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ORIGINAL ARTICLE

Antiarthritic Activity of Asiaticoside against the Freund's Complete Adjuvant-Induced Rheumatoid Arthritis in Experimental Wistar Rats

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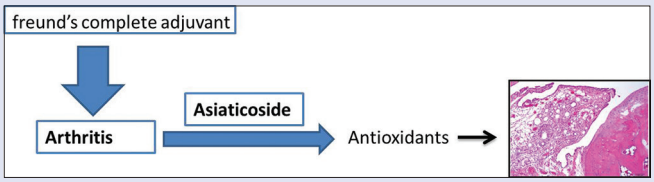
ABSTRACT

Background: Rheumatoid synovial inflammation occurs at the synovial joint, which is activated by the articular system and mediated by the immune cells. In the case of untreated rheumatoid arthritis, the inflammation may affect the extra-articular system including cardiovascular, hepatic, respiratory, circulatory, and neuromuscular systems, which may ultimately interfere with the quality of life of patients with rheumatoid arthritis. This, in turn, reduces the life expectancy of the patient. **Materials and Methods:** The effectiveness of asiaticoside was evaluated by the Freund's complete adjuvant (FCA)-induced rheumatoid arthritis model using experimental Wistar rats. According to our results, asiaticoside exhibited potent anti-arthritic activity in contrast to the control animals in the FCA-induced rheumatoid arthritic model. **Results:** The parameters of arthritis such as reduced body weight, increased paw volume and pro-inflammatory cytokines, and cartilage destruction were significantly ($P < 0.05$) affected by the administration of asiaticoside. It significantly ($P < 0.01$) increased the body weight, decreased the paw volume and pro-inflammatory cytokines, restored the blood components, increased the levels of antioxidant enzymes, and protected the cartilage. **Conclusion:** Asiaticoside exhibited potential anti-arthritic activity against FCA-induced rheumatoid arthritis in experimental rats.

Key words: Asiaticoside, Freund's complete adjuvant, inflammation, paw volume band pro-inflammatory cytokines, Wistar rats

SUMMARY

- Rheumatoid arthritis disease is one of the debilitating diseases that can develop into serious crippling deformities, disability of functions, and destruction of cartilage
- Asiaticoside exhibited a significant ($P < 0.01$) anti-arthritic activity by increasing the body weight, decreasing the paw volume, decreasing the pro-inflammatory cytokines, restoring the blood components, increasing the antioxidant enzyme level, and by protecting the cartilage.



Abbreviations used: FCA: Freund's complete adjuvant; IAEC: International Animal Ethics Committee; RBCs: Red blood cells; SGOT: Serum glutamic-oxaloacetic transaminase; SGPT: Serum glutamic-pyruvic transaminase.

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INTRODUCTION

Rheumatoid arthritis is a progressive chronic inflammatory disorder that primarily affects the joints and articular tissues. Its symptoms include energy loss, fatigue, muscle pain, joint pain, and rigidity of the joints. It usually begins at the small joints of feet and hands and finally spreads to the other larger joints. Inflammation at the joint lings or in the synovial membrane damages the articular cartilage and the tissues of the joint, thereby causing rigidity of the joints and physical disability.[1-3]

It is a seriously debilitating disease, and a chronic inflammation of the joint leads to complete loss in mobility of the patient, thereby affecting their quality of life. In addition, it damages the tissues surrounding the joints such as skin, blood vessels, and muscular tissue. The inflammation around the joints can be triggered by various types of pro-inflammatory molecules including reactive oxygen species eicosanoids and by some of the inflammatory molecules such as leukotrienes, cytokines, and prostaglandins, which are secreted by the macrophages.[4] Delay in the treatment of rheumatoid arthritis may lead to extra-articular complications related to the cardiovascular, hepatic, respiratory, circulatory, and neuromuscular systems. It can cause substantial social, personal, and economic losses; on a personal end, the patient's quality of life is reduced in addition to his/her life expectancy.[5-8]

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Among the various pathophysiological causes of rheumatoid arthritis, it is hypothesized that some of the free radicals such as nitrous oxide and superoxide radicals, which are formed as the byproducts of cellular metabolism, are the triggering agents. These free radicals, in turn, stimulate the generation of interleukin (IL) and tumor necrosis factor-alpha (TNF-α) from the thymus cells (T-cells), which may interfere with the development of growth factors and cytokines. These agents can lead to tissue destruction and chronic inflammation.[9-11] To date, there is no treatment available for rheumatoid arthritis; however, the symptomatic treatment is commonly taken care of by nonsteroidal anti-inflammatory drugs, disease-modifying anti-rheumatoid drugs, various steroids, and some biological agents such as antagonists of TNF-α and IL-1β. Despite the administration of antagonistic drugs, patients usually develop side effects such as cardiovascular disease, gastrointestinal disease, and nephropathy.[12-14]

