# The Protective Effect of Ethanolic Extract of *Sida Tiagii* Bhandari Seeds Against Complete Freund's Adjuvant Induced Experimental Animal Model of Rheumatoid Arthritis

Vaibhavi Jakhetia[1], Pankaj Khatri[1]*, Rakesh Patel[2], Lohit Badgujar, Anupriya Pandey[1]

[1]Department of Pharmacognosy, Gyan Vihar School of Pharmacy, Suresh Gyan Vihar University, Jagatpura, Jaipur, Rajasthan
[2]Department of Pharmacognosy, Ganpat University, Ahmedabad

*Corresponding author
Pankaj Khatri,
Assistant Professor,
Department of Pharmacognosy,
Gyan Vihar School of Pharmacy,
Suresh Gyan Vihar University,
Jagatpura, Jaipur
Email: pankajkhatripharma@yahoo.com
vaibhavijakhetia2002@yahoo.co.in

**Summary**

Rheumatoid arthritis is a chronic, systemic, inflammatory autoimmune disorder causing symmetrical polyarthritis of large and small joints, typically presenting between the ages of 30 and 50 years. The objective of the study was to investigate the anti arthritic activity of the ethanolic extract of *Sida tiagii* Bhandari seeds against experimental animal model of complete Freund's adjuvant (CFA) induced arthritis.

Arthritis was induced in female Sprague Dawley (SD) rats by administration of complete Freund's adjuvant in the sub plantar region of the hind paw. Indomethacin (2 mg/kg/day p.o.) was used as the standard drug. Ethanolic extract of *Sida tiagii* was administered at the following doses 30, 100 and 300 mg/kg/day p.o. The treatments were started on the day of CFA administration. The change in body weight, paw volume, diameter of the tibiotarsal joint and leukocyte count in the blood were measured at different time points.

The results demonstrate that ethanolic extract of *Sida tiagii* Bhandari dose dependently showed significant anti-arthritic activity with minimal side effect. As no gastric ulcer was seen in the animals treated with the extracts.

**Keywords:** *Sida tiagii;* Complete Freund's adjuvant induced arthritis; Immunomodulatory activity.

## Introduction

*Sida tiagii* Bhandari (Family-Malvaceae), a native species of the Indian and Pakistani desert area popularly known as "Kharanti" in India; is used in traditional and folk medicine as blood purifier, tonic, anti-arthritic and immuno-modulatory properties (1). A very less scientific literature is available on *Sida tiagii* Bhandari as compared to the other species of *Sida such as S. cordifolia, S. acuta, S. rhombifolia.* Recently, *Sida tiagii* Bhandari is reported for its antidepressant and anxioloytic activity in animals (2).

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease characterized by a distinctive pattern of bone and joint destruction. RA is also a systemic disease, and several patient subsets can be distinguished based on the presence of extra-articular manifestations (3). The incidence of RA varies across populations. Estimates from North America and Northern Europe range from 20 to 50 cases per 100,000 population. In Southern Europe, lower incidences of 9 to 24 cases per 100,000 population have been reported. The incidence of RA in developing countries is unknown (4-6). The pathogenesis in this disease has been attributed to the development of autoantibodies which infiltrate the synovial joint leading to degradation of structural macromolecules in connective tissue and proteoglycans present in the cartilage of the joint (7). The pathogenesis/reasons for development of adjuvant disease following injection of arthrogenic preparations are not fully understood despite the fact that numerous studies have contributed to the understanding of various possibilities including reactivity to cartilage proteoglycans, heat shock proteins and interactions with intestinal flora (8).

Based upon the above facts, we designed the present study to elucidate the anti arthritic activity of ethanolic extract of *Sida tiagii* Bhandari seeds. Complete Freund's adjuvant induced arthritis which mimics the human pathophysiological state was used as the animal model to investigate the activity of ethanolic extract of *Sida tiagii* Bhandari seeds in laboratory rats.

