



PHARMACOLOGICAL SCREENING OF POLYHERBAL FORMULATION ON COMPLETE FREUND'S ADJUVANT INDUCED RHEUMATOID ARTHRITIS DISEASE IN RATS

Shivakumar S. Ladde^{1*}, Prasad S. Menkudale², Pratik D. Kadere², Sachin R. Kamble²

^{*1}Head of Department of Pharmacy Practice, Channabasweshwar Pharmacy College (Degree), Latur, Maharashtra, India. 413512.

²Research Scholar, Department of Pharmacology, Channabasweshwar Pharmacy College (Degree), Latur, Maharashtra, India. 413512.

Article Received on 02 May 2026,
Article Revised on 22 May 2026,
Article Published on 01 June 2026,

<https://doi.org/10.5281/zenodo.20443856>

*Corresponding Author

Shivakumar S. Ladde

Head of Department of Pharmacy Practice, Channabasweshwar Pharmacy College (Degree), Latur, Maharashtra, India. 413512.



How to cite this Article: Shivakumar S. Ladde^{1*}, Prasad S. Menkudale², Pratik D. Kadere², Sachin R. Kamble². (2026). Pharmacological Screening Of Polyherbal Formulation On Complete Freund's Adjuvant Induced Rheumatoid Arthritis Disease In Rats. World Journal of Pharmacy and Pharmaceutical Sciences, 15(6), 842–865.

This work is licensed under Creative Commons Attribution 4.0 International license.

ABSTRACT

Rheumatoid Arthritis (RA) is a chronic, systemic, and progressive autoimmune disease that primarily affects the synovial joints, leading to inflammation, swelling, and gradual destruction of cartilage and bone. It affects nearly 0.5–1% of the world's population, with women being more commonly affected than men, and its onset usually occurs between the ages of 40 and 60 years. In addition to joint-related complications, RA is often associated with systemic manifestations such as fatigue, cardiovascular complications, and reduced quality of life. The exact cause of RA is multifactorial, involving genetic predisposition, environmental influences, and abnormal immunological responses, which together trigger persistent inflammation and tissue damage. In the present study, an attempt was made to investigate the anti-arthritic potential of a polyherbal formulation containing *Vitex Negundo* & *Boswellia Serrata*. The study was carried out using

a Complete Freund's Adjuvant (CFA)-induced arthritis model in rats, which closely mimics the pathophysiological features of human RA. The experimental design consisted of six groups: Group I served as the normal control, Group II as the disease control, Group III received the standard anti-arthritic drug, Group IV was treated with the polyherbal

suspension 100mg/kg, Group V Was treated with polyherbal suspension 200mg/kg, and Group VI was treated with polyherbal suspension 400mg/kg. The progression of arthritis and the therapeutic efficacy of the formulation were evaluated using various parameters, including changes in body weight, paw volume, and locomotor activity. The disease control group showed a significant reduction in body weight, increased paw swelling, and impaired locomotor activity, confirming the successful induction of arthritis. Treatment with the polyherbal suspension formulation produced a marked reduction in hind paw edema and significantly improved body weight and locomotor activity compared with the disease control group. The results suggest that the polyherbal suspension formulation possesses substantial anti-inflammatory and anti-arthritic activity. In conclusion, the findings of this study highlight the therapeutic potential of a polyherbal formulation containing *Vitex Negundo* & *Boswellia Serrata* in the management of rheumatoid arthritis. Its ability to reduce inflammation and improve functional outcomes indicates its usefulness as an alternative or complementary therapeutic approach. The present study has already evaluated biochemical parameters Full Blood Count (WBC, RBC, haemoglobin count, platelets count), rheumatoid factor test, C-reactive protein test, and skin irritation tests, further investigations focusing on histopathological and molecular studies are warranted to confirm the precise mechanism of action and enhance its clinical applicability.

KEYWORDS: Autoimmune, Polyherbal Formulation, Complete Freund's adjuvant, Rheumatoid Arthritis *Vitex Negundo*, *Boswellia Serrata*.

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease characterized by joint inflammation and joint damage. However, it is clear that cytokines are important in activating synovial cells and causing inflammation and joint degeneration in the joints.^[1,2] Considering their different contributions to the development of the disease, it is generally accepted that the cytokines TNF- α , IL-1, and IL-6 play a key role in the pathogenesis of RA.^[1,2] Therefore, research into new cytokines and other treatments continues. There is evidence that the cytokine IL-17 has anti-inflammatory properties and can reduce inflammation caused by other cytokines, including TNF.^[1,2] Inhibition of IL-17 slows the progression of inflammation and damage in several animal models of RA.^[1,2] T cells in the peripheral blood or synovial fluid of patients with rheumatoid arthritis express more cytokines after stimulation by IL-21.^[1,2] However, blocking IL-21 can reduce inflammatory cytokines in rheumatoid arthritis synovial cell

cultures.^[1,2] In addition, inhibition of the IL-21/IL-21R pathway has been shown to increase disease progression in animal models of RA.

Epidemiology

Rheumatoid arthritis (RA) is estimated to affect between 0.24% and 1% of the population; women are twice as likely to develop the disease as men.^[3,4] The incidence of rheumatoid arthritis neck pain increases with age by approximately 6% in both sexes.^[3,4] About 3% of women and 3% of men over the age of 65 have the disease. Juvenile RA (JRA), a disease similar to RA that can affect children under the age of 16, may also occur.^[5] Osteoarthritis is the most common type of arthritis and occurs due to wear and tear rather than an autoimmune disease.^[4,5] The disease, which is seen in children and adults (mostly between the ages of 16 and 40), is called early-onset rheumatoid arthritis. People who develop symptoms after the age of 60 are considered to have late-onset rheumatoid arthritis.^[5]

MATERIALS AND METHODS

Drug Materials: *Vitex negundo* and *Boswellia Serrata* extracts purchased from Natu Harvest Health pvt. Limited, Uttar Pradesh, India. Methotrexate procured from Ipca Laboratories, Used as Standard marketed drug.

Inducing agents: DPPH (Molychem) used as oxidant or free radical agent, Complete Freund's Adjuvant (Labware chemicals) used as inducing agent.

Chemicals: Polysorbate 80, Hydroxyethyl cellulose, Sorbitol, Peppermint, Sodium benzoate, Citric acid, Acetone, Ascorbic acid, Methanol, Ethanol used analytical grade procured from (Molychem, Research lab fine chem. Industries, labware chemicals)

List of instruments: Digital Plethysmometer, (Orchid scientific). Actophotometer, (M. M. Scientific & Surgical Co.), Digital Weighing Balance, (Wensar). Viscometer, (Brookfield Ametek). pH Meter, (Avi Science). Magnetic stirrer, (Athena technology). Vernier Caliper (Mitutoyo, Japan), Uv Spectrophotometer (Shimadzu). Micropipette (Thermo lab systems).

Methodology Phytochemical Analysis

The ethanolic extract was subjected to phytochemical analysis to identify and quantify various bioactive compounds, including alkaloids, Triterpenoids using standard qualitative and quantitative methods. Test procedures are as follows in table 1 & 2.^[6]

Table 1: Detection of terpenoids for *Boswellia Serreta* extract.

Sr. No.	Test	Observation	Inference
1	Liberman-Burchard's test: 0.1 ml extract chloroform + acetic anhydride conc. H ₂ SO ₄ along the sides of test tube	Formation of brown ring junction	Presence of terpenoids
2	Salkowski test: 0.1 ml extract + 2 drops of conc. H ₂ SO	Formation of yellow coloured lower layer	Presence of terpenoids
1	Liberman-Burchard's test: 0.1 ml extract chloroform + acetic anhydride conc. H ₂ SO ₄ along the sides of test tube	Formation of brown ring junction	Presence of terpenoids
2	Salkowski test: 0.1 ml extract + 2 drops of conc. H ₂ SO	Formation of yellow coloured lower layer	Presence of terpenoids

Table 2: Detection of alkaloids for *Vitex negundo* extracts.

