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Exploring the Antiarthritic Effects of a Polyherbal Formulation in Experimental Animal Models

Palak jain*, Dr. Jitendra Banweer 1

Department of pharmaceutical sciences, Sanjeev Agrawal Global Educational University, Bhopal, Madhya Pradesh, INDIA

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KEYWORDS

Arthritis, Fingerprint analysis,Vitex negundo, Boswellia serrata, Capsicum frutescens

ABSTRACT:

Objectives:

The aim of this study was to develop a polyherbal formulation and assess its antiarthritic properties in female Wistar rats with arthritis induced by Freund's complete adjuvant.

Materials and Methods:

Vitex negundo , Boswellia serrata , Capsicum frutescens are well-known plants commonly found across India, and they are frequently used in traditional medicine for treating various ailments, including arthritis. In this study, a polyherbal formulation was developed using ethanol extracts of the stem bark of **Vitex negundo**, the whole plant of **Boswellia serrata**, and the leaves of **Capsicum frutescens**, along with **Night Jasmine (Cestrum nocturnum)**. The polyherbal formulation was prepared with a ratio of 2:2:1 for the extracts of **Vitex negundo**, **Boswellia serrata**, and **Capsicum frutescens**, respectively. The quality of the final product was assessed according to the World Health Organization's guidelines for herbal materials quality control. Arthritis was induced in female Wistar rats using Freund's complete adjuvant (FCA), and the antiarthritic effect of the polyherbal formulation was tested at doses of 250 and 500 mg/kg. These effects were compared to those of indomethacin (10 mg/kg). At the conclusion of the experiment, blood samples were collected for biochemical and hematological analysis, and radiological evaluations were performed before the study was terminated.

Results:

The polyherbal formulation demonstrated significant antiarthritic effects at doses of 250 mg/kg and 500 mg/kg, with results comparable to those of indomethacin. Biochemical and hematological analyses further supported the antiarthritic activity of the polyherbal formulation.

Conclusion:

The polyherbal formulation exhibited significant antiarthritic effects against FCA-induced arthritis in female Wistar rats.

1. Introduction

Arthritis is a debilitating condition characterized by painful joint inflammation, commonly affecting a large segment of the population. The two most prevalent types are osteoarthritis and rheumatoid arthritis. Osteoarthritis is primarily a degenerative joint disease that typically affects older individuals, whereas rheumatoid arthritis is an autoimmune disorder of unclear origin. [1] Complementary and alternative medicine practices, such as Ayurveda (which utilizes herbs) and acupuncture, are widely used to treat various systemic diseases, including arthritis. [2] According to Chopra et al., approximately 68% of patients with chronic rheumatic conditions have turned to alternative medicine, demonstrating the clinical effectiveness of herbal treatments for osteoarthritis of the knee. [3,4]

Herbs and herbal medicines have been utilized for centuries to treat numerous diseases, including arthritis. However, the scientific validation of these uses remains incomplete. Hence, there is a need to investigate the pharmacological properties of various herbs and natural products. Plants are considered an abundant source of pharmaceutical compounds, playing a pivotal role in drug discovery. The objective of this study is to

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formulate a polyherbal formulation (PHF) and evaluate its antiarthritic activity in animal models. The PHF was developed using herbs with known antiarthritic properties, combined in a specific ratio to enhance the pharmacological activity of each plant while reducing the required dose of individual extracts. In Ayurveda, two main principles guide drug formulation: the use of single herbs or the combination of multiple herbs, as seen in polyherbal formulations (PHF). This approach, highlighted in the *Sarangdhar Samhita* from the 1300s, is designed to achieve enhanced therapeutic efficacy with minimized toxicity. [5] In traditional Indian medicine, combined extracts of multiple plants are often preferred over single-plant treatments to maximize therapeutic outcomes. [6]

The ethanolic extracts of the stem bark of **Vitex negundo**, **Boswellia serrata**, and **Capsicum frutescens** were utilized in the formulation of the polyherbal formulation (PHF). Preliminary acute toxicity studies on both the PHF and individual plant extracts showed no significant toxic effects up to a dose of 2000 mg/kg in rodents. [7] Based on the promising results from studies on the individual plant extracts, which demonstrated notable antidiabetic and antiarthritic activities, this study was designed to evaluate the antiarthritic effects of a polyherbal formulation containing the ethanolic extract of the stem bark of **Vitex negundo**, the whole plant of **Boswellia serrata**, and the leaves of **Capsicum frutescens**. These effects were tested in **Freund's complete adjuvant (FCA)**-induced arthritis in female Wistar rats.

