected, fixed with 10% buffered formalin for 72hr. The samples were then subjected to decalcification with 10% EDTA for 60 days and embedded in paraffin. The praffinized tissue was sectioned into 5 μ thickness and stained with H and E stain. The histopathological changes in the tissue sections were assessed by two trained blind observers and the joint, cartilage damage, bone destruction, infiltration of inflammatory cells were analyzed.

Statistics

The analysis was conducted in triplicates using samples randomly collected from test groups, each containing six rats. Statistical assessment was performed using GraphPad Prism version 6.02 software and results were presented as means±SD. Intergroup differences were evaluated using one-way ANOVA, while intragroup differences were assessed using the post hoc Student Newman-Keuls test. A *p*-value <0.05 was considered statistically significant.

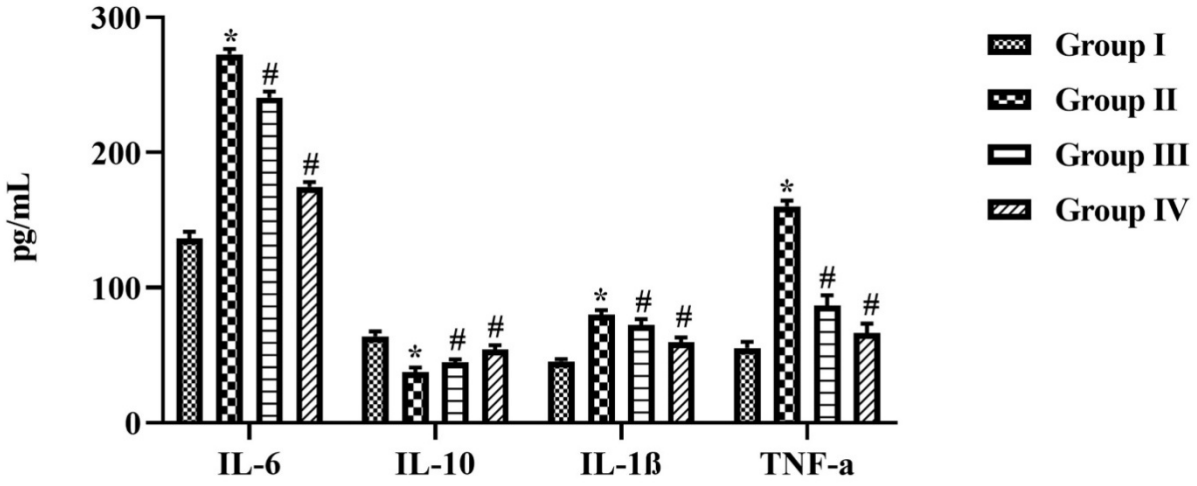

Figure 6: Effect of bavachalcone on inflammatory cytokines in arthritis induced rats. Levels of Inflammatory cytokines Interleukin 6 (IL-6), Interleukin 10 (IL-10), Interleukin 1β (IL-1β) and Tumor Necrosis Factor α (TNF-α). Analysis was conducted in triplicates using samples randomly collected from test groups, each containing six rats. Statistical assessment was performed using GraphPad Prism version 6.02 software and results were presented as means±SD. *p*-value<0.05 was considered statistically significant.

RESULTS

Impact of bavachalcone on body weight and lymphatic organs in arthritis induced rats

Figure 1A illustrates the average weight gained by the experimental rats at the end of the treatment period. The average weight of the control rats was 285±4 g whereas it is significantly decreased to 260±2 g in the arthritis induced rats. Compared to the arthritis induced untreated rats both bavachalcone (270±3) and indomethacin (280±0.9) treated shown significant increase in their body weights.

Lymphatic organs thymus and spleen weights were measured and their relative index were depicted in Figure 1B. Significant increase in both thymus (6±0.02 mg/g) and spleen (8.3±0.08 mg/g) index was observed in arthritis induced rats compared to the control thymus (4.2±0.02 mg/g) and spleen (4.3±0.01 mg/g) index. Bavachalcone treatment significantly decreased thymus index to 5.7±0.02 mg/g and spleen index 6.8±0.02 mg/g in arthritis induced rats.

Role of bavachalcone on preventing Paw edema and arthritic index in arthritis induced rats

Hind paw volume was measured in the control and experimental rats for every 5 days interval and the results were illustrated in the Figure 2A. On day 5 the hind paw volume of control rats was 1.2±0.07 mL, arthritis induced untreated rats was 2.7±0.05 mL, bavachalcone treated rats was 2.2±0.05 mL and indomethacin treated rats was 1.6±0.1 mL. The hind paw volume was significantly increased in arthritis untreated rats during the treatment period and at end of the experiment day 25th it shown about 4.1±0.07 mL whereas it remained same in the control rats. Both bavachalcone (2.5±0.06 mL) and indomethacin (2.2±0.05 mL) treated rats shown significant decrease in the paw volume compared to the arthritis untreated rats

Figure 2B represents the arthritis index calculated in the arthritis induced untreated and drugs treated rats. The arthritis index was significantly increased in the arthritis induced untreated rats from 7 on treatment day 5 to 16 on treatment day 25. On final day of treatment the bavachalcone treated rats shown arthritis index 6 and it was observed to be 3 in standard drug indomethacin treated rats.

