EFFECT OF PETROLEUM ETHER EXTRACT OF *SPHAERANTHUS INDICUS LINN.* ON COMPLETE FREUNDS ADJUVANT INDUCED ARTHRITIS IN LABORATORY RATS

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Summary

The objective of the study was to investigate the anti arthritic activity of the petroleum ether extract of the flowers of *Sphaeranthus indicus Linn.* against complete Freund's adjuvant induced arthritis in laboratory rats.
Arthritis was induced in male wistar 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. The petroleum ether extract of *Sphaeranthus indicus* (SIP) was administered at the following doses 10, 30 and 100 mg/kg/day p.o. The following parameters were measured: change in paw volume, body weight, diameter of the tibiotarsal joint and total leukocyte count in the blood.
The results demonstrate that petroleum ether extract of *Sphaeranthus indicus Linn.* at a dose of 100mg/kg/day p.o. showed significant anti arthritic activity.

Keywords: *Sphaeranthus indicus Linn.;* SIP*;* Complete Freund's adjuvant induced arthritis; Immunomodulatory activity.

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Introduction

Rheumatoid arthritis is a chronic, progressive, systemic autoimmune disease causing inflammatory erosion of synovial joints ultimately culminating to joint destruction, deformity, and disability. It affects nearly 1% of the population of the world (1). 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 (2).

Sphaeranthus indicus Linn belongs to family *Asteraceae*. The plant is commonly known as *Gorakhmundi* in Hindi. It is an annual spreading herb, which grows approximately 15-30 cm in height. The plant is distributed throughout the plains and wet lands in India, Sri Lanka and Australia (3).

All the parts of the plant have medicinal uses. In folk medicine, the plant is reportedly used in treating epileptic convulsions, mental illnesses and hemicranias (4). The whole herb is used in ayurvedic preparations to treat epilepsy and mental disorders (5). It is used to treat vitiated conditions of hemicranias, jaundice, hepatopathy, diabetes, leprosy, fever, pectoralgia, cough, gastropathy, hernia, hemorrhoids, helminthiasis, dyspepsia and skin diseases. It is also used as a nervine tonic. The oil prepared using the plant root is reportedly useful in treating scrofula and as an aphrodisiac. The external application of a paste of this herb is beneficial in treating pruritus and edema, arthritis, filariasis, gout and cervical adenopathy. It also treats piles and hepatitis (6).

The petroleum ether extract showed potent anxiolytic activity against various animal models of anxiety (16). It also showed immunemodulatory activity in vitro (17, 18).

Hence, we designed the present study to unravel the immunomodulatory and anti arthritic activity of the petroleum ether extract. Complete Freund's adjuvant induced arthritis which mimics the human pathophysiological state was used as the animal model to investigate the activity of petroleum ether extract of *Sphaeranthus indicus* (SIP) in laboratory rats.

Methods

Collection of plant material

The flowers of *S. indicus* were collected at Raigad, Maharashtra. They were authenticated by Dr. Mujumdar (Head, Dept. of Botany), Agharkar Research Institute, Pune. A voucher specimen was deposited at the herbarium in the institute.

Preparation of extract

The powdered plant material (500 g) was soaked in petroleum ether (2000 ml) and allowed to stand for 48 h, with occasional shaking. The macerate was decanted and filtered, through cloth, and then, through Whatman filter paper (No.1). This process of extraction was repeated with the same volume of petroleum ether. The macerates were pooled and evaporated to yield a dark greenish yellow, waxy mass (yield 3.2%). The residue, called 'marc,' (after the extraction with petroleum ether) was dried and extracted with ethanol (90%) by the same procedure to yield a greenish brown semisolid (yield 4.0%). The extracts of petroleum ether and alcohol were stored in air-tight glass bottles, at room temperature.

Preparation of dosage form

The emulsion of petroleum ether extract (SIP) and indomithacin 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

Male albino rats of wistar strain weighing 230-250 gms 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. Acclimatization period was two weeks**.** The animals had free access to food (Standard chow pellets, Chakan Oil Mills, Sangli) and water, *ad libitum*. The Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune approved the pharmacological and acute toxicity protocol.

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.

