MULTIDISCIPLINARY SCIENCE JOURNAL

RESEARCH ARTICLE
Published Online: June 7, 2023
https://doi.org/10.31893/multiscience.2023046

Anti-arthritic potential of ethyl acetate fraction of *Pinus roxburghii* Sargent stem bark in Freund's complete adjuvant induced arthritis in Wistar rats

Pratiksha Hajare[a] | Vaishali Rai[a] | Sonali Nipate[a] | Aishwarya Balap[b] | Bhushan Pimple[c] | Deshraj Chumbhale[d] | Asmita Gaikwad[e] | Harshal Tare[f]

[a]Department of Pharmacology, Progressive Education Society's Modern College of Pharmacy, Nigdi, Affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India.
[b]Department of Pharmaceutical Chemistry, Progressive Education Society's Modern College of Pharmacy, Nigdi, Affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India.
[c]Department of Pharmacognosy, Progressive Education Society's Modern College of Pharmacy, Nigdi, Affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India.
[d]Department of Pharmacognosy, Amrutvahini College of Pharmacy, Sangamner, Affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India.
[e]Department of Pharmaceutical Chemistry, Sharadchandra Pawar College of Pharmacy, Otur, Affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India.
[f]Department of Pharmacognosy, Sharadchandra Pawar College of Pharmacy, Otur, Tal. Junnar, Affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India.

Abstract The potential anti-arthritic effects of *Pinus roxburghii* Sargent (Pinaceae) plant have been utilized in traditional and folk medicine. However, data on its efficacy in this regard is lacking. Anti-arthritic properties have been found in the ethyl acetate stem bark of *Pinus roxburghii* Sargent in Wistar rats induced with arthritis produced by Freund's Complete adjuvant. The anti-arthritic activity was evaluated by assessing pain perception parameters, haematological parameters, rheumatoid factor, CRP, and serum parameters. As a positive control, diclofenac was administered. The ethyl acetate fraction of *Pinus roxburghii* Sargent was found to significantly inhibit pain perception parameters at doses of 250 and 500 mg/kg. Compared to the disease control group, serum levels of RF, NO, and CRP were significantly lower in the *Pinus roxburghii* Sargent group. In disease-free rats, *Pinus roxburghii* Sargent significantly increased RBC and decreased WBC, which indicated a suppressive effect on inflammatory mediators. Histopathology showed a reduction in the number of inflammatory cells. The radiological assessment showed significant reductions in several imaging parameters, including spur formation, joint spacing, and bone erosion. The authors of this study noted that *Pinus roxburghii* Sargent had a direct effect on slowing the progression of arthritis, reducing inflammation and synovitis, and protecting arthritic joints from cartilage and bone deterioration. The stem bark extract of *Pinus roxburghii* Sargent has been shown to have anti-arthritic action based on clinical, biochemical, and histological analysis. However, more research is required to determine the precise mechanism of action.

Keywords: arthritis, *Pinus* species, Wistar rats

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease that causes chronic joint discomfort and inflammation, which can result in cardiopathy, nephropathy, vasculopathy, pulmonary, and cutaneous issues (Lindler et al 2020). Arthritis is a condition characterized by joint pain and stiffness, with inflammatory arthritis referring to joint pain, swelling, redness, and heat. The cause of RA remains uncertain, although it may start with autoimmunity.

Nearly 1% of the world's population is affected by RA, which manifests as symmetric polyarticular synovitis, most commonly affecting the small joints of the hands, wrists, and feet. Current RA treatment plans typically focus on anti-inflammatory medication to inhibit the inflammatory response, aiding in the recovery process. Research into the disease's pathologic underpinnings and medications targeting these mechanisms has transformed RA treatment in the past two decades. While many of these novel drugs improve patient outcomes, they also come with potential drawbacks that can make long-term treatment difficult and even delay surgery.

Traditional plant remedies remain important in the contemporary pharmaceutical business due to their low risk of side effects and the synergistic effects of their ingredients. *Pinus roxburghii* Sargent, a tree found in Bhutan, Tibet, and some areas of North India, serves as both a beautiful specimen and a source of several medical benefits. The plant is a member of the Pinaceae family, also known as chir Pine, and can treat a variety of medical conditions, including hemostasis, stimulation, analgesia, inflammation, antioxidation, anthelmintics, digestion, liver tonic, diuresis, bronchitis, inflammation of the skin, and more (Bissa et al 2008). *Pinus roxburghii Sargent* contains numerous substances, such as terpenoids, flavonoids, tannins, and xanthones (Eckert and Hall 2006; Rawat et al 2006).

This study aimed to test the anti-arthritic effects of the ethyl acetate fraction of a *Pinus roxburghii* Sargent stem bark extract in experimental animals using advanced determination methods (Labib et al 2017; Shuaib et al 2013; Naeem et al 2010).

 Received: March 24, 2023 | Accepted: May 3, 2023

The authors found that the ethyl acetate fraction significantly inhibited pain perception parameters and reduced the number of inflammatory cells. Additionally, serum levels of rheumatoid factor, nitric oxide, and C-reactive protein were significantly lower in the *Pinus roxburghii* Sargent group compared to the disease control group. The stem bark extract was also found to slow the progression of arthritis, reducing inflammation and synovitis, and protecting arthritic joints from cartilage and bone deterioration, based on clinical, biochemical, and histological analysis. Although further research is needed to determine the precise mechanism of action, these findings suggest that *Pinus roxburghii* Sargent could be a promising anti-arthritic agent.