Nowadays, synthetic drugs not only exhibiting long-term benefits but also producing various diseases as side effects and also being as expensive.[15] The patients who suffered from persistent musculoskeletal diseases are mostly preferred the best alternative treatment strategies for treatment.[16] Although many researchers were reported the anti-inflammatory and antioxidant properties of the asiaticoside,[17-22] the previous literature discovered that there are no scientific claims or evidence for the therapeutic effects of asiaticoside against rheumatoid arthritis. Hence, the current scientific investigation was designed to investigate the curative efficiency of asiaticoside against the FCA-induced rheumatoid arthritis in the experimental animal model.

MATERIALS AND METHODS

Drugs

All the chemicals, reagents, and drugs which include asiaticoside, indomethacin, Freund's complete adjuvant (FCA), and ELISA kit was commercially procured from Sigma-Aldrich, United States of America.

Animals

The Wistar albino rats weighing from 210 to 230 g were procured and maintained in the polypropylene confines beneath the laboratory conditions (26°C ± 1°C, air moisture 60%–70%, light and dark sequence, 12 h). The animals were fed with the commercially purchased rat pelleted food with water *ad libitum*. The animals were getting used to laboratory situations for 7 days previous to the beginning of the work. Heed and treatment of the rats were completed based on the procedure of council directive of a committee for the purpose of control and supervision of experiments on animals. Every animal works in the current work that was carried out in the laboratory based on the procedures recommended by the International Animal Ethics Committee.

Experiment design and induction of arthritis

Wistar rats (male breed) were separated into five groups, and every group contains eight animals (n = 8). Group I rats were served as a control, and it was administered with standard pelleted diet; Group II rats were a rheumatoid arthritis-induced group by intraplantar injection of 0.1 ml of FCA. Group III rats were treated with a low dose of asiaticoside (25 mg/kg per day) by gavage. Group IV rats were treated with a high dose of asiaticoside (50 mg/kg per day) by gavage. Group V animals were treated with the standard drug indomethacin (3 mg/kg per day).On the 1st day, all the groups of rats except the control group were received the intraplantar administration of 0.1 ml of FCA in the right hind footpad of the experimental rats. After the experimentation, all the animals were anesthetized by using the xylazine and ketamine and sacrificed by dislocation of cervical bone. After an hour of ethanol administration, the mice were forfeited, and then, the samples were collected for the experimental analysis.

Measurement of total body weight

The precise body weight of experimental rats was weighed in before and after experiments. The earlier and final body weights of all the experimental rats were weighed and noted sensitive electronic weighing balance.

Paw volume quantity measurement

The changes in the paw volume of the experimental rats were measured and tabulated. The beginning and the end paw volumes of all the experimental rats were measured carefully by using the plethysmograph.

Estimation of hemoglobin

For the estimation of hemoglobin (Hb), the collected blood was taken in the Hb pipette with up to the 20-mm spot on the pipette, and then, the blood was expelled to the Sahli tube which contains the HCl solution up to the 3-g mark on the tube. The contents were mixed and the tube was kept at the comparator and stand for 10 min for incubation. After the incubation period of 10 min, the double-distilled water was added to the tube by drop until the developing color matched with the standard color of the comparator. The Hb level was expressed as g per 100 ml.

Estimation of red blood cells

The collected whole blood was taken in the Thoma red cell pipette up to 0.5 marks and then diluted up to 11 marks by using the Hayem's diluting fluid for the dilution of blood with 1:200 concentrations. The diluted blood was filled in red blood cell (RBC) counting chamber, and the cells were counted under the magnification of light microscope. The values are expressed as cells per cubic millimeter of the whole blood.

Estimation of white blood cells

The collected whole blood was taken in the white blood cell pipette up to 0.5 marks and then diluted up to 11 marks by using 1.5% of HCl for the dilution of blood with 1:20 concentration. The diluted blood sample was filled in the white blood cell (WBC) counting chamber, and the cells were counted under the magnification of light microscope. The values are expressed as cells per cubic millimeter of the whole blood.

Assay of tissue cytokine production

For the determination of total cytokine levels, the tissues of subcutaneous paw were collected after the scarification of anesthetized rats. The collected paw samples were kept in the phosphate buffer containing 0.1 mM of phenylmethylsulfonyl fluoride, 0.05% of tween 80, 10 mM of ethylenediaminetetraacetic acid (EDTA), 0.1 mM of benzethonium chloride, and aprotinin. Then, the tissue was homogenized and centrifuged at 4000 rpm for 12 min. The resulting upper phase was freeze and stored at – 76°C, and later, it was used for the assessment of cytokines. The specific cytokines such as IL-1β, TNF-α, and IL-6 were evaluated by using the commercially purchased specific rat immunoassay ELISA kits.