Vasoregulatory effect of bavachalcone in arthritis induced rats

Figure 3 depicts the levels of vasoregulators prostaglandin E2 and nitric oxide in arthritis induced untreated and drugs treated rats. The levels of both PGE2 and nitric oxide were increased in arthritis induced untreated group compared to the control rats. The PGE2 and nitric oxide levels were 798±10 pg/mL and 47±1.4 µM respectively in arthritis induced untreated rats whereas it is 95±1.2 pg/mL and 5±0.01 µM respectively in control rats. Bavachalcone and indomethacin treatment significantly decreased the levels of PGE2 to 587±10 pg/mL and 354±16 pg/mL respectively. Nitric oxide levels were also decreased in both bavachalcone (32±1.2 µM) and indomethacin (18±0.8 µM) treatment compared to the untreated arthritis induced rats.

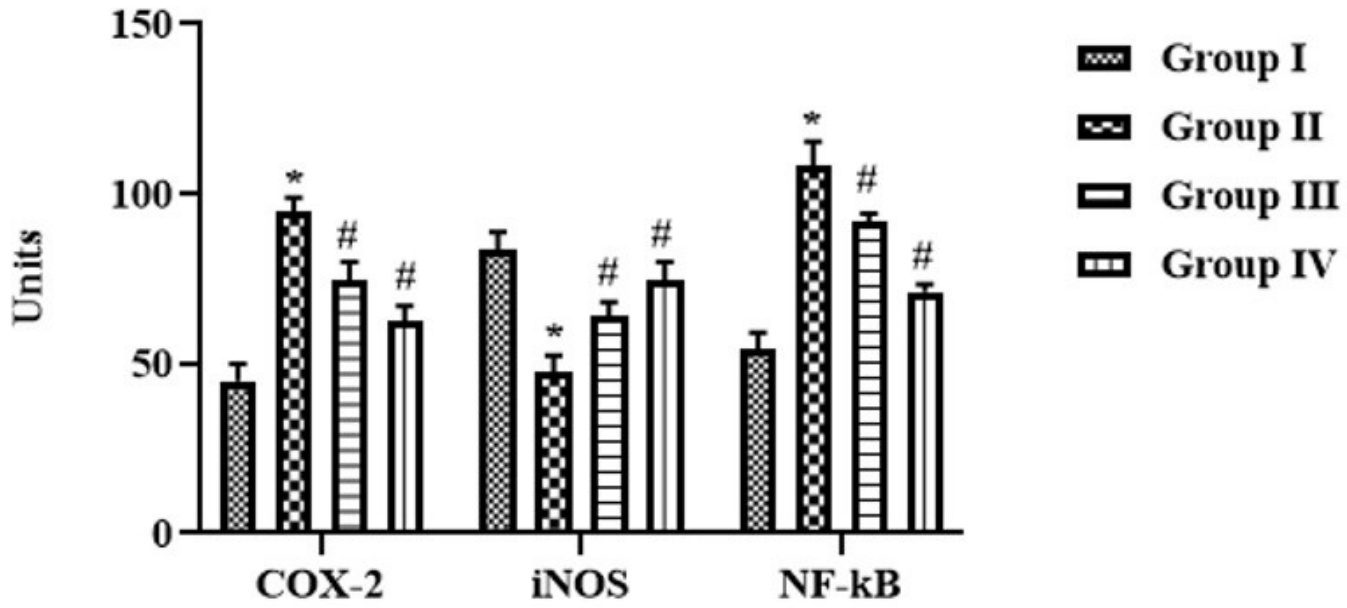

Figure 7: Anti-inflammatory mediating effect of bavachalcone in arthritis induced rats. Levels of inflammatory regulators Cyclooxygenase-2 (COX-2), inducible Nitricoxide Synthase (iNos) and Nuclear Factor-κ-B (NFκB). Analysis was conducted in triplicates using samples randomly collected from test groups, each containing six rats. Statistical assessment was performed using GraphPad Prism version 6.02 software and results were presented as means±SD. *p*-value<0.05 was considered statistically significant.

Ameliorative effect of bavachalcone against oxidative stress in arthritis induced rats

The extend of lipid peroxidation in arthritis induced rats and the impact of drugs were evaluated by estimating the MDA content and the results were represented in Figure 4A. The MDA content was significantly increased to 5.2±0.04 nmol/mg protein in arthritis induced untreated rats compared to control rats which shown 3.8±0.06 nmol/mg protein. Bavachalcone and indomethacin treatment substantially reduced the levels of MDA to 4.7±0.04 and 3.9±0.09 nmol/mg protein respectively.

Antioxidant levels were quantified in the experimental animals were presented in Figure 4B. Both enzymatic antioxidant SOD, CAT and non-enzymatic antioxidant reduce glutathione were quantified in the test samples. Arthritis induced rats shown significant decrease in the levels of the enzymatic and non-enzymatic antioxidants compared to the control rats whereas treatment with bavachalcone increased the antioxidant levels in arthritis induced rats.

Effect of bavachalcone on hepatic biomarker enzymes in arthritis induced rats

Hepatic biomarker enzymes SGOT, SGPT and ALP were quantified in the test animals and the results were illustrated in the Figure 5. Arthritis induced rat shown significant increase in the levels of hepatic biomarkers confirming the hepatic injury whereas both bavachalcone and indomethacin treatment substantially decreased the levels of hepatic biomarker enzymes. Compared to SGOT and SGPT the levels of ALP were drastically increased in arthritis induced untreated rats and treatment with bavachalcone significantly decreased the ALP level.

