Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN 0976-9595

Research Article

# EVALUATION OF ANTIARTHRITIC ACTIVITY OF RHIZOMES OF *BERGENIA CILIATA* AGAINST FREUND'S ADJUVANT INDUCED ARTHRITIS IN RATS

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## ABSTRACT

*Bergenia ciliata* (How) Sternb (*B. ciliata*) is a medicinally important perennial herb used as astringent, diuretic and tonic. It has many medicinal properties such as antibacterial, anti-inflammatory, anticancer, antidiabetic and mainly used for kidney disorders. The objective of the present study was to assess the anti-arthritic activity of isolated component and *B. ciliate* extract in complete Freund's adjuvant (CFA) induced arthritis in wistar rats. Methanolic extract of *B. ciliate* extracts (200 and 400 mg/kg. b. wt.) and isolated component (50 mg/kg. b. wt) were given orally to arthritic rats induced with Complete Freund's Adjuvant and changes in paw diameter, joint diameter, arthritic score, body weight and antioxidant parameters (LPO, SOD, GSH) were determined. Histopathological analysis was also performed. Indomethacin was taken as standard. Rats treated with extracts and isolated component displayed marked reduction in paw diameter, joint diameter, arthritic score, along with substantial enhancement in body weight. Further, biochemical assays revealed a significant enhancement in the levels of reduced glutathione, and superoxide dismutase but a significant reduction in lipid peroxidation levels with extract and isolated component treatment, as compared to arthritic control group. Histopathological findings showed significant reduction in destruction of joints and inflammation following extract and isolated component treatment. The results of the study show that extract and isolated component treatment has markedly enhanced health and reduced inflammation via lessening the pro inflammatory cytokines expression in arthritic rats. It is concluded that extract and isolated component has significant anti-arthritic activity in CFA induced arthritis in rats.

**Keywords:** *Bergenia ciliata,* Freund's adjuvant (CFA) induced arthritis, Biochemical parameters, Histopathology.

## 1. INTRODUCTION

Traditional and folklore medicines play an important role in healthcare. There exist a plethora of knowledge and benefits of herbal drugs in our ancient literature of ayurvedic and unani medicine. Herbal drugs or their extracts are prescribed widely, even when their biologically active compounds are unknown. Rheumatoid arthritis (RA) is a chronic, auto-immune disease and the causative factors that are responsible for development of disease are unknown. The pathological features of the patients who suffer from this disease display joint pain, joint inflammation, synovial tissue proliferation which leads to damage and disability of joints [1]. The mechanism by which joint destruction occurs in RA includes increased expression of cytokines and transcription factors. Interleukins namely IL-6, tumour necrosis factor (TNF)-α, IL-1β and IL-1 are the cytokines involved in progression of arthritis [2]. Four varieties of drugs are available for treatment of RA which includes steroid hormones, biological agents, immunosuppressant, anti-rheumatic drugs and anti-inflammatory drugs [3-6]. The anti-arthritic drugs even though have various potent benefits but criteria such as high costs, side effects and their efficacy towards specific target site limits their clinical use [7]. Reported side effects of RA drugs include immune deficiency, gastrointestinal tract disorders, hormonal disturbances and complications in cardiovascular system [8]. The beneficial compounds against RA are studied using various animal models to identify their therapeutic benefits which shares disease similarity with that of humans. Among other models of arthritis in rodents,

Complete Freund's Adjuvant (CFA) induced model displays various similarities with that of human arthritis which makes it most suitable model for inducing arthritis [9]. The therapeutic approach for arthritis demands the drug to be economical, have long life and minimal or less side effects. The drugs should also inhibit inflammation and pro inflammatory cytokines expression thereby preventing damage of joints. *B. ciliata* is the member of family Saxifragaceae commonly known as Pashanbheda. The plant originates between rocks and appears to break them or that it possesses lithotriptic property. It is considered as a miracle herb because it is used to cure several diseases viz; gastrointestinal problems, kidney stone, malaria etc. This plant showed the prescence of various phyto-chemicals viz; tannins, terpenoids, flavonoids, steroids, saponins, coumarins and glucosides. The rhizome is the rich source of alkaloids, tannins and coumarins. There are approximate 58 phytochemicals present in *Bergenia ciliata* species. Out of these 48 volatile organic compounds are classified into 11 categories such as phenols, terpenoids, fatty acids, carboxylic acid, flavonoids, nitro compounds, cinnamic acid, glycosides, alcohols, volatile organic compounds and sterol [10]. The present study was designed to evaluate the antiarthritic activity of extract and isolated component in complete Freund's adjuvant (CFA) induced arthritis in wistar rats supporting its traditional use in arthritis.