Estimation of arthritis index and score

The level of arthritis in the experimental rats was determined and noted in the sequence of 3 days after the administration of FCA to experimental rats. The rheumatic arthritis index level can be measured by the degree and the erythema area, swelling of the joint, and deformation of the toe of the rats after the immunization.[23] The score of the arthritis was measured by the rats with the normal joint position, mild erythema, and rear ankle swelling; rats with the erythema and rear ankle joint and tarsal

swelling; rats with the erythema and moderate metacarpal joint swellings; and rats with the serious swelling and erythema in the metatarsal ankle. Severity scores were obtained as 0 as no signs of arthritis, 1 as swelling or redness in one joint, 2 as swelling or redness in more than one joint, 3 as swelling or redness in the whole paw region, and 4 as serious swelling in the whole paw region with deformity.

Measurement of an index of immune organs

The spleen and thymus are major immune organs which play an important role in cellular and humoral immunity, respectively. For the measurement of immune organ index of the experimental rats, after the completion of the experimentation, all the animals were forfeited by the dislocation of cervical bone and the immune organs such as thymus and spleen were taken out, and then, wet weight was weighed to determine the indices of spleen and thymus. The index of the spleen and thymus was measured by using the following formula:

Weight of the organ (mg)/body weight (g) $\times 10^3$.

Estimation of alkaline phosphatase

Four milliliters of the buffered substrate was taken into the clean test tube, and then, it was incubated at 37°C for 6 min. Then, 0.2 ml of serum was mixed with the substrate solution and continued the incubation for another 15 min. After that, 1.8 ml of diluted phenol reagent was mixed. Simultaneously, the control was run with 4 ml of substrate solution with 0.2 ml sample and 1.8 ml diluted phenol reagent. Then, it was homogenized by vortex mixer and centrifuged at 3000 rpm for 6 min. Then, 4 ml of the resulting upper phase was mixed with 2 ml of sodium carbonate solution. Then, all the tubes were incubated at 37°C for 15 min; then, the developed color was read at 700 nm. Enzyme activity was depicted as μmoles of phenol per liter in the serum sample.

Determination of liver function

For the determination of liver function of the rats, the level of serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) in the serum was determined by using the ultraviolet kinetic technique. Both the enzymes such as SGOT and SGPT were analyzed based on the coupled enzymatic method, where the keto acid is developed by the amino transaminase which reacts with a system by using nicotinamide adenine dinucleotide (NAD) + hydrogen (H) (NADH) as a cofactor. Coenzyme is further oxidized to develop as nicotinamide adenine dinucleotide (NAD), and it can reduce the absorbance at 340 nm for SGOT malate dehydrogenase was reduced to malate with the same as oxidation of NADH to NAD. The oxidation rate of NADH is determined whereas SGPT was forming the pyruvate during the reaction is further concerted to the lactate by the lactate dehydrogenase.[24]

Estimation of antioxidant enzymes

Assay of superoxide dismutase enzyme activity

The effectiveness of the superoxide dismutase (SOD) enzyme in cell lysate was evaluated by the previously described procedure by Kakkar *et al*. (1984).[25] 0.2 ml of cell lysate was diluted to 0.4 ml with the double dH_2O followed by the addition of 1 ml of absolute ethanol and 0.6 ml of ice-cold chloroform. This reaction solution was continuously shaken well for 2 min at 4°C. The reaction mixture included 480 μl of sodium pyrophosphate buffer, 40 μl of phenazine methosulfate, 120 μl of nitro blue tetrazolium was diluted for enzyme preparation. A reaction was initiated by adding 80 μl of NADH. Then, the reaction medium was incubated at 30°C for 2 min. After the incubation, the reaction was terminated by the addition of 0.4 ml of acetic acid. Then, 1.6 ml of butanol was mixed into the reaction mixture and mixed by continuous shaking for 1 min and set aside. Then, the reaction mixture was centrifuged to take apart the butanol layer from the reaction medium, and the absorbance was taken 520 nm.

Assay of catalase enzyme activity

To assesses the catalase (CAT) enzyme activity in cell lysate was examined by previously described procedure by Beers and Sizer (1952).[26] The 0.1-ml cell lysate was mixed with 1.9 ml of phosphate-buffered saline solution. Then, 1 ml of hydrogen peroxide was mixed to this reaction medium. Finally, the absorbance was taken at 240 nm in 1-min interval for three min. The control solution was placed on the reference containing 0.1 ml of cell lysate and 2.9 ml of the phosphate buffer. Finally, the activity of the CAT enzyme was calculated by the extinction of molar coefficient at 4.37 nm.