Effect if bavachalcone on inflammatory cytokines in arthritis induced rats

Figure 6 represents the levels of pro-inflammatory cytokines IL6, 1β, TNF-α and anti-inflammatory cytokine IL-10 in arthritis induced untreated and drug treated rats. Proinflammatory cytokines were significantly increased in arthritis induced rats compared to control rats. Compared to IL-1β and TNFα the IL-6 levels were substantially increased in the arthritis induced untreated rats. The anti-inflammatory cytokine IL-10 was significantly decreased in the untreated rats compared to the control rats. The drugs bavachalcone and indomethacin treatment significantly increased the level of anti-inflammatory cytokine IL-10 and decreased the levels of pro-inflammatory cytokines IL6, 1β, TNF-α in arthritis induced rats.

Anti-inflammatory mediating effect of bavachalcone in arthritis induced rats

The anti-inflammatory property of bavachalcone was evaluated by assessing the levels of COX-2, iNOS and NFκB in the test animals. Bavachalcone treatment significantly decreased the

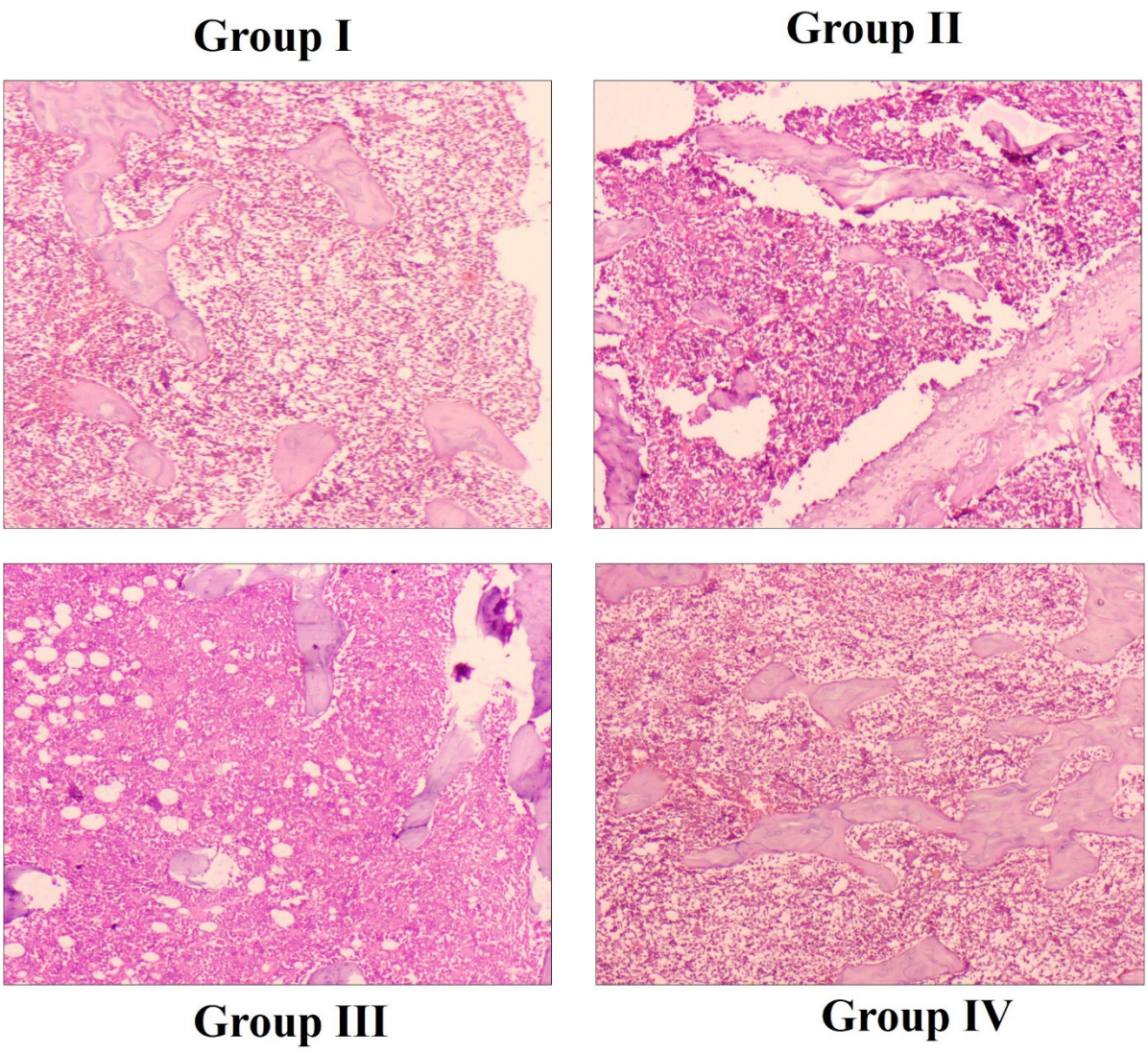

Figure 8: Osteoprotective effect of bavachalcone in arthritis induced rats. Group I) Control Group II) Arthritis induced untreated rats Group III) Arthritis induced Bavachalcone treated rats Group IV) Arthritis induced indomethacin treated rats. Synovial tissue sections were subjected to tissue processing and stained with hematoxylin, eosin stains. Representative images of H and E stained synovial tissue sections.

levels of COX-2, NFκB and increased the level of iNOS in arthritis induced rats. Arthritis induced untreated rats shown increased levels of COX-2, NFκB and decreased iNOS level confirming the inflammation induction in rats (Figure 7).