## Methods

### Preparation of dosage form

The emulsion of ethanolic extract of *Sida tiagii* Bhandari seeds and indomethacin suspension was prepared with 1% polysorbate 80 (Tween 80) in a glass mortar, with the gradual addition of water for injection (WFI), to make up the required volume.

**Animals Used**

Female albino Sprague Dawley (SD) rats of weighing 230-250 g were procured from National Toxicological centre, Pune for the present study. The animals were housed in groups of 4 in solid bottom polypropylene cages. They were maintained at 24 $^{o}$C ± 1 $^{o}$C, with relative humidity of 45-55% and 12:12 h dark/light cycle (9-17). Acclimatization period was two weeks**.** The animals had free access to food (Standard chow pellets, Chakan Oil Mills, Sangli) and water, *ad libitum*.

**Acute Toxicity Testing**

The acute oral toxicity study was carried out as per the guideline set by the Organization for Economic Co-operation and Development (OECD) received from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Acute toxicity study was performed in female swiss albino mice. The extracts were administered intraperitoneally (i.p.) at doses of 175, 550, 2000 mg/kg. They were then observed for signs of toxicity, continuously for 2 h, and for mortality up to 24 h, after injection (18).

**Induction of arthritis**

Arthritis was induced by a single intra dermal injection of Freund's complete adjuvant (FCA) containing 1.0 mg dry heat killed *Mycobacterium tuberculae* per milliliter sterile paraffin oil into the sub plantar region of the foot pad of the left hind paw of male rats (19). 1 ml tuberculine syringe with 26 gauge needle was used to administer the Freund's complete adjuvant. The rats were anesthetized with light ether inhalation prior to and during adjuvant injection.

**Experimental setup**

Animals were divided into five groups of 6 animals in each group as follows:

Group 1: Control group (1ml of 1% tween 80/day).

Group 2: Indomethacin (2 mg/kg/day in 1% Tween 80)

Group 3: ***Sida tiagii*** **Bhandari Ethanolic Extract** (30 mg/kg/day in 1% tween 80)

Group 4: ***Sida tiagii*** **Bhandari Ethanolic Extract** (100 mg/kg/day in 1% tween 80)

Group 5: ***Sida tiagii*** **Bhandari Ethanolic Extract** (300 mg/kg/day in 1% tween 80)

**Assessment of arthritis**

The progression of Complete Freund's adjuvant induced arthritis was evaluated by measuring the following parameters on 0, 4, 7, 10, 13, 14, 17, 19 and 21$^{st}$ day after adjuvant injection.

**Paw volume**

The swelling in the hind paw from the ankle was measured periodically on the days mentioned above using plethysmometer (Ugo Basile, Italy) (19).

**Arthritis score**

Rats were scored for arthritis (arthritis index) daily by a set visual criterion (19)**.**

The following scoring system was used:

| | |
|---|---|
| Normal paw | = 0 |
| Swelling and erythema of the digits | = 1 |
| Mild swelling and erythema of the digits | = 2 |
| Gross deformity and inability to use the limb | = 3 |

**Body weight**

The body weight of all the animals was recorded using electronic balance (18).

**WBC Count**

The total WBC count was measured using Neubar's chamber as an indication of the inflammatory response (20).

**Joint Diameter:**

The joint diameter was measured in millimeters with the help of vernier calipers and change in joint diameter was calculated (21).

**Statistical Analysis:**

All data are presented as Mean±SEM and analyzed by one-way ANOVA, followed by Dunnett's test. The groups treated with extracts were compared with the respective vehicle group. The indomethacin treated group was compared with vehicle 1% tween 80 solution in sterile water for injection. *P* values <0.05 were considered statistically significant (10, 11).

## Results

**Acute toxicity**

Extract was found to be safe in the dose used and there was no mortality up to a dose of 2000 mg/kg, i.p.