Assay of glutathione enzyme activity

The glutathione (GSH) level in experimental mice was assayed by the method of Sedlak and Lindsay (1968).[30] The portion of the glandular part was weighed and mixed with EDTA. Then, the double dH_2O and trichloroacetic acid (50%) were mixed with the homogenate, and then, it was centrifuged. Then, the upper layer was collected added to the tris buffer and Ellman's reagent and shaken vigorously. Finally, the absorbance was taken at 412 nm.

Histopathological analysis

The right hind limb of the experimental rats was collected and washed thoroughly with cold saline solution. Then, the small piece of the collected organ was fastened in 10% formalin and then embedded with paraffin wax. Wax-fixed hind limb was cut into small slices at the size of 5 μm. Then, the slices were stained by using eosin and hematoxylin (E and H). The histopathological analysis of the hind limb was performed by using optical microscope to detect any damages and inflammation in the hind limb tissues.

Statistical study

An obtained experimental result was depicted as mean ± standard error of mean (SEM). The statistical variations between the experimental animals were calculated by the one-way analysis of variance by the Tukey's Kramer test with the $P < 0.05$ when compared to the control group and $P < 0.01$ when compared to the FCA-treated group by using SPSS (version 19 NY, USA).

RESULTS

Effects of asiaticoside on red blood cell, white blood cell, and hemoglobin levels in Freund's complete adjuvant-induced arthritic rats

In contrast to the untreated control group of rats, the FCA-induced arthritic rats demonstrated a significantly low count of RBCs and a low percentage of Hb ($P < 0.05$) and showed a significantly elevated count of WBCs ($P < 0.05$). However, administration of asiaticoside (25 and 50 mg/kg) in FCA-induced arthritic rats caused a significant increase in the count of RBCs and increased the Hb

levels ($P < 0.01$) and caused a significant decrease in the count of WBCs ($P < 0.01$) [Table 1]. Thus, it shows that asiaticoside restored the blood components that were affected due to the induction of inflammation by FCA.

Effects of asiaticoside on the body weight of the Freund's complete adjuvant-induced rheumatoid arthritic rats

The FCA-induced arthritic rats exhibited a significant decrease in their body weight when compared to the untreated control group of rats ($P < 0.05$). However, the administration of asiaticoside (25 and 50 mg/kg) in FCA-induced rheumatoid arthritic rats demonstrated a significant increase in their relative body weight ($P < 0.01$) [Figure 1]. Furthermore, the administration of indomethacin in FCA-induced arthritic rats significantly increased the body weight when compared to the untreated control group of rats ($P < 0.01$).

Effect of asiaticoside on organ weight of the Freund's complete adjuvant-induced rheumatoid arthritic rats

The weight of the spleen and thymus of the FCA-induced arthritic rats was found to be significantly elevated when compared with the untreated control group of rats ($P < 0.05$). On the contrary, the administration of asiaticoside and indomethacin (25 and 50 mg/kg, respectively) in FCA-induced rheumatic arthritis rats significantly decreased the weight of both thymus and spleen when compared with the untreated control group of rats [Figure 1] ($P < 0.01$).

Effects of asiaticoside on the hind paw volume of Freund's complete adjuvant-induced rheumatoid arthritic rats

In the hind paw of FCA (0.1 mL)-induced arthritic rats, we observed a progressive increase in the volume of the paw volume from 5 to 10, 15, 20, and 25 days after induction. The volume was significantly increased when compared with the untreated control group of animals ($P < 0.05$). On the contrary, the administration of asiaticoside (25 and 50 mg/kg) and indomethacin in FCA-induced rheumatic arthritis rats significantly decreased the relative volume in the hind paw [Figure 2] ($P < 0.01$).

Effects of asiaticoside on the arthritic index score of the Freund's complete adjuvant-induced rheumatoid arthritic rats

Compared to the control animals, there was a gradual and significant increase in the arthritic score of FCA-induced arthritic rats, which was evaluated on 5, 10, 15, 20, and 25 days after induction ($P < 0.05$). The administration of asiaticoside (25 and 50 mg/kg) and indomethacin significantly decreased the relative arthritic index score [Figure 2] ($P < 0.01$).