## 2. MATERIAL AND METHODS

### 2.1. Plant materials

The rhizomes of *B. ciliata were* collected from District Udhampur, Himalayan region of Jammu and Kashmir. The sample was identified by senior Botanist Dr. Zia-Ul-Hassan, Professor and head department of Botany, Safia College of Arts and Science, Bhopal. A herbarium of plants was submitted to the specimen library of Safia College of Arts and Science, Bhopal and The specimen voucher no. of *B. ciliata* is 119/Bot/Saf/18.

### 2.2. Chemicals and reagents

Complete freund's adjuvant (CFA) was procured from Sigma Aldrich chemicals Pvt. Ltd, Hyderabad, India. Indomethacin was obtained from Akums Drugs and Pharmaceuticals, India. All other chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), SRL Pvt. Ltd. (Mumbai, India) and Merck Life Sci. Private Ltd. (Mumbai, India).Kits for biochemical estimation were procured from Span Diagnostics, Pvt. Ltd. Navsari, Gujarat, India. All other chemicals used in this study were obtained commercially and were of analytical grade and triple distilled water was used for whole experiment was generated in house.

### 2.3. Extraction of Plant Material

#### *2.3.1. Soxhlet Extraction*

The rhizomes of *B. ciliata* were collected, washed and rinsed properly. They were dried in shade and powdered mechanically. 1kg of the powder rhizomes was successive extracted with different organic solvents viz; Pet. ether, ethyl acetate and methanol and allow to stored for 72 hours in ice cold condition for the extraction of phytochemicals. At the end of the third day extract was filtered using whatmann No.1 filter paper to remove all un-extractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent. The entire extract was concentrated to dryness using rotary flash evaporator under reduced pressure and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts [11]. Phytochemical analysis, quantitative phytochemical assay was performed by calculating total phenolic content (TPC) and total flavonoids content (TFC), result showed that methanolic extract of *B. ciliata* has highest methanolic extractive percentage compare to other extracts [12].

#### *2.3.2. Animals*

*Albino* Wistar rats (5-6 weeks, either sex, 200±20 gm) were selected randomly from animal house of Pinnacle Biomedical Research Institute (PBRI), Bhopal, India and further divided into various treatment groups randomly and kept in propylene cage with sterile husk as bedding. Relative humidity of 30.7 % at 22±2°C and 12:12 light and dark cycle was maintained in the animal house and fed with standard pellets (Golden Feeds, New Delhi, India) and water was available *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. Animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal (Reg No. 1824/PO/ERe/S/15/CPCSEA). Protocol Approval Reference No. PBRI/IAEC/PN-19024.

#### 2.3.3. Acute Oral Toxicity

Acute toxicity study of the prepared extracts of *B. ciliata* was carried out according to the Organization for Economic Co-Operation and Development (OECD) Guidelines-423 [13]. The animals were fasted for 4 h, but allowed free access to water throughout. As per the OECD recommendations, the starting dose level should be that which is most likely to produce mortality in some of the dosed animals; and when there is no information available on a substance to be tested in this regard; for animal welfare reasons, The dose level to be used as the starting dose is selected from one of four fixed levels 5, 50, 300 and 2000 mg/kg body weight. Acute toxicity was determined as per reported method [14].

### 2.4. Complete Freund's adjuvant (CFA) Induced Arthritis

**Experimental design**

Group I: Arthritic control
Group II: Normal control
Group III: Standard (Indomethacin 10mg/kg)
Group IV: *B. ciliata* extract treated (200 mg/kg. b.wt.)
Group V: *B. ciliata* extract treated (400 mg/kg. b.wt.)
Group VI: Isolated component (50 mg/kg. b. wt)