**Paw volume**

A significant increase in the paw volume was seen in CFA treated animals up to 4th day, which can be attributed to its inflammatory response. Further, a decrease in the paw volume was seen upto 10th day as compared to the 4th day of the treatment which was again started to increase and reached the plateau on the 14th day post inoculation exhibiting a late inflammatory response in all the groups of animals. Administration of ethanolic extract of *Sida tiagii* Bhandari at a dose of 100 and 300 mg/kg/day for a period of 21 days to arthritic animals dose dependently, suppressed the chronic phase of inflammation significantly ($p<0.01$) when compared with the control group of animals. A similar pattern was observed in the animals treated with indomethacin at a dose of 2 mg/kg/day. However, at lower dose (30 mg/kg), of ethanolic extract of *Sida tiagii* Bhandari did not show any inhibition in the acute phase of inflammation. Indomethacin was found more effective than 300 mg/kg ethanolic extract of *Sida tiagii* Bhandari. Figure 1 portrays the change in the paw volume during the entire treatment schedule of 21 days (figure 1).

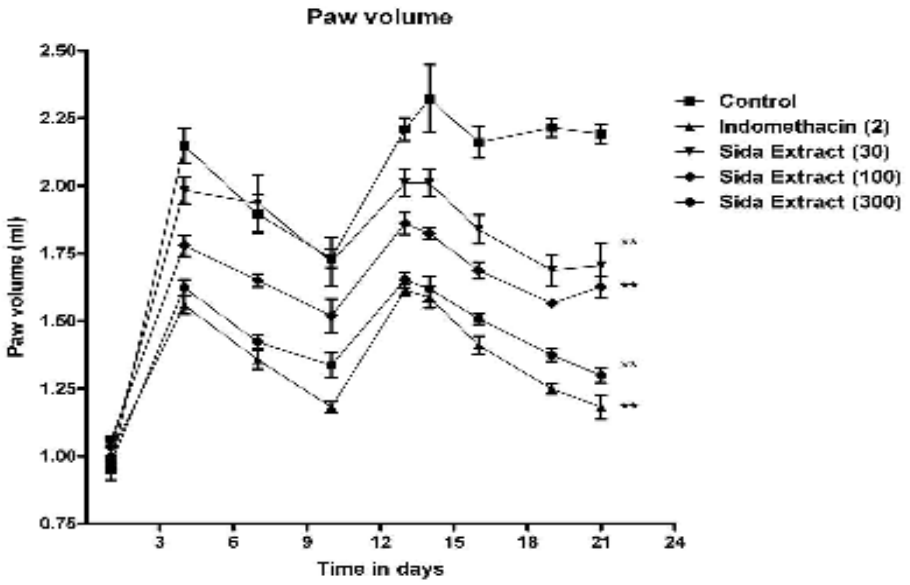


**Figure 1.** Effect of ethanolic extract of *Sida tiagii* Bhandari on change in rat paw volume. Results are expressed as mean±SEM, n = 6 in each group. *P<0.05, as compared to control.