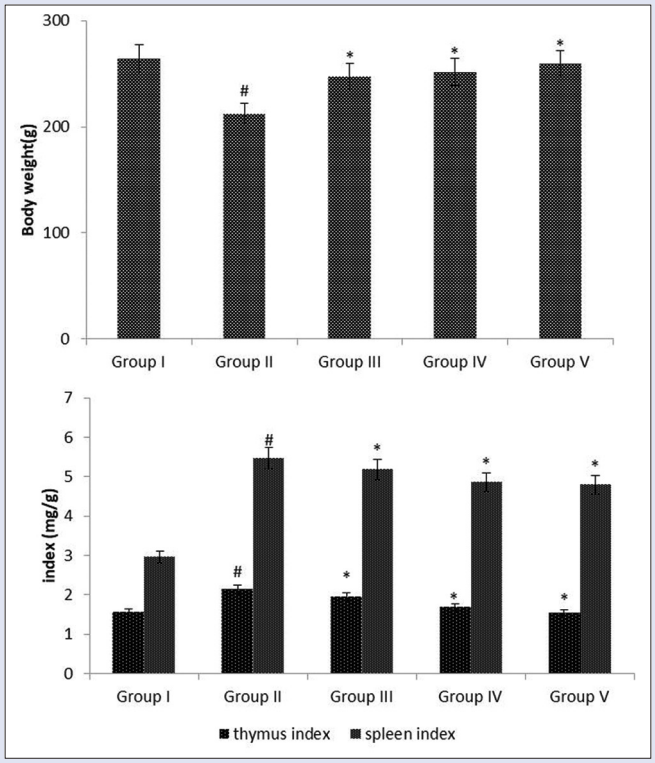

Figure 1: Effects of asiaticoside on the body weight and organ index of the Freund's complete adjuvant-induced rheumatoid arthritis in rats. Results are depicted as mean ± standard error of mean of eight animals ($n = 8$). Note: $^{\#}P < 0.05$ as compared to the control group. $^{*}P < 0.01$ as compared to the Freund's complete adjuvant-treated group

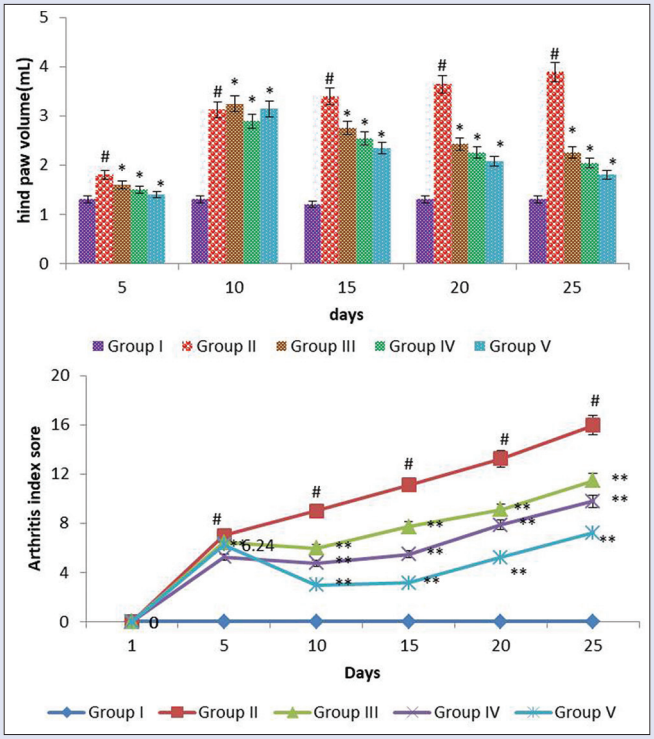

Figure 2: Effects of asiaticoside on the hind paw volume and arthritic index score of the Freund's complete adjuvant-induced rheumatoid arthritis in rats. Results are depicted as mean ± standard error of mean of eight animals ($n = 8$). Note: $^{\#}P < 0.05$ as compared to the control group. $^{*}P < 0.01$ as compared to the Freund's complete adjuvant-treated group.

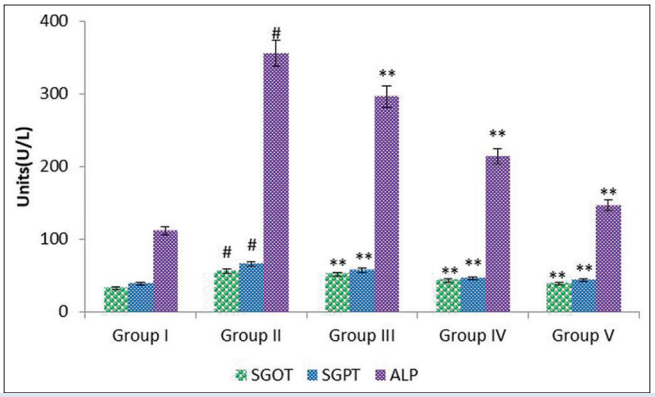

Figure 3: Effects of asiaticoside on the serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, and alkaline phosphatase of the Freund's complete adjuvant-induced rheumatoid arthritis in rats. Results are depicted as mean ± standard error of mean of eight animals ($n = 8$). Note: $^{\#}P < 0.05$ as compared to the control group. $^{**}P < 0.01$ as compared to the Freund's complete adjuvant-treated group