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)

Group3: SIP 10 (10 mg/kg/day in 1% tween 80)

Group4: SIP 30 (30 mg/kg/day in 1% tween 80)

Group5: SIP 100 (100 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 21st 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 (20)**.**

The following scoring system was used:

Normal paw	= 0
Swelling and erythema of the digits	= 2
Mild swelling and erythema of the digits	= 3
Gross deformity and inability to use the limb	= 4

Body weight

The body weight of all the animals was recorded using electronic balance (21).

WBC Count

The total WBC count was measured using Neubar's chamber as an indication of the inflammatory response (22, 23).

Joint Diameter:

The joint diameter was measured in millimeters with the help of vernier calipers and change in joint diameter was calculated (24).

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 vehicle1% tween 80 solution in sterile water for injection. *P* values <0.05 were considered statistically significant.

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:

The change in paw volume elicited a biphasic response. It was maximum on 4^{th} day showing an early inflammatory response and on the 14^{th} day post inoculation exhibiting a late inflammatory response in all the groups of animals. A chronic phase of inflammation reached a plateau on the nineteenth and twenty first days. Administration of SIP at a dose of 100 mg/kg/day for a period of 21 days to arthritic animals 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 in the animals treated with indomethacin at a dose of 2 mg/kg/day. However, at lower doses of 10 and 30 mg/kg, SIP did not inhibit the acute and chronic phases of inflammation and calculated percentage inhibition showed a dose dependent effect of SIP. When compared to control, SIP 100 mg/kg showed significant reduction of paw volume. Indomithacin was prove effective than SIP 100 mg/kg. Figure 1 portrays the change in the paw volume during the entire treatment schedule of 21 days (figure 1).

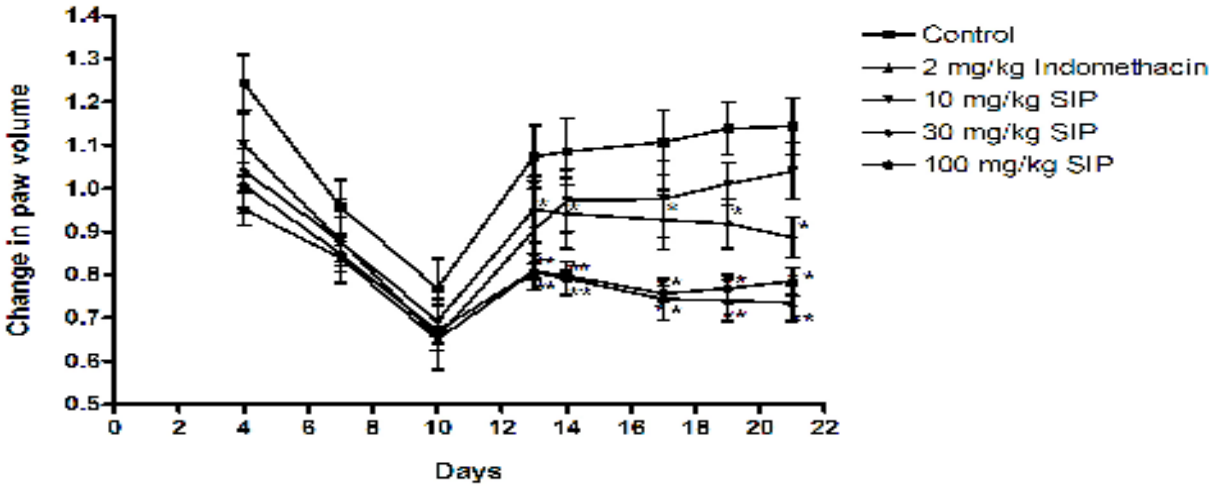


Figure 1. Effect of petroleum ether extract of *Sphaeranthus indicus Linn.* on change in rat paw volume. Results are expressed as mean±SEM, n = 6 in each group. *P<.05, **P<.01 when compared to vehicle. (One-way ANOVA followed by Dunnett's test.).

Arthritis score:

The pattern of amelioration in the arthritis was evaluated according to the above mentioned scoring system. Indomithacin 2 mg/kg and SIP 100 mg/kg significantly reduced (p<o.o1) arthritis score compared to control group (figure 2).

