

Development, Characterization, and Evaluation of *Asystasia Gangetica*-Loaded Phytosomes Against Freund's Complete Adjuvant-Induced Rheumatoid Arthritis in Rats

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DOI: <https://doi.org/10.63001/tbs.2026.v21.i04.pp72-86>

KEYWORDS

Asystasia gangetica;
Phytosomes; Rheumatoid arthritis; Freund's Complete Adjuvant; Anti-arthritis activity; Anti-inflammatory activity; Antioxidant activity; Phospholipid delivery system; Wistar rats; Phytosomal formulation.

Received on: 15/09/2026

Revised on: 26/09/2026

Accepted on: 05/10/2026

Published on: 12/10/2026

Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disorder characterized by persistent joint inflammation, progressive cartilage and bone damage, and systemic inflammatory alterations. The present study aimed to develop, characterize, and evaluate *Asystasia gangetica*-loaded phytosomes for their anti-arthritis activity against Freund's Complete Adjuvant (FCA)-induced rheumatoid arthritis in rats. A hydroalcoholic extract of *A. gangetica* was incorporated into a phytosomal delivery system using the thin-film hydration method with soya lecithin and cholesterol. The formulation was optimized using Central Composite Design (CCD) based on particle size, polydispersity index (PDI), and zeta potential. The optimized formulation exhibited a particle size of 150 nm, PDI of 0.2109, and zeta potential of -15.86 mV. FTIR and DSC studies supported the compatibility and incorporation of the plant extract into the lipid matrix. The anti-arthritis activity was evaluated in FCA-induced arthritis in Wistar rats. Treatment with *A. gangetica* extract and its phytosomal formulation improved body weight and significantly reduced paw volume and arthritic score compared with the disease control group. The treatments also improved hematological parameters, including WBC, RBC, and hemoglobin, and reduced elevated serum ALT, AST, ALP, and CRP levels. Furthermore, the phytosomal formulation enhanced antioxidant status by increasing SOD and GSH levels and reducing MDA levels. Histopathological examination demonstrated marked protection of ankle joint architecture, with the phytosome-treated group showing nearly normal joint morphology. Overall, *A. gangetica*-loaded phytosomes demonstrated superior anti-arthritis, anti-inflammatory, and antioxidant effects compared with the conventional extract, with efficacy comparable to the standard treatment. These findings suggest that phytosomal delivery may enhance the therapeutic potential of *A. gangetica* in the management of rheumatoid arthritis.

1. INTRODUCTION

Rheumatoid arthritis (RA) is a long-term systemic autoimmune disease characterized by persistent inflammation of the synovial joints, which can progressively lead to cartilage and bone damage, joint deformities, and impaired physical function. It mainly involves the peripheral joints and is associated with the presence of autoantibodies, particularly rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA). These immune responses play an important role in sustaining inflammation and promoting joint tissue damage.¹ The global prevalence of RA is estimated to be approximately 0.24–1%, with women being affected more frequently than men. In India, the reported prevalence is approximately 0.28–0.7%.^{2,3}

The development and progression of rheumatoid arthritis involve complex interactions among immune cells and inflammatory mediators. Activated immune cells stimulate the production of

several pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF- α), interleukins (ILs), and interferon-gamma (IFN- γ). These inflammatory mediators contribute to abnormal proliferation of the synovial tissue, formation of pannus, degradation of articular cartilage, and progressive erosion of the underlying bone.⁴ Pharmacological management of RA commonly involves non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, and disease-modifying antirheumatic drugs (DMARDs). Although these medications can reduce disease activity and symptoms, their prolonged use may be associated with various adverse effects. Consequently, increasing attention has been directed toward naturally occurring bioactive compounds from medicinal plants as potential therapeutic options for the management of RA.^{5,6}

Herbal medicines have been an important component of traditional and complementary healthcare systems worldwide. The World

Health Organization (WHO) has reported that a substantial proportion of the global population relies on medicinal plants and herbal preparations for primary healthcare needs. Several medicinal plants and their bioactive constituents have demonstrated anti-inflammatory effects in experimental animal studies, suggesting their potential role in the management of inflammatory disorders such as rheumatoid arthritis (RA).⁷ Phytochemicals obtained from medicinal plants represent a wide variety of biologically active compounds with potential therapeutic applications. Compared with some synthetic agents, these naturally derived compounds are often investigated for their favorable safety profiles, affordability, and diverse pharmacological activities. Their ability to interact with multiple biological targets has also encouraged their investigation as potential sources for the discovery and development of new therapeutic agents.⁸

Asystasia Gangetica (L.) T. Anderson subsp. *micrantha* (Nees) Ensermu, belonging to the family *Acanthaceae*, is a rapidly growing herbaceous plant commonly found as a spreading groundcover. The plant generally attains a height of approximately 300–600 mm and possesses opposite, green, oval to broadly shaped leaves with rounded leaf bases. Previous pharmacological investigations have reported several biological activities of *A. Gangetica*, including analgesic and anti-inflammatory⁹, antihypertensive¹⁰, antiulcer¹¹, antibacterial¹², antioxidant¹³, and antiasthmatic effects. The therapeutic potential and traditional applications of this plant are considered to be associated with the diverse phytochemical constituents present in its various parts. In addition to its medicinal importance, *A. Gangetica* has been consumed as a food source during periods of food scarcity and has traditionally been employed in the management of various ailments and health conditions.¹⁴

Phytosomes are lipid-based delivery systems developed to improve the therapeutic performance of biologically active compounds obtained from medicinal plants. In this approach, plant-derived phytoconstituents are molecularly associated with phospholipids, particularly phosphatidylcholine, resulting in the formation of a phospholipid-phytoconstituent complex. The amphiphilic nature of phospholipids facilitates interaction with both aqueous and lipid environments, which can enhance the incorporation and transport of phytoconstituents across biological membranes. Consequently, phytosomal systems may improve membrane permeability, absorption, bioavailability, and overall therapeutic activity compared with conventional plant extracts. They can also provide protection to sensitive constituents against degradation and may contribute to improved stability and pharmacokinetic behavior. Thus, phytosome technology represents a potential approach for addressing the limited absorption and bioavailability of certain herbal constituents and for enhancing the therapeutic potential of plant-based formulations.¹⁵

The current study aims to assess the therapeutic potential of *Asystasia Gangetica* using a hydroalcoholic extract, as well as to formulate, characterise, and evaluate the efficacy of an *Asystasia gangetica*-loaded phytosome formulation against arthritic disease using Freund's Complete Adjuvant (FCA)-induced adjuvant arthritis in a rat model.

2. MATERIALS AND METHODS

Chemicals and Reagents

Asystasia Gangetica aerial extract (Active phytoconstituent) was prepared from authenticated plant material obtained and verified

by Durva Herbal Centre (authentication certificate attached). Freund's Complete Adjuvant (FCA) (analytical grade), used for induction of rheumatoid arthritis, was procured from a certified supplier. Soya lecithin (phosphatidylcholine) and cholesterol were used as lipid components for phytosome preparation and were of analytical grade. All other chemicals and reagents used in the study were of analytical grade and obtained from the institutional chemical store. HPLC-grade solvents including methanol and chloroform were used for formulation and analytical procedures. Distilled water was used throughout the experimental work.

Instrumentation and Equipment

Asystasia Gangetica extract-loaded phytosomes (AGP) were prepared using a rotary evaporator (Buchi Rotavapor R-300 equipped with Heating Bath B-300 Base and Vacuum Pump V-100). UV-visible spectrophotometric analysis was performed using Shimadzu UV-1800 and UV-1900 spectrophotometers with UV-Probe software. Particle size, polydispersity index (PDI), and zeta potential were determined using a Malvern Zetasizer Nano ZS90 (Malvern Instruments, UK). High-speed refrigerated centrifuge (Kubota, Japan) was used for separation processes. FT-IR spectra were recorded using an Agilent Cary 630 FTIR spectrophotometer.