Sr. No.	Test	Observation	Inference
1	Mayer's test: 1 ml filtrate + 2 drops of Mayer's reagent along sides of tube	Presence of cream colour ppt	Presence of alkaloids
2	Wagner's test- 1ml filtrate + 2 drops of Wagner's reagent along sides of tube	Presence of reddish brown ppt	Presence of alkaloids

Experimental study

Selection and procurement of animals

Prior to starting the experimentation, approval from the Institutional Animal Ethics Committee (Approval No: CPCSEA/CBPL/AH/94) was acquired for the study plan. A total of 36 Wistar Albino rats of either sex, weighing between 150 and 200g, for carrying out experimental investigation. Throughout the study period, the rats were cared for and the experiments were carried out in accordance with the standards set by the Committee for Control and Supervision of Experimentation on Animals (CCSEA, formerly CPCSEA), India, for the use and care of laboratory animals.

Housing facilities

Throughout the research period, rats were housed in air-conditioned animal house with 12 – 15 cycles of filtered fresh air at a temperature of 22 ± 3 °C and a relative humidity of 30-70%. Animals were kept under 12 hours: 12-hour light/dark (natural day and light cycle) cycle and rodent feed was provided in the form of standard rodent pellet diet. Water was provided ad libitum. The rats were acclimatized to laboratory conditions for 7 days before beginning of study.

Formulation of suspension

The formulae for preparing of a suspension of extracts of *Vitex negundo* & *Boswellia serrata*.

Was taken in the ratio of (1:1) Suspension was prepared by using extracts of selected plant materials trituration method in mortar and pestle by using Both the powder drugs were mixed by trituration using mortar and pestle. Subsequently, Further, water was added into the powdered drug mixture. Finally, Hydroxyethyl cellulose (HMC), Polysorbate 80 were added, followed by sodium benzoate, sorbitol, citric acid and peppermint oil were added, along with final volume make up to with water. The preparation was filled into amber coloured bottle.^[7]

Evaluation parameter test of suspension

- 1 pH - The pH of suspension was determined using pH meter.
- 2 Viscosity - The viscosity of the sample was determined at room temperature using Brook field viscometer.
- 3 Sedimentation- Settling of particles over time was measured.
- 4 Flow rate- Measures the flow rate of suspension was measured.
- 5 Microbial test- Microbial growth in the suspension during study period was measured.
- 6 Physical Evaluation- The physical evaluation of suspension was evaluated

Anti-Oxidant Study of polyherbal formulation containing *Vitex negundo* & *Boswellia serrata* Using DPPH Assay

The DPPH radical scavenging activity of polyherbal formulation containing *Vitex negundo* & *Boswellia serrata* was tested by standard method of Blois. Free radical scavenging activity of *Vitex negundo* & *Boswellia serrata* were measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH). Briefly, 0.1 mM solution of DPPH was prepared by dissolving 0.004 g of DPPH crystalline solid in 100 mL of analytical grade methanol and stored at 4 °C. A 4 mg of the plant extract was dissolved in 10 mL of methanol in order to prepare 400 µg/mL stock solutions and then serial dilution with methanol was performed to prepare the required concentrated solutions (50, 100,150, 200, 250, 300 µg/mL). A 2 mL of plant extract solution from each concentration was taken in a test tube and then, 3 mL of DPPH solution was added in each test tube. After 120 min incubation in the dark, the absorbance at 517 nm was recorded using a UV–Vis Spectrophotometer. Reference standard compound being used was ascorbic acid. A stock solution of 800 µg/mL was prepared by dissolving 1.5 ml ascorbic acid in 13.5 mL of distilled water. Then, serial dilution with different concentrated solution was prepared (50, 100,150, 200, 250, 300 µg/mL). DPPH solution was used as the blank for respective extracts. A mixture of 3 mL of 0.1 mM DPPH and (100 µL MeOH for methanol extract) was used as control.

All determinations were performed in triplicate. The percent of inhibition were plotted against concentration from which IC₅₀ values were calculated. where, A control is the mixture of methanol and DPPH solution, and A sample is the mixture of sample extract and DPPH solution.

$$\text{DPPH radical scavenging activity \%} = (\text{control OD} - \text{sample OD} / \text{control OD}) \times 100^{[8]}$$

Acute oral toxicity study

An acute oral toxicity study was performed according to the Organization of Economic Co-operation and Development (OECD) guideline 425 for testing a chemical. Five female mice were used. A test suspension of in purified water was orally administered only once at a single dose of 2000 mg/kg, whereas the control group only received 2 mL of purified water as a vehicle. All mice were then granted free access to food and water, observed for any signs of acute toxicity for 24 h with special attention to the first 4 h.^[9]

Preparation of dosages

A preliminary test was determined the possible protective dose of *Boswellia serrata* & *Vitex Negundo*. The doses 100, 200, and 400 mg/kg were examined and administered orally in the form of suspension prepared in double distilled water containing Hydroxyethyl cellulose (1% w/v, HEC) based on previous studies, according to its results, *Boswellia serrata* & *Vitex Negundo* at doses 100, 200 and 400 mg/kg in combination was select for the main study. CFA was injected with 0.1ml of (1% w/v in water) into the sub-plantar area of left hind paw.^[10]

Grouping of animals

Before initiation of experiment 36 animals were randomly divide into following six groups in table 6.2.1

Table 3: Grouping of animals.

Group I	Normal control group	Normal saline
Group II	Disease control group	0.1 ml of complete Freund's adjuvant
Group III	Standard treatment group	Methotrexate (1mg/kg) (p.o) + 0.1 ml of complete Freund's adjuvant
Group IV	Low Dose group (1:1)	Polyherbal Formulation of <i>Vitex negundo</i> & <i>Boswellia serrata</i> 100mg/kg (p.o) + 0.1 ml of complete Freund's adjuvant
Group V	Medium Dose group(1:1)	Polyherbal Formulation of <i>Vitex negundo</i> & <i>Boswellia serrata</i> 200 mg/kg (p.o) + 0.1 ml of complete Freund's adjuvant

Group VI	High Dose group (1:1)	Polyherbal Formulation of <i>Vitex negundo</i> & <i>Boswellia serrata</i> 400mg/kg (p.o) + 0.1 ml of complete Freund's adjuvant
----------	-----------------------	---

Complete Freund's Adjuvant

The rats were injected with 0.1ml of Complete Freund's Adjuvant (1% w/v) into the sub-plantar Region of left hind paw.^[11]

Procedure

On day zero, Animals are injected into the sub plantar region of the left hind paw with 0.1 ml of complete Freund's adjuvant (FA). This consist of 6mg Mycobacterium butyricum suspended in heavy paraffin oil by through grinding with motor and pestle to give a concentration of 6mg/ml. On the 8th day of arthritic induction, simultaneously on 0, 8th, 13th, 18th, 23rd, 28th the Behavioral parameters were conducted & Inflammatory activities. Dosing with the standard compound was administered orally 6 times in 20 days and test compounds continued for 20 days according to the following schedule. On 28th day blood was collected & Hematology study was conducted. Evaluation was done by comparing certain parameters between the control and treatment.^[12-15]

Measuring paw volume

Digital plethysmometer instrument

A digital plethysmometer was used to detect the paw volume of both left and right hind paws on the 0, 8th, 13th, 18th, 23rd and 28th day of arthritic induction, where the right paw worked as a negative control. The rise in paw volume was determined by calculating the difference among the paw volumes of the left injected paw and right non-injected paw.^[16]

Behavioral Parameters Locomotor activity

Assessment of Locomotor Activity by used Actophotometer instrument, the movement of animals in the Actophotometer was measured on the 0, 8th, 13th, 18th, 23rd and 28th day of experiment for the assessment of locomotor activity.^[17]

Measuring paw thickness Vernier Caliper

The measurements of paw thickness on day 0, 8th, 13th, 18th, 23rd and 28th day of the experiment was done by Vernier caliper.^[17]

Haematology Study Full blood count (FBC)

A full blood count, also known as a complete blood count (CBC), evaluates the cells that

make up blood. This includes your white blood cells, red blood cells, and platelets. Rats orally administered with standard treatment and polyherbal formulation was decreased in WBC count, ESR and increased in Hb and RBC count, when compared with arthritic control groups. The blood was collected from Retro orbital puncture and sample was sent to “Med plus clinical laboratory, Solapur, Maharashtra.”^[18]

Rheumatoid Arthritis (RA) factor test

Rheumatoid factors are immune system proteins that sometimes attack the healthy tissue in body. Auto antibodies termed as “Rheumatoid Factor is the most useful prognostic marker for the diagnosis of rheumatoid arthritis. The blood was collected from Retro orbital puncture and sample was send to “Med plus clinical laboratory, Solapur, Maharashtra.”^[19]

C-reactive protein test (CRP)

The C-reactive protein (CRP) test is a blood test that checks for inflammation in body. Levels of CRP start to increase very soon after any inflammation or infection affects body. Rats orally administered with standard treatment and polyherbal formulation was decreased in inflammation. The blood was collected from Retro orbital puncture and sample was send “Med plus clinical laboratory, Solapur, Maharashtra.”^[19]

RESULTS

Physico-chemical parameters of drugs

A) *Boswellia serrata* (Boswellic acid 85%)

Table 4: Physico-chemical parameters of *Boswellic serrate*.