Complete Freund's adjuvant (CFA) model was performed to evaluate the anti-Arthritic Activity. Animals were divided into six groups. Group I animals received CFA (0.01 ml *Freund's* adjuvant) injected intra peritoneally into the plantar region of the right hind paw of each rat served as an arthritic control, Group III animals received Indomethacin (10 mg.kg-1 p.o.) served as reference standard, Group II animals received normal saline (1 ml.kg-1) served as control and Group IV and V animals received the crude extract of *B. ciliata* (200 mg.kg-1and 400mg.kg-1), Group VI animals received isolated component (50mg/kg. b.wt). All were compared to standard Indomethacin and evaluated. The paw volume is considered as an indicator of arthritic condition. To assess the anti-arthritic activity of the extracts were given to the animal 30 minutes before the administration of Freund's adjuvant and continued till 21$^{th}$ day. Paw diameter, joint diameter, arthritic score, body weight measurements was carried out on days 3$^{rd}$, 7$^{th}$, 14$^{th}$, 21$^{st}$. After completion of protocol on the 21$^{th}$ day blood (terminal) was collected by puncturing the retro-orbital plexus into heparanized vials [15, 16].

### 2.5. Evaluation of Arthritis

#### 2.5.1. Arthritic score

The experimental animals were subjected to macroscopical examination to measure the arthritic score by observing the degree of swelling and redness of joints, edema of periarticular tissues in the injected and non-injected paw [17].

#### 2.5.2. Bodyweight examination

Change in body weight was measured with the help of digital weighing balance from the day of CFA immunization and then subsequently on 3th, 7th, 14th, and 21$^{st}$ days [18].

#### 2.5.3. Estimation of paw diameter

Volumes of injected hind paws of rats were recorded before and at 7 days interval after adjuvant inoculation by using water displacement plethysmograph. The percentage change in paw volume was calculated.

#### 2.5.4. Paw Thickness (Joint diameter)

Joint diameter was measured using a digital vernier caliper (Mitutoyo digimatic caliper, Japan) before adjuvant administration. The joint diameter was measured again on day (1, 3, 7, 14 and 21).

### 2.6. Evaluation of *In-Vivo* Antioxidant Activity

At the last day of treatment rats were sacrificed and liver of individual rats were isolated and washed in ice cold saline. Tissue homogenates were prepared with 0.1 M tris-HCl buffer (pH 7.4). The supernatant obtained was used to lipid peroxidation (LPO) [19] and antioxidant enzymes such as superoxide dismutase (SOD)[20] and reduced glutathione (GSH) content [21].

#### 2.6.1. Histopathological Examination

For histopathology, on the 21$^{th}$day at the end of the experiment, all animals were anesthetized under light ether anesthesia and sacrificed by cervical decapitation. Then, the right hind (arthritis induced) limb was removed just distal to the knee, washed with saline and stored in 10% formalin. The fixed tissues were decalcified in 5% formic acid, processed for paraffin embedding, sectioned at 5µm thickness, and subsequently stained with haematoxylin-eosin [22] for examination under a light microscope with 10x magnifications. Sections were observed for the presence of hyperplasia of the synovium, pannus formation, soft tissue swelling, bone demineralization, cartilage erosion and destruction of the joint space.

### 2.7. Statistical Analysis

The data is expressed as mean ± Standard Deviation (SD). Results were analyzed using one-way ANOVA followed by Bonferroni test. Differences were considered as statistically significant at $P < 0.05$, $P<0.001$ when compared with control.