Formulation of *Asystasia Gangetica* Loaded Phytosomes

Preparation (Thin-Film Hydration Method)

The *Asystasia Gangetica* extract-loaded phytosomes were prepared using the thin-film hydration method. Briefly, soya lecithin and cholesterol in varying ratios were dissolved in a chloroform:methanol mixture (2:1 v/v), followed by the addition of *Asystasia Gangetica* extract. The solvent was evaporated using a rotary evaporator under reduced pressure at 40 °C to form a thin lipid film, which was further dried in a desiccator to remove residual solvents. The dried film was then hydrated with distilled water and rotated to obtain a milky phytosomal suspension. Different formulations were prepared by varying the concentrations of soya lecithin and cholesterol, and their effects on vesicle characteristics were evaluated.¹⁶

Characterization and optimization of *Asystasia Gangetica* Loaded Phytosomes

Selection of Optimized Phytosomal Suspension

The phytosomal formulations prepared using Central Composite Design (CCD) were evaluated based on particle size, polydispersity index (PDI), and zeta potential. These parameters were considered critical quality attributes for optimization. The formulation showing the most desirable physicochemical characteristics was selected as the optimized phytosomal suspension and used for further characterization and pharmacological studies.¹⁷

Particle Size, Polydispersity Index (PDI), and Zeta Potential

The mean particle size, PDI, and zeta potential of *Asystasia gangetica*-loaded phytosomes were determined using dynamic light scattering (DLS) technique with a Malvern Zetasizer Nano ZS90. Samples were appropriately diluted with distilled water to avoid multiple scattering effects and placed in a cuvette. Measurements were carried out at a fixed scattering angle at 25 °C. Each sample was analyzed in triplicate, and average values were reported.¹⁸

Encapsulation Efficiency (% EE)

The encapsulation efficiency (%EE) of the *Asystasia gangetica*-loaded phytosomes was determined using UV-visible spectrophotometry. A calibration curve of the extract was prepared

by measuring the absorbance of standard solutions at the λ_{max} of the extract. The phytosomal suspension was centrifuged at 12,000–15,000 rpm for 60 minutes, and the supernatant containing the untrapped extract was collected and analyzed spectrophotometrically. The amount of free extract was quantified using the calibration curve, and the encapsulation efficiency was calculated using the following equation:

$$\text{EE (\%)} = \frac{W_{\text{total}} - W_{\text{free}}}{W_{\text{total}}} \times 100$$

where W_{total} is the total amount of extract used and W_{free} is the amount of untrapped extract present in the supernatant.¹⁹

Fourier Transform Infrared (FTIR) Spectroscopy

Fourier Transform Infrared (FT-IR) spectroscopy was employed to investigate potential drug–excipient interactions and to assess the compatibility of the formulation components. The spectra of pure extract, soy lecithin, and cholesterol were recorded using the potassium bromide (KBr) pellet method. Briefly, each dried sample was finely triturated with spectroscopic-grade KBr and compressed into transparent pellets under hydraulic pressure. For the optimized phytosomal formulation, a small quantity of the dried phytosomal sample was similarly mixed with KBr and pelletized for analysis. All samples were scanned over the spectral range of 4000–400 cm^{-1} using an Agilent Cary 630 FTIR spectrometer. The obtained spectra of pure drug, individual excipients, and the optimized phytosomal formulation were compared to identify any significant shifts, disappearance, or broadening of characteristic peaks, which could indicate possible chemical interactions or incompatibility. The absence of major spectral changes confirmed the compatibility of extract with the selected lipid components. FT-IR analysis was carried out at Ashta's Annasaheb Dange College of B. Pharmacy.²⁰

Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) was employed to evaluate the thermal behavior and compatibility of the plant extract and its formulation. The analysis was performed using a TA Instruments calorimeter. Approximately 2 ± 0.1 mg of accurately weighed sample (extract and formulation) was placed in sealed aluminium crucibles, while an empty aluminium pan served as the reference. All measurements were conducted under a nitrogen atmosphere with a constant flow rate of 50 mL min^{-1} to maintain an inert environment. The samples were subjected to a heating program from 25°C to 300°C at a controlled heating rate of 10°C min^{-1} . Thermograms obtained were analyzed using TA Instruments software to determine phase transitions such as melting point, crystallization and possible interactions between components.²¹

In Vivo Anti-Arthritic Activity Study

Healthy adult Wistar rats (150–250 g) were procured from the animal house facility of KLE College of Pharmacy, Belagavi. Animals were acclimatized for one week under standard laboratory conditions, maintained at a temperature of $24 \pm 1^\circ\text{C}$, relative humidity of $45 \pm 5\%$, and a 12 h light/dark cycle, with free access to standard pellet diet and water. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of KLE College of Pharmacy, Belagavi, in accordance with the guidelines of Committee for the Control and Supervision of Experiments on Animals (CCSEA).

FCA model

A total of 36 animals were randomly divided into six groups ($n = 6$ per group). Rheumatoid arthritis was induced by a single subcutaneous injection of Freund's Complete Adjuvant (FCA) (0.1 mL) into the sub-plantar region of the left hind paw on day 0.²² Group I served as the normal control and received standard food and water, while Group II received a single subcutaneous injection of Freund's Complete Adjuvant (FCA, 0.1 mL) into the right hind paw to induce rheumatoid arthritis. Group III received FCA along with Indomethacin (10 mg/kg, p.o.) as the standard treatment. Groups IV and V received FCA along with hydroalcoholic extract of *Asystasia Gangetica* at doses of 200 and 400 mg/kg, respectively, for 21 days. Group VI received FCA along with *Asystasia Gangetica* phytosome formulation (200 mg/kg, p.o.) for 21 days.²³

At the end of the treatment period, the animals were fasted overnight and anesthetized, and blood samples were collected through retro-orbital plexus puncture. Blood samples were first analyzed for hematological parameters, including red blood cell (RBC) count, white blood cell (WBC) count, and hemoglobin (Hb) concentration, using an automated hematology analyzer.

The remaining blood samples were centrifuged at 3000 rpm for 10 minutes to obtain serum. The separated serum was used for the estimation of biochemical and inflammatory biomarkers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and C-reactive protein (CRP), using AGD clinipak diagnostic kit according to the manufacturer's instructions.

Following blood collection, the animals were sacrificed, and the ankle joints along with the surrounding skeletal muscle tissues were excised and washed with ice-cold normal saline. The skeletal muscle tissues were homogenized in phosphate buffer (pH 7.4) for the estimation of oxidative stress markers, including superoxide dismutase (SOD), reduced glutathione (GSH), malondialdehyde (MDA), and catalase.

Finally, the ankle joint tissues were preserved in 10% formalin for histopathological examination. Histological evaluation was performed using Hematoxylin and Eosin (H&E) staining to assess rheumatoid arthritis-induced tissue alterations and the protective effects of *Asystasia Gangetica* extract and its phytosomal formulation.

Statistical Analysis

Each and every result was displayed as Mean $\pm$ SEM. Using GraphPad Prism version 8.02, the data were subjected to a one-way ANOVA, two-way ANOVA and Tukey's Multiple Comparison Test to determine statistical significance; a value of $P < 0.001$ was deemed significant.