Test	Specification	Result	Testing Method/Reference
Appearance	Creamish To Off White Color	Complies	Visual
Odor & Taste	Characteristics	Complies	Visual
Identification Test	Positive	Complies	By TLC Method
Extractive Value in Water	NLT 80% (W/W)	84.51%	In House
Extractive Value In 60% V/V Method	NLT 80% (W/W)	82.41%	In House
Sieve Analysis	95% Pass 40 Mesh	Complies	40 Mesh Screens
Loss On Drying	NMT 7% (W/W)	3.38%	5g/105 °C/2hrs
Bulk Density	40-.50 Gm/100ml.	Complies	In House
pH (1% W/V Aqueous Solution)	Between 4-7	5.36	In House
Active Component Assay (Boswellic Acid)	NLT 85 %	85.76%	By Titration

B) *Vitex Negundo* (Nirgundi Leaves)**Table 5: Physico-chemical parameters of *Vitex Negundo*.**

Test	Specification	Result	Testing method/reference
Appearance	Brown colour	Complies	Visual
Odor & Taste	Characteristics	Complies	Visual
Identification Test	Positive	Complies	By TLC Method
Extractive value in Water	NLT 80% (w/w)	84.46%	In house
Extractive value in 60% v/v Method	NLT 80% (w/w)	82.24%	In house
Sieve Analysis	95% pass 40 mesh	Complies	40 mesh screens
Loss on drying	NMT 7% (w/w)	3.28%	5g/105 °C/2hrs
Bulk Density	40-.50 gm/100ml.	Complies	In house
pH (1% w/v aqueous solution)	Between 4-7	5.46	In house
Active component Assay (Alkaloids)	NLT 0.15%	0.64%	By UV

Detection of terpenoids for *Boswellia Serreta* extract & Detection of alkaloids for *Vitex negundo* extracts

**Fig 1: Phytochemical test for *Boswellia Serreta* & *Vitex negundo*.****Polyherbal formulation****Table 6: Formulation of PHF.**

Sr. No	Parameter	Observations
1	Nature	Liquid
2	Colour	Brownish
3	Odor	Pleasant
4	Taste	Sweet

Table 7: Physical Evaluation for polyherbal suspension.

Sr. No	Parameter	Observations
1	Sedimentation volume	1.3
2	Flow Rate	5ml/25.3sec
3	Viscosity	59 cp
4	pH	6±85
5	Microbial growth	No

Table 8: Formulation study for polyherbal formulation.

Contents	Quantity
<i>Vitex negundo</i>	5g
<i>Boswellia serrata</i>	5g
polysorbate 80	1ml
Hydroxyethyl cellulose	1gm
Sorbitol	3gm
sodium benzoate	0.2gm
citric acid	0.2gm
peppermint oil	0.1ml Qt. sufficient up to 100 ml
Distilled water	

Determination of antioxidant activity polyherbal formulation containing *Boswellia serrata* & *Vitex negundo* by DPPH assay

The antioxidant activity of polyherbal formulation has been studied by its ability to reduce DPPH. Interaction of antioxidant compounds with DPPH is based on the transfer of hydrogen atom or electron to DPPH radical and converts it to 1, 1- diphenyl-2- picrylhydrazine. The result of reduction DPPH radicals causes discoloration from purple colour to yellow pale colour which demonstrates the scavenging activity. The antioxidant activity of the solvents extracts and ascorbic acid against DPPH assay was tested with concentrations ranging from 50 to 300 µg/ ml as the results shown in Table No.9 & 10 the MeOH extracts were displayed and significant concentration- dependent free radical scavenging activity from 44.2, 46.81, 59.08, 65.84, 80.42, 38, 41.52, 54.47, 59.01, 71.61,

Table 9: DPPH scavenging activity of standard compound.

Sr.No.	Concentration	Control	Absorbance	% of inhibition	IC ₅₀ (µg/mL)
1	50	2.1807	1.2168	44.2	106.8148
2	100	2.1807	1.1597	46.81	
3	150	2.1807	0.8922	59.08	
4	200	2.1807	0.7449	65.84	
5	300	2.1807	0.4268	80.42	

Table 10: DPPH scavenging activity of test compound.

Sr.No.	Concentration	Control	Absorbance	% of inhibition	IC ₅₀ (µg/mL)
1	50	2.1807	1.352	38	120.4239
2	100	2.1807	1.2765	41.52	
3	150	2.1807	0.9941	54.47	
4	200	2.1807	0.8925	59.01	
5	300	2.1807	0.6186	71.61	

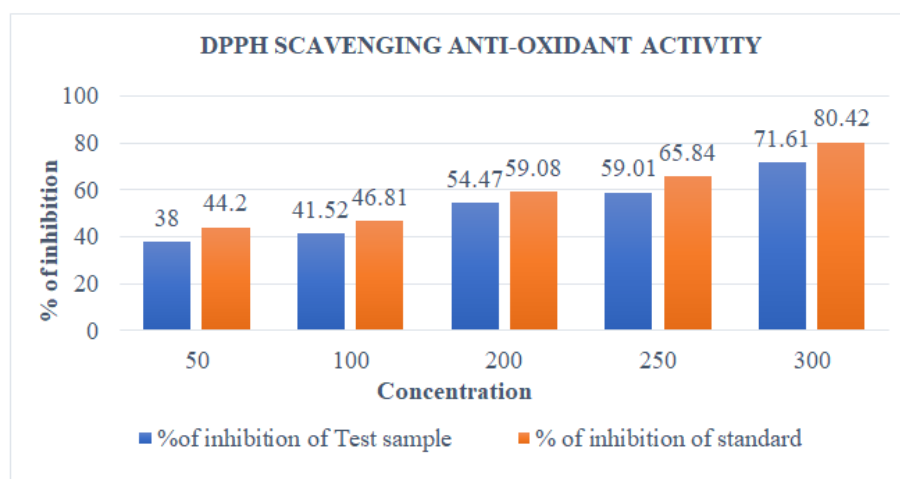


Fig 2: % of inhibition of anti-oxidant DPPH free radical Scavenging activity of standard and test compound Acute oral Toxicity Studies.

In acute oral toxicity studies, it shows that there is no behavioural change, mortality observed up to a dose of 2000 mg/kg PHF, hence the acute oral toxicity of PHF is more than 2000 mg/kg. The 1/20, 1/10, 1/5 of dose of 2000 mg/kg of PHF is considered as Low Dose (100 mg/kg), Medium Dose (200 mg/kg) and High Dose (400 mg/kg) for evaluation of Anti-arthritic activity of PHF Suspension.

Complete Freund's Adjuvant

The rats were injected with 0.1ml of Complete Freund's Adjuvant (1% w/v) into the sub-plantar Region of left hind paw.



Fig 3: CFA induced in Disease control group.

Fig 4: CFA induced in standard treatment group.



Fig 5: CFA induced in PHF 1 group.

Fig 6: CFA induced in PHF 2 group.



Fig 7: CFA induced in PHF 3.