2. Materials and methods

Animals

In this study, Wistar rats were chosen for inducing arthritis due to their tendency to develop chronic joint swelling, characterized by the infiltration of inflammatory cells and the erosion of joint cartilage, resulting in bone destruction. [8]

Adult female Wistar rats weighing 180 g ± 10 g were sourced from the veterinary college in Mhow, Indore. The animals were housed in large, spacious polyacrylic cages under controlled environmental conditions with a 12-hour light/dark cycle and free access to food and water. Standard pellet rat feed, purchased from a veterinary supplier in Indore, India, was provided throughout the experiment. The study received approval from the Institutional Animal Ethics Committee of Sanjeev Agrawal Global Education University, Bhopal. All experimental procedures were conducted in accordance with the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Plant Materials

The leaves of **Vitex negundo**, **Boswellia serrata**, and **Capsicum frutescens** used in this study were taxonomically identified and collected from a regional nursery in Indore District. The collected plant materials were authenticated at the Department of Botany, JNKVV, Jabalpur. A voucher specimen of each plant was deposited at the Department of Pharmaceutics, SAGE University, Bhopal, for future reference.

Preparation of Plant Extracts

The air-dried stem bark of **Vitex negundo**, the whole plant of **Boswellia serrata**, and the leaves of **Capsicum frutescens** were powdered and extracted using absolute ethanol (80%) in a Soxhlet apparatus. The resulting extracts were concentrated under reduced pressure at 60°C and stored at 4°C until further use. The yield of the extracts from **Vitex negundo** stem bark, **Boswellia serrata** whole plant, and **Capsicum frutescens** leaves were found to be 25.6%, 25.6%, and 5.34% (w/w, dry weight basis), respectively.

Phytochemical Evaluation

The ethanolic extracts of the stem bark of **Vitex negundo**, **Boswellia serrata**, and **Capsicum frutescens** were reconstituted with ethanol and subjected to phytochemical screening to test for the presence of various bioactive compounds, including carbohydrates, proteins, sterols, alkaloids, tannins, glycosides, flavonoids, phenolic compounds, and saponins. [9]

Preparation of Polyherbal Formulation

The polyherbal formulation (PHF) was prepared by combining the ethanolic extracts of **Vitex negundo**, **Boswellia serrata**, and **Capsicum frutescens** in a 2:2:1 ratio. The formulation was developed into capsules using the wet granulation method, with a 20% solution of lactose used as a binder. Each 750 mg herbal capsule contained the following extract quantities: **Vitex negundo** (100 mg), **Boswellia serrata** (100 mg), **Capsicum frutescens** (50 mg), and excipients (q.s.).

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Standardization of the Formulation

The physicochemical properties of the raw materials used in the polyherbal formulation were assessed according to the guidelines provided by the World Health Organization (WHO) for the quality control of herbal materials. The following parameters were determined: moisture content, total ash value, water-soluble ash, acid-insoluble ash, heavy metals, water-soluble extractive, alcohol-soluble extractive, and pH. [10,11]

High Performance Thin Layer Chromatography (HPTLC) Fingerprint Analysis

A 20 mg sample from randomly selected capsules was reconstituted with ethanol and filtered through a 0.46 µm membrane. The mobile phase used for chromatographic separation consisted of toluene:ethyl acetate:methanol (7:2:1). A 2 µl sample was spotted (8 mm band) on the plate, which was developed in a 20 cm × 10 cm twin trough glass chamber saturated with the mobile phase. After exposure for 20 minutes, the chromatogram was scanned using a densitometer at wavelengths of 254 nm, 366 nm, and 520 nm. Rf values, peak areas, and fingerprint data were recorded using WinCATS 1.4.3 software (Camag Scientific Inc, United States).