3. RESULTS

Characterization and optimization of *Asystasia Gangetica* Loaded Phytosomes

The phytosomal formulation was optimized using Central Composite Design (CCD) to evaluate the effect of soy lecithin (A) and cholesterol (B) on particle size and zeta potential. A total of nine experimental runs were generated and analyzed. The observed particle size ranged from 150 nm to 207.4 nm, whereas zeta potential values ranged from -15.86 mV to -58.27 mV.

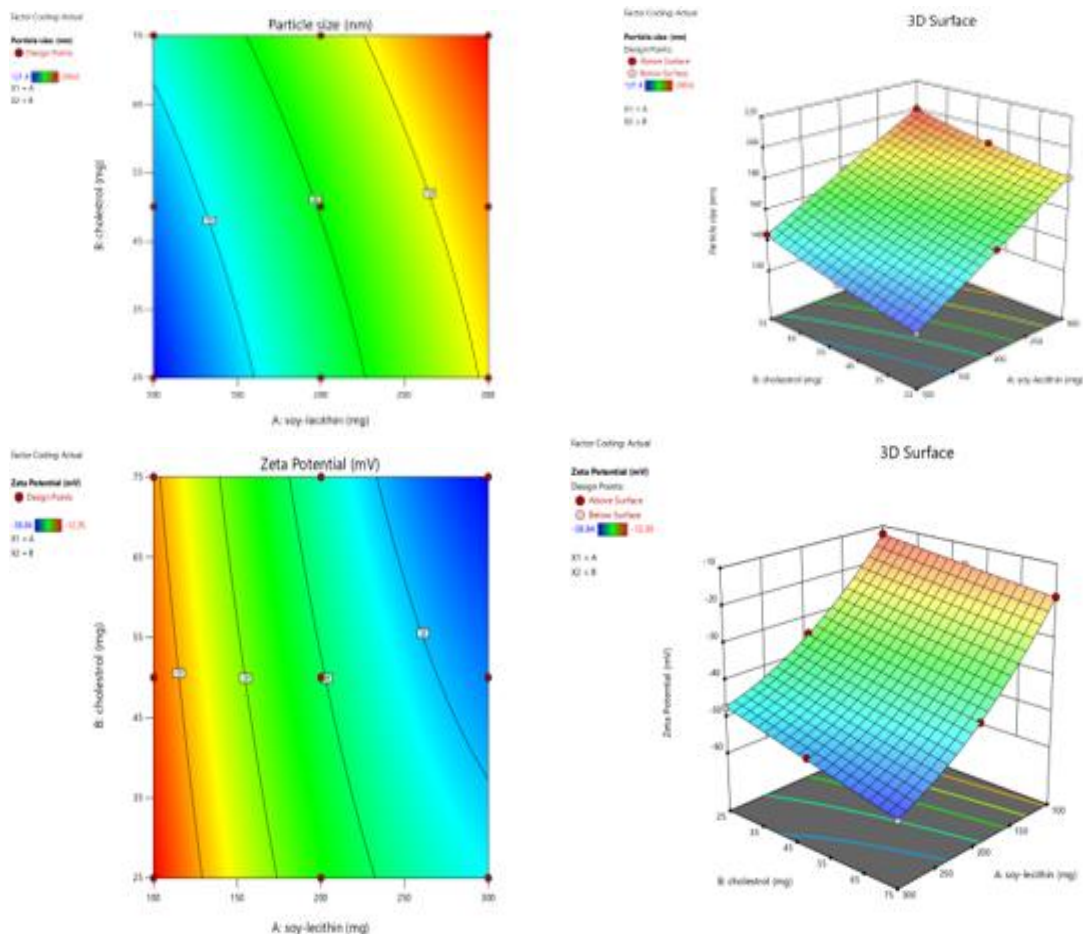


Figure 1: Experimental design matrix and response surface plots illustrating the effect of Soy Lecithin (X₁) and Cholesterol (X₂) on the characterization parameters of phytosomal formulation optimized using the Box–Behnken design. Upper panels represent the effect on vesicle size, while lower panels represent the effect on zeta potential through contour and 3D surface plots

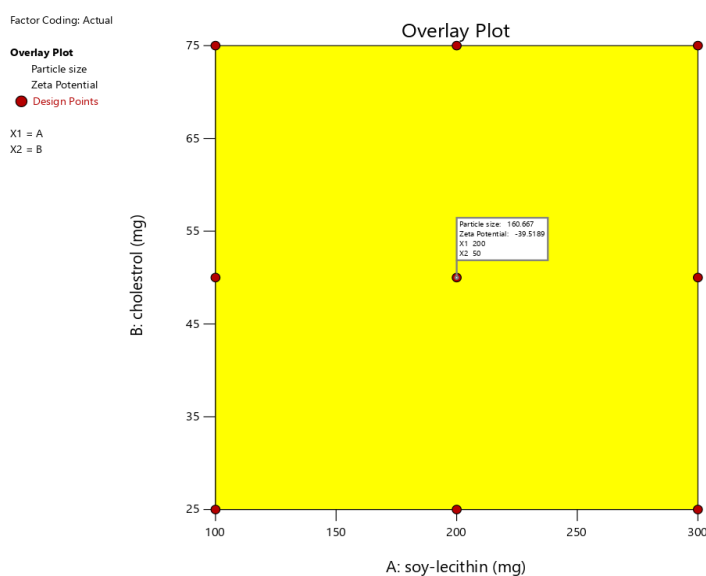
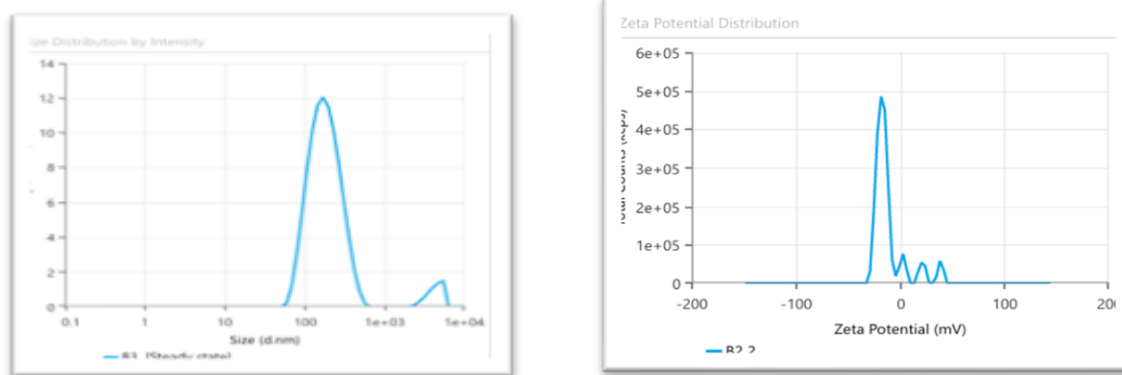


Figure 2: Overlay plot of optimized batch of phytosomes

Selection of Optimized Phytosomal Suspension based on Particle Size, Polydispersity Index (PDI), and Zeta Potential

Among all the prepared formulations, F1 was identified as the optimized batch due to its desirable physicochemical characteristics. The formulation exhibited a particle size of 150 nm, indicating successful formation of nanosized phytosomal vesicles. The PDI value of 0.2109 suggested a uniform and

homogeneous particle size distribution. Furthermore, the zeta potential of -15.86 mV indicated satisfactory physical stability of the formulation. The optimized formulation met the desired criteria for particle size, homogeneity, and stability. Therefore, F1 was selected as the optimized phytosomal suspension for further characterization and pharmacological evaluation.