Osteoprotective effect of bavachalcone in arthritis induced rats

Figure 8 depicts the representative images of H and E stained synovial tissue section of arthritis induced drugs treated and untreated rats. Control group shown clear articular cavity, the synovial cells were arranged ordered with no pathological findings and infiltration of inflammatory cells (Group I). The arthritis induced group shown a severe synovial damage with thickened matrix, synovial, collagen hyperplasia and excessive infiltration of inflammatory cells (Group II). Compared to arthritis induced untreated rats the bavachalcone treated rats shown decreased number of inflammatory cells infiltration and reduce synovial damage (Group III). The synovial damage, collagen matrix thickness and infiltration of inflammatory cells were further reduced in indomethacin treated rats (Group IV) compared arthritis induced untreated and bavachalcone treated rats.

DISCUSSION

Rheumatoid arthritis is a multifactorial rampant autoimmune disease which has a severe impact on global health. The incidence varies geographically and it is prevalent in industrialized countries. The rate of incidence is rapidly increased over the past decades and it is estimated about 1% of global population are suffering with rheumatoid arthritis.[1,28] Both modifiable risk factors such

as life style, smoking habits, environmental pollutants and non-modifiable risk factors such as genetic predisposition and sex plays an interconnected role in triggering rheumatoid arthritis development and progression in individuals.[29] Morning stiffness, joint pains, swelling of small joints are the common symptoms observed in RA patients but due to delayed intervention and treatment the severity of diseases increases causes bone deformity, internal organ damage and even premature deaths. At present RA patients treated with non-steroidal anti-inflammatory drugs such as Ibuprofen, Naproxen and disease modifying anti-rheumatic drugs such as Methotrexate, JAK inhibitors, IL-1, IL-17 inhibitors. The efficacy of these drugs are questionable and they are not cost effective also causes undesirable side effects.[30]

Traditional Chinese Medicine (TCM) which has long promising history of treating various diseases is now gaining ever-increasing acceptance by the global population. TCM treats diseases based on the identifying the root cause of the disease and these targets the multifactorial etiology therefore renders minimal side effects and prevents reoccurrence of the disease.[31] Malarial drug artemisinin, TCM-arsenic trioxide combination drug for treating acute promyelocytic leukemia are few examples of TCM excellency. Various herbal formulations, extracts such as Guizhi-Shaoyao-Zhimu decoction, Zheng Qing Feng Tong Ning (ZQFTN) Tablets, Tripterygium wilfordii Hook-F, Stephania tetrandra S Moore were prescribed to treat rheumatoid arthritis.[32,33] Even though these drugs are effective in treating RA lack scientific data with international standards for the potency, safety and quality prevents them in usage of global market. In this study we assessed the mechanism of action of a Chinese herbal compound bavachalcone in arthritis induced rats.

Lymphatic organs spleen and thymus plays a vital role in progression of rheumatoid arthritis. Excessive antibodies were generated by spleen were observed in the adjuvant treated arthritis models.[34] A significant increase in the both thymus and spleen weight was observed in the arthritis induced untreated rats which confirms the increased cellularity. Treatment with bavachalcone significantly decreased the spleen and thymus weights this may be due to suppression of lymphocytes in arthritis which was also earlier reported with -sitosterol-loaded solid lipid nanoparticles[35] and Nyctanthes arbor-tristis treatment.[36] Decrease in body weight was observed in arthritis induced rats this may be due to the FCA treatment which decreases the intestinal nutrient absorption.[37] Bavachalcone suppressed the FAC effect and increased the body weight in arthritis induced rats.

Edema is the common preliminary symptom observed in the arthritis patients it occurs due to invasion of inflammatory cells to the targeted site.[38] Bavachalcone treatment had effectively prevented the edema in paws of arthritis induced rats from the day 5 of the treatment and it potentially inhibited edema at end of the treatment period compared to the untreated rats. The arthritic index score also confirms the inhibition arthritis in bavachalcone treated rats. The pathophysiology of arthritis was previously believed to be a gradual process resulting from prolonged biomechanical disturbance due to abnormal joint edema and mechanical stress. Hence the factors such as sex, age, body mass index and physical strain are the predicted factors which promotes arthritis.[39,40] In recent times research evidences had proven both mechanical and the biochemical factors are involved in development of arthritis.[41] Osteoarthritic chondrocytes stimulates production of inflammatory mediators such as cytokines, vasoregulators such as prostaglandins and nitric oxide.[42]

