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# Effect of hydroalcoholic leaf extract of *Cassia fistula* L. on type Ⅱ collagen-induced arthritis in rats

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## ABSTRACT

**Objective:** To explore the effect of *Cassia fistula* on collagen Ⅱ-induced arthritis in rats.

**Methods:** The effect of 250 and 500 mg/kg chloroform and hydroalcoholic extract of *Cassia fistula* leaf on collagen Ⅱ-induced arthritis was investigated by evaluating paw volume, arthritis index, spleen index, and biochemical parameters. Histopathological analysis and docking study were also performed.

**Results:** A dose-dependent reduction in paw volume, arthritic index, and spleen index was observed following oral administration of the chloroform and hydroalcoholic extracts. Treatment with *Cassia fistula* extracts reduced tumor necrosis factor-α, interleukin (IL)-1β, IL-6, prostaglandin $E_2$, aspartate aminotransferase, alanine aminotransferase, total leucocyte count, and erythrocyte sedimentation rate while increasing IL-10 level. In addition, *Cassia fistula* extracts improved joint architecture, and prevented cartilage and bone destruction. Docking analysis demonstrated that the physcion, 1-octacosanol, 5,3',4'-trihydroxy-6-methoxy-7-*O*-α-*L*-rhamnopyranosyl-(1,2)-*O*-β-*D*-galactopyranoside and scopoletin may be responsible for the anti-arthritic effect of *Cassia fistula*.

**Conclusions:** *Cassia fistula* suppresses the progression of collagen Ⅱ-induced arthritis by lowering the inflammatory factors, decreasing paw volume and arthritic index, and alleviating joint architecture. However, further studies are required to confirm the bioactive molecule responsible for the anti-arthritic potential of *Cassia fistula*.

**KEYWORDS:** *Cassia fistula*; Rheumatoid arthritis; Collagen Ⅱ; Inflammatory cytokines; Docking

## 1. Introduction

Rheumatoid arthritis (RA) is a painful auto-immune disorder characterized by chronic and progressive inflammation which eventually leads to the destruction of cartilage and bone, followed by deformity, disability, immobility of joints of feet, hands, knees, and premature death[1]. Globally, the situation pervasiveness diverges between 0.3% and 1% between the ages of 20 and 40. Although the pathogenesis of RA is poorly understood, accumulated evidence from pre-clinical and clinical settings revealed the involvement

**Significance**

*Cassia fistula* has been used for the treatment of rheumatoid arthritis in several traditional medicinal systems, however, relevant experimental data supporting its use is lacking. In the present study, *Cassia fistula* extracts mitigate collagen Ⅱ-induced arthritis, as evidenced by decreased inflammatory factors, paw volume and arthritic index, and improvement of joint architecture. The detailed mechanism for the anti-arthritic activity of *Cassia fistula* and its bioactive compounds need to be investigated further in future study.

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of auto-antibodies, T cells, B cells, prostaglandin $E_2$ ($PGE_2$), and cytokines, predominantly tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-10 in the pathogenesis and progression of RA[2].

$PGE_2$ is an inflammatory mediator produced by prostaglandin endoperoxide synthase and is known to produce inflammation, apoptosis, and structural changes in the cartilage and thus contributing to the pathogenesis of RA[3]. Activated macrophages in the synovial membrane of the RA patient produce TNF-α, which is known to play a pivotal role in the pathogenesis of RA[4]. Literature reports the potential of TNF-α to induce cartilage and bone destruction during RA[5], and its ability to produce other pro-inflammatory cytokines like IL-1β, and IL-6 which further contribute to disease progression[6]. IL-1β induces the release of more cytokines from synovial mononuclear cells and metalloproteinases from fibroblasts, which further contributes to the destruction of bone and cartilage[7]. Moreover , IL-6 plays a key role by modulating auto-immune response in the pathogenesis of RA[8]. The role of IL-6 becomes evident from the findings where anti-IL-6 receptor antibodies demonstrated beneficial effects during RA[9]. IL-10 further contributes to the pathogenesis of RA by downregulating class Ⅱ MHC molecule, promoting differentiation and survival of B cells, preventing the release of inflammatory mediators, inhibiting T-cell activation, and release of cytokines from T lymphocytes[10].

Most of the synthetic drugs that are being used in the management of RA are associated with severe side effects and toxicity, which confines their effective use and full therapeutic benefits during RA. Therefore it is essential to develop some alternative measures with better safety profiles. Recent trends have shown that 60%-90% of patients suffering from arthritis choose complementary and alternative medicines such as chiropractic and herbal therapy[11]. *Cassia fistula* (*C. fistula*) L., commonly known as Golden Shower and Indian-Laburnum, is a semi-wild plant native to South-East Asia[12]. It is an ethnomedicinal plant and its various parts are being used by various tribes across the world for the treatment of a variety of ailments, including fungal infections[13], nasal infections[14], inflammation, rheumatism, fever, flu, heart, and liver ailments, eye ailments, demulcent, purgative, chest pain[15-17], hematemesis, pruritus, diabetes[18], microbial infections[19], diarrhea, abdominal pain, and leprosy[20]. To date, the anti-arthritic potential of this plant lacks scientific validation. Therefore, the present study aimed at evaluating the effect of *C. fistula* on type Ⅱ collagen-induced arthritis (CIA) in rats and predicting the potential mechanism.