Effect on Body Weight

Disease control group shows significant reduction ($p < 0.001$) in body weight throughout the study when compared to the Normal control group. Methotrexate and Polyherbal suspension (medium and high doses) groups significantly prevented body weight loss, especially at 400 mg/kg ($p < 0.001$ by Day 28). 100 mg/kg dose demonstrated mild protective effects, becoming statistically significant from Day 13 onwards. Dose-dependent improvement is evident with increasing doses of the polyherbal suspension. Effect on body weight enlisted in table 11

Effect on Paw Volume using Digital Plethysmometer

Disease control group showed highly significant increase in paw volume compared to normal control ($p < 0.001$). Methotrexate and Polyherbal suspension (dose-dependent) reduced paw volume significantly from Day 13 onwards.

400 mg/kg dose produced the most pronounced anti-arthritic effect, showing very highly significant reduction ($p < 0.001$) by Day 18 onwards. Effect on paw volume enlisted in table 12

Effect on Locomotor activity using Actophotometer

CFA-induced arthritis caused a significant reduction in locomotor activity in the disease control group ($p < 0.001$) compared to the normal control. Methotrexate and polyherbal suspension groups showed significant dose-dependent improvement in locomotor activity from Day 13 onward. The 400 mg/kg dose showed very high significance by Day 23 and Day 28 ($p < 0.001$), restoring activity levels near normal, Shown in table 13

Effect on Paw thickness using Digital Vernier calliper

Disease control group showed significant progressive paw swelling compared to normal control ($p < 0.001$). Methotrexate and polyherbal groups exhibited progressive reduction in paw thickness starting Day 13. 400 mg/kg dose showed very highly significant reduction ($p < 0.001$) by Day 13 onwards, approaching normal values by Day 28, Shown in table 14.

Haematology Study Full blood count (FBC)

CFA-induced arthritis significantly increased WBC and platelets and decreased RBC and haemoglobin in the disease control group ($p < 0.001$ vs. Normal control). Methotrexate and polyherbal treatment groups restored haematological parameters dose-dependently, with 400 mg/kg producing effects comparable to the methotrexate group. High dose (400 mg/kg) achieved very highly significant reductions in WBC ($p < 0.001$) and improvements in RBC and haemoglobin ($p < 0.01$) by Day 28, Shown in table 15.

Rheumatoid Arthritis (RA) Factor test

CFA-induced arthritis significantly increased RF levels ($p < 0.001$) compared to normal rats. Methotrexate and polyherbal treatments significantly decreased RF levels compared to the disease control group. 400 mg/kg dose showed the most effective reduction ($p < 0.001$) in RF level, comparable to methotrexate, Shown in table 16.

C-reactive protein (CRP) Test

CFA-induced arthritis led to a very highly significant increase in CRP levels ($p < 0.001$) compared to the Normal control group. Methotrexate significantly reduced CRP levels ($p < 0.001$) compared to Disease Control. Polyherbal treatment groups showed dose-dependent

decreases in CRP, with 400 mg/kg producing very highly significant reductions ($p < 0.001$) approaching the efficacy of methotrexate, Shown in table 17.

Table 11: Effect of polyherbal formulation containing *Boswellia serrata* & *Vitex negundo* on Body Weight.

Treatment	Paw Volume in ml (Mean $\pm$ SD)					
	Day 0	Day 8	Day 13	Day 18	Day 23	Day 28
Normal control	0.39 $\pm$ 0.06	0.40 $\pm$ 0.06	0.41 $\pm$ 0.11	0.42 $\pm$ 0.09	0.42 $\pm$ 0.05	0.42 $\pm$ 0.04
Disease control	0.38 $\pm$ 0.06	1.19 $\pm$ 0.10	1.29 $\pm$ 0.04	1.39 $\pm$ 0.05	1.44 $\pm$ 0.03	1.49 $\pm$ 0.04
Methotrexate	0.39 $\pm$ 0.06	1.17 $\pm$ 0.06 ns	1.08 $\pm$ 0.07**	0.89 $\pm$ 0.05**	0.74 $\pm$ 0.04**	0.59 $\pm$ 0.03**
PHF 100mg/kg	0.39 $\pm$ 0.02	1.14 $\pm$ 0.04 ns	1.04 $\pm$ 0.08**	0.89 $\pm$ 0.07**	0.69 $\pm$ 0.07**	0.54 $\pm$ 0.04**
PHF 200mg/kg	0.38 $\pm$ 0.05	1.16 $\pm$ 0.05 ns	0.94 $\pm$ 0.08**	0.74 $\pm$ 0.05***	0.59 $\pm$ 0.04***	0.44 $\pm$ 0.05***
PHF 400mg/kg	0.39 $\pm$ 0.02	1.15 $\pm$ 0.06ns	0.84 $\pm$ 0.05**	0.59 $\pm$ 0.06***	0.44 $\pm$ 0.03***	0.25 $\pm$ 0.12***

All values are expressed as mean $\pm$ SD, n=6. All data are subjected to one Way ANOVA followed by Dunnet 't' test.

Different dose of Polyherbal Formulation and Standard Drug Treated are compared with Disease Control group

* $P > 0.05$, ** $P > 0.01$, *** $P > 0.001$, NS; Not Significant

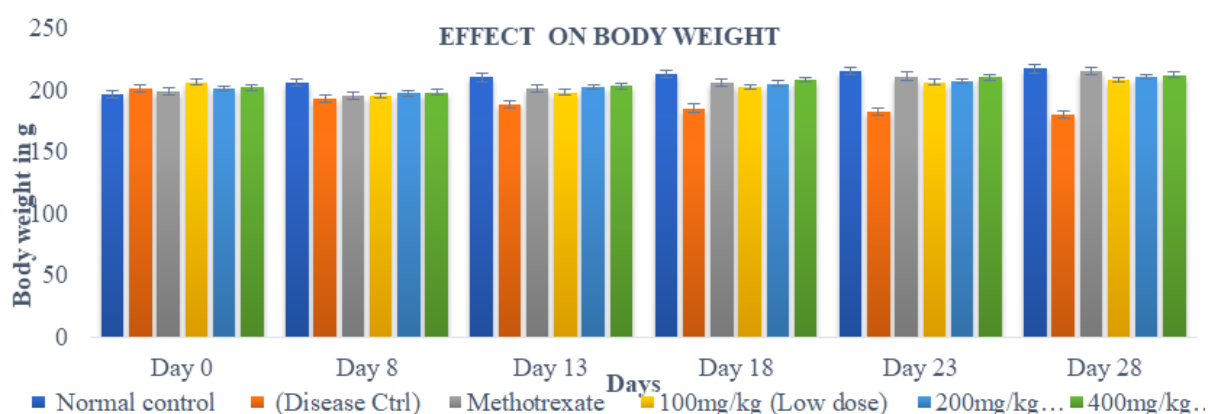


Fig 8: Effect of polyherbal formulation containing *Boswellia serrata* & *Vitex negundo* on body weight.

Table 12: Effect of polyherbal formulation containing *Boswellia serrata* & *Vitex negundo* on Paw Volume.

Treatment	Paw Volume in ml (Mean $\pm$ SD)					
	Day 0	Day 8	Day 13	Day 18	Day 23	Day 28
Normal control	0.39 $\pm$ 0.06	0.40 $\pm$ 0.06	0.41 $\pm$ 0.11	0.42 $\pm$ 0.09	0.42 $\pm$ 0.05	0.42 $\pm$ 0.04
Disease control	0.38 $\pm$ 0.06	1.19 $\pm$ 0.10	1.29 $\pm$ 0.04	1.39 $\pm$ 0.05	1.44 $\pm$ 0.03	1.49 $\pm$ 0.04
Methotrexate	0.39 $\pm$ 0.06	1.17 $\pm$ 0.06 ns	1.08 $\pm$ 0.07**	0.89 $\pm$ 0.05**	0.74 $\pm$ 0.04**	0.59 $\pm$ 0.03**
PHF 100mg/kg	0.39 $\pm$ 0.02	1.14 $\pm$ 0.04 ns	1.04 $\pm$ 0.08**	0.89 $\pm$ 0.07**	0.69 $\pm$ 0.07**	0.54 $\pm$ 0.04**
PHF 200mg/kg	0.38 $\pm$ 0.05	1.16 $\pm$ 0.05 ns	0.94 $\pm$ 0.08**	0.74 $\pm$ 0.05***	0.59 $\pm$ 0.04***	0.44 $\pm$ 0.05***
PHF 400mg/kg	0.39 $\pm$ 0.02	1.15 $\pm$ 0.06ns	0.84 $\pm$ 0.05**	0.59 $\pm$ 0.06***	0.44 $\pm$ 0.03***	0.25 $\pm$ 0.12***