Antiarthritic Activity of Polyherbal Formulation

Male Wistar rats were divided into five groups, each consisting of six animals:

Group I: Normal control

Group II: Arthritic control (subcutaneous FCA injection, 0.1 ml)

Group III: Polyherbal formulation (250 mg/kg b.w, orally)

Group IV: Polyherbal formulation (500 mg/kg b.w, orally)

Group V: Indomethacin (10 mg/kg b.w, orally)

Acute toxicity studies revealed no significant toxic signs up to 2000 mg/kg in rodents. Therefore, the study used 250 mg/kg and 500 mg/kg doses for further investigation.

Arthritis was induced by injecting FCA (0.1 ml) into the right hind paw of the rats. The test groups received the respective doses of the polyherbal formulation or indomethacin 24 hours before FCA injection. The control group received 0.1 ml of liquid paraffin (Incomplete Freund's adjuvant). Treatment continued once daily for 20 days after FCA injection.

Paw volume was measured daily using a liquid displacement plethysmometer to assess the extent of erythema and edema, indicating inflammation severity. Body weight changes and paw edema were also recorded. At the study's conclusion, blood samples were collected for hematological and biochemical analysis. Hematological parameters such as hemoglobin (Hb), red blood cell (RBC) count, white blood cell (WBC) count, and erythrocyte sedimentation rate (ESR) were measured manually. Serum samples were analyzed for liver markers (AST, ALT, ALP, creatinine) using an auto-analyzer and enzymatic kits. C-reactive protein (CRP) levels and serum copper levels were estimated using ELISA kits and the bathocuproin disulfonate method, respectively. [12,13,14,15,16]

Radiographic Analysis

At the conclusion of the experiment, all rats were anesthetized with an intraperitoneal injection of 40 mg/kg sodium thiopental. The animals were then positioned on X-ray plates, and projections of the left ankle joint were captured. Radiographic evaluation was conducted using parameters such as:

- **Erosion**: Destruction of bony structures, resulting in an irregular bone surface.
- **Periosteal reaction**: A fine ossified line paralleling the normal bone, leading to bone thickening.
- **Increase in soft tissue**: Manifested as an increased width of soft tissue and potential calcification.

In addition, X-rays of the knee joints were taken to confirm and assess the severity of arthritis in FCA-induced rats.

Statistical Analysis

The data are presented as mean ± standard error of the mean (SEM). The statistical differences between the normal control and arthritic control groups, as well as between the control and treatment groups, were analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison tests. Statistical

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significance was considered at a p-value of < 0.05. Data analysis was performed using GraphPad Prism software.

3. Results

Phytochemical Analysis

Phytochemical screening of the ethanolic extracts revealed the following active compounds in each plant:

- **Vitex negundo**: Alkaloids, tannins, flavonoids, and saponins
- **Boswellia serrata**: Alkaloids, flavonoids, saponins, and phenolic compounds
- **Capsicum frutescens**: Alkaloids, tannins, flavonoids, and phenolic compounds

These compounds are known for their potential therapeutic properties, which may contribute to the antiarthritic effects observed in this study.

Preformulation Studies

The preformulation studies of the polyherbal formulation (PHF) capsules demonstrated uniformity in both content and weight. Further analysis included the evaluation of moisture content, microbial load, heavy metal limits, monographic analysis of plant materials, pH, and disintegration time, all of which were within acceptable limits. The presence of heavy metals was tested by comparing the opalescence produced by the sample with a standard. The results indicated that the test solution showed less opalescence than the standard, confirming that heavy metals were within the prescribed limits. Additionally, the formulation complied with the World Health Organization (WHO) standards for microbial load, ensuring its safety for internal use.

High Performance Thin Layer Chromatography (HPTLC) Fingerprint Analysis

Fingerprint analysis at 366 nm yielded better separation of compounds compared to 254 nm and 520 nm. The chromatograms of the polyherbal formulation revealed the presence of active compounds found in the individual herbs. The Rf values and peak areas observed in the formulation were identical to those of the individual extracts, indicating that the marker compounds in the herbal extracts were retained in the final formulation. This confirmed the consistency of active compounds across the formulation and its constituent herbs.

Effect of Polyherbal Formulation on Body Weight of Arthritic Rats

Body weight changes were monitored as an indicator of arthritis progression, with weight loss being a common symptom in the early stages of arthritis. The arthritic control group exhibited a significant loss in body weight by the end of the study. In contrast, animals treated with the polyherbal formulation (PHF) and indomethacin showed a significant increase in body weight, suggesting improvement in their overall health and a reduction in the severity of arthritis symptoms [Table 1].