## 2. Materials and methods

### 2.1. Chemicals and animals

All the chemicals, reagents, and kits used in the present study were procured from Sigma Aldrich unless mentioned specifically. Female Sprague-Dawley rats weighing 200-250 g were used in the study. Animals were procured from the National Institute of Pharmaceutical Education and Research, Mohali, India, after the ethical clearance for conducting this experimentation (Protocol No. IPS/AH/222) by Institute Animals Ethics Committee, Kurukshetra University, Haryana, India. Animals were kept under pathogen-free conditions in the Animal House of Kurukshetra University, Kurukshetra, and were maintained at room temperature (25 ± 2) ℃, (55 ± 5)% humidity, and a 12 h light-dark cycle with free access to food and water.

### 2.2. Plant collection and extraction

Leaves of the plant were collected from the campus of Kurukshetra University, Kurukshetra, and were authenticated as *C. fistula* Linn. (Fabaceae) by Dr. B. D. Vashistha, Chairman, Department of Botany, Kurukshetra University, Kurukshetra (voucher specimen no. KUK/BOT/IPS-01). Collected leaves were cleaned under tap water to remove adhering impurities and were dried in the shade for 5 weeks. After milling dried leaves into a coarse powder, 1 200 g of powder was subjected to successive extraction with chloroform and hydro-alcohol (water:ethanol; 30:70) in a Soxhlet apparatus at a temperature of 55 ℃ for 72 h each. The obtained extract was concentrated to a semi-solid mass by using a rotary evaporator (Heidolph 4011, USA) and then lyophilized. The crude yield of the lyophilized chloroform and hydroalcoholic extract was 13.8% and 29.52% *w/w*, respectively. The lyophilized extract was then stored at 4 ℃ until used for further study.

### 2.3. Preparation of test samples and animals

Both extracts were administered orally at 250 and 500 mg/kg doses, once daily. The dose of the extracts was reconstituted by suspending the required quantity of the chloroform and hydroalcoholic extract in Tween 80 (5% *v/v* in normal saline). All the doses were prepared freshly before use. Animals were brought to the experiment lab 24 h before the start of experimentation to make them familiar with the laboratory conditions and were randomly divided into 7 groups ($n$=10).

### 2.4. Induction of type Ⅱ CIA and treatments

Bovine type Ⅱ collagen (CⅡ) (Sigma Aldrich) was dissolved in 0.1 M acetic acid to obtain the concentration of 2 mg/mL and was stored overnight at 4 ℃ followed by its emulsification with an equal volume of chilled incomplete Freund's adjuvant (IFA) (Merck Specialties Private LTD) to give final CⅡ-IFA concentration of 1 mg/mL. On the first day of the experiment, each animal was given 0.5 mL collagen by intradermal injection at 5 different sites; one

injection at the base of four paws and one injection at the back of the neck. Each animal received a booster dose of 0.5 mL CⅡ-IFA at the same places 14 days after primary immunization. On day 20, the hind paw volume of each animal was measured by a plethysmometer (Ugo Basile No. 7140, Italy), and animals having paw volume >1.8 were used for further study. The normal control group was separated before the experiment and received no immunization.

All the treatments were given orally, once daily till day 40. Group 1 (normal) and group 2 (arthritic control) were treated with 10 mL/kg vehicle (5% *v/v* Tween 80 in normal saline). The standard group was treated with 3 mg/kg dexamethasone. Treatment groups (group 4-7) were given 250 and 500 mg/kg doses of the chloroform and hydroalcoholic extract in a vehicle[12,13].

## 2.5. Evaluation of arthritis

### 2.5.1. Arthritis score

Rats were assessed for the severity of arthritis every 2 days after injecting a booster dose by different researchers to eliminate the personal bias of the individual researcher. A well-established and widely accepted 5-point scoring scale was used for measuring arthritis scores: 0 = normal paw, 1 = minor inflammation and sign involving ankle/wrist, 2 = inflammation and signs involving ankle and tarsal of the hind paw or/and wrist, and carpals of fore paw, 3 = severe inflammation and arthritic signs extended to metatarsals and metacarpals, 4 = severe disease involving entire hind and fore paw or/and subcutaneous arthritic nodules. The maximum arthritic score was set at 16 per rat (4 points × 4 paws).

### 2.5.2. Hind paw swelling

The volume of hind paw swelling was measured by using a plethysmometer on 20, 25, 30, 35, and 40 days of the study. Each volume was measured 3 times and its average was taken as the final volume.

## 2.6. Measurement of spleen index

At the end of the experiment, all rats were sacrificed by cervical dislocation. Spleens were removed and weighed immediately after dissection. The spleen index was measured by using the following equation.

$$\text{Spleen index} = \frac{\text{Spleen weight of rats}}{\text{Body weight of rats}}$$

## 2.7. Measurement of serum biochemical parameters

Blood was collected from all the rats by cardiac puncture under anesthesia before sacrifice and was allowed to clot for 30 min. Serum was obtained by centrifugation of clotted blood at 3 000 rpm for 15 min and was stored at −20 ℃. Serum TNF-α, IL-1β, IL-6, IL-10, and $PGE_2$ concentration was estimated by commercially available kits according to the manufacturer's protocol.

Total leucocyte count (TLC) and erythrocyte sedimentation rate (ESR) were determined by standard laboratory methods. Blood concentration of serum marker enzymes including aspartate aminotransferase (AST), and alanine aminotransferase (ALT) was estimated by commercially available kits according to the manufacturer's protocol.