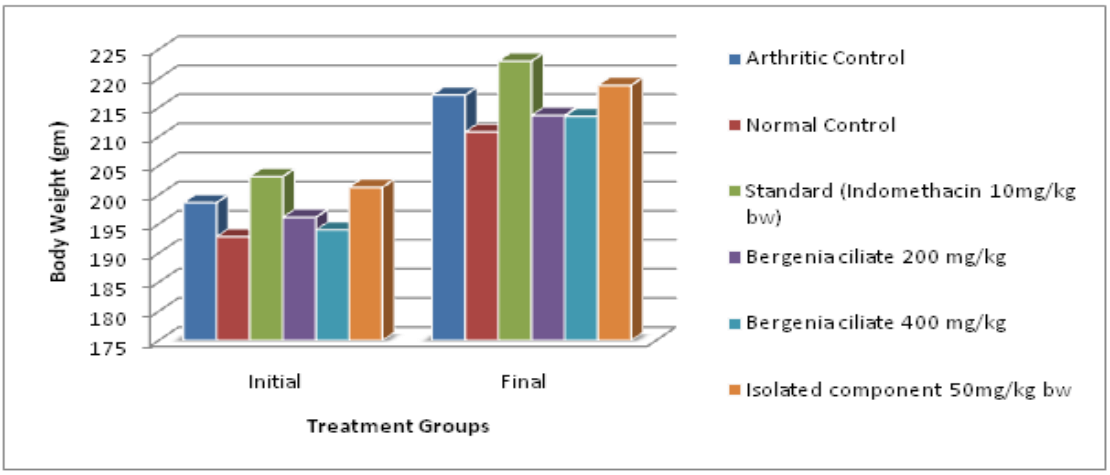
## 3. RESULTS AND DISCUSSION

To determine the safety of *B. ciliata* for human use, toxicological evaluation is carried out in experimental animals. In the acute toxicity study, no signs of toxicity were found up to the dose of 2000 mg/kg body weight. Hence 1/10th and 1/5th doses i.e. 200 mg/kg and 400 mg/kg have been fixed as ED50 for present study Table 1. The body weight of CFA induced rats was markedly decreased when compared with control rats. Treatment with extract (200, 400 mg/kg per day) and isolated component (50 mg/kg. b. wt) showed marked increase in body weight in CFA when compared with CFA alone induced group Fig.1. Paw diameter of group I rats was increased markedly than group II. Extract (200, 400 mg/kg per day) and isolated component (50 mg/kg. b. wt) produced significant and dose-dependent decreases from day 14 to day 21 in comparison to arthritic rats Fig. 2. Following CFA administration there was an increase in joint diameter which was maximum on day 7. After that, there was a progressive decrease in joint diameter in all the groups except the arthritic-treated where it was increased up to some extent after the 14$^{th}$ day. Treatment with indomethacin and extract resulted in a remarkable decline in joint diameter and the decline was statistically significant in comparison to arthritic treated group Fig. 3. The arthritic score does not materialize in normal controls, but in the groups that received CFA, it is very well materialized. Arthritic control groups are more affected from 7$^{th}$ day until the end of treatment. Nevertheless, animals treated with different extracts showed a decrease in arthritic score values until the end of treatment. However, animals treated with indomethacin and extract presented a significant ($p<0.05$) reduction in the physical value of this materialization at the end of treatment Fig. 4. Arthritic rats showed reduction in liver SOD and GSH level and increased MDA level compared to that in liver of healthy control. Oral administration of isolated component (50 mg/kg, bw) showed increase in SOD and GSH level respectively, while MDA level was significantly decreased in the liver of arthritic rats. Treatment of extract (200 and 400mg/kg) showed dose dependent increase in SOD and GSH level respectively, while MDA level was decreased in the liver of arthritic rats. Indomethacin also produced an increase in SOD activity and a reduction of MDA level in the liver of arthritic rats Table 2. Fig. 5 represents the changes observed in the hind paw of the experimental groups. Group 1: Chronic inflammation involve synovial hyperplasia Group 2: Normal control rat showing normal articular cartilage, absence of damage in the synovium; Group 3: Inflammation with increased vascularity, oedematous inflammation, inflammatory cell infiltrate (involve T-lymphocytes); Group 4: Synovial membrane structure re-establishing with the less oedema and inflammatory cell; Group 5: Moderate healing in the synovial membrane structure; Group 6: Decrease in inflammation with decrease in oedematous spaces, restructuring of synovial membrane with noticeable reduction of histological injury.

**Table1: Acute oral toxicity of *B. ciliata* extract**

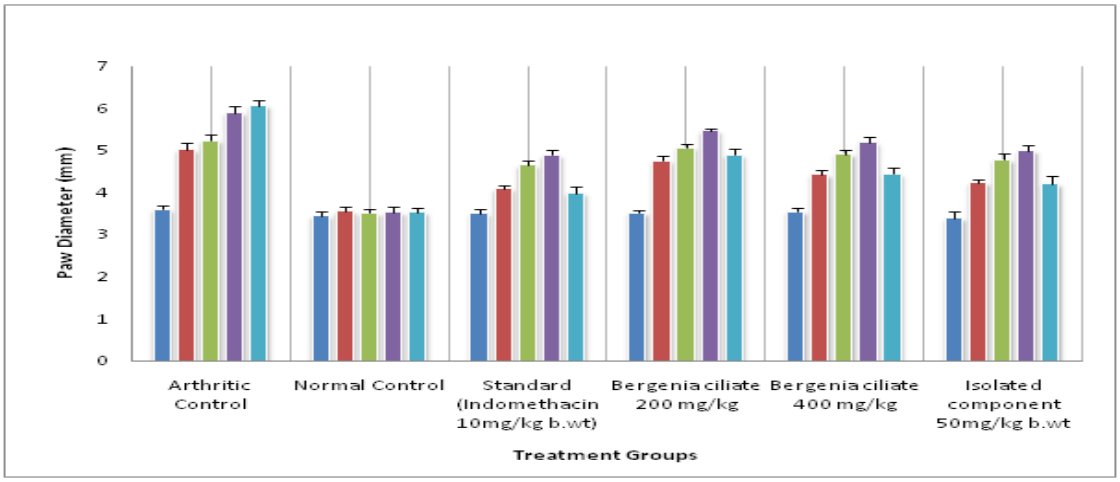
| Groups | Observations/ Mortality |
|---|---|
| 5 mg/kg Bodyweight | 0/3 |
| 50 mg/kg Bodyweight | 0/3 |
| 300 mg/kg Bodyweight | 0/3 |
| 2000 mg/kg Bodyweight | 0/3 |