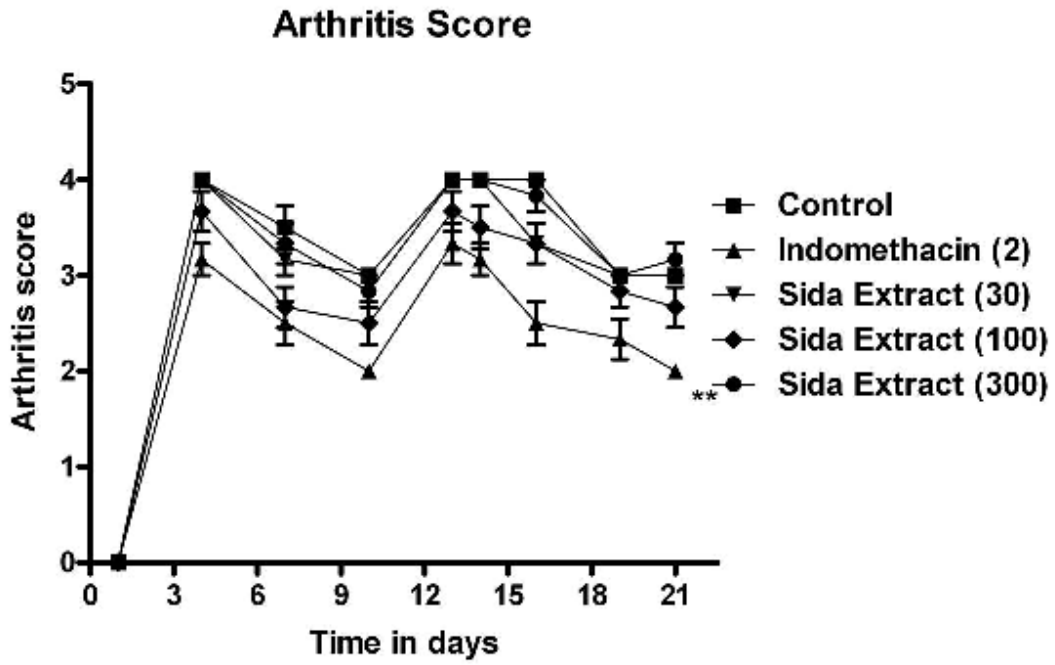


**Figure 2.** Effect of ethanolic extract of *Sida tiagii* Bhandari on change on arthritis score. Results are expressed as mean±SEM, n = 6 in each group. *P<0.05, as compared to control.

**Joint Diameter**

In control group the tibiotarsal joint was increased while it was significantly decreased ($p<0.01$) in indomethacin 2 mg/kg and ethanolic extract of *Sida tiagii* Bhandari (100 and 300 mg/kg) treated groups. However, lower dose of ethanolic extract of *Sida tiagii* Bhandari (30 mg/kg) was found ineffective (figure 3)