## 2.8. Histological analysis

For histological examination of the rat's knee joint, the left hind limb was removed and fixed in 10% phosphate-buffered formalin for 24 h. The limb was decalcified in 10% ethylene diamine tetra acetic acid for 15 d, embedded in paraffin, and sections of 6-8 µm were prepared using a microtome. All the prepared sections were stained with hematoxylin and eosin before their observation by a pathologist.

## 2.9. Docking study

Docking, an *in-silico* technique, is used to predict the activity of a bioactive molecule for its potential against a specific protein target. A docking study of all the reported constituents of *C. fistula* L. was performed using Molegro Virtual Docker on 1KV2 PDB. The X-ray structure of 1KV2 was accessed from the protein data bank (PDB) (www.rcsb.org).

### 2.9.1. Ligand preparation

The structures of all the reported constituents of *C. fistula* L. were constructed with the help of Marvin sketch software. The energies of all the structures were minimized and the conformation with the lowest energy was used for the study. The objective behind the energy minimization was to enable the docking program to identify conformation with minimum energy (most stable form). Further ligand preparation wizard of the Molegro virtual docker was used to assign missing charges, bonds, bond order, and hybridization states of important ligands.

### 2.9.2. Protein preparation

X-ray crystal structure of anti-arthritic PDB (PDB ID: 1KV2) was obtained from the protein data bank and all the missing charges and the protonation state of the receptor were assigned by using the Molegro algorithm which also detected the active cavities of the receptor based on the hydrophobicity. The water of crystallization was removed from 1KV2 PDB with the help of the protein

preparation wizard of Molegro virtual docker to prevent it from interfering with our final result.

### 2.9.3. Molecular docking

Final docking was performed through the docking wizard of Molegro virtual docker by using 5 potential cavities and 10 runs per structure. Molegro virtual docker utilizes MolDock score for the evaluation of the interaction between the receptor and the ligand. Final results were recorded in the form of MolDock score energy, number of interactions between the receptor and the ligand, bond length, and amino acids involved, which were further utilized to determine the anti-arthritic potential of the compounds.

### 2.10. Statistical analysis

All the results are expressed as mean ± SD. The statistical significance of the results was analyzed by one-way ANOVA followed by Dunnett's *t*-test using GraphPad Prizm software. Results were considered statistically significant at $P < 0.05$.

### 2.11. Ethical statement

The animal experiment was approved by Institute Animals Ethics Committee, Kurukshetra University, Haryana, India (Reg. No. 562/GO/02/a/CPCSEA). The experimental protocol and procedure used in the present study were in accordance with the guidelines of CPCSEA, Ministry of Environment, Govt. of India, and New Delhi.

## 3. Results

### 3.1. Effects of the chloroform and hydroalcoholic extract of C. fistula on paw swelling and arthritis score

Effects of the chloroform and hydroalcoholic extract of *C. fistula* on hind paw volume are depicted in Table 1. The effect of extracts was evaluated once every 5 days from the 20th to the 40th day post-primary immunization. In our study, we observed a significant increase in paw volume in arthritic control rats compared with normal rats ($P < 0.001$). Treating animals with the hydroalcoholic extract of *C. fistula* resulted in a dose-dependent decrease in the paw volume on the 25th day post-immunization ($P < 0.05$), which continued till the 40th day. Initially, we observed a slight increase in the paw volume of rats treated with the chloroform extract (250 and 500 mg/kg) on the 25th day, which was followed by a significant ($P < 0.05$) reduction in paw volume compared to arthritic control. Rats treated with 500 mg/kg hydroalcoholic extract demonstrated the most significant reduction in paw edema throughout the study.

Signs of arthritis started to appear after the 14th day of primary immunization, although minor inflammation was observed in a few rats initially. The first indication of the disease was erythema of small distal joints with progressive involvement of ankle joints, tarsal, metatarsal, carpal, metacarpal, and interphalangeal joint. The results of the arthritic score are shown in Figure 1. The arthritis score was similar for all the groups till the 20th day of the study, after which the effect of various treatments became evident. The arthritis score of the arthritic control group increased gradually throughout the study with a slight reduction from the 36th to the 40th day. Rats orally administered the chloroform and hydroalcoholic extract at either 250 or 500 mg/kg displayed a significant reduction in arthritic score when compared with the arthritic control group.

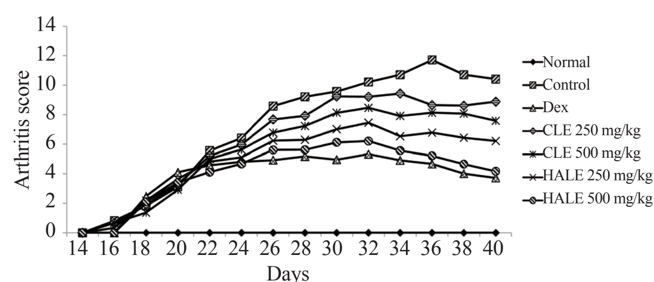


**Figure 1.** Effect of the chloroform and hydroalcoholic extract of *Cassia fistula* on arthritis score. CLE: chloroform extract; HALE: hydroalcoholic extract; Dex: dexamethasone.