2. Material and Methods

2.1. Extract Preparation

The dried and powdered stem bark of *Pinus roxburghii* Sargent, weighing 650 g, was defatted in distilled water for 72 hours prior to extraction with 95% ethanol for 48 hours. The resulting concentrate was dried in a low pressure hoover and decanted into an ambient-temperature desiccator. To prepare the fraction, 350 ml of ethyl acetate was added to 350 ml of distilled water in a separating funnel, resulting in a final extract concentration of 35% w/w. As reported in the literature, increasing the solvent concentration from 30% to 70% significantly increased the extract's total phenolic content and antioxidant activity (Tsvetkov et al 2019). The lower layer was then collected and subjected to evaporation in a rota-vapor at 40-50°C and reduced pressure to complete the layer separation process. The resulting ethyl acetate fraction was extracted and stored at 16% w/w (Satyal et al 2013).

2.2. Drug and chemicals

Freund's complete adjuvant was supplied by Sigma Aldrich Pvt. Ltd. The standard drug given was marketed preparations of Diclofenac sodium. All chemicals and reagents used were of analytical quality and were purchased from SRL, E.Merck, and other reputable sources in India. The biochemical and haematological parameters kits were purchased from pathozyme laboratories ltd in Kolhapur.

2.3. Instruments

The paw volume up to the thio-tarsal articulation was measured using a plethysmometer from VJ Instruments in Nagpur. Radiographs were taken using an X-ray apparatus (Siemens-60MA., Germany) and industrial X-ray film from Fuji photo film in Japan. The X-ray apparatus was operated at 220 V with a 40 V peak, 0.2 second exposure time, and a 60 cm tube to film distance for anterior-posterior projection.

2.3.1. Animals

Healthy Wistar rats (150-170 g) of both sexes were purchased from Crystal Biological Solutions in Pune, Maharashtra, India and were acclimatized to their new environment in our animal house prior to the study. During the study, the animals were kept in a room with a temperature of 22 ± 1°C and a light/dark cycle of 12 hours. They had free access to commercial food and water at all times. The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) in India ensured that the animals were cared for, used, and experimented on in accordance with their rules and procedures. The Institutional Animal Ethics Committee (IAEC) approved the experimental plan with the reference number MCP/IAEC/11/2018.

2.3.2. Acute toxicity study

Toxicity studies were conducted as per internationally accepted protocol drawn under OECD Guidelines 425 in Albino Wistar rats.

2.4. Anti-rheumatoid activity evaluation

2.4.1. FCA-induced arthritis

Experimental rats had arthritis induced by injecting a 0.1 ml (0.1% w/v) slurry of deceased Mycobacterium tuberculosis bacteria homogenised in liquid paraffin (Fruend's complete adjuvant) into their right hind paws. On day 14, following the induction of chronic disease, drug treatment began and lasted for 28 days (Patel et al 2021, Nipate et al 2015).

2.4.2. Evaluation of hematological, biochemical, and other parameters

Paw edema and arthritic scores were recorded on days 7, 14, 21, and 28 of the trial to determine the extent of inflammation. Pain perception metrics, including the dorsal flexion pain test, the stair ascending test, and the motility test, were conducted on the same days as the trial. The participants' weight was measured on an electronic balance on days 7, 14, 21, and 28. On day 28, blood was collected from all groups via retro-orbital vein puncture while the animals were under ketamine anesthesia, and biochemical parameters, including hemoglobin content, total white blood cell count, total red blood

cell count, and ESR, were evaluated. Serum C-reactive protein (CRP) was measured using latex-enhanced nephelometry, and Rheumatoid factor was measured using quantitative latex-enhanced immune turbidometric pathology. Lysosomal enzymes in the serum, including glutamate pyruvate transaminase (SGPT), glutamate oxaloacetate transaminase (SGOT), and alkaline phosphatase (ALP), were measured using standard analysis kits. Antioxidant enzyme levels, such as superoxide dismutase (SOD) and catalase (CAT), were measured. Histological analysis and radiological evaluation were performed on several animals that were sacrificed.

2.4.3. Statistical data analysis

Using Graph Pad Prism 8.0, we performed a one-way analysis of variance (ANOVA) on the data and then a Dunnett's test to determine statistical significance.

3. Results

3.1. Acute toxicity test of plant extract

Ethyl acetate extract of plant *Pinus roxburghi* Sargent was found safe at 5,000 mg/kg according to OECD guidelines 425. To ensure that the doses are both safe and effective, the doses chosen for further investigations should be below the MTD and above the NOAEL. As part of the conventional fixed-dosage technique, a fixed dose wis selected as a fraction of the MTD, such as 1/10 and 1/20 of the MTD.

3.2. Effect of ethyl acetate fraction of Pinus roxburghii Sargent stems bark on dorsal flexion pain

In the present study, it was observed that Test 1 (250 mg/kg) and Test 2 (500 mg/kg) oral treatment with ethyl acetate fraction of *Pinus roxburghii* Sargent stem bark showed significant ($p<0.001$) reduction in dorsal flexion score on 28th by (0.66±0.33) at the dose of 250 mg/kg, (0.33±0.21) at the dose 500 mg/kg in comparison with disease control group (Table1).

Table 1 Effect of ethyl acetate fraction of *Pinus roxburgii* Sargent stems bark on pain perception parameters.

Groups	Dorsal Flexion pain				Stair Climbing Activity Score				Motility test			
Days	7th Day	14th Day	21th Day	28th Day	7th Day	14th Day	21th Day	28th Day	7th Day	14th Day	21th Day	28th Day
NC	0	0	0	0	3.00 ±00	3.00 ±00	2.80 ±0.6	3.00 ±00	0	0	0	0
DC	1.66 ±0.16	1.83 ±0.16	2.00 ±0.00	2.00 ±0.00	2.00 ±00	1.50 ±0.3	1.16 ±0.16	1.00 ±0.21	1.44 ±0.16	1.90 ±0.16	2.00 ±0.00	2.00 ±0.00
STD	1.66 ±0.16	1.66 ±0.21	1.33 ±0.21	0.66 ±0.33**	1.80 ±0.4	1.40 ±0.16	2.00 ±0.16	2.80 ±00***	1.23 ±0.26	1.60 ±0.21	1.33 ±0.21	0.50 ±0.22***
Test 1	1.66 ±0.16	1.66 ±0.21	1.50 ±0.22	0.66 ±0.33**	1.30 ±0.6	1.20 ±0.5	1.50 ±0.34	2.30 ±00***	1.60 ±0.21	1.90 ±0.21	1.50 ±0.22	0.66 ±0.21**
Test 2	1.66 ±0.16	1.66 ±0.21	1.33 ±0.21	0.33 ±0.21***	1.0 ±0.54	0.83 ±0.6	1.8 ±0.16	2.5 ±00***	1.62 ±0.16	1.66 ±0.21	1.00 ±0.00	0.33 ±0.21***