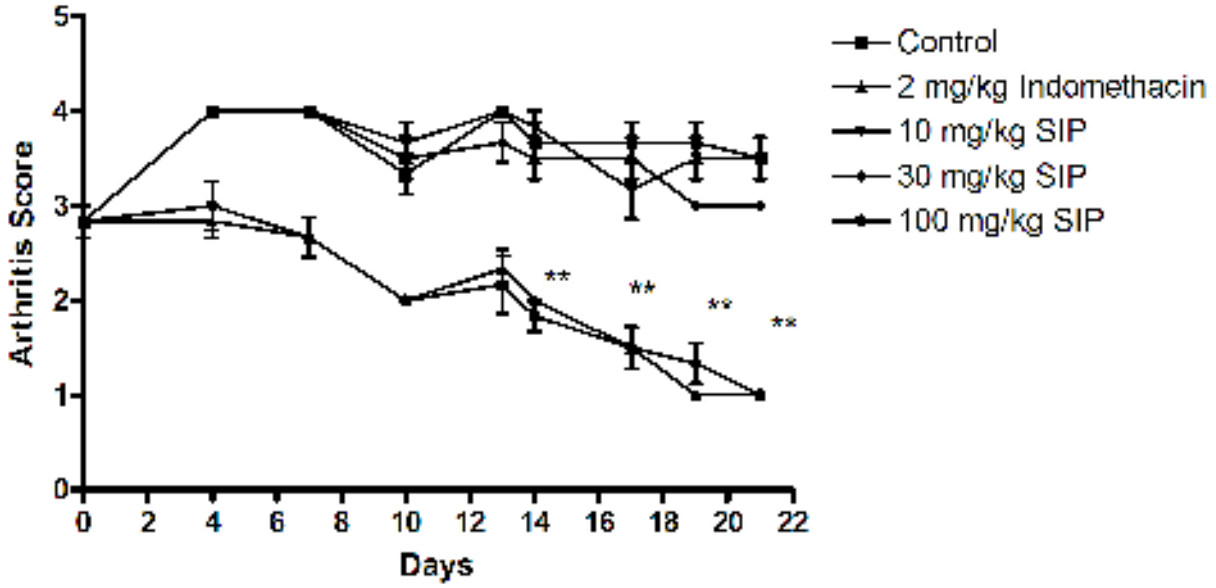


Figure 2. Effect of petroleum ether extract of *Sphaeranthus indicus Linn.* on arthritis score. Results are expressed as mean±SEM, n = 6 in each group. *P<.05, **P<.01 when compared to vehicle. (One-way ANOVA followed by Dunnett's test.).

Joint Diameter:

In control group the tibiotarsal joint was increased while it was significantly decreased ($p<0.01$) inindomithacin 2 mg/kg and SIP 100 mg/kg treated groups as illustrated in the (figure 3).

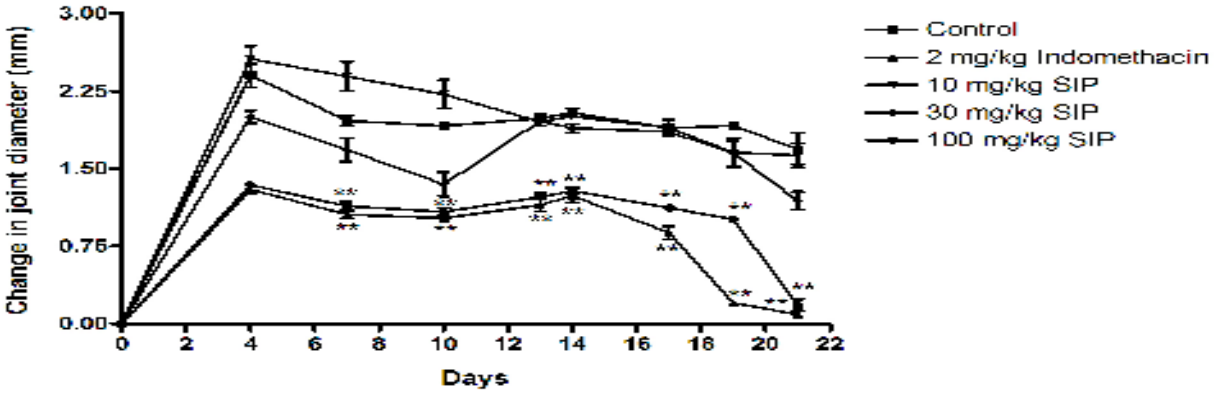


Figure 3. Effect of petroleum ether extract of *Sphaeranthus indicus Linn.* on rat joint diameter. Results are expressed as Mean±SEM, n = 6 in each group. *P<.05, **P<.01 when compared to vehicle. (One-way ANOVA followed by Dunnett's test.).