All values are expressed as mean $\pm$ SD, n=6. All data are subjected to one Way ANOVA followed by Dunnet 't' test. Different dose of Polyherbal Formulation and Standard Drug Treated are compared with Disease Control group

*P>0.05, **P>0.01, ***P>0.001, NS; Not Significant

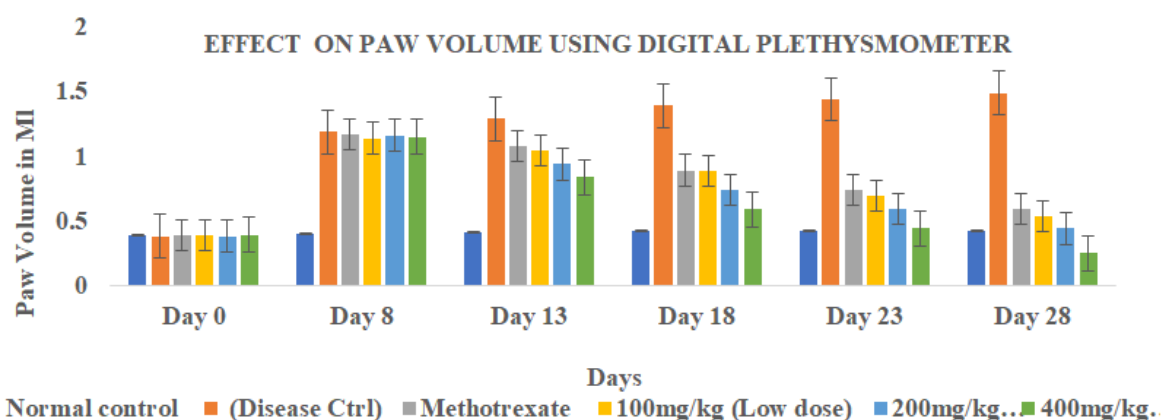


Fig 9: Effect of polyherbal formulation containing *Boswellia serrata* & *Vitex negundo* on paw volume.

Table 13: Effect of polyherbal formulation containing *Boswellia serrata* & *Vitex negundo* on Locomotor activity.

Treatment	Body weight in gram (Mean $\pm$ SD)					
	Day 0	Day 8	Day 13	Day 18	Day 23	Day 28
Normal control	196 $\pm$ 2.58	206 $\pm$ 2.59	210 $\pm$ 4.54	213 $\pm$ 3.69	215 $\pm$ 1.99	217 $\pm$ 1.86
Disease control	201 $\pm$ 2.29	193 $\pm$ 4.38	188 $\pm$ 1.75	185 $\pm$ 2.44	182 $\pm$ 1.53	180 $\pm$ 1.76
Methotrexate	199 $\pm$ 2.56	195 $\pm$ 2.43 ns	201 $\pm$ 3.15*	206 $\pm$ 2.26**	211 $\pm$ 1.64**	215 $\pm$ 1.34**
PHF 100mg/kg	206 $\pm$ 1.20	195 $\pm$ 1.70 ns	198 $\pm$ 3.20*	202 $\pm$ 2.94*	206 $\pm$ 2.82**	208 $\pm$ 1.85**

PHF 200mg/kg	201±2.18	197±2.26 ns	202±3.29**	205±2.05**	207±1.91**	210±2.02***
PHF 400mg/kg	202±0.88	198±2.40ns	203±2.14**	208±2.77**	210±1.55***	220±5.12***

All values are expressed as mean $\pm$ SD, n=6. All data are subjected to one Way ANOVA followed by Dunnet 't' test. Different dose of Polyherbal Formulation and Standard Drug Treated are compared with Disease Control group *P>0.05, **P>0.01, ***P>0.001, NS; Not Significant.

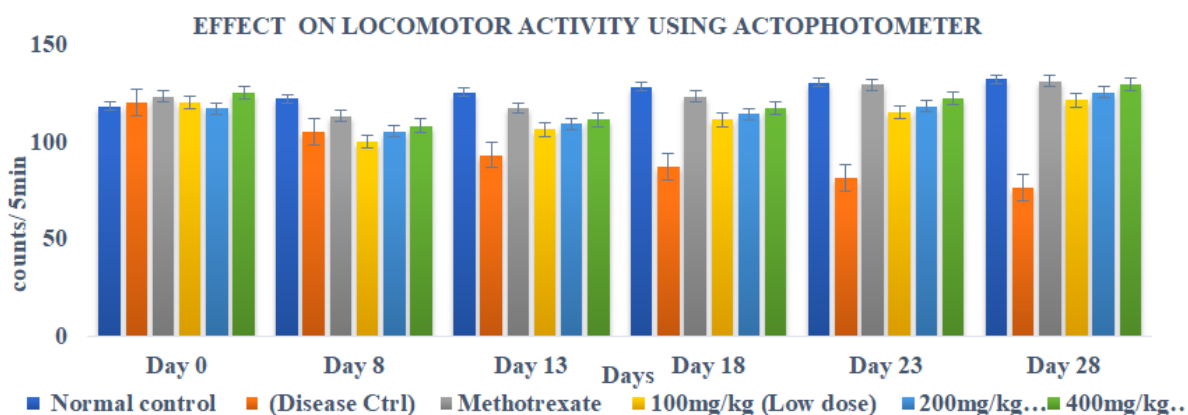


Fig 10: Effect of polyherbal formulation containing *Boswellia serrata* & *Vitex negundo* on locomotor activity.

Table 14: Effect of polyherbal formulation containing *Boswellia serrata* & *Vitex negundo* on Paw thickness.

Treatment	Locomotor activity in Score (Mean $\pm$ SD)					
	Day 0	Day 8	Day 13	Day 18	Day 23	Day 28
Normal control	118±7.22	122±1.28	125±3.0	128±1.87	130±3.30	132±2.62
Disease control	120±3.12	105±2.33	93±3.74	87±3.16	81±2.46	76±0.82
Methotrexate	123±3.24	113±2.54 ns	117±1.97**	123±2.42**	129±2.36**	131±3.52**
PHF 100mg/kg	120±2.58	100±3.22 ns	106±2.85**	111±4.72**	115±4.13**	121±3.17**
PHF 200mg/kg	117±1.72	105±2.79 ns	109±1.79**	114±1.35**	118±3.23**	125±2.06***
PHF 400mg/kg	125±1.83	108±2.71 ns	111±2.51**	117±2.12**	122±1.63***	129±1.63***

All values are expressed as mean $\pm$ SD, n=6. All data are subjected to one Way ANOVA followed by Dunnet 't' test.

Different dose of Polyherbal Formulation and Standard Drug Treated are compared with Disease Control group

*P>0.05, **P>0.01, ***P>0.001, NS; Not Significant

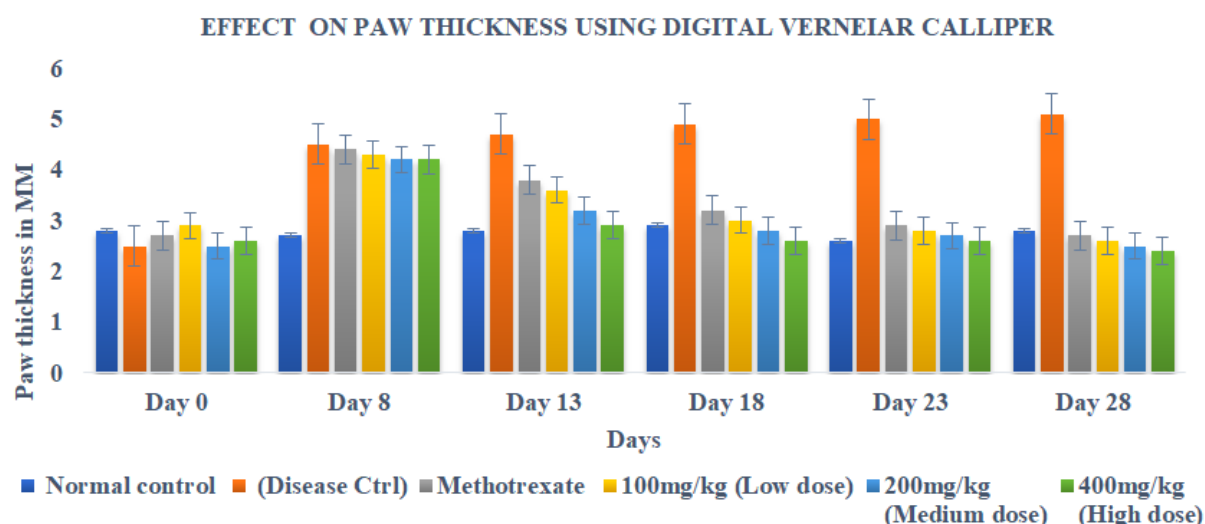


Fig 11: Effect of polyherbal formulation containing *Boswellia serrata* & *Vitex negundo* on paw thickness.