Values are expressed in mean ± SEM (n=6); *p<0.05, **p<0.01, ***p<0.001 vs. Control group. The data were analysed by a One-way ANOVA test followed by Dunnett's multiple test for comparison. Treatment of various groups are as follows, DC: Disease Control, STD: Diclofanec sodium. Test 1: (250 mg/kg) ethyl acetate fraction of Stem bark of *Pinus roxburghii* Sargent. Test 2: (500 mg/kg) ethyl acetate fraction of *Pinus roxburghii* Sargent.

3.3. Effect of ethyl acetate fraction of Pinus roxburghii Sargent stems bark on stair climbing activity score

In the present study, it was observed that Test 1 (250 mg/kg) and Test 2 (500 mg/kg) oral treatment with ethyl acetate fraction of *Pinus roxburghii* Sargent stem bark showed significant ($p<0.001$) increased the stair climbing score on 28th by (2.3±00) at the dose of 250 mg/kg, (2.5±00) at the dose 500 mg/kg in comparison with the disease control group (Table 1).

3.4. Effect of ethyl acetate fraction of Pinus roxburghii Sargent stems bark on motility test

In the present study, it was observed that Test 1 (250 mg/kg) and Test 2 (500 mg/kg) oral treatment with ethyl acetate fraction of *Pinus roxburghii* Sargent stem bark showed significant ($p<0.001$) reduction in the motility test on 28th by (0.66±0.21) at the dose of 250 mg/kg, (0.33±0.21) at the dose 500 mg/kg in comparison with disease control group (Table 1).

3.5 Effect of ethyl acetate fraction of Pinus roxburghii Sargent stems bark on body weight

In the present study, it was observed that Test 1 (250 mg/kg) and Test 2 (500 mg/kg) oral treatment with ethyl acetate fraction of *Pinus roxburghii* Sargent stem bark showed significant ($p<0.001$) increased in the body weight on 28th by (169.3±3.40) at the dose of 250 mg/kg, (180.8±1.85) at the dose 500 mg/kg in comparison with disease control group (Table 2).

Table 2 Effects of ethyl acetate fraction of *Pinus roxburgii* Sargent stem bark on body weight.

Groups	Body Weight (G)			
Days	7th	14th	21th	28th
NC	141.8±7.9	151±8.2	165.7±8.9	160±9.04
DC	166.5±9.3	161.3±8.2	158.8±11.19	155 ±3.97
STD	168.2±8.0	172.2±7.0	179.3±4.16*	185.7±3.16***
Test 1	159.8±8.3	156.3±8.9	168.8±7.80	169.3±3.40**
Test 2	161±3.96	158 ±4.6	170.8±2.34	180.8±1.85***

Values are expressed in mean ± SEM (n=6); *p<0.05, **p<0.01, ***p<0.001 vs. Control group. The data were analysed by a One-way ANOVA test followed by Dunnett's multiple test for comparison. Treatment of various groups are as follows, DC: Disease Control, STD: Diclofanec sodium, Test 1: (250mg/kg) ethyl acetate fraction of Stem barks ofPinus roxburghiiSarent Test 2: (500mg/kg) ethyl acetate fraction of *Pinus roxburghii* Sargent.

3.6. Effect of ethyl acetate fraction of Pinus roxburghii Sargent stems bark on Rheumatoid factor and C reactive protein

In the present study, it was observed that Test 1 (250 mg/kg) and Test 2 (500 mg/kg) oral treatment with ethyl acetate fraction of *Pinus roxburghii* Sargent stem bark showed significant (p<0.001) decreased the raised CRP and RF to (1.60±0.3, 4.0±0.46) respectively at the dose of 250 mg/kg (1.68±0.27, 2.84±0.46) at the dose 500mg/kg in comparison with the disease control group (Table 3).

Table 3 Effect of ethyl acetate fraction of *Pinus roxburgii Sargent* stems bark on Rheumatoid factor and C reactive protein.

Exp. G	RF(IU/ml)	CRP (mg/L)
NC	0.63±0.11	0.70±0.15
DC	5.78±0.41	3.25±0.48
STD	2.14±0.29***	1.35±0.8**
Test 1	4.0±0.46*	1.60±0.3**
Test 2	2.84±0.46***	1.68±0.27*

Values are expressed in mean ± SEM (n=6); *p<0.05, **p<0.01, ***p<0.001 vs. Control group. The data were analysed by a One-way ANOVA test followed by Dunnett's multiple test for comparison.

3.7. Effect of ethyl acetate fraction of Pinus roxburghii Sargent stems bark on hematological parameters

In the present study, it was observed that Test 1 (250 mg/kg) and Test 2 (500mg/kg) oral treatment with ethyl acetate fraction of *Pinus roxburghii* Sargent stem bark showed significant (p<0.001) decreased the raised WBC count and ESR to normal level (14.2±0.57, 3.6±0.3) respectively at the dose of 250 mg/kg (13.3±0.54, 3.06±0.4), at the dose 500mg/kg along with significantly increased in RBC, Hb count by (8.31±0.17,13.48±0.18), respectively at the dose of 250 mg/kg (8.54±0.25, 13.60±0.16) at the dose 500mg/kg. There was a slight change in HCT count (40.13±0.26) at the dose of 250 mg/kg (40.93±0.38) at the dose of 500 mg/kg in comparison with the disease control group (Table 4).