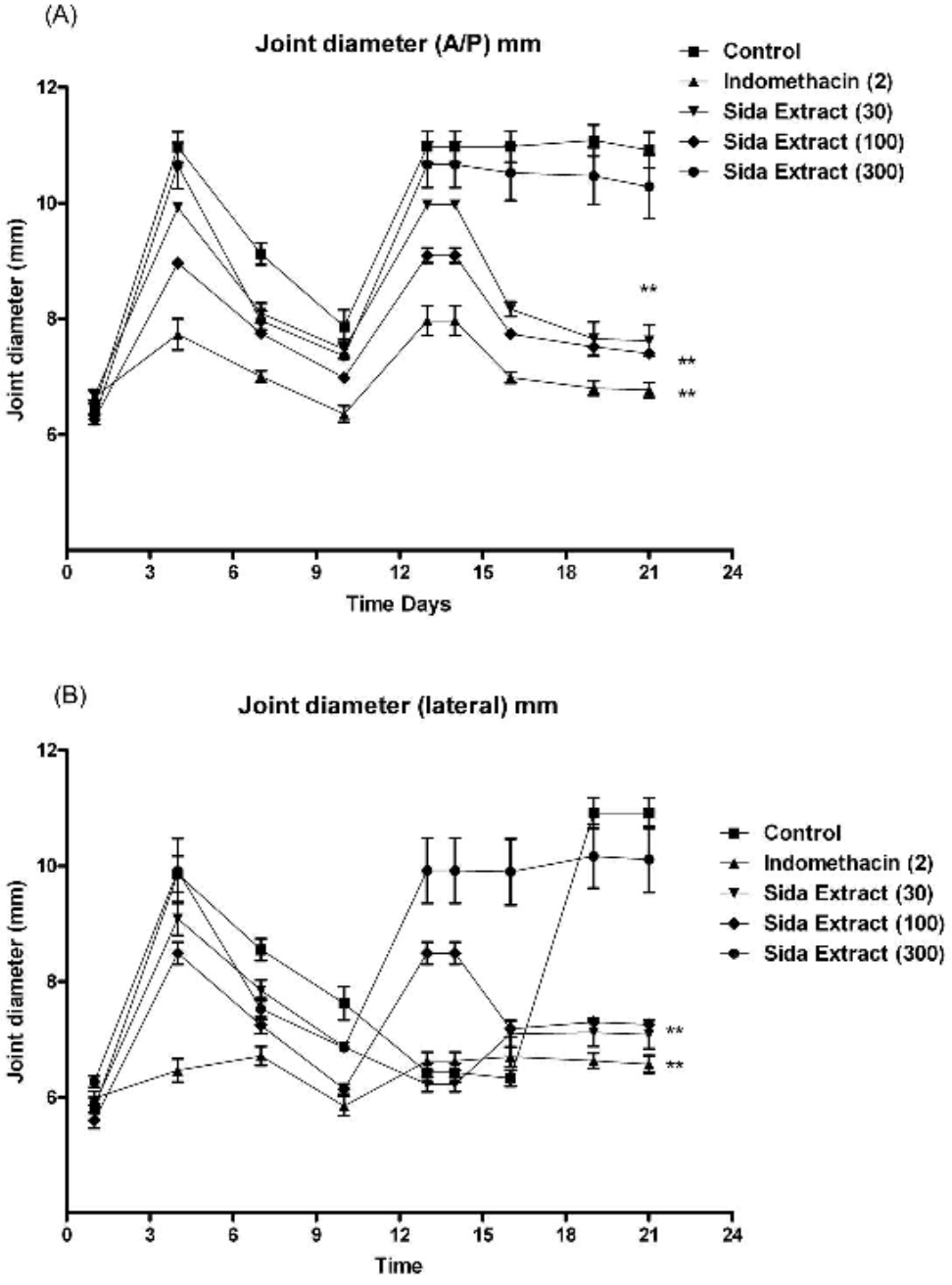


**Figure 3.** Effect of ethanolic extract of *Sida tiagii* Bhandari on change on rat joint diameter (A) anterior-posterior and (B) Lateral. Results are expressed as mean±SEM, n = 6 in each group. *P<0.05, as compared to control.

**Total leukocyte (WBC) count:**

The total leukocyte count showed a steep rise during both the acute and delayed inflammatory response in the control group. Whereas, in the indomethacin and ethanolic extract of *Sida tiagii* Bhandari treated groups a significant attenuation in elevated WBC count was ($p<0.01$) observed in the chronic phase of inflammation. However, lower dose of ethanolic extract of *Sida tiagii* Bhandari (30 mg/kg) was found ineffective (figure 4).