Effect of Polyherbal Formulation on Primary Response of Arthritic Rats (Injected Paw)

One day after the FCA injection, primary arthritis was induced in the right hind paw of the rats, and inflammation was consistently maintained for 21 days. The peak swelling occurred between the 5th and 7th day, with the maximum increase in paw volume reaching 1.21 ml in the arthritic control group. There was a significant increase in paw edema in all FCA-induced arthritic groups compared to the normal control.

Table 1. Percentage increase body weight compared to poly herbal formulation and indomethacin

Group	Treatment	Body weight (g)		% increase in body weight
		Initial	Final	
I	Normal control	210.21±1.674	228.21±4.214	8.5
II	Arthritic control	208.07±3.562	196.23±3.624	−5.76
III	PHF 250 mg/kg	212.05±1.640	225.02±3.325	6.13
IV	PHF 500 mg/kg	206.58±2.314	220.46±3.014	6.42
V	Indomethacin	218.04±2.250	236.16±2.940	8.25

PHF: Polyherbal formulation

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However, in animals treated with the polyherbal formulation (PHF) at both 250 mg/kg and 500 mg/kg, as well as in the indomethacin (10 mg/kg) group, the swelling began to subside gradually ($P < 0.001$) compared to the arthritic control. Notably, the anti-edematous effect of PHF was observed to be significant as early as the 2nd day post-FCA injection, and this effect was maintained throughout the duration of the study.

The results, as shown in **Table 2**, demonstrate that the polyherbal formulation exhibited a synergistic effect in reducing paw volume and alleviating primary inflammatory responses in the FCA-induced arthritis model. This suggests that PHF has potent anti-inflammatory properties that help in controlling edema in the affected paw.

Effect of Polyherbal Formulation on Biochemical Parameters of Arthritic Rats

The biochemical analysis revealed significant alterations in the liver enzymes and inflammatory markers in the arthritic rats. Inflammation induced by adjuvant resulted in elevated levels of liver enzymes such as **AST**, **ALT**, and **ALP** in all arthritic groups compared to the normal control rats. However, animals treated with the polyherbal formulation (PHF) at doses of 250 mg/kg and 500 mg/kg exhibited a significant ($P < 0.001$) reduction in the elevated levels of these liver enzymes, suggesting hepatoprotective and anti-inflammatory effects of the formulation. The treated groups showed a more pronounced reduction in **AST**, **ALT**, and **ALP** levels compared to the control, further demonstrating the antiarthritic efficacy of PHF.

C-reactive protein (CRP), an acute-phase reactant, is known to increase during inflammatory conditions. The results indicated a significant ($P < 0.001$) reduction in CRP levels in the PHF-treated groups, supporting the formulation's anti-inflammatory properties.

Ceruloplasmin, a copper-containing enzyme synthesized in the liver, plays a critical role in binding copper ions and preventing oxidative damage caused by free copper ions. Elevated serum copper levels are often associated with inflammation. In this study, **serum copper concentration** was significantly elevated in the arthritic rats, indicating an inflammatory condition. However, the copper levels were significantly ($P < 0.001$) reduced in the PHF-treated rats, suggesting that the polyherbal formulation may help in mitigating oxidative damage by regulating copper levels during inflammation.

These findings confirm that PHF exerts beneficial effects on various biochemical markers associated with inflammation, supporting its potential as an effective antiarthritic agent.

Results for Radiographic Analysis

The radiographic examination of the knee joints for all rat groups is shown in **Figure 1**. The results clearly demonstrate that the rats in the adjuvant-treated (arthritic) group exhibited significant pathological changes in their knee joints, including **periosteal reaction**, **irregular joint space**, **soft tissue swelling**, and **reduction in joint space**. These findings indicate severe arthritis-induced damage to the joint structures.

In contrast, rats treated with the polyherbal formulation (PHF) and the standard drug (indomethacin) showed normal joint structures. No periosteal reaction was observed, the joint space appeared intact, and there was no swelling or joint space reduction in these groups, suggesting that the polyherbal formulation effectively protected the joints from the destructive effects of arthritis. The radiographs of these treated groups appear almost identical to those of the normal control rats, indicating a significant protective effect of the PHF on joint health [Figures 1 and 2].