Table 4 Effects of ethyl acetate fraction of *Pinus roxburgii Sargent* stem bark on hematological parameters.

Exp.Grp	Hb (g/dl)	RBC (x10^6 uL)	WBC (x10^{3}uL)	ESR (mm)	HCT (%)
NC	12.4 ± 0.19	8.97 ± 0.29	8.91 ± 0.48	2.05 ± 0.34	42.38 ± 0.64
DC	11.37 ± 0.25	6.91 ± 0.19	17.3 ± 1.38	4.0 ± 0.5	38.77 ± 0.62
STD	12.60 ± 0.12	8.32 ± 0.19	11.86 ± 0.45	2.8 ± 0.3	39.82 ± 0.38
Test 1	13.48 ± 0.18	8.31 ± 0.17	14.2 ± 0.57	3.6 ± 0.3	40.13 ± 0.26
Test 2	13.60 ± 0.16	8.54 ± 0.25	13.3 ± 0.54	3.06 ± 0.4	40.93 ± 0.38

Values are expressed in mean ± SEM (n=6); *p<0.05, **p<0.01, ***p<0.001 vs. Control group. The data were analysed by a One-way ANOVA test followed by Dunnett's multiple test for comparison.

3.8. Effect of ethyl acetate fraction of Pinus roxburghii Sargent stems bark on serum transaminase

In the present study, it was observed that Test 1 (250 mg/kg) and Test 2 (500 mg/kg) oral treatment with ethyl acetate fraction of *Pinus roxburghii* Sargent stem bark showed significant (p<0.001) decreased the raised level of serum transaminase like SGOT, SGPT, and ALP on 28th by(149.2±9.26, 130.8±7.30 and126.3±4.55) at the dose of 250mg/kg (144.6±5.87, 124.3±7.04 and 115.2±5.40) at the dose 500mg/kg in comparison with the disease control group (Figure 1).

3.9. Effects of ethyl acetate fraction of Pinus roxburghii Sargent stem bark on antioxidant enzymes level

In the present study, it was observed that Test 1 (250 mg/kg) and Test 2 (500 mg/kg) oral treatment with ethyl acetate fraction of *Pinus roxburghii* Sargent stem bark showed significant (p<0.001) increased the level of SOD and CAT on 28th by (7.40±0.46, 53.11±2.54) at the dose of 250 mg/kg (9.35±0.29, 56.10±2.43) at the dose of 500 mg/kg respectively along with

decreased the raised level of MDA (206.7±6.30) at the dose of 250 mg/kg (201.2±8.43) at the dose of 500 mg/kg in comparison with the disease control group (Figure 2).

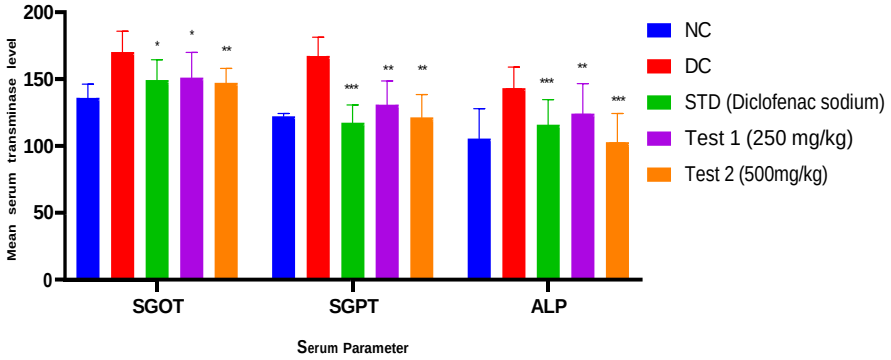

Figure 1 Effect of ethyl acetate fraction of *Pinus roxburgii Sargent* stems bark on serum transaminase. Values are expressed in mean ± SEM (n=6); *p<0.05, **p<0.01, ***p<0.001 vs. Control group. The data were analysed by a One-way ANOVA test followed by Dunnett's multiple test for comparison.

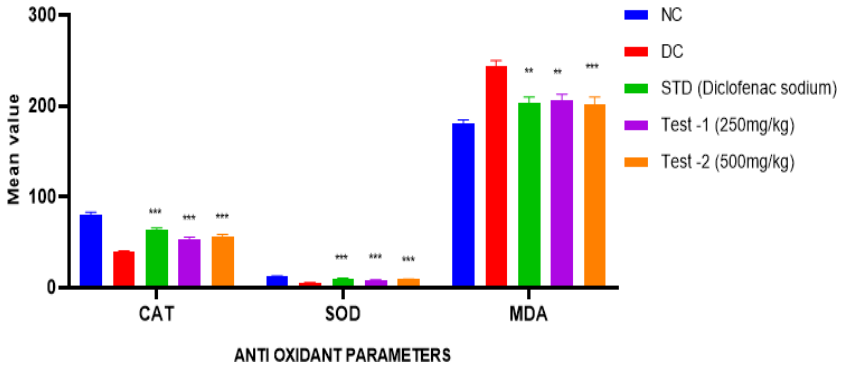

Figure 2 Effect of ethyl acetate fraction of *Pinus roxburgii Sargent* stems bark on antioxidant enzymes level. Values are expressed in mean ± SEM (n=6); *p<0.05, **p<0.01, ***p<0.001 vs. Control group. The data were analysed by a One-way ANOVA test followed by Dunnett's multiple test for comparison.

3.10. Effects of ethyl acetate fraction of Pinus roxburghii Sargent stem bark on histopathology of the ankle joint

Disease control arthritic rat joints revealed edoema, degeneration, partial cartilage erosion, and bone marrow loss.

- Standard drug-treated rat joints had normal bone marrow with fewer cellular infiltrates.
- Test 1 (250 mg/kg) exhibited cellular infiltrates on the articular surface and reduced cartilage degradation.
- Test 2 (500 mg/kg) demonstrated minimal inflammation, including sparse cellular infiltration, no edoema, and normal bone marrow (Figures 3.1 to 3.5).