Table 15: Effect of polyherbal formulation containing *Boswellia serrata* & *Vitex negundo* on FBC counts,

Treatment	FBC (Mean $\pm$ SD)			
	WBC ($\times 10^3/\mu\text{L}$)	RBC ($\times 10^6/\mu\text{L}$)	Haemoglobin (g/dL)	PLT ($\times 10^3/\mu\text{L}$)
Normal control	8.5 $\pm$ 0.6	8.0 $\pm$ 0.4	15.0 $\pm$ 0.5	750 $\pm$ 60
Disease control	15.5 $\pm$ 1.2	6.5 $\pm$ 0.3	12.8 $\pm$ 0.6	950 $\pm$ 80
Methotrexate	9.2 $\pm$ 0.7***	7.8 $\pm$ 0.4**	14.20.6**	770 $\pm$ 65**
(PHF100mg/kg)	12.6 $\pm$ 0.8**	7.0 $\pm$ 0.3*	13.5 $\pm$ 0.4*	870 $\pm$ 70**
(PHF 200mg/kg)	10.5 $\pm$ 0.7***	7.4 $\pm$ 0.4**	14.0 $\pm$ 0.3**	800 $\pm$ 55**
(400mg/kg)	9 $\pm$ 0.5***	7.9 $\pm$ 0.4**	14.6 $\pm$ 0.5**	760 $\pm$ 50**

All values are expressed as mean $\pm$ SD, n=6. All data are subjected to one Way ANOVA followed by Dunnet 't' test.

Different dose of Polyherbal Formulation and Standard Drug Treated are compared with Disease Control group.

*P>0.05, **P>0.01, ***P>0.001, NS; Not Significant

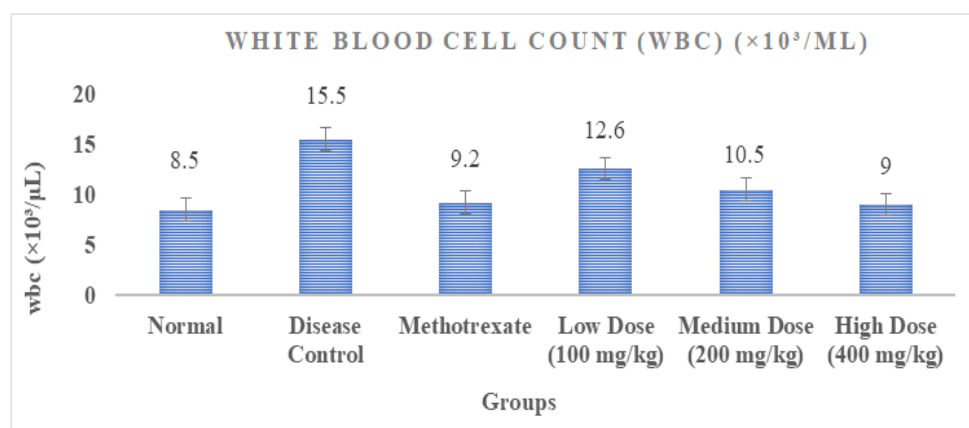


Fig 12: Effect of polyherbal formulation containing *Boswellia serrata* & *Vitex negundo* on WBC counts.

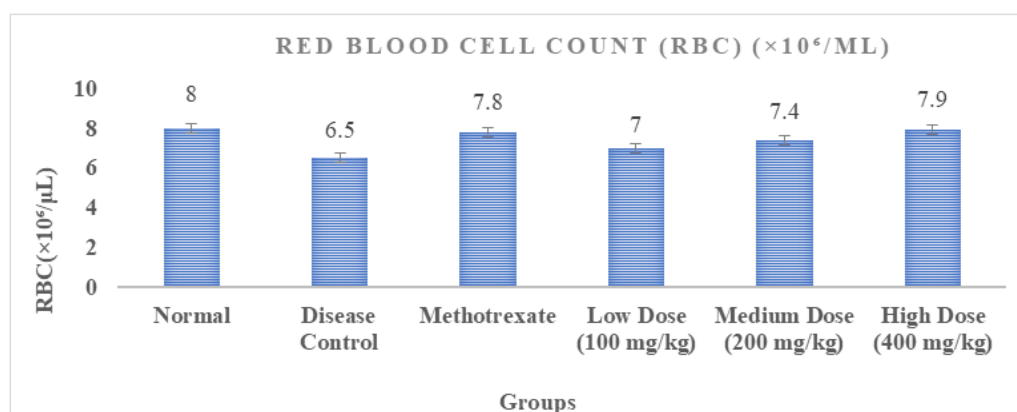


Fig 13: Effect of polyherbal formulation containing *Boswellia serrata* & *Vitex negundo* on RBC counts.

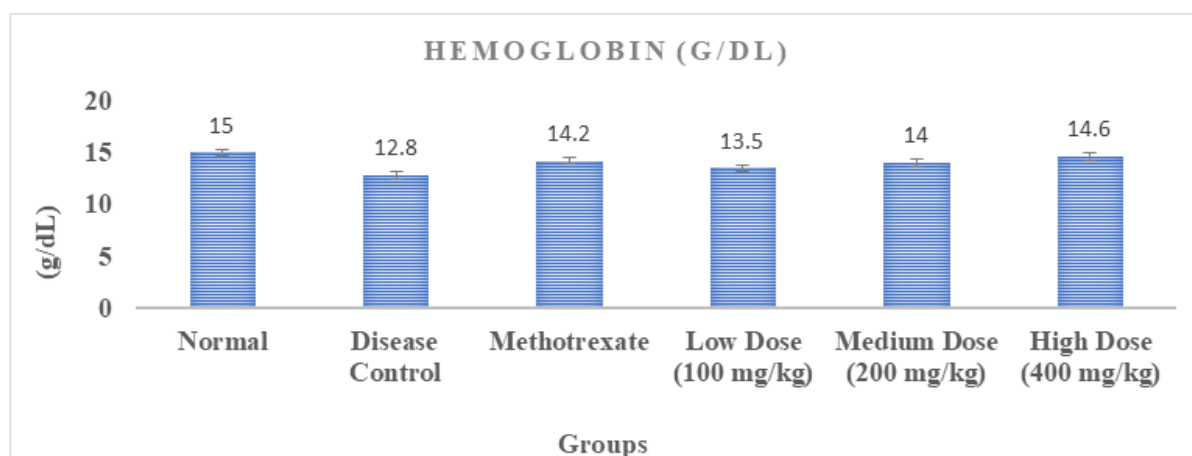


Fig 14: Effect of polyherbal formulation containing *Boswellia serrata* & *Vitex negundo* on Hb count.