Table 2: Antiarthritic activity of polyherbal formulation compared with indomethacin in injected paw volume

Treatment	Paw volume (ml)±SEM on injected paw										
Days	1	3	5	7	9	11	13	15	17	19	21
Normal control	0.11±0.00	0.10±0.00	0.10±0.00	0.10±0.00	0.10±0.00	0.11±0.00	0.10±0.00	0.10±0.00	0.10±0.00	0.11±0.00	0.10±0.00
Arthritic control	0.78±0.00	0.88±0.01	1.07±0.00###	1.21±0.01###	1.10±0.00###	0.99±0.01###	0.81±0.01###	0.82±0.01###	0.84±0.01###	0.87±0.01###	0.92±0.01###
PHF 250 mg/kg	0.79±0.01	0.81±0.01	0.84±0.01***	0.91±0.01***	0.86±0.01*	0.70±0.00***	0.67±0.01***	0.65±0.01***	0.61±0.01***	0.58±0.00***	0.45±0.01***
PHF 500 mg/kg	0.78±0.01	0.81±0.00	0.87±0.01***	0.89±0.01***	0.85±0.01***	0.80±0.00***	0.64±0.00***	0.62±0.01***	0.56±0.01***	0.51±0.01***	0.40±0.01***
Indomethacin, 10 mg/kg	0.77±0.00	0.82±0.00	0.87±0.00***	0.88±0.00***	0.83±0.01***	0.79±0.00***	0.61±0.00***	0.64±0.00***	0.54±0.00***	0.51±0.01***	0.43±0.01***

Values are expressed as mean±SEM (n=6). *P<0.05; ***P<0.001compared with arthritic control, ###P<0.001 compared with normal control. Data was analyzed using one-way ANOVA followed by Dunnett's t-test. ANOVA: Analysis of variance, SEM: Standard error of the mean, PHF: Polyherbal formulation

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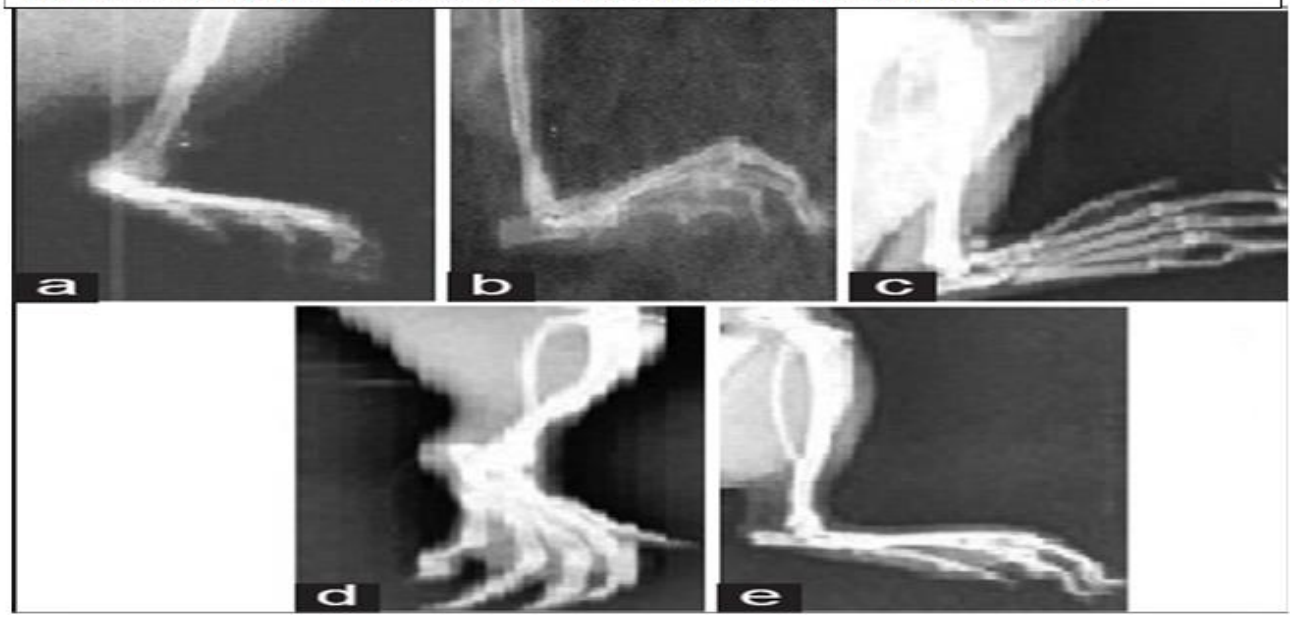
These radiographic findings further support the antiarthritic potential of the polyherbal formulation, demonstrating its ability to mitigate joint damage in the FCA-induced arthritis model.