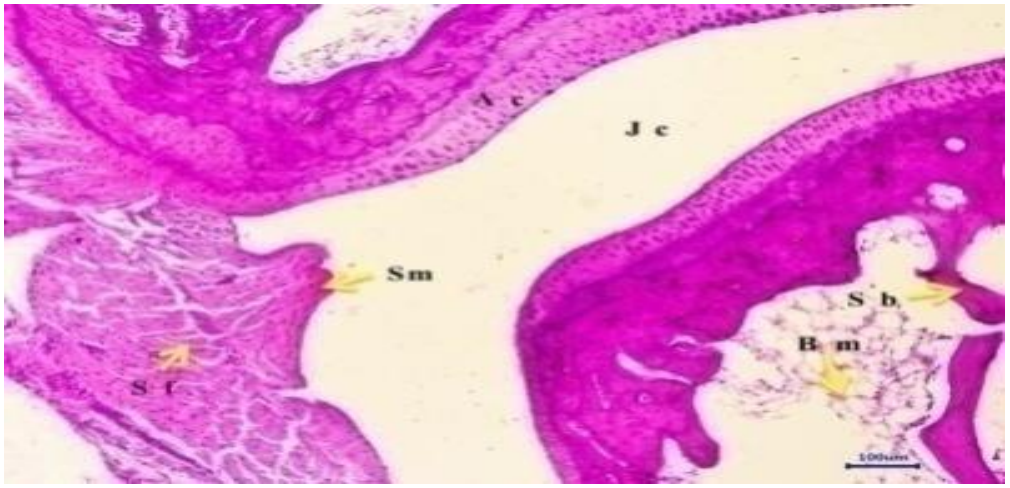

Figure 3.1 Histopathology of the ankle joint of the normal control group.

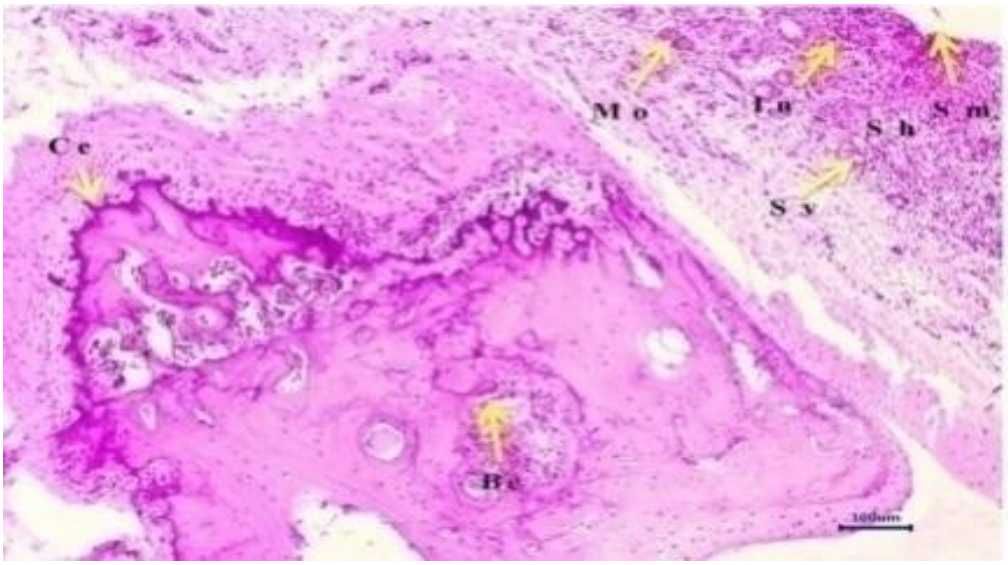

Figure 3.2 Histopathology of the ankle joint of rat from the disease control group.

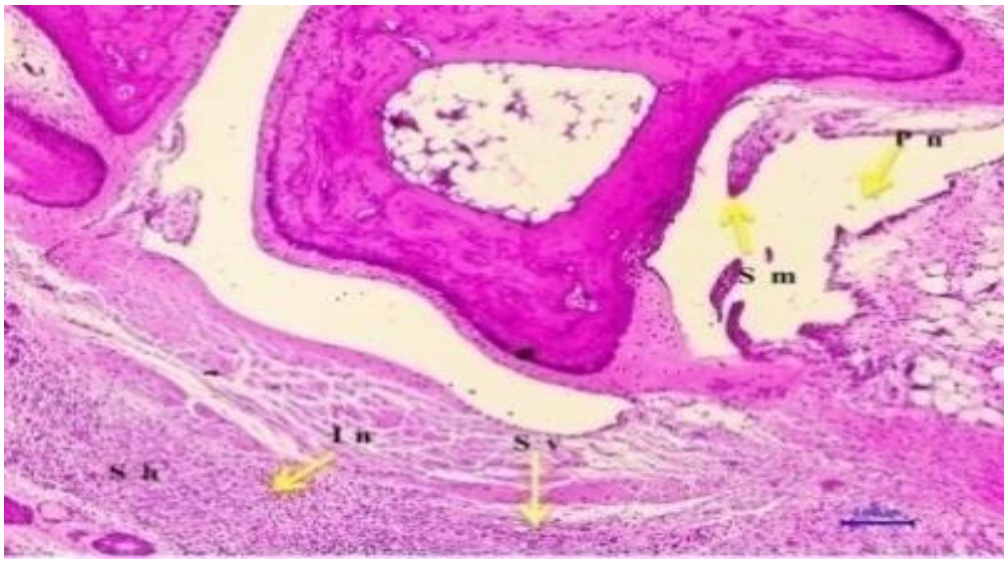

Figure 3.3 Histopathology of the ankle joint of rat treated with standard.