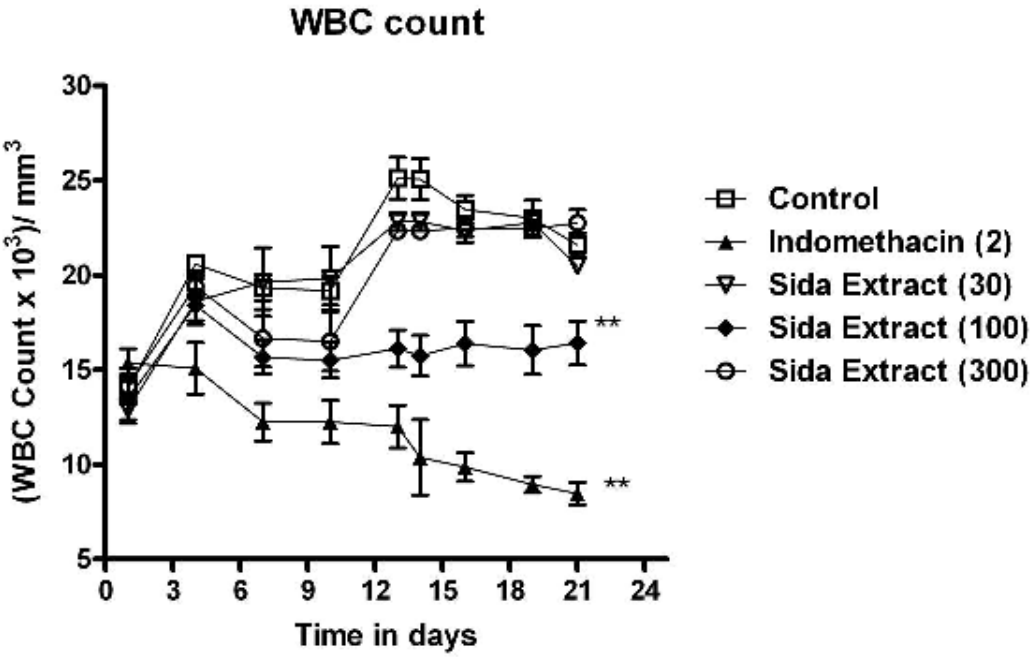


**Figure 4.** Effect of ethanolic extract of *Sida tiagii* Bhandari on change on leukocyte count. Results are expressed as mean±SEM, n = 6 in each group. *P<0.05, as compared to control.

**Body Weight:**

A continuous loss in body weight was observed throughout the period of 21 days in CFA treated control group. However, there was no significant alteration in body weight was observed in the indomethacin and extract treated group. However, lower dose of ethanolic extract of *Sida tiagii* Bhandari (30 mg/kg) was found ineffective (figure 5).

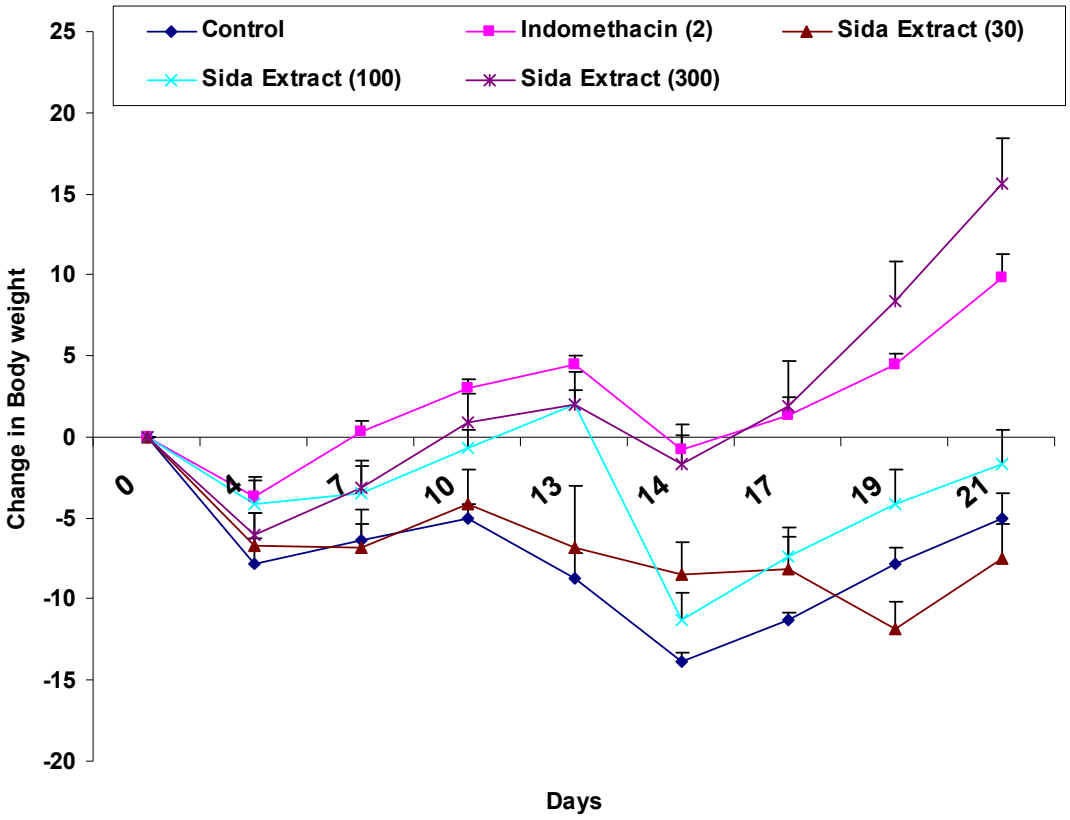


**Figure 5.** Effect of ethanolic extract of *Sida tiagii* Bhandari on change on rat body weight. Results are expressed as mean±SEM, n = 6 in each group. *P<0.05, as compared to control.