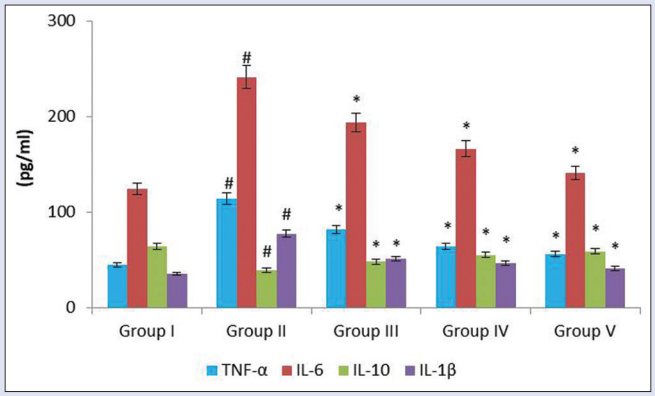

Figure 4: Effects of asiaticoside on the interleukin-6, interleukin-1β, interleukin-10, and tumor necrosis factor-alpha in the Freund's complete adjuvant-induced rheumatoid arthritis in rats. Results are depicted as mean ± standard error of mean of eight animals ($n = 8$). Note: $^{\#}P < 0.05$ as compared to the control group. $^{*}P < 0.01$ as compared to the Freund's complete adjuvant-treated group.

Table 1: Effect of asiaticoside on red blood cells, white blood cells, and hemoglobin levels of Freund's complete adjuvant-induced arthritis in rats

Groups	RBC ($\times 10^6$/μl)	WBC ($\times 10^3$/μl)	Hb (g/dl)
Group I	10.57±0.56	12.35±0.40	13.83±0.81
Group II	7.42±0.63$^{\#}$	25.56±0.59$^{\#}$	6.74±0.59$^{\#}$
Group III	8.33±0.91*	20.97±0.74*	10.52±0.63
Group IV	8.93±0.48*	15.36±0.30*	11.89±0.76*
Group V	9.26±0.57*	13.15±0.97*	12.39±0.10*

$^{\#}P<0.05$ as compared to the control group,$^{*}P<0.01$ as compared to the FCA-treated group. Results are depicted as mean±SEM of eight animals (n=8). RBC: Red blood cell; WBC: White blood cell; Hb: hemoglobin; FCA: Freund's complete adjuvant; SEM: Standard error of mean

Effects of asiaticoside on the levels of serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, and alkaline phosphatase in the serum of Freund's complete adjuvant-induced rheumatoid arthritic rats

The levels of SGOT, SGPT, and alkaline phosphatase (ALP) were significantly higher than that of the control rats ($P < 0.05$). However, the FCA-induced rheumatoid arthritic rats treated with asiaticoside (25 and 50 mg/kg) and indomethacin significantly reduced the relative concentration of SGOT, SGPT, and ALP ($P < 0.01$) [Figure 3].

Effects of asiaticoside on the serum levels of interleukin-6, interleukin-1β, interleukin-10, and tumor necrosis factor-alpha in the Freund's complete adjuvant-induced rheumatoid arthritic rats

Compared to the control animals, the FCA-induced arthritic rats showed a significant increase in the levels of IL-6, IL-1β, and TNF-α ($P < 0.05$) and showed a significant decrease in the IL-10 levels ($P < 0.05$). However, the administration of asiaticoside (25 and 50 mg/kg) showed a significant reduction in the levels of IL-6, IL-1β, and TNF-α ($P < 0.05$) and showed a significant increase in the IL-10 levels ($P < 0.05$) when compared to FCA-induced control rats [Figure 4]. Indomethacin significantly decreased the levels of IL-6, IL-1β, and TNF-α and increased the levels of IL-10 when compared to the FCA-induced control rats ($P < 0.01$).

Effects of asiaticoside on oxidative stress of the Freund's complete adjuvant-induced rheumatoid arthritic rats

The presence of an elevated level of malondialdehyde (MDA) is an indication of increased amount of lipid peroxidation and oxidative stress. Herein, the FCA-induced arthritic rats showed significantly higher levels of MDA as compared to the control group animals ($P < 0.05$). However, asiaticoside (25 and 50 mg/kg) significantly reduced the relative concentrations of MDA when compared to the FCA-induced control rats ($P < 0.01$) [Figure 5]. However, the FCA-induced rats showed a significant decrease in the levels of SOD, CAT, and GSH ($P < 0.05$) than that of the control group animals. However, asiaticoside and indomethacin showed contradictory results. They significantly elevated the levels of relative SOD, CAT, and GSH ($P < 0.01$) [Figure 5].