Radiographic Analysis of Polyherbal Formulation (PHF)

- **(a) Normal Control Animal**: The radiographic image shows the normal knee bone structure without any abnormalities. The joint space is well-maintained, and the bone structures appear intact, indicating healthy joints.
- **(b) Animal with Freund's Complete Adjuvant (FCA)-Induced Arthritis**: The radiograph of the FCA-induced arthritis group displays significant joint damage, including reduced joint space, irregular bone contours, soft tissue swelling, and a periosteal reaction. These findings are indicative of the destructive effects of inflammation and arthritis.
- **(c) Animal with FCA-Induced Arthritis Treated with PHF (250 mg/kg)**: The animal treated with PHF at a dose of 250 mg/kg shows noticeable improvements. While some mild changes are present, the joint space appears better preserved compared to the FCA-induced arthritis group. There is a reduction in paw edema, suggesting the anti-inflammatory and protective effects of the PHF.
- **(d) Animal with FCA-Induced Arthritis Treated with PHF (500 mg/kg)**: The animal treated with PHF at a dose of 500 mg/kg exhibits a more significant reduction in joint space reduction and paw edema. The joint structure looks almost normal, with reduced inflammation and signs of repair in the bone and soft tissues.
- **(e) Animal with FCA-Induced Arthritis Treated with Indomethacin (10 mg/kg)**: The radiograph of the group treated with indomethacin, a standard anti-inflammatory drug, shows some improvement in joint health, including a slight reduction in the fractional tibial epiphysis and better-maintained joint space compared to the FCA-induced arthritis group.

These radiographic findings collectively demonstrate that the polyherbal formulation (PHF) at both 250 mg/kg and 500 mg/kg doses has a significant protective effect against joint damage, with results comparable to the standard anti-inflammatory treatment (indomethacin). The PHF effectively reduces inflammation and preserves joint integrity in FCA-induced arthritis.

Figure 1. Radiographic analysis of poly herbal formulation (PHF) (a) Normal control animal (b) Animal with Freund's complete adjuvant (FCA) induced Arthritis (c) Animal with FCA induced Arthritis treated with PHF (250mg/kg) (d) Animal with FCA induced Arthritis treated with PHF (500mg/kg) (e) Animal with FCA induced Arthritis treated with Indomethacin (10mg/kg)



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4. Discussion

In this study, the polyherbal formulation (PHF) at doses of 250 mg/kg and 500 mg/kg demonstrated significant antiarthritic activity, comparable to the standard anti-inflammatory drug **indomethacin**. PHF not only reduced inflammation and joint damage but also significantly increased the body weight of the animals when compared to the arthritic control group. This suggests that PHF has a potent therapeutic effect in mitigating the symptoms of arthritis and improving overall health status in the affected animals.

The polyherbal formulation was developed using the **ethanolic extracts** of the stem bark of **Vitex negundo**, the whole plant of **Boswellia serrata**, and the leaves of **Capsicum frutescens**, combined in a 2:2:1 ratio. Each of these plants has demonstrated individual antiarthritic properties. For instance, **Vitex negundo** has been shown to possess significant antiarthritic and antidiabetic effects in animal models, particularly in **FCA-induced arthritis** and **streptozotocin-induced diabetes** at doses of 400 and 800 mg/kg, respectively.[20] Similarly, **Capsicum frutescens** (specifically its aqueous leaf extract) has demonstrated significant **anti-inflammatory** activity both in **in vivo** and **in vitro** studies.[21,22] **Boswellia serrata** is well-known for its antiarthritic effects, with **ethanolic extracts** of the whole plant showing remarkable efficacy in **FCA-induced arthritis** at doses of 250 and 500 mg/kg.[1] Some studies have also highlighted its **antidiabetic** potential, further emphasizing its therapeutic versatility.[1]