**Figure 3:** Particle Size and Zeta potential Distribution of Optimized F1 formulation

Fourier Transform Infrared (FTIR) Spectroscopy

The extract shows phenolic O–H, ester C=O, and aromatic C=C peaks, confirming phytochemical presence. Cholesterol displays hydroxyl and steroid skeletal vibrations. Soya lecithin highlights ester and phosphate groups typical of phospholipids. The

formulation integrates all signals, with broad O–H and ester/phosphate peaks, indicating successful incorporation of extract into the lipid matrix via hydrogen bonding and lipid packing.

Functional Group (Assignment)	EXTRACT	CHOLESTEROL	SOYA LECITHIN	FORMULATION
O–H stretching (~ 3400 cm^{-1})	3303 cm^{-1}	3321 cm^{-1}	3322 cm^{-1}	3302 – 3507 cm^{-1} (broad)
Aliphatic C–H stretching (2920 – 2850 cm^{-1})	$2926, 2855$ cm^{-1}	$2922, 2855$ cm^{-1}	$2926, 2857$ cm^{-1}	$2894, 2771$ cm^{-1}
Ester C=O stretching (~ 1735 cm^{-1})	1726 cm^{-1}	1734 cm^{-1}	1742 cm^{-1}	1712 – 1736 cm^{-1}
Aromatic / C=C stretching (1650 – 1450 cm^{-1})	1619 cm^{-1}	1648 – 1592 cm^{-1}	$1599, 1517$ – 1455 cm^{-1}	1628 – 1493 cm^{-1}
C–H bending / Ester C–O stretching (1400 – 1000 cm^{-1})	$1396, 1337, 1026$ cm^{-1}	$1444, 1332, 1158, 1038$ cm^{-1}	$1375, 1235, 1161, 1017$ cm^{-1}	$1403, 1329$ – $1272, 1223, 1056$ cm^{-1}
Phosphate C–O stretching (1000 – 1100 cm^{-1})	1026 cm^{-1} (overlap)	1038 cm^{-1}	1235 – 1161 cm^{-1} (P=O, C–O–P)	$1223, 1056$ cm^{-1}
Fingerprint region (<900 cm^{-1})	898 – 777 cm^{-1}	$902, 828, 684$ cm^{-1}	$830, 697$ cm^{-1}	$909, 816$ – $770, 703$ – 652 cm^{-1}

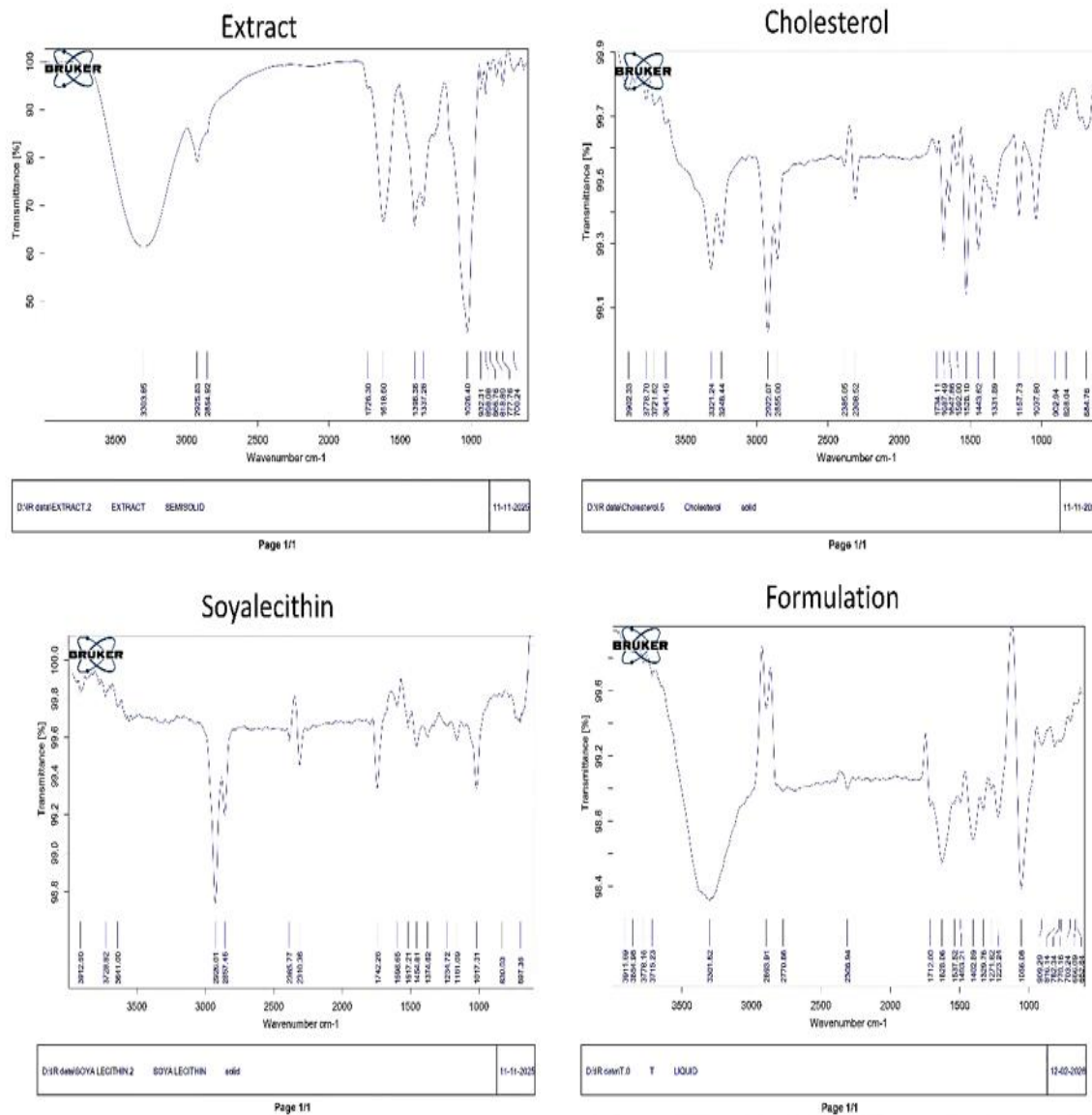


Figure 4: FTIR spectra of extract, cholesterol, soya lecithin, and formulation

In Vivo Anti-Arthritic Activity

Effect of hydroalcoholic extract of *Asystasia Gangetica* on body weight in FCA induced rheumatoid arthritis in rats

On days 1, 7, 14, and 21, the body weights of animals in different groups were measured. The disease control group's body weight change decreased steadily from day 1 to day 21, indicating that the status of rheumatoid arthritis is severe. However, treatment with *Asystasia Gangetica* at

doses of 200 mg/kg, 400 mg/kg, and its phytosomal formulation resulted in a significant increase in body weight by day 21 compared to the disease control group. Throughout the experiment, the typical control group's weight gradually increased. Body weight changes were observed to be improved when compared to the disease control group, but the phytosome treated group experienced the greatest rise, achieving near-normal values by day 21.

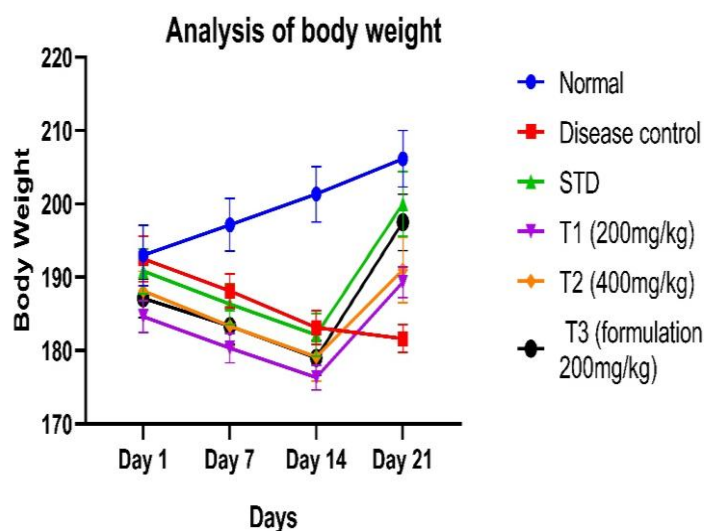


Figure 5: Effect of hydroalcoholic extract of *Asystasia Gangetica* on body weight in FCA induced rheumatoid in rats.