Effect of asiaticoside in histopathology of the hind paw of control and experimental rats

The histopathological evaluation of hind footpad of the FCA-induced rats displayed notable indications in the infiltrations of inflammatory cells, synovial hyperplasia, the formation of pannus, and partial destruction of joint bone and cartilage when compared to the control rats. On the contrary, asiaticoside was found to provide significant protection on synovial and cartilages of the hind limb in the FCA-treated rats [Figure 6]. Notably, the administration of a low dose of asiaticoside (25 mg/kg) showed mild tissue destruction, whereas the high dose (50 mg/kg) gave significant protection for the cartilage and joint bones of the hind limb.

DISCUSSION

So far, the effectiveness and safety quality of many drugs used to treat autoimmune diseases have been in question; therefore, many researchers are focusing their study on the development of novel drugs with lesser side effects. In line with this research goal, many biologically active compounds from natural sources are being tested against various autoimmune diseases.[27,28]

Modern lifestyle lacking in physical activities and improper dietary intake are the primary reasons for the development of musculoskeletal diseases and arthritis, which leads to permanent disability or even to premature death.[29] Hence, we intended to investigate the anti-arthritic

activity of asiaticoside by using FCA-induced rheumatoid arthritis in the experimental animal model. Recent studies have shown that pro-inflammatory and anti-inflammatory cytokines play a crucial part in the progression of rheumatoid arthritis. The FCA-induced rheumatoid arthritis in animal model explains various features of pathogenesis of rheumatoid arthritis in humans.[30]

Since the paw swelling assessment is an easy, susceptible, and fastest method of determining the level of inflammation and efficacy of the drug, the FCA-induced arthritic model is a closer model resembling human arthritis. Eventually, this inflammatory alteration causes swelling in the soft tissues around the joints and gradually destroys the joint tissues.[31]

In this study, the FCA-induced arthritic rats showed elevated levels of hind paw volume and arthritic score than that of the control group animals. However, asiaticoside was found to reverse the conditions triggered by the induction of inflammation by FCA [Figure 2]. Indomethacin was also found to significantly reduce the hind paw volume and arthritic score in FCA-induced animals.

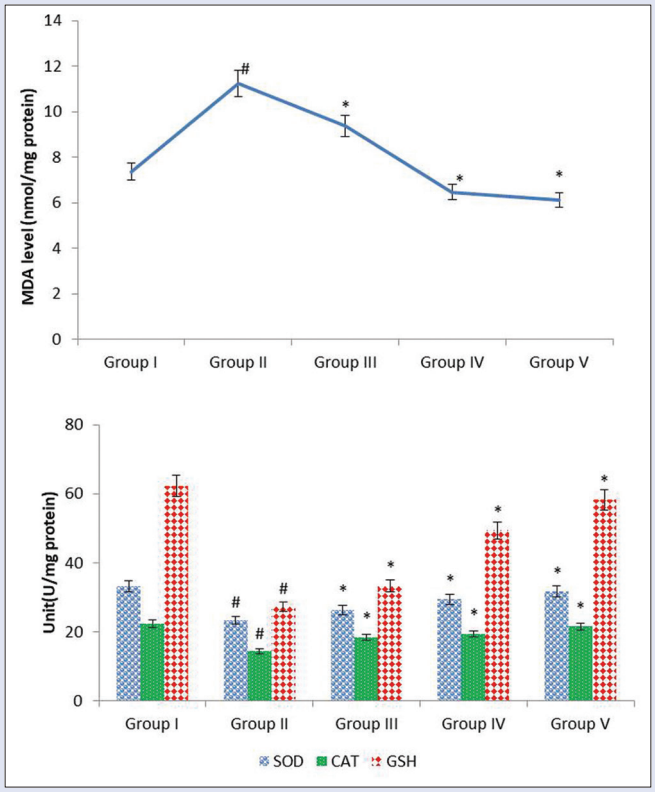

Figure 5: Effects of asiaticoside on the oxidative stress of the Freund's complete adjuvant-induced rheumatoid arthritis in rats. Results are depicted as mean ± standard error of mean of eight animals ($n = 8$). Note: #$P < 0.05$ as compared to the control group. *$P < 0.01$ as compared to the Freund's complete adjuvant-treated group