CFA is commonly used for preclinical studies of NSAIDs and anti-rheumatic drugs and this model is most appropriate as like human arthritis [23-24]. In arthritis different inflamantory mediators were involved which are the products of arachadonic acid metabolism, histamine, 5-HT, bradykinin, cytokines, and nitric acid. CFA produces a characteristic inflammation and associated hyperalgesia, which can be used to quantify the anti-inflammatory or anti-hyperalgesic actions of drugs [25]. Mediators, like bradykinin, which are released from injured tissue directly stimulate nociceptors and stimulate tumour necrosis factor alpha (TNF-α) release. The TNF-α in turn, stimulates the release of interleukin-1beta (IL-1β) and interleukin-6 (IL-6), promoting the initiation of cycloxygenase enzymes, which convert arachidonic acid to prostaglandins [26]. Tumour necrosis factor-alpha (TNF-α) also stimulates the release of cytokine-induced neutrophil chemo attractant (CINC-1) in rats or interleukin-8 (IL-8) in humans. Cytokines, like IL-1β, TNF-α and IL-6, plays imp role in rhumatoid arthritis [27], these cytokines plays imp role in hyperalgesia by sensitizing peripheral nociceptors, decreasing the peripheral nociceptor threshold [28].



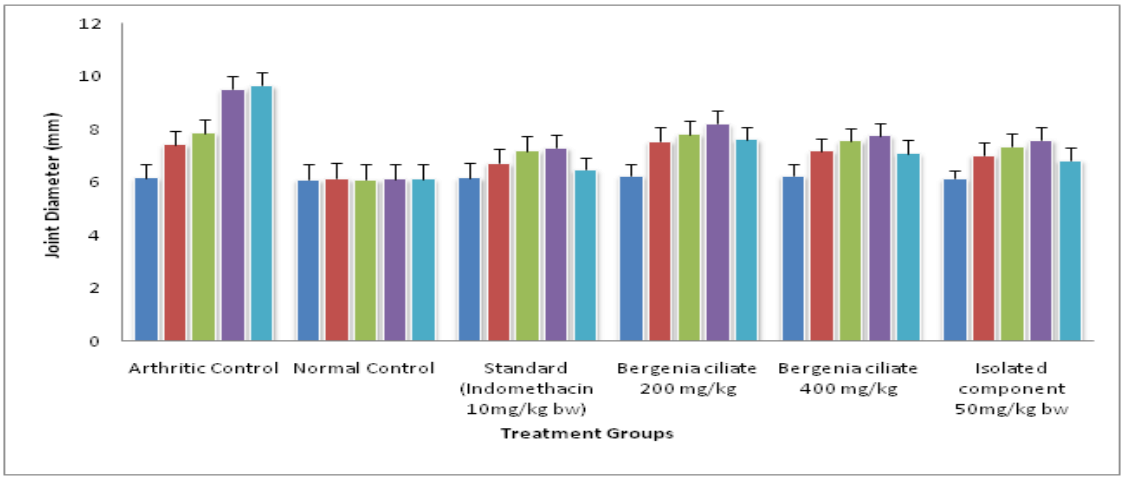

*n = 6, Mean ± SD*

**Fig. 1: Effect of *B. ciliata*and Indomethacin drug on rat body weight**



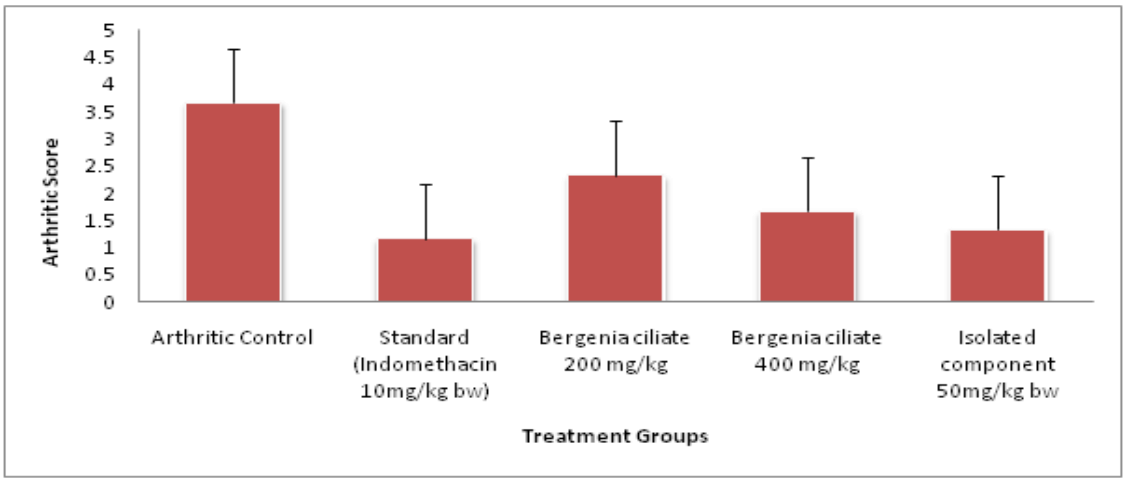

*Mean ± SD, n = 6, P<0.05 as compared to control group*