Total leukocyte (WBC) count:

The total leukocyte count showed a steep rise during both the acute and delayed inflammatory response in the control group and the animals treated with SIP at doses of 10 and 30 mg/kg of animals. Whereas in the indomethacin and SIP treated group the elevation of the WBC count was inhibited significantly ($p<0.01$) in the chronic phase of inflammation (figure 4).

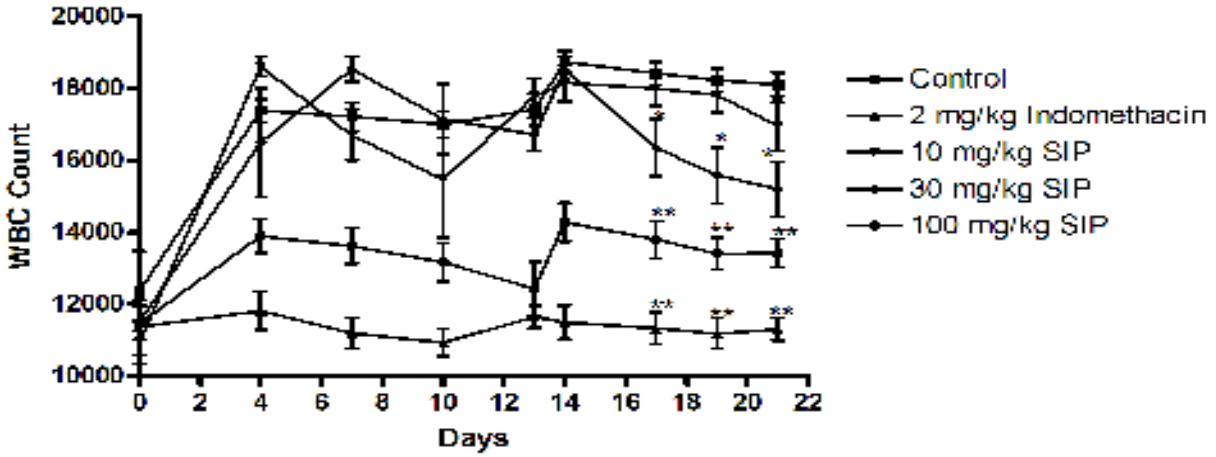


Figure 4. Effect of petroleum ether extract of *Sphaeranthus indicus Linn.* on rat leukocyte count. Results are expressed as mean±SEM, n = 6 in each group. *P<.05, **P<.01 when compared to vehicle. (One-way ANOVA followed by Dunnett's test.).

Body Weight:

The animals in the control group suffered continuous loss in body weight throughout the period of 21 days whereas the animals in the indomethacin and SIP treated group did not lose weight significantly when compared to control (figure 5).