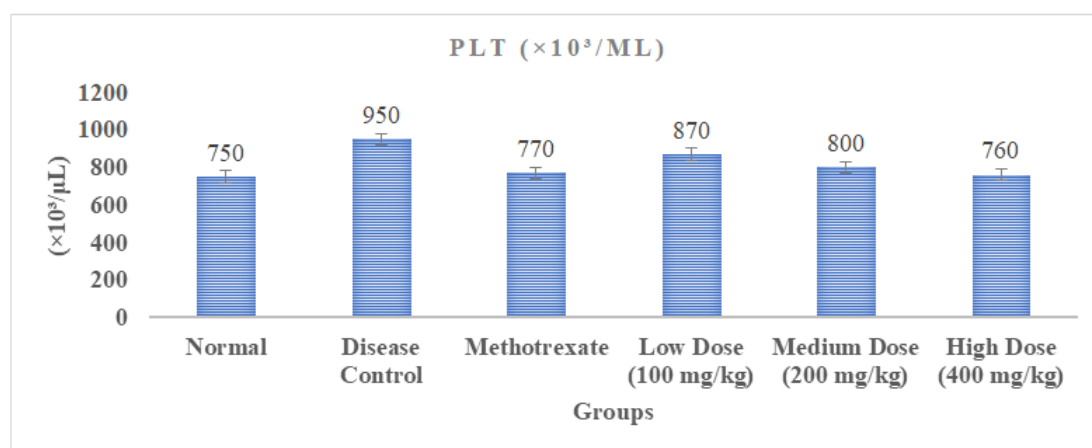


Fig. 15: Effect of polyherbal formulation containing *Boswellia serrata* & *Vitex negundo* on RA factor test Platelets count.

Table 16: Effect of polyherbal formulation containing *Boswellia serrata* & *Vitex negundo* on.

RA Factor (Mean $\pm$ SD)	
Treatment	RA Factor (IU/mL)
Normal control	6.5 $\pm$ 1.1
Disease Control (CFA)	24.3 $\pm$ 2.2
Methotrexate	9.1 $\pm$ 1.3***
100 mg/kg	17.5 $\pm$ 1.8**
200 mg/kg	12.0 $\pm$ 1.5**
400 mg/kg	8.2 $\pm$ 1.2***

All values are expressed as mean $\pm$ SD, n=6. All data are subjected to one Way ANOVA followed by Dunnet 't' test. Different dose of Polyherbal Formulation and Standard Drug Treated are compared with Disease Control group *P>0.05, **P>0.01, ***P>0.001, NS; Not Significant.

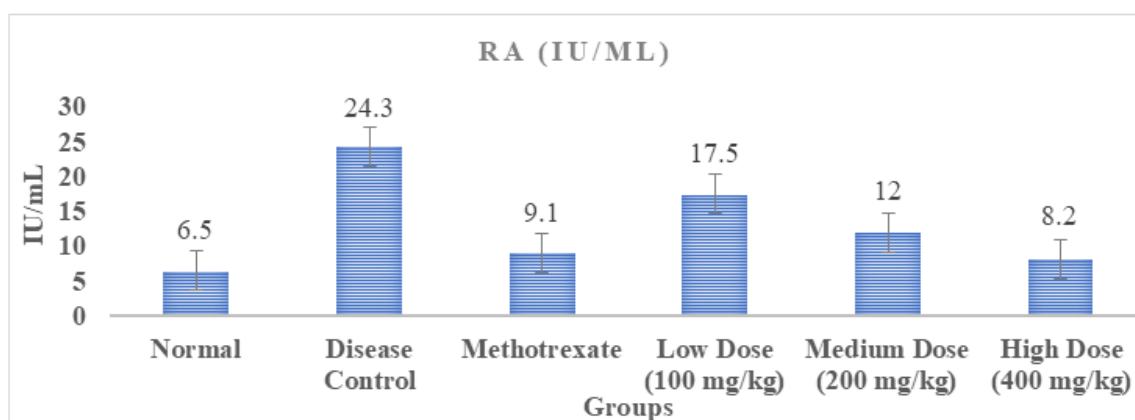


Fig 16: Effect of polyherbal formulation containing *Boswellia serrata* & *Vitex negundo* on RA factor test.

Table 17: Effect of polyherbal formulation containing *Boswellia serrata* & *Vitex negundo* on CRP test.

CRP (Mean $\pm$ SD)	
Treatment	CRP Level (mg/L)
Normal control	0.9 $\pm$ 0.2
Disease Control (CFA)	58.0 $\pm$ 4.5
Methotrexate	10.5 $\pm$ 1.2***
100 mg/kg	35.4 $\pm$ 2.9**
200 mg/kg	20.3 $\pm$ 2.4**
400 mg/kg	11.2 $\pm$ 1.5***

All values are expressed as mean $\pm$ SD, n=6. All data are subjected to one Way ANOVA followed by Dunnet 't' test. Different dose of Polyherbal Formulation and Standard Drug Treated are compared with Disease Control group *P>0.05, **P>0.01, ***P>0.001, NS; Not Significant.

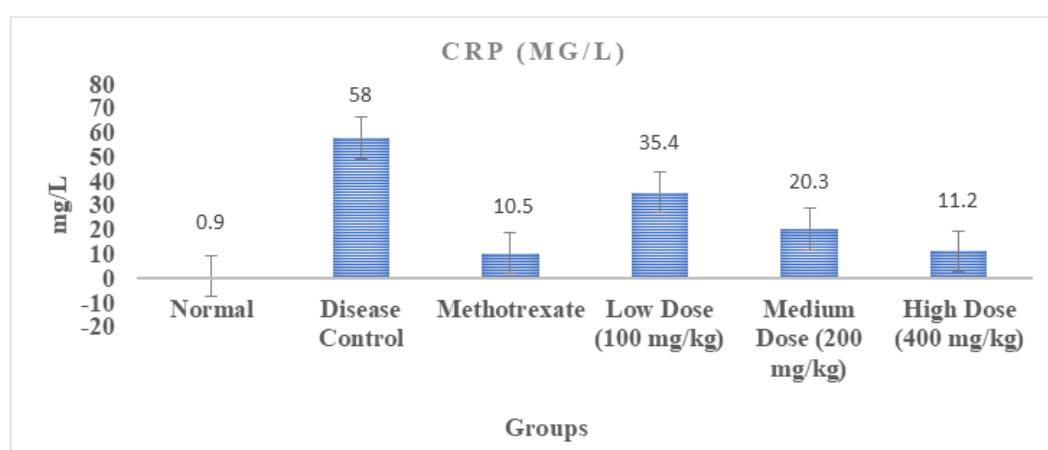


Fig 17: Effect of polyherbal formulation containing *Boswellia serrata* & *Vitex negundo* on C-reactive protein level.

DISCUSSION

Arthritis, particularly rheumatoid arthritis and osteoarthritis, is a debilitating inflammatory disorder that progressively damages joint cartilage and bone, significantly impairing daily life activities.^[20] While conventional treatments provide symptomatic relief, they are frequently accompanied by adverse effects, leading to a growing interest in herbal alternatives with better safety profiles. This study explored the *in vivo* anti-arthritic potential of *Vitex negundo* and *Boswellia serrata*, both well-established in Ayurvedic medicine for their anti-inflammatory, analgesic, and immunomodulatory activities.^[21]

Phytochemical screening of the plant extracts confirmed the presence of bioactive

compounds such as alkaloids, terpenoids all of which are known for their pharmacological actions against inflammation and oxidative stress. The results were positive, confirming the therapeutic basis of the herbal extracts used in the study. The anti-arthritic activity was assessed using animal models of arthritis, specifically induced through Freund's Complete Adjuvant (FCA).^[18] Experimental groups treated with the herbal extracts showed a marked reduction in paw edema, joint swelling, and improved locomotor activity compared to the disease control group. These improvements were quantitatively evaluated using digital plethysmometer for paw volume, vernier caliper for joint diameter, and actophotometer for locomotor performance, ensuring objective measurement of therapeutic response. A significant observation during the treatment period was the progressive increase in body weight of the treated animals, in contrast to the weight loss commonly observed in arthritic control groups.^[22] This weight gain correlated positively with the administered dosage form, suggesting not only symptomatic improvement but also enhanced overall health and nutritional status of the animals. It supports the systemic benefits of the polyherbal formulation beyond joint protection. Additionally, biochemical markers such as C-reactive protein (CRP) and rheumatoid factor (RF) were significantly reduced in treated groups, reflecting systemic anti-inflammatory activity.^[23]

As discussed with my guide, this work marks an essential first step in bridging traditional knowledge with scientific validation. The use of multiple assessment tools and consistent therapeutic outcomes confirms the reliability of this polyherbal approach. Looking ahead, future work will aim to: Isolate and characterize individual phytoconstituents responsible for anti-arthritic effects Investigate underlying molecular pathways such as NF- κ B, COX-2, and TNF- α inhibition Develop standardized dosage forms for clinical use Conduct toxicity profiling, pharmacokinetic studies, and eventually human clinical trials. This novel combination of *Vitex negundo* and *Boswellia serrata* shows promise as a plant-based disease-modifying anti-rheumatic drug (DMARD), offering a safer, sustainable, and effective alternative for arthritis therapy.