2. Effect of Hydroalcoholic Extract of *Asystasia Gangetica* on Paw Volume and Arthritic Score in FCA-Induced Rheumatoid Arthritis in Rats

The disease control group showed a significant increase in paw volume and arthritic score ($p < 0.001$) compared to the normal control group, confirming the successful induction of rheumatoid arthritis by Freund's Complete Adjuvant (FCA). Paw edema progressively increased from day 1 to day 21 in the disease control group, indicating persistent inflammation. Treatment with *Asystasia Gangetica* extract at 200 mg/kg and 400 mg/kg showed a reduction in paw volume and arthritic score compared to the

disease control group, with the 400 mg/kg dose producing greater improvement than the 200 mg/kg dose. The phytosomal formulation significantly reduced paw volume and arthritic score compared to the disease control group ($p < 0.001$). The reduction was dose-dependent, with the phytosomal formulation exhibiting the greatest anti-arthritic effect, followed by the high-dose extract. Indomethacin also produced a significant decrease in paw edema and arthritic score. These findings suggest that *Asystasia Gangetica*, particularly in phytosomal form, effectively attenuates inflammation and joint damage associated with rheumatoid arthritis.

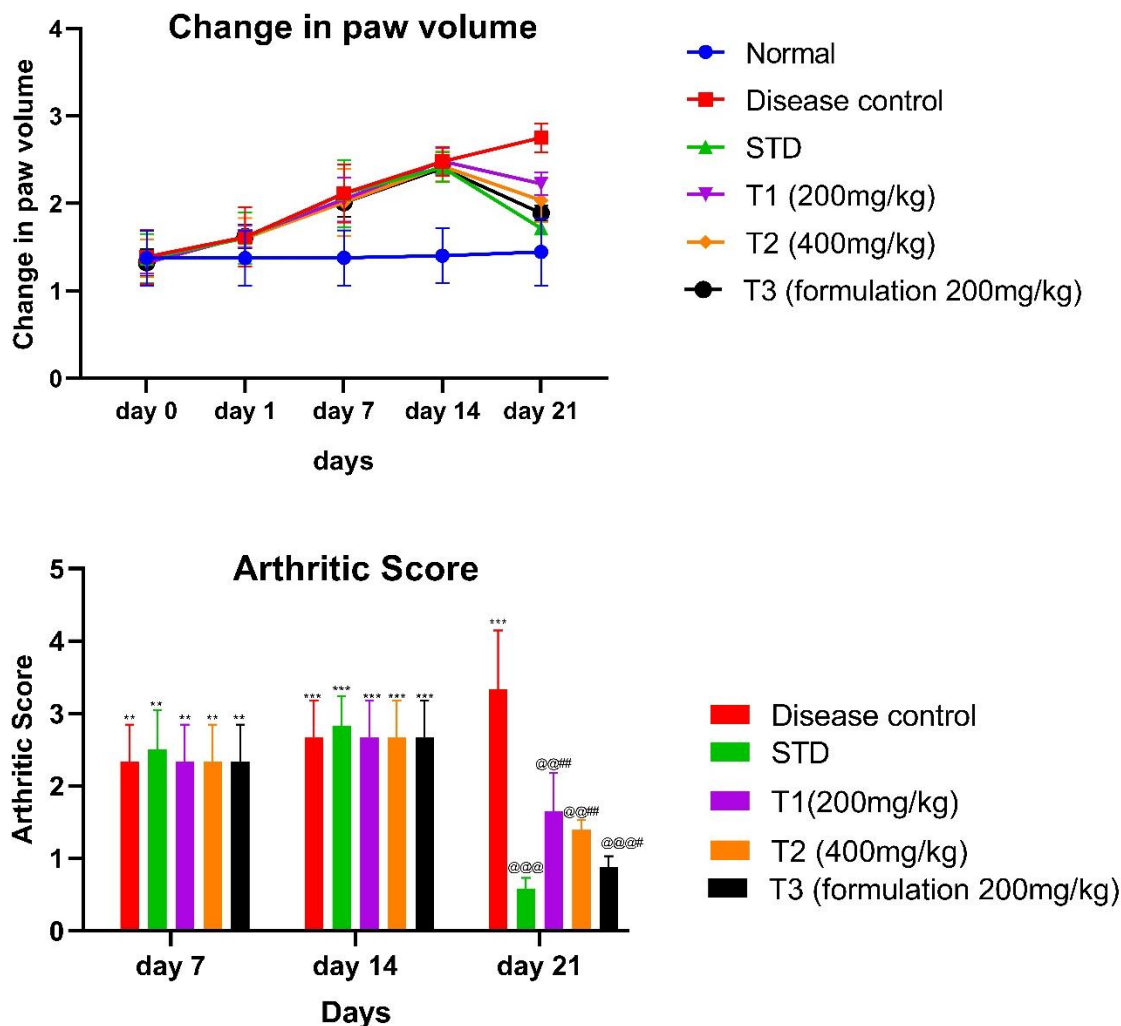


Figure 6: Effect of Hydroalcoholic Extract of *Asystasia Gangetica* on Paw Volume and Arthritic Score in FCA-Induced Rheumatoid Arthritis in Rats

All data are expressed as Mean $\pm$ SEM (n = 6 per group). Statistical analysis was performed using two-way Analysis of Variance (Two-way ANOVA) followed by Tukey's multiple comparison test. *p < 0.05, **p < 0.01, ***p < 0.001 compared with the Normal Control group; @p < 0.05, @@p < 0.01, @@@p < 0.001 compared with the Disease Control group; #p < 0.05, ###p < 0.01, ####p < 0.001 compared with the Standard Control group.

3. Effect of hydroalcoholic extract of *Asystasia Gangetica* on spleen weight in FCA induced rheumatoid arthritis in rats

In the FCA-induced rheumatoid arthritis model, the disease control group exhibited a significantly increased spleen weight ratio compared to the normal control group. Treatment with the hydroalcoholic extract of *Asystasia Gangetica* at 200 mg/kg and 400 mg/kg, as well as its phytosomal formulation, reduced the spleen weight ratio when compared with the disease control group. Phytosomal formulation, and standard treatment groups showed a more pronounced reduction (@@p < 0.01). Among the treatment groups, the phytosomal formulation demonstrated greater efficacy than the extract and exhibited a response comparable to that of the standard treatment group.

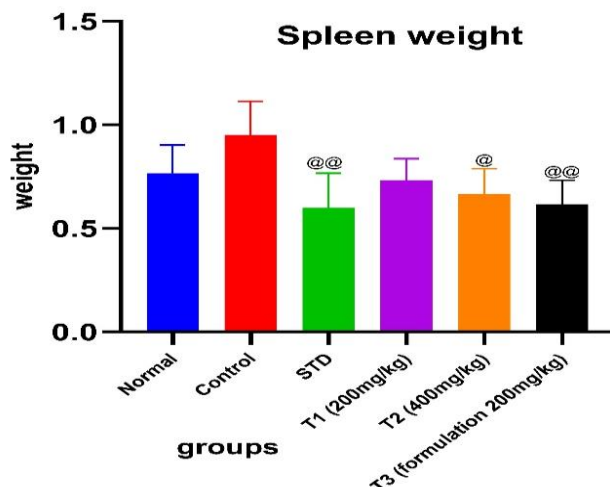


Figure 7: Effect of hydroalcoholic extract of *Asystasia Gangetica* on spleen weight in FCA induced rheumatoid in rats.