**Table 1.** Effect of the chloroform and hydroalcoholic extract of *Cassia fistula* on left hind paw volume of rats.

| Group | 20th day | 25th day | 30th day | 35th day | 40th day |
|---|---|---|---|---|---|
| Normal | 1.20±0.01 | 0.98±0.02 | 1.12±0.02 | 1.06±0.01 | 1.20±0.01 |
| Arthritic control | 1.88±0.04* | 2.26±0.30*** | 2.31±0.12*** | 2.57±0.07*** | 2.85±0.11*** |
| Dex (3 mg/kg) | 1.94±0.08 | 1.77±0.07# | 1.76±0.08## | 1.62±0.03### | 1.42±0.08### |
| CLE (250 mg/kg) | 1.86±0.03 | 2.18±0.06 | 2.00±0.13 | 1.81±0.05### | 1.73±0.07### |
| CLE (500 mg/kg) | 1.91±0.08 | 2.01±0.08 | 1.89±0.10# | 1.79±0.09### | 1.68±0.07### |
| HALE (250 mg/kg) | 2.15±0.42 | 1.95±0.05 | 1.81±0.07## | 1.62±0.02### | 1.51±0.04### |
| HALE (500 mg/kg) | 1.89±0.61 | 1.84±0.06 | 1.77±0.05## | 1.56±0.06### | 1.45±0.05### |

Values are represented as mean ± SD and analyzed by one-way ANOVA followed by Dunnett's *t*-test. *$P < 0.05$, and ***$P < 0.001$ compared with the normal group. #$P < 0.05$, ##$P < 0.01$ and ###$P < 0.001$ compared with the arthritic control. CLE: chloroform extract; HALE: hydroalcoholic extract; Dex: dexamethasone.

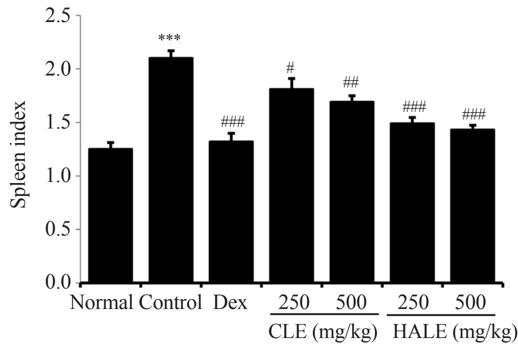


**Figure 2.** Effect of the chloroform and hydroalcoholic extract of *Cassia fistula* on the spleen index. Values are represented as mean ± SD and analyzed by one-way ANOVA followed by Dunnett's *t*-test. $^{***}P < 0.001$ compared with the normal group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ compared with the arthritic control group.

## 3.2. Effects of the chloroform and hydroalcoholic extract of C. fistula on the spleen index

The effect of the chloroform and hydroalcoholic extract of *C. fistula* L. on the spleen index of CIA rats was examined (Figure 2). The spleen index of arthritic control rats was significantly ($P < 0.001$) higher in comparison to the normal control. Treatment with the chloroform and hydroalcoholic extract of *C. fistula* at 250 and 500 mg/kg significantly lowered the spleen index in arthritic control rats ($P < 0.05$).

## 3.3. Effects of the chloroform and hydroalcoholic extract of C. fistula on biochemical parameters

The levels of TNF-α, IL-1β, IL-6, and $PGE_2$ in the arthritic control group were significantly ($P < 0.01$) higher than those of normal control rats with significantly reduced levels of IL-10. TNF-α levels in rats treated with *C. fistula* extracts were lower than those in arthritic control rats, especially in 500 mg/kg chloroform extract ($P < 0.05$) and 250 and 500 mg/kg hydroalcoholic extract treated groups ($P < 0.01$). The hydroalcoholic extract-treated group also showed a remarkable reduction in the serum levels of IL-1β and IL-6 compared to the arthritic control group ($P < 0.001$; $P < 0.01$). Treatment with the chloroform extract at 500 mg/kg could markedly