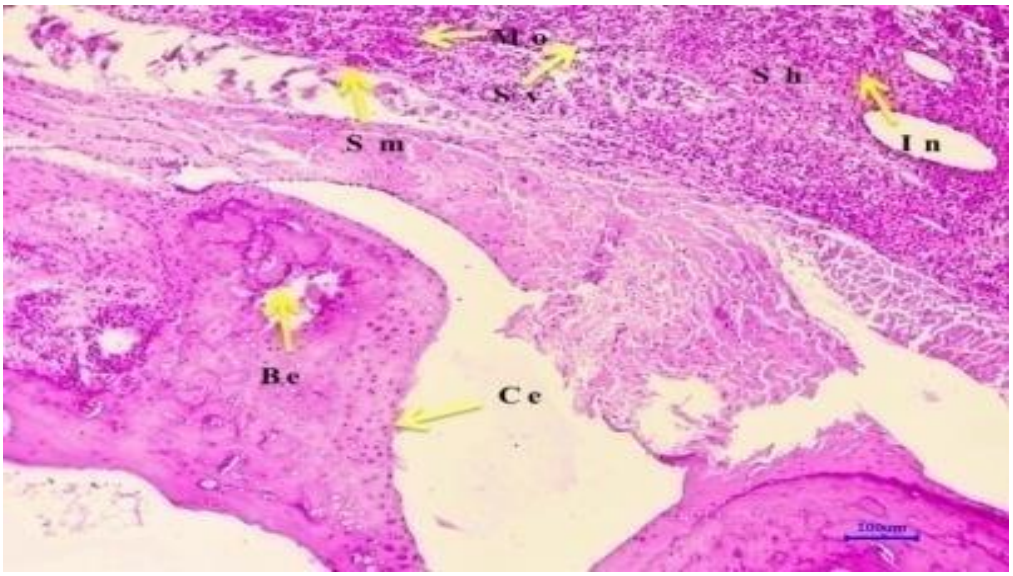

Figure 3.4 Histopathology of the ankle joint of rat treated with *Pinus roxburgi extract* (250 mg/kg).

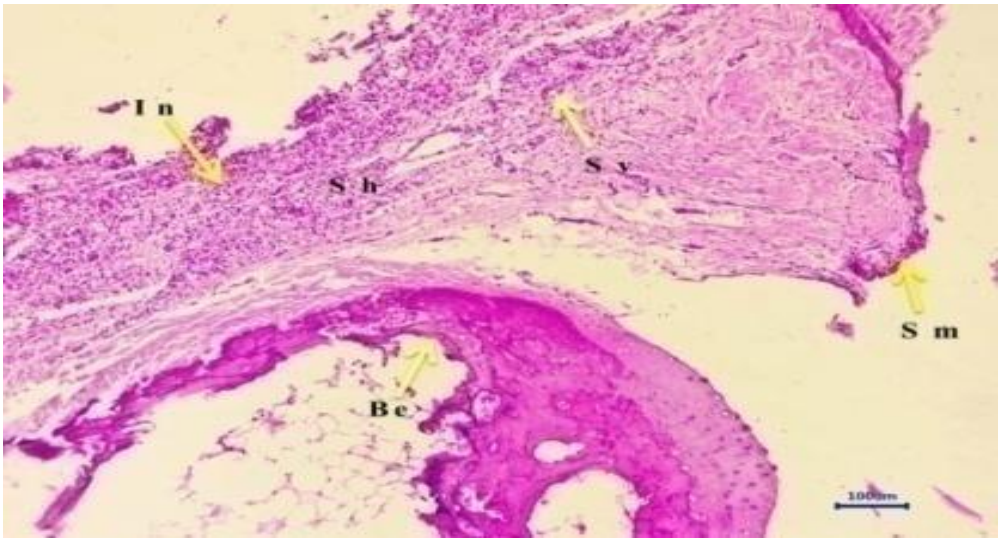

Figure 3.5 Histopathology of the ankle joint of rat treated with *Pinus roxburgi extract* (500 mg/kg).

Imaging metrics such spur formation, interspacing between the joints, bone degradation, and connective tissue edoema around the joints were significantly reduced in the Test-1 (250 mg/kg), Test-2 (500 mg/kg), and standard (Diclofenac sodium) groups compared to the disease control group (Figure 4).