All data are expressed as Mean $\pm$ SEM (n = 6 per group). Statistical analysis was performed using one-way Analysis of Variance (ANOVA) followed by Tukey's multiple comparison test. @p < 0.05 and @@p < 0.01 compared with the Disease Control group.

4. Effect of hydroalcoholic extract of *Asystasia Gangetica* on haematological parameters in FCA induced rheumatoid arthritis in rats

The disease control group exhibited a significant increase in WBC count along with a marked decrease in RBC count and

haemoglobin (Hb) levels compared to the normal control group (p < 0.001), indicating systemic inflammation and anemia associated with rheumatoid arthritis. Treatment with *Asystasia Gangetica* extract at different doses and its phytosomal formulation significantly reduced WBC counts while restoring RBC and Hb levels toward normal values when compared with the disease control group. Among the treatment groups, the phytosomal formulation showed the most pronounced improvement in haematological parameters, followed by the high-dose extract group. The standard treatment also produced significant normalization of WBC, RBC, and Hb levels, demonstrating comparable efficacy to the phytosomal formulation.

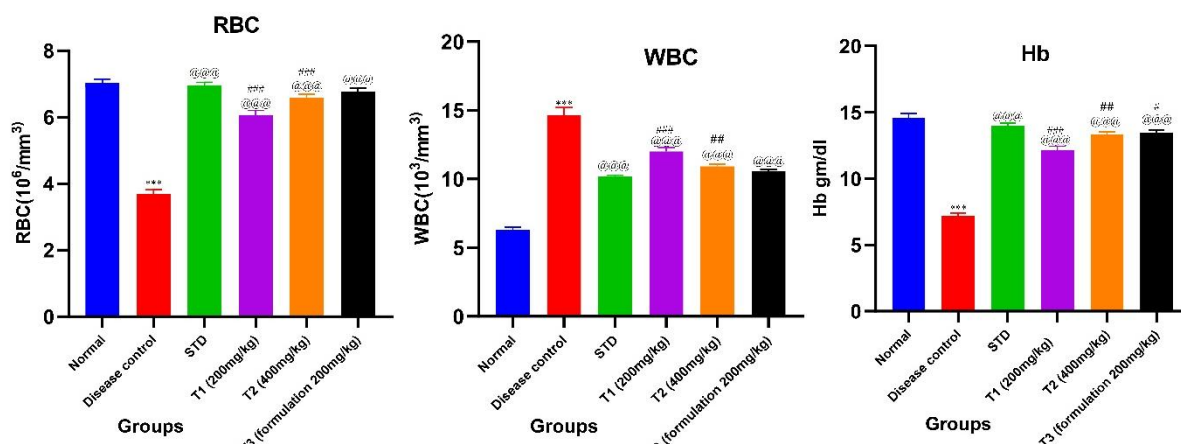


Figure 8: Effect of hydroalcoholic extract of *Asystasia Gangetica* on haematological parameters in FCA induced rheumatoid in rats.

All values are expressed as Mean $\pm$ SEM (n = 6 per group). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. *p < 0.05, **p < 0.01, ***p < 0.001 compared with the Normal Control group; @p < 0.05, @@p < 0.01, @@@p < 0.001 compared with the Disease Control group; #p < 0.05, ##p < 0.01, ###p < 0.001 compared with the Standard Treatment group.

Biochemical Parameters

Effect of hydroalcoholic extract of *Asystasia Gangetica* on serum biomarkers in FCA induced rheumatoid arthritis in rats

In the FCA-induced rheumatoid arthritis model, the disease control group exhibited significantly elevated serum levels of ALT, ALP, AST, and CRP (p < 0.001) compared to the normal control group,

indicating systemic inflammation, tissue injury, and liver dysfunction associated with rheumatoid arthritis. Treatment with the hydroalcoholic extract of *Asystasia Gangetica* at both dose levels and its phytosomal formulation significantly reduced the levels of these biomarkers ($p < 0.001$) when compared with the disease control group. Among the treatment groups, the

phytosomal formulation and high-dose extract demonstrated greater effectiveness in restoring biomarker levels toward normal values. The standard drug treatment also produced significant improvements, and no statistically significant difference was observed between the standard and treatment groups, indicating comparable therapeutic efficacy.

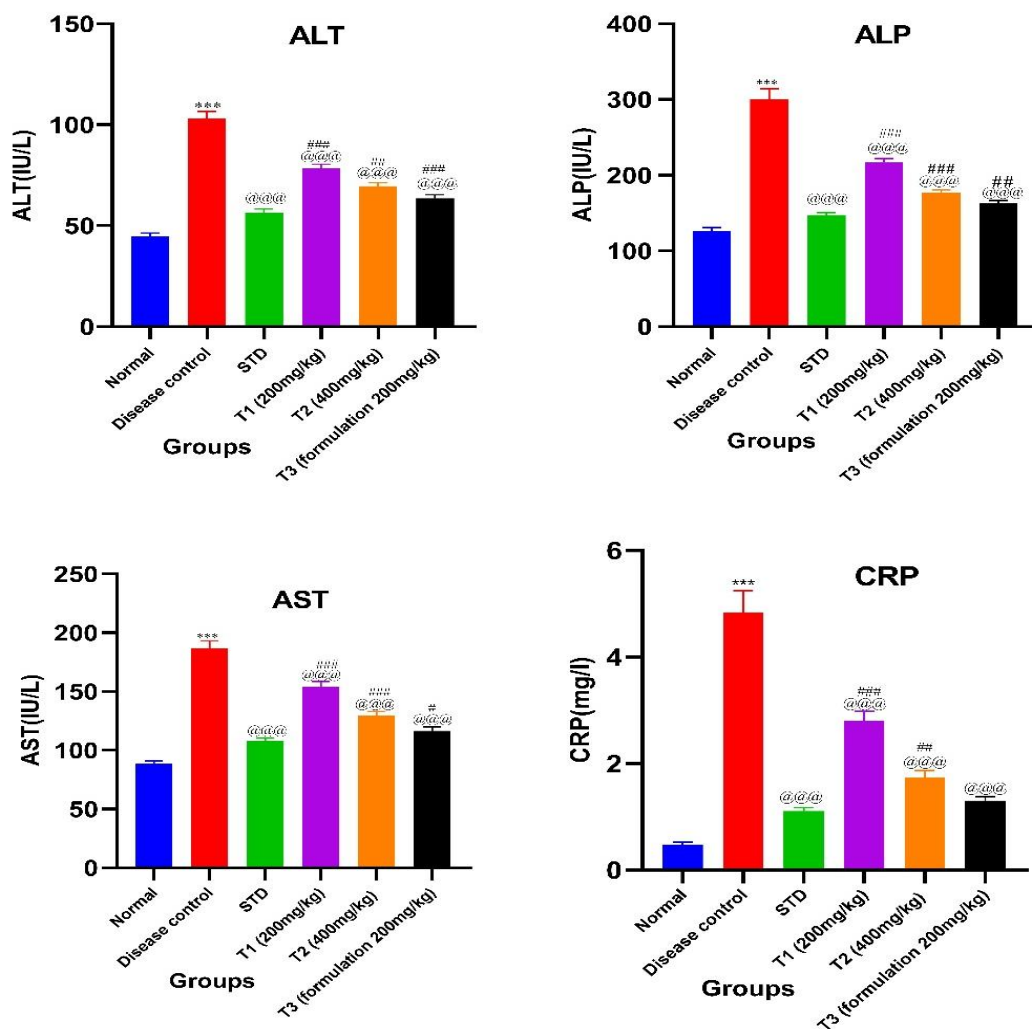


Figure 9: Effect of hydroalcoholic extract of *Asystasia Gangetica* on the serum biomarker enzymes in FCA induced rheumatoid in rats.