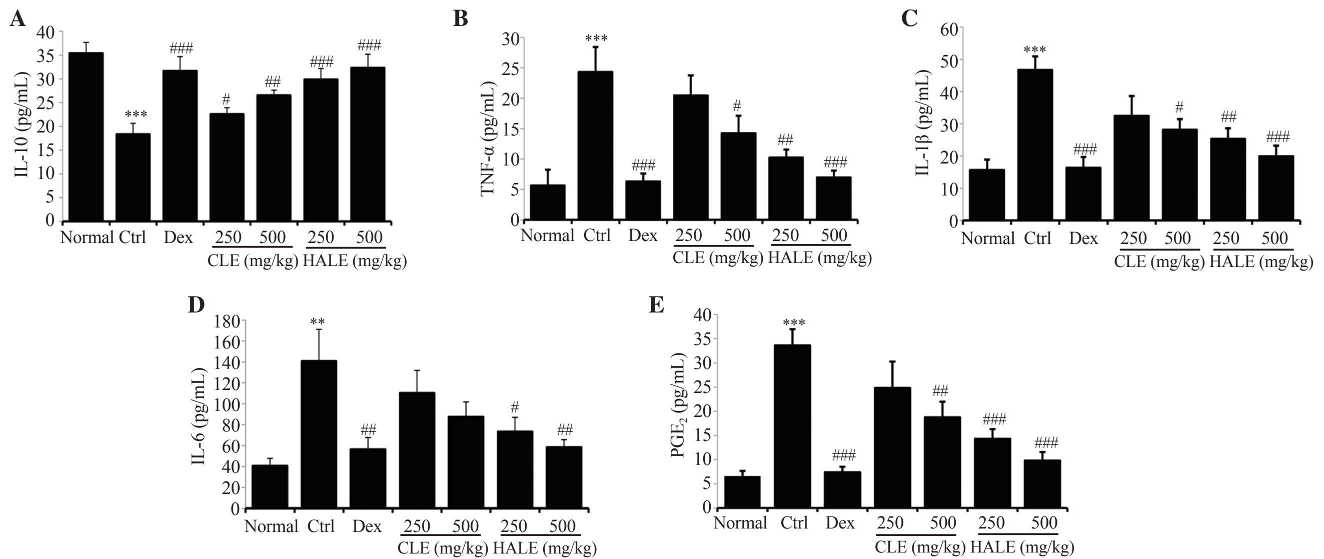


**Figure 3.** Effect of the chloroform and hydroalcoholic extract of *Cassia fistula* on serum inflammatory indicators. A: IL-10; B: TNF-α; C: IL-1β; D: IL-6; E: $PGE_2$. Values are represented as mean ± SD and analyzed by one-way ANOVA followed by Dunnett's *t*-test. $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with the normal group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ compared with the arthritic control group. $PGE_2$: prostaglandin $E_2$; Ctrl: arthritic control.

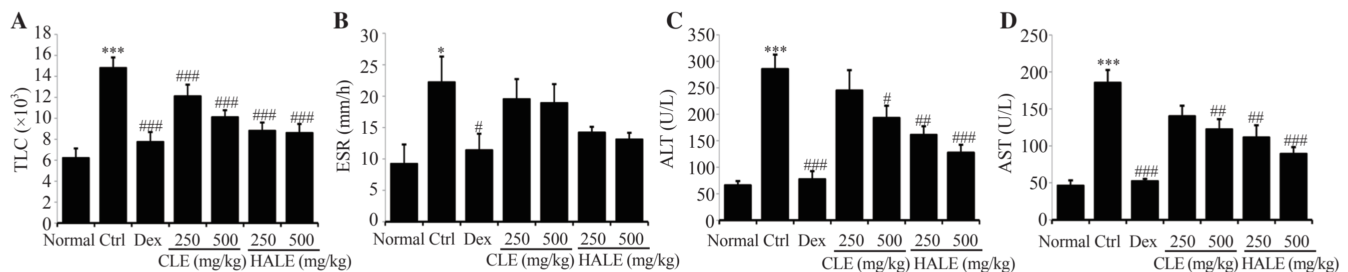


**Figure 4.** Effect of the chloroform and hydroalcoholic extract of *Cassia fistula* on (A) TLC, (B) ESR, (C) ALT, and (D) AST. Values are represented as mean ± SD and analyzed by one-way ANOVA followed by Dunnett's *t*-test. $^{*}P < 0.05$ and $^{***}P < 0.001$ compared with the normal control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ compared with the arthritic control group. TLC: total leucocyte count; ESR: erythrocyte sedimentation rate; AST: aspartate aminotransferase; ALT: alanine aminotransferase.

decrease the IL-1β level ($P < 0.05$). However, both doses of the chloroform extract had no significant effect on lowering IL-6 levels. Both doses of the hydroalcoholic and chloroform extract prominently elevated serum levels of IL-10 ($P < 0.05$). In addition, rats treated with 250 and 500 mg/kg hydroalcoholic extract as well as 500 mg/kg chloroform extract markedly lowered the $PGE_2$ level ($P < 0.05$) (Figure 3).

Arthritic control rats demonstrated a significant elevation in TLC, ESR, ALT, and AST compared to normal control ($P < 0.05$). Treatment with 250 and 500 mg/kg hydroalcoholic extract as well as 500 mg/kg chloroform extract significantly reduced these parameters ($P < 0.05$). Furthermore, all treatments reduced ESR but with no significant differences compared with the arthritic control group ($P > 0.05$) (Figure 4).

### 3.4. Effects of the chloroform and hydroalcoholic extract of *C. fistula* on histopathological changes

The arthritic control group revealed enormous synovial hyperplasia, cartilage proliferation, partial bone destruction, inflammatory cell infiltration, and fibrosis in the synovial membrane (Figure 5). The normal control group showed normal synovial lining without any evidence of inflammation and cartilage or bone destruction. Pathological signs were reduced in rats treated with the hydroalcoholic extract (250 and 500 mg/kg) and dexamethasone (3 mg/kg). Rats treated with the chloroform extract (500 mg/kg) demonstrated a reduction in synovial hyperplasia, inflammatory cell infiltration, and joint fibrosis but this was not as evident as in the hydroalcoholic extract-treated group.

### 3.5. Molecular docking results

All the above results suggest the effectiveness of *C. fistula* L. leave extract on the pathogenesis and progression of CIA-induced arthritis. However, the active constituents in the extract responsible for its activity are not clear. To predict the active moiety that may be responsible for the anti-arthritic activity of the plant, an *in-silico* docking analysis of each reported constituent of *C. fistula* L. was performed. In our study, we evaluated biomolecules with anti-arthritic potential compared to internal standard ligand, B96_391A, and the results of the best 4 compounds are depicted in Figure 6. The docking study predicted physcion as the most promising molecule with MolDock score of −151.568 and demonstrated 12 interactions with the receptor. 1-Octacosanol, 5,3',4'-trihydroxy-6-methoxy-7-*O*-α-*L*-rhamnopyranosyl-(1,2)-*O*-β-*D*-galactopyranoside, and