Prostaglandin E2 and nitric oxide are the pleiotropic inflammatory mediators overexpressed in the arthritis condition.[43,44] These mediators are derived from the enzymes COX2 and iNOS which were found in the cartilages and synovial tissues. The expression of iNOS and COX2 were regulated by the inflammatory cytokines IL1β and TNF-α. The upregulation of COX-2 in inflamed cartilages synthesis various eicosanoids such as PGF1α, PGF2α and PGE2 which in turn triggers inflammatory response.[45] Therefore drugs inhibiting COX-2 activity renders 90% PGE2 reduction and shown effective prevention in osteoarthritis affected individuals.[46] Inhibition of osteoclastogenesis by bavachalcone was reported by various research studies.[47,48] Bavachalcone effectively inhibits the MEK, ERK and AKT signaling pathway pathways and decreased the NFκB synthesis.[49,50] In our study we assessed the potency of bavachalcone in inhibiting the synthesis of PGE2 and nitric oxide. Bavachalcone treatment significantly reduced the PGE2 and nitric oxide this may be due to inhibition of COX-2 and NFκB activity. It also increased the levels of iNOS and decreased the levels of pro-inflammatory cytokines IL1β and IL-6. The anti-inflammatory potency of bavachalcone was confirmed with the IL-10 cytokines in arthritis induced rats.

Increased production of reactive oxygen species in chondrocytes is one of the major contributors of arthritis pathogenesis.[51,52] Increased infiltration of inflammatory cells in synovium induces the generation of ROS which turn activates the proinflammatory cytokines and damages the cartilage matrix. The chondrocytes proteoglycan synthesis is inhibited by the hydrogen peroxide.[53-55] and rheumatoid arthritis patients are present with increased lipid peroxidation.[56] The interplay of reduced antioxidant levels and increased lipid peroxidation were reported in RA condition.[23,57] Bavachalcone has reported to decrease mitochondrial oxidative stress by increasing the MnSOD expression and stimulating the AMPK activity.[11] This correlates with our results bavachalcone effectively increased the antioxidants SOD, catalase and glutathione thereby prevented lipid peroxidation in arthritis induced rats.

Hepatoxicity with varied severity was observed with NSAID which was prescribed for RA patients.[58-60] Therefore we evaluated the hepatic biomarker enzymes in bavachalcone treated arthritis induced rats. Bavachalcone treatment significantly decreased

the hepatic biomarker enzymes in arthritis induced rats thereby confirmed the hepatoprotective property. The findings of vasoregulatory, antioxidant, anti-inflammatory, anti-arthritic potency of bavachalcone was further confirmed with our histopathological findings.

CONCLUSION

Rheumatoid arthritis an autoimmune disorder affects quality life of millions of individuals worldwide. It is a global threat to social economy since it affects the productive of the individual and the treatment are required for long term and cost effective. Traditional Chinese medicines are potent economical safe therapy which ameliorates the root cause of the disease. In this study we assess the anti-arthritic potency of TCM compound bavachalcone in arthritis induced rats. Bavachalcone exhibited vasoregulatory, antioxidant, anti-inflammatory, hepatoprotective and anti-arthritic potency in arthritis induced rats. Our findings suggest bavachalcone is an ideal molecule which can be subjected to further research to be formulated as drug to treated rheumatoid arthritis patients.

ACKNOWLEDGEMENT

This work was supported by Huai'an Hospital of Traditional Chinese Medicine, Huai 'an 223001, China.

FUNDING

Science and technology project of Huai'an City, Jiangsu Province: Study on the molecular mechanism of external application of anti-inflammatory analgesic herbs to improve knee arthritis in middle-aged and elderly patients (HAB202131).

ETHICAL APPROVAL

This work has approved by the institutional animal ethical committee by Huai 'an Hospital of Traditional Chinese Medicine, Huai'an 223001, China.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

TCM: Traditional Chinese medicine; **PGE2:** Prostaglandin E2; **COX-2:** Cyclooxygenase-2; **iNOS:** Inducible nitric oxide synthase; **NF-κB:** Nuclear factor-kappa B.

SUMMARY

Rheumatoid arthritis is presented as symmetrical polyarthritis caused due to chronic inflammation. Traditional Chinese Medicine (TCM) may be potent and most trusted alternative for treating various autoimmune disorders. Bavachalcone treatment significantly reduced the PGE2 and nitric oxide this may be due to inhibition of COX-2 and NFκB activity. It also increased the levels of iNOS and decreased the levels of pro-inflammatory cytokines IL1β and IL-6. The anti-inflammatory potency of bavachalcone was confirmed with the IL-10 cytokines in arthritis induced rats.