The pro-inflammatory cytokines are the key factors involved in the pathophysiology of rheumatoid arthritis. The T-cell activation generates cytokines such as interferon-gamma and IL-2, which are necessary for the stimulation of inflammatory response in the body. There are various pro-inflammatory cytokines and chemokines produced during continual inflammation and destruction of cartilage and bones at the joints that are involved in the pathogenesis of rheumatoid arthritis. This includes IL-1β, IL-6, and TNF-α and pro-inflammatory enzymes such as cyclooxygenase-1 (COX-1), 5-lipoxygenase, COX-2, and matrix metalloproteinase.[32-35] The immune complexes which are deposited in synovial joints can increase the levels of pro-inflammatory cytokines by the stimulation of mononuclear cells. These immune composites in blood circulation can induce the macrophages and peripheral polymorphonuclear leukocytes to produce pro-inflammatory cytokines such as TNF-α. The activation of synovial macrophages may lead to the hypersecretion of TNF-α and IL-1β and other major pro-inflammatory cytokines such as IL-6, IL-15, and IL-12.[36-38]

In this study, the FCA-induced experimental rats demonstrated a significant elevation in the levels of IL-6, IL-1β, and TNF-α when compared with the untreated control group of rats ($P < 0.05$). On the contrary, asiaticoside-treated rats (25 and 50 mg/kg) showed a significant ($P < 0.05$) reduction in the levels of IL-6, IL-1β, and TNF-α when compared with untreated control group of rats [Figure 6].

The continuous production of pro-inflammatory cytokines in the case of autoimmune diseases[39] leads to the continuous presence of inflammatory condition which triggers the initiation and progression of rheumatoid arthritis, which may be interlinked with serious oxidative stress and adverse inflammatory reactions.[40] The existing drugs, namely ocrelizumab, veltuzumab, and rituximab, for the treatment of rheumatoid arthritis target specific cytokines by means of particular monoclonal antibodies; however, these drugs are banned due to their detrimental effects.[41] However, there is a great need for the development of novel herbal-based drugs to treat rheumatoid arthritis efficiently.[42]

Therefore, we tested asiaticoside to treat arthritis in an animal model. In this study, the FCA-induced arthritic rats showed a significant reduction in the level of antioxidant enzymes such as SOD, CAT, and GSH when compared to the control group ($P < 0.05$). However, asiaticoside (25 and 50 mg/kg) was found to counter these effects; it significantly elevated the levels of SOD, CAT, and GSH in FCA-induced rats ($P < 0.01$) [Figure 5]. The pathways of pivotal cytokines are illustrated in which activation of the T-cells, dendritic cells, B-cells, and macrophages underlying the deregulated expression of the cytokines. This, in turn, decreases the induction of the effector cells, which includes mast cells, endothelial cells, neutrophils, and synovial fibroblasts.[43,44]

The untreated control group of rats demonstrated a normal structure of the connective tissue structure with the absence of necrosis in the tibiotarsal joint. However, the histopathological assessment of joints in the FCA-induced rats revealed the remarkable signs of cellular infiltration, synovial hyperplasia, pannus formation, partial cartilage, and bone destruction. Interestingly, the asiaticoside-treated rats protected the knee joint, cartilage, and bones of the hind limb from necrosis when

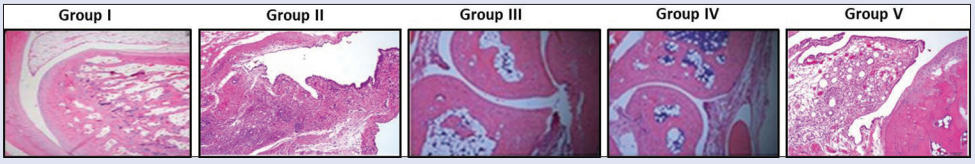

Figure 6: Result of histopathological examination of hind paw of control and experimental rats

compared to the FCA-induced control group of rats [Figure 6]. Moreover, asiaticoside significantly decreased the levels of cellular infiltration, hyperplasia, and bone destruction and improved histopathology in treated rats ($P < 0.05$). Hence, it is clear that asiaticoside demonstrated an excellent anti-arthritic activity against FCA-induced rheumatoid arthritis in experimental rats.

CONCLUSION

In summary, our findings clearly demonstrate that asiaticoside shows excellent anti-arthritic activity against the FCA-induced rheumatoid arthritis in the experimental animal model. Hence, future development of novel anti-arthritic drug-containing asiaticoside will improve patient health efficiently. Therefore, further clinical research is required to evaluate the complete mechanism of action of asiaticoside and understand the therapeutic effects on rheumatoid arthritis.

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Conflicts of interest

There are no conflicts of interest.

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