The **Freund's complete adjuvant (FCA)-induced arthritis model** is widely used to study **rheumatoid arthritis** and to test potential therapeutic agents. This model simulates the rapid progression of arthritis, characterized by **joint erosion**, **inflammation**, and **bone destruction**. The **bacterial peptidoglycan** and **muramyl dipeptide** in FCA are responsible for initiating the inflammatory cascade, leading to arthritis in animal models.[1,23]

In this study, **arthritic rats** exhibited reduced red blood cell (RBC) counts, lower hemoglobin (Hb) levels, and elevated **erythrocyte sedimentation rate (ESR)**, all of which are indicative of **anemia**, a common feature in patients with chronic arthritis. The ESR, which reflects the concentration of acute-phase proteins such as **fibrinogen** and **β-globulins**, rises during inflammatory processes, signaling ongoing disease activity. Treatment with the polyherbal formulation improved **RBC count**, **Hb levels**, and **ESR**, bringing them closer to normal levels, indicating recovery from both the anemic condition and the progression of arthritis. This demonstrates that the formulation has a significant therapeutic role in managing the hematological abnormalities associated with arthritis.[1]

White blood cell (WBC) count, another important marker in inflammatory conditions, was elevated in the arthritic rats, reflecting the immune response to the inflammation. **WBC count** is often used as an indicator for infections and inflammatory diseases.[24] In arthritis, an increase in WBCs is commonly observed due to the inflammatory processes. The increase in WBCs can also be linked to the activation of various inflammatory pathways, including the release of **prostaglandins**, **cyclooxygenase (COX)** products, and free radicals, which contribute to the progression of arthritis. The treatment with PHF was able to control this increase in WBCs, further supporting its anti-inflammatory and immunomodulatory effects.

The **radiographic analysis** of the knee joints from both the arthritic and treated groups showed significant improvements. In the arthritic rats, the joint spaces were reduced, and the bone structures were damaged. However, in the groups treated with PHF, **indomethacin**, or other plant extracts, the joint spaces appeared almost normal, with minimal periosteal reactions and bone damage. This radiological evidence strongly supports the **dose-dependent antiarthritic efficacy** of PHF in protecting against joint destruction, further confirming the therapeutic benefits observed in the **paw edema** and **biochemical** parameters.

The results from this study suggest that **polyherbal formulation** offers enhanced antiarthritic efficacy compared to the individual plant extracts alone. The formulation's **synergistic effect** could be attributed to the complementary properties of the selected herbs, which work together to provide a more potent therapeutic response against arthritis.[1,5] The marked reduction in joint space and inflammation observed in the **radiographic** analysis further substantiates the significant potential of PHF to manage arthritis.

However, this study is limited to **preclinical** testing, and additional **chronic toxicity** and **pharmacokinetic**

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studies are required to confirm the **safety** and **efficacy** of PHF. Furthermore, **clinical trials** are essential to validate the findings of this study and to establish the formulation's **safety** and **clinical applicability** in human subjects.

Figure 2. Compare poly herbal formulation and different doses with standard anti-inflammatory drug Indomethacin to demonstrate significant anti arthritic activity

5. Conclusion

The polyherbal formulation demonstrated significant antiarthritic activity by effectively modulating the pathogenesis of **FCA-induced arthritis** in **female Sprague-Dawley rats**. The formulation exhibited antiarthritic potential comparable to the standard drug **indomethacin**, as evidenced by reductions in **paw volume** and **serum CRP levels**. These findings suggest that the polyherbal formulation could serve as a potent therapeutic agent for managing arthritis, offering a promising alternative to conventional treatments.

6. Conclusion

The polyherbal formulation demonstrated significant antiarthritic activity by effectively modulating the pathogenesis of **FCA-induced arthritis** in **female Sprague-Dawley rats**. The formulation exhibited antiarthritic potential comparable to the standard drug **indomethacin**, as evidenced by reductions in **paw volume** and **serum CRP levels**. These findings suggest that the polyherbal formulation could serve as a potent therapeutic agent for managing arthritis, offering a promising alternative to conventional treatments.

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