**Gastrointestinal side effects:**

The animals sacrificed after the complete therapeutic schedule of 21 days did not show the presence of ulcers or hemorrhages in extract treated groups which suggest that the present extract is having relatively safer profile as compared to the standard drug (indomethacin). Ulcers were seen in the indomethacin treated group (data not shown).

## Discussion

Rheumatoid arthritis (RA) is a systemic inflammatory disorder that affects approximately 1% of the population worldwide. Even after decades of research, our understanding of the pathogenesis of the disease and the underlying mechanisms remains rudimentary. Multiple factors including immune, genetic and environmental factors

govern the progression of the disease (22, 23). Rat adjuvant arthritis is an experimental model of polyarthritis which has been widely used for preclinical testing of numerous anti-arthritic agents which are either under preclinical or clinical investigation or are currently used as therapeutics in this disease (24, 25). The hallmarks of this model are reliable onset and progression of robust, easily measureable, polyarticular inflammation, marked bone resorption and periosteal bone proliferation. Cartilage destruction occurs but is disproportionally mild in comparison to the inflammation and bone destruction that occurs. In this study, CFA administration, showed soft tissue swelling in the tibio tarsal joint, along with increase in paw volume and elevation in the serum total leukocyte count, in a biphasic pattern, representing an acute inflammatory (days 1 to 7) and chronic autoimmune state (days 8 to 21).

The increase in paw volume and joint diameter in the chronic inflammatory phase were significantly and dose dependently attenuated in ethanolic extract of *Sida tiagii* Bhandari

The acute phase response can be attributed to its anti-inflammatory activity. As it is well known that the chronic phase can be reversed by a potent immunomodulator. This activity could be attributed to the presence of various steroids in this extract which possess potent immunomodulatory as well as anti inflammatory activity (26). Anti-inflammatory properties have already been reported for several compounds (27, 28). It is well known that the secondary chronic phase in arthritis induced by Freund's adjuvant is due to production of auto anti-bodies. Hence, it appears that the inhibition in the chronic inflammatory response was exhibited due to the immunomodulatory property of the steroids present in *Sida tiagii* Bhandari

Further, the immunomodulatory property of the ethanolic extract could be explained by the inhibition of the serum total leucocytes count in chronic phase of inflammation. The animals in the control group continued to lose weight during the entire treatment period whereas the animals in the indomethacin and *Sida tiagii* extract treated group lost weight non-significantly. The loss in weight in animals is closely associated with the arthritic status of the animals. Hence; this observation might well be explained by the anti inflammatory activity of *Sida tiagii* Bhandari. However, these activities cannot be attributed to a single phytoconstituent, but due to a synergistic action of all the

steroidal saponins and alkaloids present in *Sida tiagii* Bhandari. The anti-arthritic potential of the compound is well reported for *Sida tiagii* Bhandari in Indian traditional system and Ayurveda.

In this study, even after chronic administrations of ethanolic extract of *Sida tiagii* Bhandari for 21 days at all the three doses there was no gastro intestinal damage was seen to the animals. This gives *Sida tiagii* Bhandari an edge over the clinically prescribed Non steroidal anti-inflammatory drugs, which cause severe ulcers on chronic administration at higher doses.

In conclusion, the present investigation demonstrates that ethanolic extract of *Sida tiagii* Bhandari possesses potent anti arthritic potential against complete Freund's adjuvant induced experimental arthritis at a dose of 100 and 300 mg/kg/day p.o. unraveling a novel facet in its pharmacological profile.

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