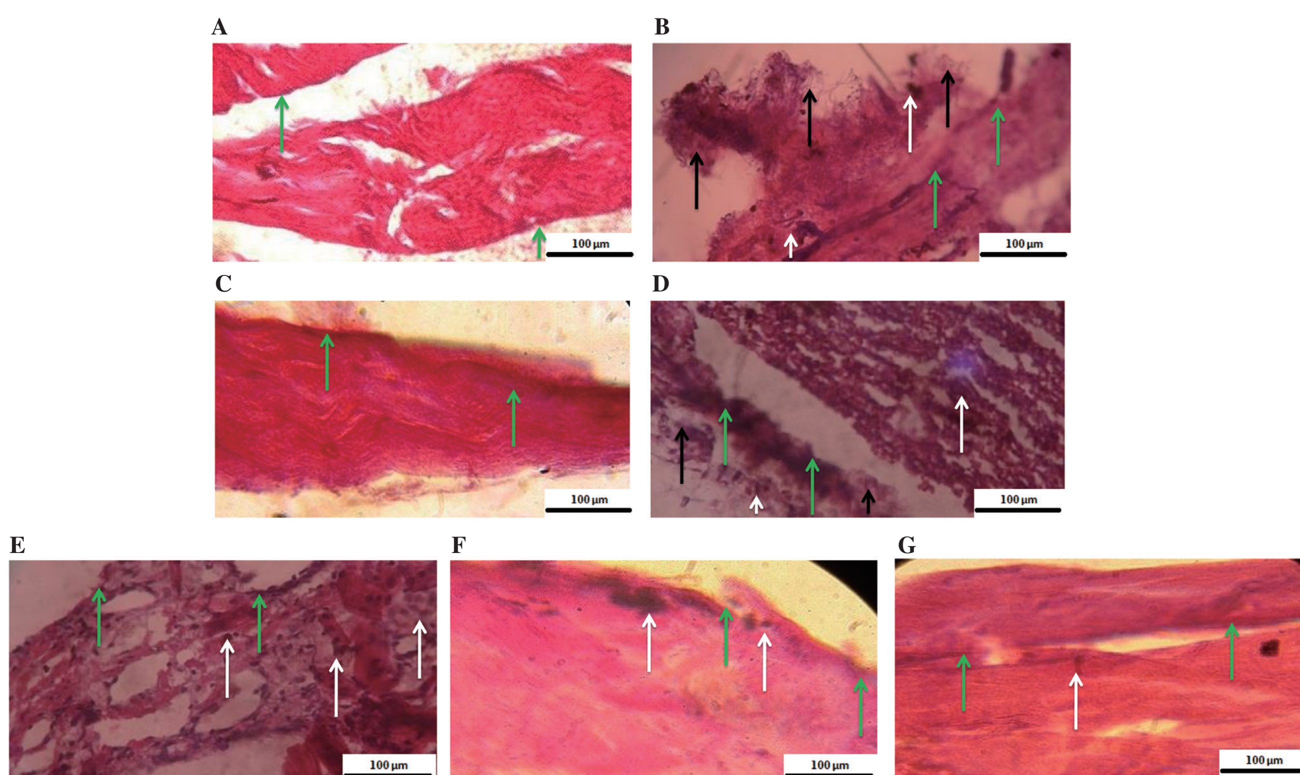


**Figure 5.** Effect of the chloroform and hydroalcoholic extract of *Cassia fistula* on joint histopathology in CIA rats. A: normal control + vehicle, B: CIA + vehicle, C: CIA + dexamethasone 3 mg/kg, D: CIA + chloroform extract 250 mg/kg, E: CIA + chloroform extract 500 mg/kg, F: CIA + hydroalcoholic extract 250 mg/kg and G: CIA + hydroalcoholic extract 500 mg/kg (scale bar: 100 μm, magnification: 400×). The black arrow indicates the fibrosis in the joint, the white arrow shows monocyte infiltration and the green arrow depicts the synovial membrane (ruptured or normal) in normal, CIA, and treated rats. CIA: collagen Ⅱ-induced arthritis.

scopoletin demonstrated MolDock score of −149.333, −138.961, and −137.687, respectively. These results suggest that the beneficial effects of *C. fistula* on RA may be attributed to the presence of physcion, 1-octacosanol, 5,3’,4’-trihydroxy-6-methoxy-7-*O*-α-*L*-rhamnopyranosyl-(1,2)-*O*-β-*D*-dgalactopyranoside, and scopoletin.

## 4. Discussion

RA is a chronic cytokine-mediated inflammatory disorder characterized by severe joint inflammation, impaired physiology, and the impaired functioning of joints[5,10]. Recent research work conducted on RA indicates the existence of a strong interdependent relationship between RA and high levels of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, and IL-10[5,10,21]. In the present study, the CIA model in female rats was used to explore the anti-arthritic effect of *C. fistula* L. leaf extract. We specifically used female rats because females are having a higher risk of developing RA when compared to males. CIA is one of the widely used experimental models for exploring the pathogenesis and screening of new drugs for the treatment of RA. Moreover, it is known to possess similarities with the immunological and pathological presentation of human RA. CIA is characterized by chronic synovitis, inflammatory cell infiltration, pannus formation, and destruction of joint function and physiology[22,23].

*C. fistula* L. is an ethnomedicinal plant that has been used for the treatment of rheumatism, gout, and inflammation in the Ayurvedic and Unani systems of traditional medicines[24,25]. Researchers have explored and reported this plant to be effective in relieving pain[26], inflammation, and fever[27]. However, scientific justification for its anti-arthritic potential has not been fully explored. In the present study, we investigated this and found that oral administration of the hydroalcoholic extract of *C. fistula* effectively suppressed the symptoms and progression of CIA which was comparable to suppression induced by the standard drug, dexamethasone.