All values are expressed as Mean $\pm$ SEM ($n = 6$ per group). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with the Normal Control group; @ $p < 0.05$, @@ $p < 0.01$, @@@ $p < 0.001$ compared with the Disease Control group; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ compared with the Standard Treatment group.

In-vivo Antioxidant Assay

Effect of hydroalcoholic extract of *Asystasia Gangetica* on antioxidant enzymes in FCA induced rheumatoid arthritis in rats

A. Superoxide Dismutase (SOD)

SOD levels were considerably lower ($p < 0.001$) in the disease control group compared to the normal group. Administration of *Asystasia Gangetica* extracts at 200 mg/kg, 400 mg/kg, and in the form of a phytosome formulation resulted in a substantial increase ($p < 0.001$) in SOD levels compared to the disease control group. The phytosome formulation increased SOD levels more than the high-dose group, indicating a stronger antioxidant response. In addition, SOD levels increased significantly for the conventional medicines, but there was no significant difference between the treatment groups and the standard drug group.

B. Reduced Glutathione (GSH)

The disease control group showed a significant ($p < 0.001$) decrease in GSH compared to the normal group. At varied concentrations, *Asystasia Gangetica* extract significantly increased GSH levels ($p < 0.001$) compared to the disease control group. Phytosome formulation restored GSH levels better than other treatment groups, indicating antioxidant action. Standard drug group were found to increase GSH levels. However, no significant differences could be found between the standard and treatment groups.

C. Malondialdehyde (MDA)

MDA, an indication of lipid peroxidation, increased significantly ($p < 0.001$) in the disease control group compared to the normal group. The administration of *Asystasia Gangetica* extracts at doses of 200 mg/kg, 400 mg/kg, and the phytosome formulation significantly ($p < 0.001$) lowered MDA levels compared to the disease control group. Among the treatments, the phytosome formulation had the greatest reduction in MDA levels. The standard treatment significantly reduced MDA levels, and the effect of the phytosomal formulation was comparable to that of the standard treatment, with no significant difference between the groups.

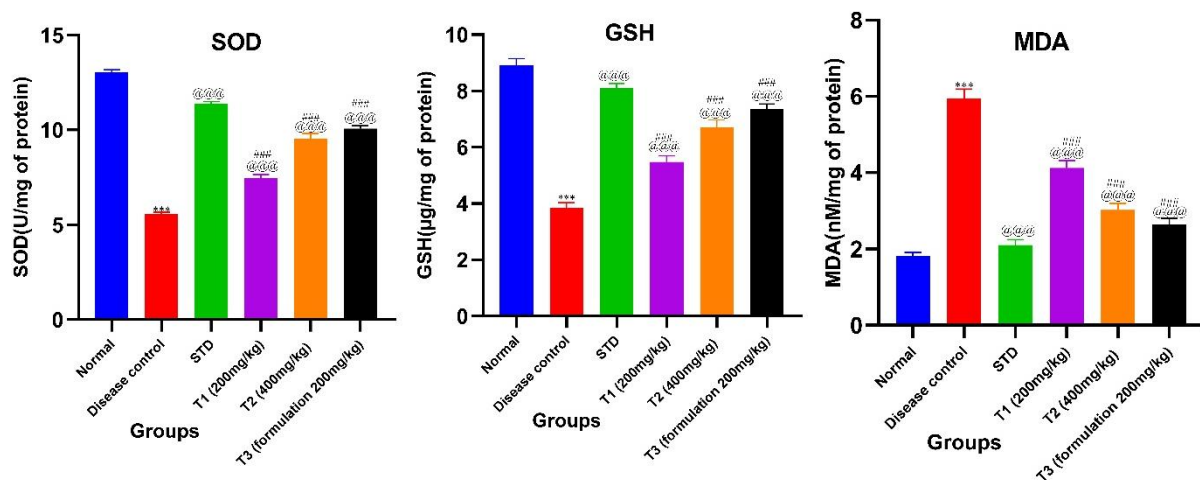


Figure 10: Effect of hydroalcoholic extract of *Asystasia Gangetica* on antioxidant parameters in FCA induced rheumatoid in rats.

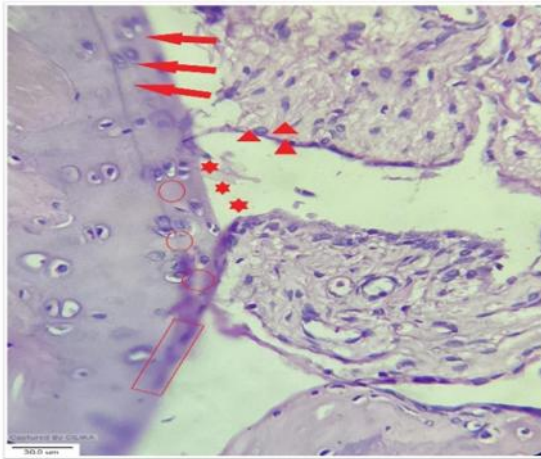
All values are expressed as Mean $\pm$ SEM ($n = 6$ per group). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with the Normal Control group; @ $p < 0.05$, @@ $p < 0.01$, @@@ $p < 0.001$ compared with the Disease Control group; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ compared with the Standard Treatment group.

Histopathology of ankle joint

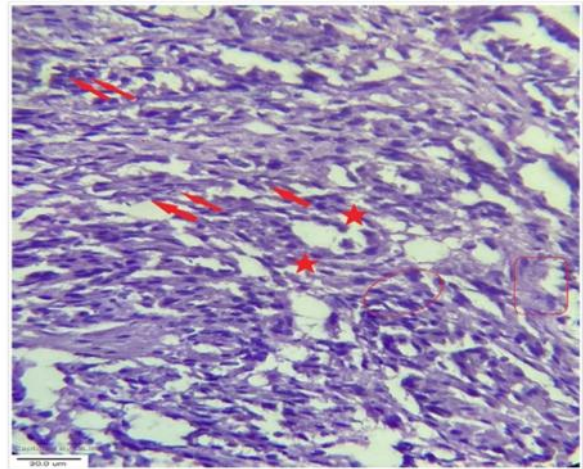
Histopathological examination showed normal ankle joint architecture in the normal group, while the disease control

group exhibited severe synovitis, lymphocytic infiltration, cartilage and bone erosion, and pannus formation. The standard-treated group showed marked protection with only mild synovial hyperplasia. Treatment I (200 mg/kg) demonstrated mild inflammatory changes without cartilage or bone damage, whereas Treatment II (400 mg/kg) showed reduced joint damage with minimal cartilage and bone erosion. The phytosomal formulation group exhibited nearly normal joint architecture with no pathological alterations, indicating superior anti-arthritis activity.