**Fig. 2: Anti-arthritic activity of *B. ciliata* on rat paw diameter**




*Mean ± SD, n = 6, P<0.05 as compared to control group*

**Fig. 3: Anti-arthritic activity of *B. ciliata* on rat joint diameter**




*Mean ± SD, n = 6, P<0.05 as compared to control group*

**Fig. 4: Effect of *B. ciliata* extract on arthritis score of arthritic rats**

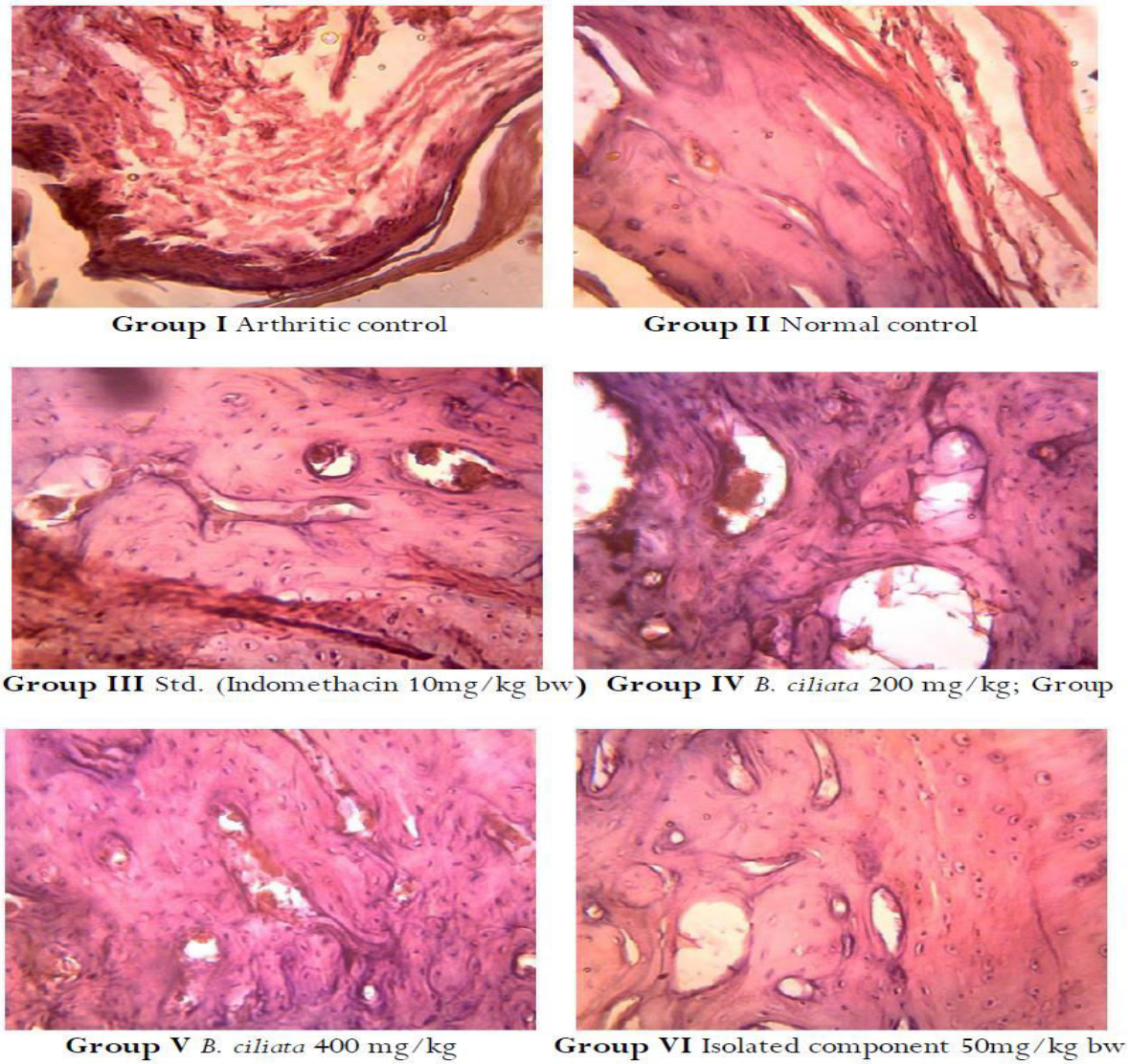



**Fig. 5: Hematoxylin and eosin staining of sections from the hind paws of rat variations groups**

**Table 2: Effect of standard, extracts and isolated component on LPO, SOD and GSH activity**

| Groups | LPO (nmol MDA/mg tissue) | SOD (unit/mg tissue) | GSH (mmol/mg tissue) |
|---|---|---|---|
| Arthritic Control | 72.61 ± 3.987 | 45.75 ± 6.697 | 0.37 ± 0.037 |
| Normal Control | 25.14 ± 3.688 | 196.90 ± 27.146 | 1.00± 0.015 |
| Std. (Indomethacin 10mg/kg bw) | 34.25 ± 2.806 | 159.72 ± 12.728 | 0.87 ± 0.080 |
| *B. ciliate* 200 mg/kg | 59.58 ± 2.447 | 69.44 ± 12.27 | 0.49 ± 0.006 |
| *B. ciliata* 400 mg/kg | 55.03 ± 2.447 | 87.42 ± 12.466 | 0.60 ± 0.046 |
| Isolated component 50mg/kg bw | 44.50 ± 5.806 | 124.18 ± 18.055 | 0.55 ± 0.054 |

*Values are expressed as mean± SD of 6 rats in each group*

As per the results isolated component and *B. ciliate* extract were significant to treat CFA induced arthritis. Free radicals production that occurs during development of arthritis in the articular cartilage leads to decreased GSH and SOD levels, increased ROS levels in rhumatoid arthritis may result in a prooxidation environment, which in turn could result in increased MDA