REFERENCES

1. WHO. Rheumatoid arthritis; Jun 2023. Available from: https://www.who.int/news-room/fact-sheets/detail/rheumatoid-arthritis.
2. Slobodin G. Rheumatoid arthritis. In: Slobodin G, Shoenfeld Y, editors. Rheumatic disease in geriatrics: diagnosis and management. Cham: Springer; 2020. p. 173-83.
3. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med. 2011;365(23):2205-19. doi: 10.1056/NEJMra1004965.
4. Papakonstantinou D. Work disability and rheumatoid arthritis: predictive factors. Work. 2021;69(4):1293-304. doi: 10.3233/WOR-213550.
5. GBD. Rheumatoid arthritis collaborators. Glob Reg Natl Burden Rheumatoid Arthritis. 2021; 5(10): e594-e610: 1990-2020 and projections to 2050: a systematic analysis of the Global Burden of Disease Study 2021. Lancet Rheumatol. 2023 Sep 25.
6. Hsieh PH, Wu O, Geue C, McIntosh E, McInnes IB, Siebert S. Economic burden of rheumatoid arthritis: a systematic review of literature in biologic era. Ann Rheum Dis. 2020;79(6):771-7. doi: 10.1136/annrheumdis-2019-216243.
7. Yu HC, Lu MC. The roles of anti-citrullinated protein antibodies in the immunopathogenesis of rheumatoid arthritis. Tzu Chi Med J. 2019;31(1):5.
8. Alsaber A, Pan J, Al-Herz A, Alkandary DS, Al-Hurban A, Setiya P, *et al.* Influence of ambient air pollution on rheumatoid arthritis disease activity score index. Int J Environ Res Public Health. 2020;17(2):416. doi: 10.3390/ijerph17020416.
9. Firestein GS, McInnes IB. Immunopathogenesis of rheumatoid arthritis. Immunity. 2017;46(2):183-96. doi: 10.1016/j.immuni.2017.02.006.
10. Mateen S, Moin S, Shahzad S, Khan AQ. Level of inflammatory cytokines in rheumatoid arthritis patients: correlation with 25-hydroxy vitamin D and reactive oxygen species. PLOS ONE. 2017;12(6):e0178879. doi: 10.1371/journal.pone.0178879.
11. Mateen S, Moin S, Khan AQ, Zafar A, Fatima N. Increased reactive oxygen species formation and oxidative stress in rheumatoid arthritis. PLOS ONE. 2016;11(4):0152925. doi: 10.1371/journal.pone.0152925.
12. Shah D, Wanchu A, Bhatnagar A. Interaction between oxidative stress and chemokines: possible pathogenic role in systemic lupus erythematosus and rheumatoid arthritis. Immunobiology. 2011;216(9):1010-7. doi: 10.1016/j.imbio.2011.04.001.
13. Ramiro S, Sepriano A, Chatzidionysiou K, Nam JL, Smolen JS, van der Heijde D, *et al.* Safety of synthetic and biological DMARDs: a systematic literature review informing the 2016 update of the EULAR recommendations for management of rheumatoid arthritis. Ann Rheum Dis. 2017;76(6):1101-36. doi: 10.1136/annrheumdis-2016-210708.
14. Schiff M, Keiserman M, Codding C, Songcharoen S, Berman A, Nayiager S, *et al.* Clinical response and tolerability to abatacept in patients with rheumatoid arthritis previously treated with infliximab or abatacept: open-label extension of the ATTEST Study. Ann Rheum Dis. 2011;70(11):2003-7. doi: 10.1136/annrheumdis-2011-200316.
15. O'Neil LJ, Alpízar-Rodríguez D, Deane KD. Rheumatoid arthritis: the continuum of disease and strategies for prediction, early intervention and prevention. J Rheumatol. 2024;51(4):337-49. doi: 10.3899/jrheum.2023-0334.
16. Lee WY, Chen HY, Chen KC, Chen CY. Treatment of rheumatoid arthritis with traditional Chinese medicine. BioMed Res Int. 2014; 2014:1-11. doi: 10.1155/2014/528018.
17. Lu C, Zha Q, Chang A, He Y, Lu A. Pattern differentiation in traditional Chinese medicine can help define specific indications for biomedical therapy in the treatment of rheumatoid arthritis. J Altern Complement Med. 2009;15(9):1021-5. doi: 10.1089/acm.2009.0065.
18. Huang MC, Pai FT, Lin CC, Chang CM, Chang HH, Lee YC, *et al.* Characteristics of traditional Chinese medicine use in patients with rheumatoid arthritis in Taiwan: A nationwide population-based study. J Ethnopharmacol. 2015;176:9-16. doi: 10.1016/j.jep.2015.10.024.
19. Murunikkara V, Rasool MK. Trikatu, a herbal compound mitigates the biochemical and immunological complications in adjuvant-induced arthritic rats. Int J Rheum Dis. 2017;20(3):298-308. doi: 10.1111/1756-185X.12535.
20. Wong YF, Zhou H, Wang JR, Xie Y, Xu HX, Liu L. Anti-inflammatory and analgesic effects and molecular mechanisms of JCICM-6, a purified extract derived from an anti-arthritic Chinese herbal formula. Phytomedicine. 2008;15(6-7):416-26. doi: 10.1016/j.phymed.2008.02.008.
21. Guo Q, Mao X, Zhang Y, Meng S, Xi Y, Ding Y, *et al.* Guizhi-Shaoyao-Zhimu decoction attenuates rheumatoid arthritis partially by reversing inflammation-immune system imbalance. J Transl Med. 2016;14(1):165. doi: 10.1186/s12967-016-0921-x.
22. Guo Q, Zheng K, Fan D, Zhao Y, Li L, Bian Y, *et al.* Wu. Tou Decoction in rheumatoid arthritis: integrating Network Pharmacology and*in vivo* Pharmacological Evaluation. Front Pharmacol. 2017;8:230. doi: 10.3389/fphar.2017.00230.
23. Dang Y, Ling S, Duan J, Ma J, Ni R, Xu JW. Bavachalcone-induced manganese superoxide dismutase expression through the AMP-activated protein kinase