In the present study, the progression of arthritis and inflammation was observed throughout the study by evaluating paw volume and arthritic score at regular intervals. Vehicle-treated rats showed a progressive increase in paw volume and arthritic index during the study. These findings are in line with the previous reports where

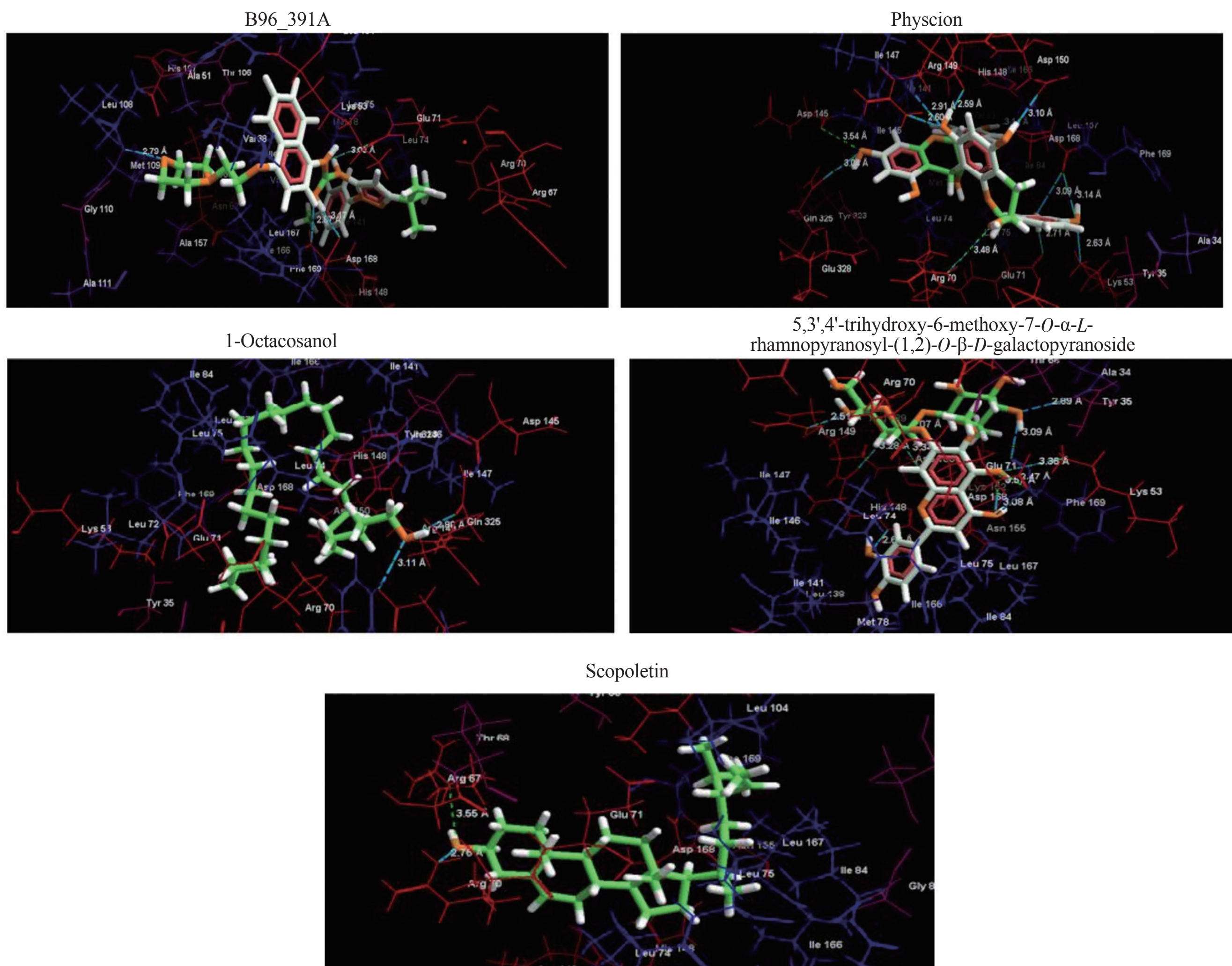


**Figure 6.** Docking interaction of biomolecules of *Cassia fistula* against 1KV2 PDB.

collagen treatment has demonstrated a similar effect on rodent paws[28]. The hydroalcoholic extract efficiently reduced the paw volume and arthritis score. Our results are consistent with the literature where a reduction in the arthritic score and paw volume was reported with the improvement in the progression of RA[28].

The spleen is an organ that is known to store and rapidly distribute monocytes into the blood during the inflammatory response of the body. Therefore, it is obvious that the workload of the spleen, measured as the spleen index, is increased during the arthritic challenge[29]. In our study, arthritic control showed enormous elevation in spleen index which was effectively countered by oral administration of the hydroalcoholic extract in a dose-dependent manner. This result suggests that extract treatment might have reduced the immune response load of the spleen by reducing the number of monocytes, thereby causing a reduction in the spleen index.