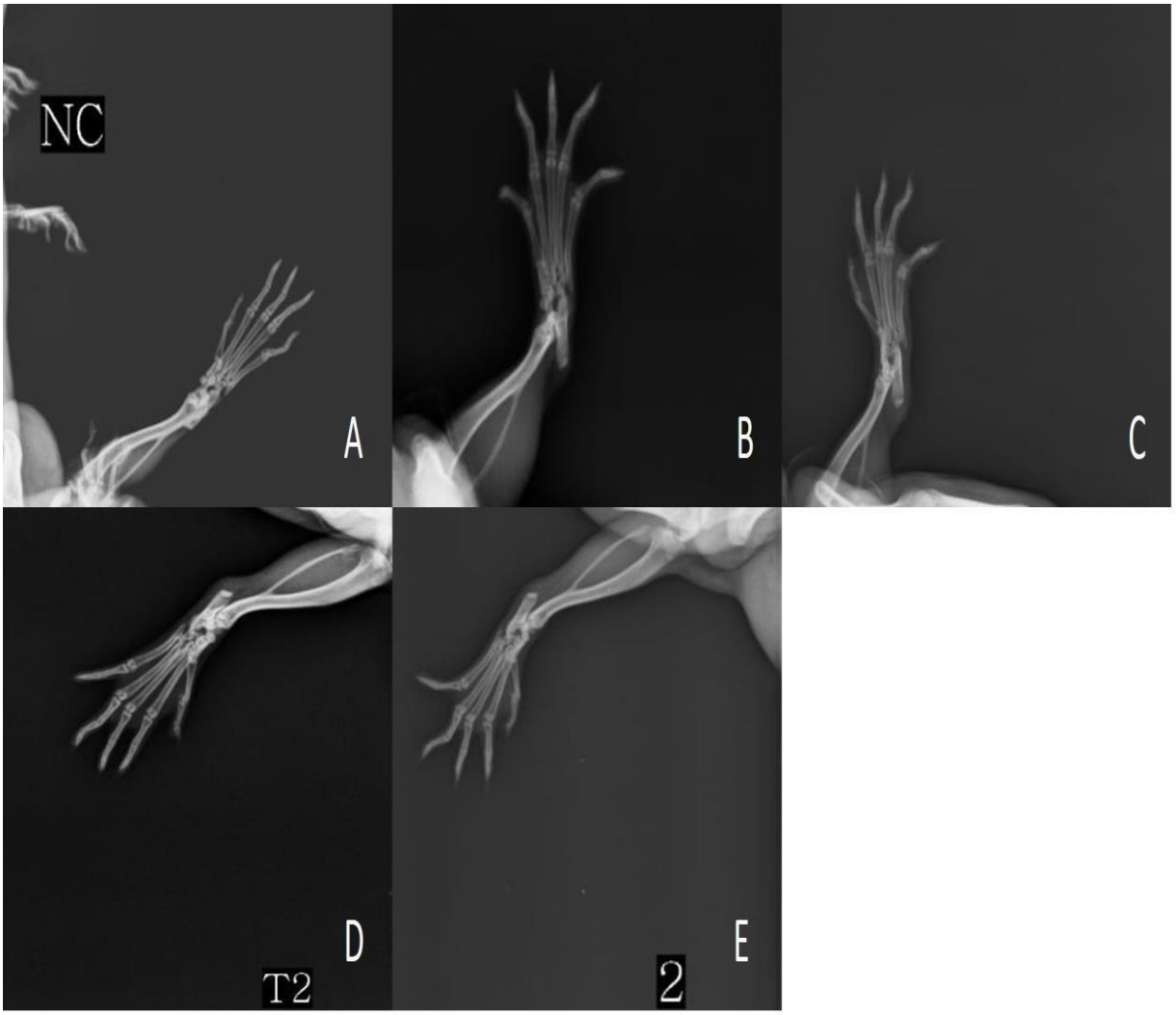

Figure 4 Effect of ethyl acetate fraction of *Pinus roxburgii Sargent* stems bark on radiology of ankle joint - A: Normal control; B: Disease control; C: Standard (diclofenac sodium); D: *Pinus roxburghiii* (250 mg/kg); E: *Pinus roxburghiii* (500 mg/kg).

Image Title	Description
NC	**Paw: Normal control:** Showing normal histology, synovial membrane (Sm), synovial folds (Sf), articular cartilage (Ac) joint cavity (Jc), spongy bone (Sb), bone marrow (Bm) {H& E, 100X}.
DC	**Paw: Disease control:** Showing enlarged synovial membrane (Sm), hyperplastic synovium (Sh), increased synovial vascularity (Sv), inflammation (In), Cartilage erosion (Ce), bone erosion (Be), increased macrophage/osteoclast (Mo) {H& E, 100X}.
STD	**Paw: Standard:** Showing enlarged synovial membrane (Sm), hyperplastic synovium (Sh), increased synovial vascularity (Sv), inflammation (In), Pannus formation (Pn), Cartilage erosion (Ce), bone erosion (Be), increased macrophage/osteoclast (Mo) {H& E, 100X}.
T1	**Paw: Test 1:** Showing enlarged synovial membrane (Sm), hyperplastic synovium (Sh), increased synovial vascularity (Sv), inflammation (In), Cartilage erosion (Ce), bone erosion (Be), increased macrophage/osteoclast (Mo) {H& E, 100X}.
T2	**Paw: Test 2:** Showing enlarged synovial membrane (Sm), hyperplastic synovium (Sh), increased synovial vascularity (Sv), inflammation (In), bone erosion (Be) {H& E, 100X}.

4. Discussion

It is widely recognized that lysosomal enzymes, including SGOT, SGPT, and ALP, play critical roles in the onset and maintenance of acute and chronic inflammation. Most anti-inflammatory medications work by either preventing the release of inflammatory enzymes from lysosomes or by stabilizing the membranes surrounding lysosomes. It was demonstrated in this study that pain perception parameters such as dorsal flexion, and motility, were significantly inhibited and the stair climbing score was significantly increased in rats treated with the ethyl acetate fraction of *Pinus roxburgh*ii Sargent at doses of 250 and 500 mg/kg, compared with the disease control group. SGOT, SGPT, and ALP serum levels were measured in both normal and experimental rats. The ethyl acetate fraction of *Pinus roxburghii* Sargent at doses of 250 and 500 mg/kg significantly lowered serum levels of RF, NO, and CRP compared to the disease control group. Additionally, this study's findings suggest a link between inflammation levels and an individual's ability to lose weight.

Hemoglobin and red blood cells (RBCs) were found to be diminished in disease-free rats' RBC counts, whereas white blood cell (WBC) counts, HCT counts, and erythrocyte sedimentation rates (ESR) varied slightly (ESR). These symptoms point to anemia in arthritic rats. Anemia can result from bone marrow failure and improper iron storage in the reticuloendothelial system and synovial tissue. The immune-modulating activity of *Pinus roxburghii* Sargent was demonstrated by a significant increase in leukocyte count in the treated groups and a corresponding decrease in the ethyl acetate fraction in the untreated groups. The ethyl acetate fraction of *Pinus roxburghii* Sargent stems bark and the standard gold medicine diclofenac sodium significantly reduced the elevated ESR count seen in the disease control group, demonstrating the latter's important function in arthritic diseases (Kaushik et al 2013, Ghangale et al 2021). Thus, in disease-free mice, treatment with the ethyl acetate fraction of *Pinus roxburghii* Sargent dramatically increased RBC and decreased WBC, indicating a suppressive action on the mediators of inflammation.