CONCLUSION

The study thus demonstrated the anti-arthritic activity of polyherbal formulation were found to be effective in the functional recovery of the Rheumatoid arthritis. The extracts promote inflammatory closure of excision inflammatory as compared to control group. The result may be attributed to the phytoconstituents such as Vitexin and Boswellic acid present

in it which may be due to their enhanced inflammatory healing and provided scientific evidence to the arthritic medicinal futures of polyherbal formulations. The study concludes that the herbal extract *Vitex negundo* and Boswellic acid obtained from Nirgundi leaf and *Boswellia serrata* gum have anti-arthritic activity. Incorporation of herbal extracts into polyherbal formulation demonstrated ideal physiochemical characteristics.

These findings could justify the inclusion of this formulation in the management of arthritis. Polyherbal formulation containing *Vitex negundo* (Alkaloids Nlt 0.64%) and *Boswellia serrata* (Boswellic acid Nlt 85%) against complete adjuvant induced arthritis in rats. From the results observed in the current investigation, it may be concluded that the polyherbal formulation (100 mg/kg, 200 mg/kg and 400 mg/kg) can be employed as potential anti-arthritic formulation since it was active in the inflammation models and adjuvant. The significant change is also observed by the detailed study and interpretation of values obtained from study of behavioral parameters of the experimental animals which indicates the anti-arthritic activity of the formulation.

REFERENCE

1. Guo, Qiang, Yuxiang Wang, Dan Xu, Johannes Nossent, Nathan J. Pavlos, and Jiake Xu. "Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies." *Bone research*, 2018; 6(1): 15.
2. Shrivastava, Amit Kumar, and Aparna Pandey. "Inflammation and rheumatoid arthritis." *Journal of physiology and biochemistry*, 2013; 69: 335-347.
3. Macfarlane, Eugenie, Markus J. Seibel, and Hong Zhou. "Arthritis and the role of endogenous glucocorticoids." *Bone research*; 8(1): 33.
4. McMurray, John J., and Simon Stewart. "Epidemiology, aetiology, and prognosis of heart failure." *Heart*, 2000; 83(5): 596-602.
5. Lee, Kyung-Shik, Su-Hyun Bae, Seung Hwa Lee, Jungun Lee, and Dong Ryul Lee. "New reference data on bone mineral density and the prevalence of osteoporosis in Korean adults aged 50 years or older: the Korea National Health and Nutrition Examination Survey 2008-2010." *Journal of Korean medical science*, 2014; 29(11): 1514-1522.
6. Qualitative tests for preliminary phytochemical screening: A Junaid R Shaikh, Matsya Gandha Kishanrao Patil overview. March 2020 *International Journal of Chemical Studies*, 8(2): 603-608.
7. Ijajahamad S. Rukadikar, Amol A. Dixit, Dnyaneshwari T. Raykar, Rutuja R. Sakpal,

- Prasad S. Repal. Formulation and Evaluation of Herbal Suspension by Using Factorial Design *Journal of Drug Delivery & Therapeutics*, 2024; 14(8): 28-32.
8. Endris Muhie Hussen and Sisay Awoke Endalew, In vitro antioxidant and free radical scavenging activities of polar leaf extracts of *Vernonia amygdalina* Hussen and Endalew *BMC Complementary Medicine and Therapies*.
 9. Rakesh H. Nikam, Vaishali R. Undale, Amol A. Rasal, Dr. Ashok V. Bhosale, Acute & subacute toxicity studies of polyherbal anti-arthritic formulation *World Journal of Pharmaceutical Research*, 2(5): 1563-1576.
 10. Maya Krishna Gupta, Dr. Bhuwanendra Singh, Yash Pratap Singh, Rana anti-arthritic study of aqueous alcoholic extracts of *Nyctanthes arbor tristis*, *Vitex negundo*, *Boswellia serrata*, *Cyperus scariosus* and *Commiphora Mukul*, Sep - Oct – 2022; 9(5).
 11. Mohd Noh ASa, Tan DCb, A Review on Complete Freund's Adjuvant-Induced Arthritic Rat Model: Factors Leading to its Success *IMJM*, October 2022; 21(4).
 12. Newbould BB. Chemotherapy of arthritis induced in rats by mycobacterial adjuvant. *Br J Pharmacol Chemotherapy*, 1963; 21: 127-36.
 13. Nair V, Singh S, Gupta YK. Anti-arthritic and disease modifying activity of *Terminalia chebula* Retz. In experimental models. *J Pharm Pharmacol.*, 2010; 62: 1801-6.
 14. Surender Singh, Rohit Kumar, Hitesh Jain, Y. K. Gupta. Anti-inflammatory and antiarthritic activity of UNIM-301 (a polyherbal Unani formulation) in Wistar rats *Pharmacognosy Research*, April-June 2015; 7(2).
 15. ZHEN-ZHEN WANG, FEI LIU, YONG-FANG GONG, TIAN-YU HUANG, XIAO-MING ZHANG, AND XUE-YING HUANG Antiarthritic Effects of Sorafenib in Rats with Adjuvant-Induced Arthritis *THE ANATOMICAL RECORD* 00:00–00 (2018).
 16. Kiran Mashaal, Arham Shabbir, Muhammad Shahzad Amelioration of Rheumatoid Arthritis by *Fragaria nubicola* (Wild Strawberry) via Attenuation of Inflammatory Mediators in Sprague Dawley Rats. *Medicina*, 2023; 59: 1917.
 17. Ashish Singhai, Yusra Ahmad, Umesh Kumar Patil, Phyto-therapeutic Potential of *Aconitum ferox* Roots in CFA-induced Arthritis in Rat Model /*Indian Journal of Pharmaceutical Education and Research*, Oct-Dec, 2022; 56(4 Suppl).
 18. Roshni Patel, Shahrukh Kadri, Priyanshee Gohil, Amelioration of complete Freund's adjuvant-induced arthritis by *Calotropis procera* latex in rats. Patel et al. *Future J Pharm Sci.*, 2021; 7: 213.
 19. A. Ruckmani, Vinayak Meti, R. Vijayashree, R. Arunkumar Anti-rheumatoid activity of ethanolic extract of *Sesamum indicum* seed extract in Freund's complete adjuvant induced

- arthritis in Wistar albino rats *Journal of Traditional and Complementary Medicine*, 2018; 8: 377-386.
20. Moumita Mandal, Aparajita Dasgupta, Debashis Dutt Quantification of health-related quality of life among patients with rheumatoid arthritis: An institution-based study in Kolkata, West Bengal *Journal of Family Medicine and Primary Care* August 2020; 9(8).
21. Dr. K. M. Nadkarni's *Indian Materia Medica: With Ayurvedic, Unani-tibbi, Siddha, Allopathic, Homeopathic, Naturopathic & Home Remedies, Appendices & Indexes, Volume 2* Popular Prakashan, 1994 - Botany.
22. Min-Seok Kim 1, Seung-Ho Lee Anti-Inflammatory Effects of Step Electrical Stimulation on Complete Freund's Adjuvant (CFA) Induced Rheumatoid Arthritis Rats *J Nanosci Nanotechnology*, 2019 Oct 1; 19(10): 6546-6553. doi: 10.1166/jnn.2019.17077.
23. Aisha Mobashar, Arham Shabbir, Muhammad Shahzad, Preclinical Rodent Models of Arthritis and Acute Inflammation Indicate Immunomodulatory and Anti-Inflammatory Properties of *Juglans regia* Extracts *Hindawi Evidence-Based Complementary and Alternative Medicine*, 2022.