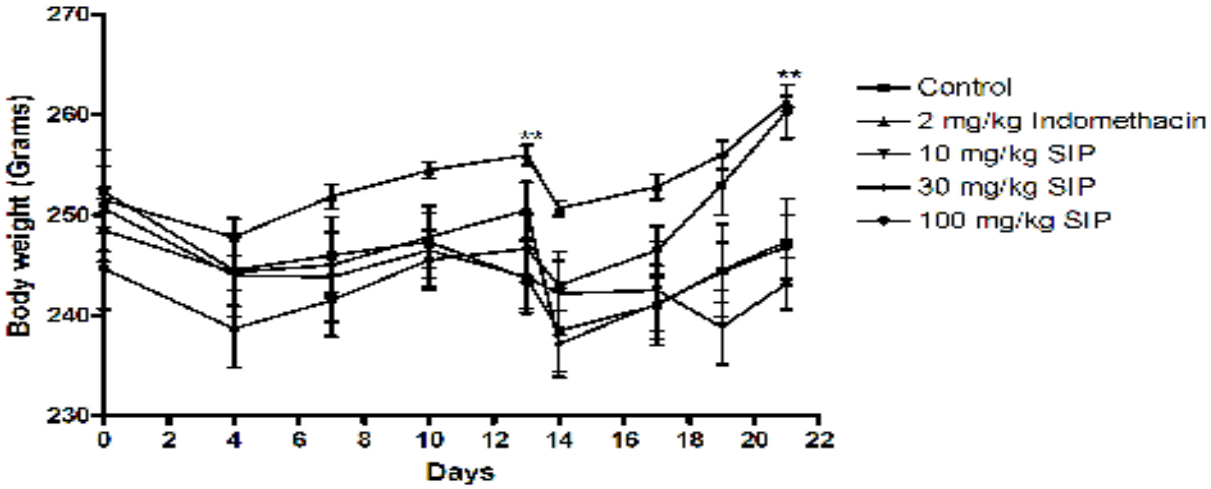
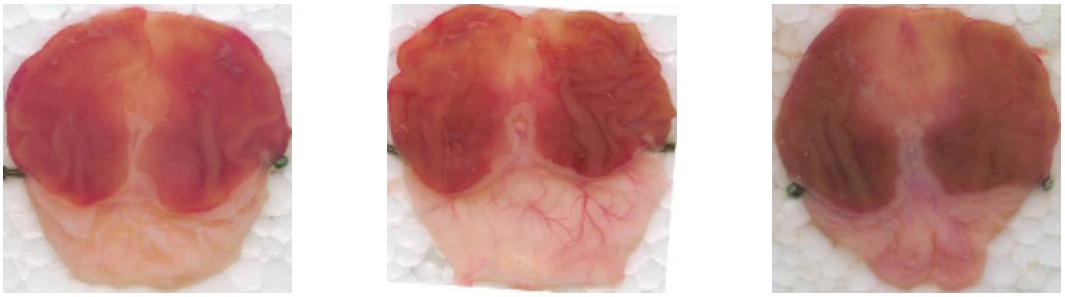


Figure 5. Effect of petroleum ether extract of *Sphaeranthus indicus Linn.* on rat body weight. Results are expressed as Mean±SEM, n = 6 in each group. *P<.05, **P<.01 when compared to vehicle. (One-way ANOVA followed by Dunnett's test.).

Gastrointestinal side effects:

The animals sacrificed after the complete therapeutic schedule of 21 days did not show the presence of ulcers or hemorrhages (figure 6) in both the indomethacin as well as all the test groups.



Control Group (1% tween 80/day) | Indomethacine (2mg/kg in 1% tween 80/day) | SIP 100 (100mg/kg in 1% tween 80/day)

Figure 6. Gastrointestinal side effect of Control (vehicle0, Indomethacin and SIP (petroleum ether extracts of *Sphaeranthus indicus Linn)* treated group on laboratory rats.

Discussion

In the present investigation, after FCA administration, the rats showed soft tissue swelling in the tibio tarsal joint, along with 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).

SIP (100mg/kg) inhibited the elevation in paw volume and joint diameter in the chronic inflammatory phase. This activity could be attributed to the presence of various sesquiterpenes and steroids like stigmasterol and β sitosterol in this extract which possess potent immunomodulatory as well as anti inflammatory activity (16-18). 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 SIP.

The immunomodulatory activity of the steroids could explain the inhibition of the serum total leucocytes count in chronic phase of inflammation. Previous investigations have elucidated the modulation of both humoral and cellular immunity in vitro (17, 18). It has also been found that the bioactive fraction of SIP protected the animals against cyclophosphamide induced suppression of humoral immunity by modulation of delayed type hypersensitivity (17, 18).

The animals in the control group continued to lose weight during the entire treatment period whereas the animals in the indomethacin and SIP treated group lost weight nonsignificantly. The loss in weight in animals is closely associated with the arthritic status of the animals(**21**).Hence, this observation might well be explained by the immunomodulatory and anti inflammatory activity of SIP.However, the anti inflammatory and immunomodulatory activity cannot be attributed to a single phytoconstituent, but due to a synergistic action of all the steroidal saponins present in SIP. Our study is in accordance with the previous workers and corroborates the imunomodulatory potential of SIP in vivo for the first time.

In this study, it is worth noticeable that even after chronic administration of SIP for 21 days at all the three doses there was no gastro intestinal damage to the animals. This gives SIP an edge over the clinically prescribed NSAIDs, which cause severe ulcers on chronic administration at higher doses.

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

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