pathway in human endothelial cells. Pharmacology. 2015;95(3-4):105-10. doi: 10.1159/000375452.
24. Song HS, Jang S, Kang SC. Bavachalcone from Cullen corylifolium induces apoptosis and autophagy in HepG2 cells. Phytomedicine. 2018;40:37-47. doi: 10.1016/j.phymed.2017.12.030.
25. Patel DK. Therapeutic application of Bavachalcone in chronic diseases: A concise report on medicinal importance and pharmacological activities. Curr Chin Sci. 2022;2(4):257-62. doi: 10.2174/2210298102666220302094731.
26. Xu W, Huang M, Zhang Y, Li H, Zheng H, Yu L, *et al.* Extracts of Bauhinia championii (Benth.) Benth. attenuate the inflammatory response in a rat model of collagen-induced arthritis. Mol Med Rep. 2016;13(5):4167-74. doi: 10.3892/mmr.2016.5070.
27. Pan T, Cheng TF, Jia YR, Li P, Li F. Anti-rheumatoid arthritis effects of traditional Chinese herb couple in adjuvant-induced arthritis in rats. J Ethnopharmacol. 2017;205:1-7. doi: 10.1016/j.jep.2017.04.020.
28. Smith E, Hoy DG, Cross M, Vos T, Naghavi M, Buchbinder R, *et al.* The global burden of other musculoskeletal disorders: estimates from the Global Burden of Disease 2010 study. Ann Rheum Dis. 2014;73(8):1462-9. doi: 10.1136/annrheumdis-2013-204680.
29. Cai Y, Zhang J, Liang J, Xiao M, Zhang G, Jing Z, *et al.* The burden of rheumatoid arthritis: findings from the 2019 global burden of diseases study and forecasts for 2030 by bayesian age-period-cohort analysis. J Clin Med. 2023;12(4):1291. doi: 10.3390/jcm12041291.
30. Gao Y, Zhang Y, Liu X. Rheumatoid arthritis: pathogenesis and therapeutic advances. MedCommComm (2020). 2024;5(3):e509. doi: 10.1002/mco2.509.
31. Pan HD, Xiao Y, Wang WY, Ren RT, Leung EL, Liu L. Traditional Chinese medicine as a treatment for rheumatoid arthritis: from empirical practice to evidence-based therapy. Engineering. 2019;5(5):895-906. doi: 10.1016/j.eng.2019.01.018.
32. Zhang P, Li J, Han Y, Wei Yu XW, Qin L. Traditional Chinese medicine in the treatment of rheumatoid arthritis: a general review. Rheumatol Int. 2010;30(6):713-8. doi: 10.1007/s00296-010-1370-0.
33. Zhang L, Cao Z, Yang Y, Tan X, Mao J, Su L. Traditional Chinese medicine on treating active rheumatoid arthritis: A protocol for systematic review and meta-analysis. Med (Baltim). 2020;99(24):e20642. doi: 10.1097/MD.0000000000020642.
34. Khanfar E, Olasz K, Gajdócsi E, Jia X, Berki T, Balogh P, *et al.* Splenectomy modulates the immune response but does not prevent joint inflammation in a mouse model of RA. Clin Exp Immunol. 2022;209(2):201-14. doi: 10.1093/cei/uxac052.
35. Zhang F, Liu Z, He X, Li Z, Shi B, Cai F. β-sitosterol-loaded solid lipid nanoparticles ameliorate complete Freund's adjuvant-induced arthritis in rats: involvement of NF-κB and HO-1/Nrf-2 pathway. Drug Deliv. 2020;27(1):1329-41. doi: 10.1080/10717544.2020.1818883.
36. Sharma A, Goel A, Lin Z. Analysis of anti-rheumatic activity of Nyctanthes arbor-tristis via*in vivo* and pharmacovigilance approaches. Front Pharmacol. 2023;14:1307799. doi: 10.3389/fphar.2023.1307799.
37. Allawadhi P, Khurana A, Sayed N, Godugu C, Vohora D. Ameliorative effect of cerium oxide nanoparticles against Freund's complete adjuvant-induced arthritis. Nanomedicine (Lond). 2022;17(6):383-404. doi: 10.2217/nnm-2021-0172.
38. Bose M, Chakraborty M, Bhattacharya S, Bhattacharjee P, Mandal S, Kar M, *et al.* Suppression of NF-κB p65 nuclear translocation and tumor necrosis factor-α by Pongamia pinnata seed extract in adjuvant-induced arthritis. J Immunotoxicol. 2014;11(3):222-30. doi: 10.3109/1547691X.2013.824931.
39. Vina ER, Kwoh CK. Epidemiology of osteoarthritis: literature update. Curr Opin Rheumatol. 2018;30(2):160-7. doi: 10.1097/BOR.0000000000000479.
40. Sacitharan PK. Ageing and osteoarthritis. Subcell Biochem. 2019;91:123-59. doi: 10.1007/978-981-13-3681-2_6.
41. Grässel S, Zaucke F, Madry H. Osteoarthritis: novel molecular mechanisms increase our understanding of the disease pathology. J Clin Med. 2021;10(9):1938. doi: 10.3390/jcm10091938.
42. Jiang H, Ji P, Shang X, Zhou Y. Connection between osteoarthritis and nitric oxide: from pathophysiology to therapeutic target. Molecules. 2023;28(4):1683. doi: 10.3390/molecules28041683.