SOD and CAT were found to be decreased in rats with RA due to increased oxidative stress and cellular lysis, consistent with the association between acute and chronic inflammation and oxidative stress and reactive oxygen species. Treatment with *Pinus roxburghii* Sargent increased serum total antioxidant capacity and reduced lipid peroxidation and malondialdehyde (MDA) levels (Baqer et al 2021). The ethyl acetate fraction of *Pinus roxburghii* Sargent stems bark at doses of 250 and 500 mg/kg prevented ankle joint injury in the histopathological and radiographic investigation. In the disease control group, ankle joints showed dense cellular infiltration, synovial hyperplasia, and pannus formation. In contrast, the ethyl acetate fraction of stem bark from *Pinus roxburghii* Sargent at doses of 250 and 500 mg/kg reduced cellular infiltration, synovial hyperplasia, and pannus development in the ankle joint of arthritic rats, suggesting that these doses may slow disease progression (Kadhim et al 2020).

A radiographic alteration can indicate the severity of RA. Early radiographic symptoms of arthritis include swelling of soft tissues, but significant radiographic changes such as bone erosion and narrowing of joint space appear later in the course of the disease. The ethyl acetate fraction of *Pinus roxburgh.*

5. Conclusion

The direct effect of reducing the development of arthritis, inflammation, and synovitis, as well as protecting against cartilage and bone deterioration in arthritic joints, has been demonstrated by the study's findings concerning *Pinus roxburghii* Sargent. Anti-arthritic activity in FCA has been evidenced by clinical, biochemical, and histological data in relation to the stem bark extract of *Pinus roxburghii* Sargent. Further research will be conducted to determine the exact mechanism of action.

6. List of abbreviations

RBC stands for Red blood cells, RA stands for Rhematoid arthritis, FCA stands for Freund’s complete adjuvant, HB stands forHaemoglobin, WBC stands for White blood cells.

Ethical considerations

The study was permitted by Institutional Animal Ethical Committee and approved by CPCSEA with Reference Number MCP/IAEC/11/2018.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

This research did not receive any financial support.

References

Baqer MS, Mohammed MM (2022). Evaluation of the anti-inflammatory effect of curcumin as an additive therapy to meloxicam in management of knee osteoarthritis. International Journal of Drug Delivery Technology 12:310-315.

Bissa S, Bohra A, Bohra A (2008) Antibacterial potential of three naked-seeded (gymnosperm) plants.Natural Product Radiance 7:420–425.

Eckert AJ, Hall BD (2006) Phylogeny, historical biogeography, and patterns of diversification for pinus (pinaceae): phylogenetic tests of fossil-based hypotheses. Molecular phylogenetics and evolution 40:166-182.

Ghangale GD, Bhawar SB (2021) Microscopic and Macroscopic Study of Leaves of *Echinops echinatus* roxb. Journal of Plant Science Research 37: 321-324.

Kadhim HM, Hamad MN, Kadhim YM (2020) Isolation and identification of two cucurbitacins b ande, and detection of phytosterols in *Cucurbita pepo* l. var. pepo (pumpkin) leaves extract. International Journal of DrugDelivery Technology 10:369-373.

Kaushik P, Kaushik D, Khokra SL (2013) Ethnobotany and phytopharmacology of *Pinus roxburghii Sargent*: a plant review. Journal of integrative medicine 11:371-376.

Labib RM, Youssef FS, Ashour ML, Abdel-Daim MM, Ross SA (2017) Chemical composition of *Pinus roxburghii* bark volatile oil and validation of its anti-inflammatory activity using molecular modelling and bleomycin-induced inflammation in albino mice. Molecules 22:1384.

Lindler BN, Long KE, Taylor NA, Lei W (2020) Use of herbal medications for the treatment of osteoarthritis and rheumatoid arthritis. Medicines 7:67.

Mythilypriya R, Shanthi P, Sachdanandam P (2008) Therapeutic effect of Kalpaamruthaa, a herbal preparation on adjuvant induced arthritis in wistar rats. Inflammopharmacology 16:21-35.

Naeem I, Taskeen A, Mubeen H, Maimoona A (2010) Characterization of flavonols present in barks and needles of *Pinus wallichiana* and *Pinus roxburghii*. Asian Journal of Chemistry 22:41.

Nipate SS, Hurali PB, Ghaisas MM (2015) Evaluation of anti-inflammatory, anti-nociceptive, and anti-arthritic activities of Indian Apis dorsata bee venom in experimental animals: biochemical, histological, and radiological assessment. Immunopharmacology and immunotoxicology 37:171-184.

Patel R, Kadri S, Gohil P, Deshpande S, Shah G (2021) Amelioration of complete Freund's adjuvant-induced arthritis by *Calotropis procera* latex in rats. Future Journal of Pharmaceutical Sciences 7:1-11.

Rawat U, Srivastava B, Semwal S, Sati OP (2006). Xanthones from *Pinus roxburghii*. Journal of the Indian Chemical Society 83:391-392.

Satyal P, Paudel P, Raut J, Deo A, Dosoky NS, Setzer WN (2013) Volatile constituents of *Pinus roxburghii* from Nepal. Pharmacognosy Research 5:43.

Shuaib M, Ali M, Ahamad J, Naquvi KJ, Ahmad MI (2013) Pharmacognosy of *Pinus roxburghii*: a review. Journal of Pharmacognosy and Phytochemistry 2:262-268.

Srinivasan V, Palanisamy P (2021) Nanotherapy technology based treatment and drug delivery managementfor osteoarthritis: a review. International Journal of Pharmaceutical Quality Assurance 12:318-334.

Tsvetkov DE, Kumar R, Devrani R, Dmitrenok AS, Tsvetkov YE, Chizhov AO, ... Nifantiev NE (2019) Chemical constituents of the extracts of the knotwood of *Pinus roxburgh*ii Sarg. and their antioxidant activity. Russian Chemical Bulletin 68:2298-2306.