levels. As a result, lipid peroxidation may have a role in the pathogenesis of the rhumatoid arthritis [29]. Pathogenesis of arthritis is associated predominantly with the formation of free radicals at the site of inflammation. In rheumatic condition oxidative injury and inflammatory status was confirmed by increased levels of prostaglandins in serum and synovial fluid compared to controls. T cells isolated from the synovial fluid of patients with rheumatoid arthritis showed signs of decreased intracellular GSH level [30]. In the present study, the levels of SOD and GSH were increased, while the level of LPO was reduced by isolated component and *B. ciliate* extract.

## 4. CONCLUSION

These findings suggest that treatment with an isolated component and *B. ciliata* extract improved the parameters to a normal level, indicating significant recovery in rheumatoid arthritis. A significant effect of *B. ciliata* extract in the *in vivo* model confirmed the result of the *in vitro* study. The possible mechanisms may be due to inhibition of release of mediators of inflammation and antioxidant activity. This activity may be due to the availability of active phytocompounds. Further isolation of responsible phytocompound for activity is underway.

## 5. ACKNOWLEDGMENTS

We are thankful to Dr. Mohd. Azaz Khan, Director and Dr. Megha Jha, Manager (Research and Development), Pinnacle Biomedical Research Institute (PBRI) Bhopal, India to provide us all lab facilities.

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