43. Fermor B, Weinberg JB, Pisetsky DS, Misukonis MA, Fink C, Guilak F. Induction of cyclooxygenase-2 by mechanical stress through a nitric oxide-regulated pathway. Osteoarthr Cartil. 2002;10(10):792-8. doi: 10.1053/joca.2002.0832.
44. Rahmati M, Mobasheri A, Mozafari M. Inflammatory mediators in osteoarthritis: A critical review of the state-of-the-art, current prospects and future challenges. Bone. 2016;85:81-90. doi: 10.1016/j.bone.2016.01.019.
45. Ferrer MD, Busquets-Cortés C, Capó X, Tejada S, Tur JA, Pons A, *et al.* Cyclooxygenase-2 inhibitors as a therapeutic target in inflammatory diseases. Curr Med Chem. 2019;26(18):3225-41. doi: 10.2174/0929867325666180514112124.
46. Wang X, Liu Z, Peng P, Gong Z, Huang J, Peng H. Astaxanthin attenuates osteoarthritis progression via inhibiting ferroptosis and regulating mitochondrial function in chondrocytes. Chem Biol Interact. 2022;366:110148. doi: 10.1016/j.cbi.2022.110148.
47. Shan L, Yang S, Zhang G, Zhou D, Qiu Z, Tian L, *et al.* Comparison of the inhibitory potential of Bavachalcone and Corylin against UDP-Glucuronosyltransferases. Evid Based Complement Alternat Med. 2014;2014(1):958937. doi: 10.1155/2014/958937.
48. Patel DK. Therapeutic application of Bavachalcone in chronic diseases: A concise report on medicinal importance and pharmacological activities. Curr Chin Sci. 2022;2(4):257-62. doi: 10.2174/2210298102666220302094731.
49. Park CK, Lee Y, Chang EJ, Lee MH, Yoon JH, Ryu JH, *et al.* Bavachalcone inhibits osteoclast differentiation through suppression of NFATc1 induction by RANKL. Biochem Pharmacol. 2008;75(11):2175-82. doi: 10.1016/j.bcp.2008.03.007.
50. Park JY, Jeong HJ, Kim YM, Park SJ, Rho MC, Park KH, *et al.* Characteristic of alkylated chalcones from Angelica keiskei on influenza virus neuraminidase inhibition. Bioorg Med Chem Lett. 2011;21(18):5602-4. doi: 10.1016/j.bmcl.2011.06.130.
51. Ansari MY, Khan NM, Ahmad I, Haqqi TM. Parkin clearance of dysfunctional mitochondria regulates ROS levels and increases survival of human chondrocytes. Osteoarthr Cartil. 2018;26(8):1087-97. doi: 10.1016/j.joca.2017.07.020.
52. Bolduc JA, Collins JA, Loeser RF. Reactive oxygen species, aging and articular cartilage homeostasis. Free Radic Biol Med. 2019;132:73-82. doi: 10.1016/j.freeradbiomed.2018.08.038.
53. Altindag O, Erel O, Aksoy N, Selek S, Celik H, Karaoglanoglu M. Increased oxidative stress and its relation with collagen metabolism in knee osteoarthritis. Rheumatol Int. 2007;27(4):339-44. doi: 10.1007/s00296-006-0247-8.
54. Zhang Q, Lenardo MJ, Baltimore D. 30 years of NF-κB: A blossoming of relevance to human pathobiology. Cell. 2017;168(1-2):37-57. doi: 10.1016/j.cell.2016.12.012.
55. Ahmadinejad F, Geir Møller S, Hashemzadeh-Chaleshtori M, Bidkhori G, Jami MS. Molecular mechanisms behind free radical scavengers function against oxidative stress. Antioxidants. 2017;6(3):51. doi: 10.3390/antiox6030051.
56. Helli B, Mowla K, Mohammadshahi M, Jalali MT. Effect of sesamin supplementation on cardiovascular risk factors in women with rheumatoid arthritis. J Am Coll Nutr. 2016;35(4):300-7. doi: 10.1080/07315724.2015.1005198.
57. Phull AR, Nasir B, Haq IU, Kim SJ. Oxidative stress, consequences and ROS mediated cellular signaling in rheumatoid arthritis. Chem Biol Interact. 2018;281:121-36. doi: 10.1016/j.cbi.2017.12.024.
58. Bessone F. Non-steroidal anti-inflammatory drugs: what is the actual risk of liver damage? World J Gastroenterol. 2010;16(45):5651-61. doi: 10.3748/wjg.v16.i45.5651.
59. Meunier L, Larrey D. Recent advances in hepatotoxicity of Non steroidal anti-inflammatory drugs. Ann Hepatol. 2018;17(2):187-91. doi: 10.5604/01.3001.0010.8633.
60. Zoubek ME, Lucena MI, Andrade RJ, Stephens C. Systematic review: ibuprofen-induced liver injury. Aliment Pharmacol Ther. 2020;51(6):603-11. doi: 10.1111/apt.15645.

Cite this article: Xiao X, Liu S, Chen G, Cao C. Anti-Arthritic Effect of Traditional Chinese Medicinal Compound Bavachalcone on Freund's Complete Adjuvant-Induced Rheumatoid Arthritis in Rats. Indian J of Pharmaceutical Education and Research. 2025;59(2):727-37.