Previously reported studies demonstrate the crucial role of pro-inflammatory cytokines and $PGE_2$ in the inflammation and joint destruction in RA[30–32]. It has been well established that interleukins and TNF-α are expressed highly at the disease site in CIA[32]. Thus the present RA therapy aims to halt the chronic disease progression by interfering with inflammation and subsequent destructive responses through the use of TNF-α blockers, anti-IL-6 receptor antibodies, and anti-B-cell agents[32,33]. In the current study, it was demonstrated that oral administration of plant extract, especially the hydroalcoholic extract, targets the pro-inflammatory cytokines which are responsible for eliciting inflammatory responses and further complications. Plant extract significantly elevated serum IL-10 and lowered the serum level of TNF-α, IL-1β, and IL-6, therefore, suppressing cytokine-mediated inflammatory response which could be beneficial in RA therapy.

$PGE_2$ is an inflammatory mediator produced at the site of inflammation by prostaglandin endoperoxide synthases. It is responsible for inflammation and structural changes in cartilage and joint during RA[34]. Nonsteroidal anti-inflammatory drugs inhibit enzymes COX-1 and COX-2 which are responsible for the generation of prostaglandins, and thus are the most widely used drugs in RA. COX-1 and COX-2 play crucial roles in pain, inflammation, and fever[5,10,35]. In the present study, a significant reduction in serum level of $PGE_2$ was observed after oral administration of the hydroalcoholic extract. Our results suggest that the extracts might prevent inflammation and cartilage destruction by inhibiting the $PGE_2$ generation in the joint.

Previous studies demonstrate that RA induces noticeable changes in the blood profile of animals. Elevated TLC, ERS, ALT, and AST levels in the blood during experimentally induced arthritis have previously been reported[36], which also comply with our study. All the treatment groups showed a significant reduction in TLC levels. The reduction was also observed in the levels of ESR for all the treatment groups but was non-significant compared to the arthritic control. The hydroalcoholic extract showed a dose-dependent lowering effect on the serum level of ALT and AST enzymes.

Histopathological examination of the joints during CIA has provided strong evidence to evaluate the effect of treatments on the progression of RA[31,32,37]. In line with the previous reports, our histopathological studies showed that vehicle-treated CIA rats experienced enormous inflammatory cell infiltration, synovial destruction, fibrosis, cartilage hyperplasia, and/or destruction and bone erosion. Treatment of rats with *C. fistula* extracts showed a dose-dependent improvement in joint physiology. The hydroalcoholic extract showed a marked reduction in infiltration, fibrosis, synovial and cartilage destruction, and improved joint physiology.

Docking studies have been used to predict lead molecules against various targets, including that for RA[38,39]. Herbal extracts possess a large number of compounds and thus it is difficult to determine which compound is responsible for the activity studied. We used this technique to predict which bioactive molecules of the *C. fistula* extract may be responsible for its anti-arthritic effect. Based on the recorded MolDock score, number of interactions between the compound and the receptor, ligand involved, bond length, and energy of most stable conformation, we predicted 4 potential anti-arthritic compounds [physcion, 1-octacosanol, 5,3',4'-trihydroxy-6-methoxy-7-*O*-α-*L*-rhamnopyranosyl-(1,2)-*O*-β-*D*-galactopyranoside, and scopoletin], which were reported to be present in the extracts in a separate study conducted in our lab. Physcion showed 12 interactions with the receptor and MolDock score of −151.568 and thus is predicted to be the most potent anti-arthritic compound in *C. fistula* L. Interestingly, our results are in agreement with the literature which suggests that physcion has anti-inflammatory potential[40], and the medicinal plants having physcion as their constituent also demonstrate good anti-inflammatory potential[41]. 5,3,4'-Trihydroxy-6-methoxy-7-*O*-α-*L*-rhamnopyranosyl-(1,2)-*O*-β-*D*-galactopyranoside, on the other hand, also has 12 interactions and the MolDock score was observed to be −138.961, suggesting it as a strong candidate which can be attributed to the anti-arthritic activity of the extract. 1-Octacosanol and scopoletin showed just 2 interactions with the receptor protein but possessed high MolDock scores of −149.333 and −137.687, respectively. Thus their anti-arthritic potential cannot be neglected as well. In addition, plants having these molecules have been demonstrated to possess anti-inflammatory potential[42–45]. Therefore, these molecules are crucial for the management of the development and progression of arthritis and need to be investigated extensively.

The findings of the present study provide experimental validation for the traditional use of *C. fistula* in RA. However, these findings are from rodent models that have been used extensively for screening drugs for RA through one particular pathological pathway, which cannot mimic the clinical presentation of RA completely. Moreover, we have predicted bioactive molecules through docking studies that could be responsible for the beneficial effect of *C. fistula* during RA and these molecules need to be studied further. Therefore, extensive

research is still needed to reach a decisive conclusion regarding the potential clinical application of *C. fistula* in the management of RA.

In conclusion, the leaf extract of *C. fistula* L. possessed good anti-arthritic potential in the CIA rat model, which could be attributed to downregulation of TNF-α, IL-6, IL-1β and $PGE_2$ levels and upregulation of IL-10 in CIA rats. Anti-arthritis potential of the plant extract was also supported by the results of biochemical, histopathological, and docking analysis in this study. Moreover, docking analysis predicted 4 potential lead compounds which may be responsible for the anti-arthritic potential of *C. fistula*. However, extensive study is still required.

## Conflict of interest statement

All the authors declare no conflict of interest.

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## Authors' contributions

VM contributed to study design and data interpretation, performed experimental work, and drafted the first version of this manuscript. PN contributed to data analysis, and critical revision of the manuscript. AP provided technical inputs, and contributed to data analysis and critical revision of the manuscript. MC contributed to study design, data analysis and interpretation, editing and finalizing the manuscript.

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