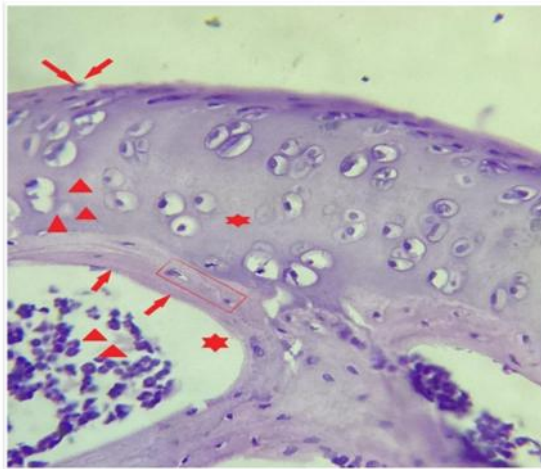
Normal group



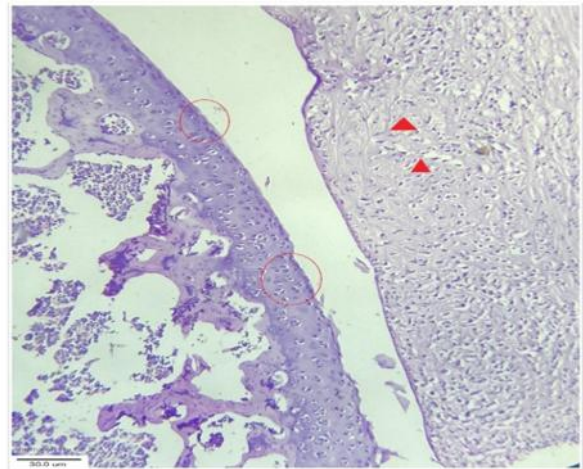
Disease control group



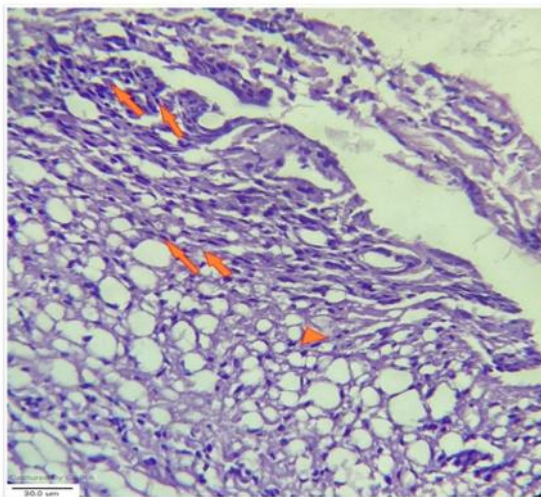
Standard treatment group



Treatment 1



Treatment 2



Treatment 3

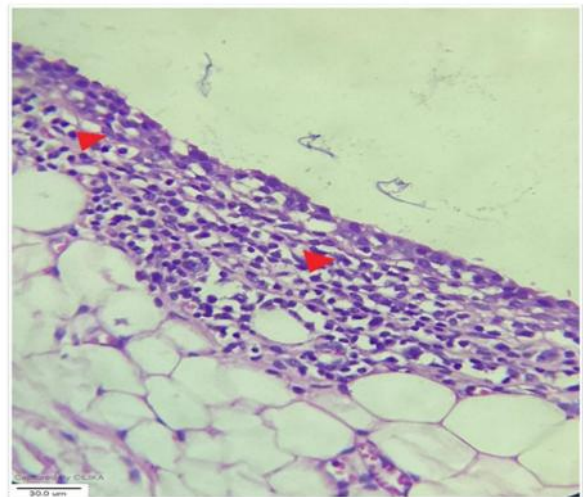


Figure 11: Effect of drug on histopathology of ankle joint Annotation symbols: Arrows (→) indicate synovial hyperplasia, Arrowheads (▲) indicates synovial lymphocytic infiltration Asterisks (★) indicates pannus formation, Circles (○) indicates cartilage erosion, Bracket (□) indicates Bone erosion

5. DISCUSSION

In the present study, the phytosomal formulation of *Asystasia Gangetica* demonstrated superior anti-arthritis activity compared to the extract in the FCA-induced rheumatoid arthritis model. The enhanced efficacy of the phytosomal formulation may be attributed to improved bioavailability and absorption of the phytoconstituents. The formulation produced greater improvements in clinical, haematological, biochemical, oxidative stress, and histopathological parameters, with effects that were comparable to those of the standard treatment group. This suggests that phytosomal delivery enhanced the therapeutic potential of *Asystasia Gangetica* against rheumatoid arthritis.²⁴

The phytosomal formulation was successfully developed and optimized using Central Composite Design (CCD). The optimized formulation exhibited a particle size of 150 nm, low polydispersity index (0.2109), and a zeta potential of -15.86 mV, indicating a stable nanosized vesicular system. Nanometric particle size and uniform distribution are known to enhance intestinal absorption, cellular uptake, and bioavailability of phytoconstituents.²⁵ FTIR analysis confirmed the compatibility of the extract with phospholipid components, while the DSC thermogram suggested successful incorporation of phytoconstituents into the lipid matrix without significant chemical interaction. These findings support the formation of a stable phytosomal complex capable of improving the therapeutic performance of *A. Gangetica*.²⁶

In FCA-induced arthritis, animals in the disease control group exhibited progressive loss of body weight, which is a common manifestation of chronic inflammation and metabolic disturbances associated with rheumatoid arthritis.⁴ Treatment with *A. Gangetica* extract and phytosomal formulation significantly restored body weight, suggesting improvement in overall health status and reduction of inflammatory burden. The phytosomal formulation produced the greatest improvement, indicating enhanced therapeutic efficacy resulting from improved bioavailability.²⁷

Paw edema and arthritic score are important indicators of inflammatory severity and joint damage in experimental arthritis. FCA administration produced a significant increase in paw volume and arthritic score, confirming successful induction of arthritis.²⁸ Treatment with *A. Gangetica* significantly reduced these parameters in a dose-dependent manner. The phytosomal formulation showed the most pronounced reduction, comparable to the standard drug indomethacin. These findings suggest that the anti-inflammatory activity of *A. Gangetica* may be attributed to its phytochemical constituents, particularly polyphenols and flavonoids, which are known to suppress pro-inflammatory mediators such as TNF- α , IL-1 β , and IL-6.²⁹

The spleen weight ratio was significantly elevated in arthritic animals, reflecting immune activation and systemic inflammation. Administration of *A. Gangetica* extract and phytosomal formulation significantly reduced spleen enlargement, indicating suppression of immune-mediated inflammatory responses. The effect observed with the phytosomal formulation was comparable to that of the standard treatment, suggesting effective modulation of the immune system.³⁰

Haematological alterations including elevated white blood cell count and decreased red blood cell count and haemoglobin levels were observed in the disease control group. These changes are

characteristic features of rheumatoid arthritis and are associated with chronic inflammation and anemia of inflammatory disease. Treatment with *A. Gangetica* significantly normalized these haematological parameters. The phytosomal formulation demonstrated the greatest restoration, suggesting improved control of systemic inflammation and protection against inflammatory anemia.³¹

Serum biochemical markers such as ALT, AST, ALP, and CRP were significantly elevated in FCA-treated animals, indicating systemic inflammation, tissue injury, and hepatic dysfunction. Administration of *A. Gangetica* extract and phytosomal formulation significantly reduced these biomarkers. The reduction in CRP further supports the anti-inflammatory activity of the plant, while normalization of liver enzymes suggests protection against inflammation-induced hepatic damage. The phytosomal formulation exhibited efficacy comparable to the standard drug, highlighting the advantage of phytosomal delivery.³²

Oxidative stress plays a crucial role in the progression of rheumatoid arthritis by promoting lipid peroxidation, cytokine production, and joint destruction. In the present study, arthritic rats showed significant reductions in endogenous antioxidant enzymes (SOD and GSH) and increased levels of MDA, a marker of lipid peroxidation.³³ Treatment with *A. Gangetica* significantly increased antioxidant enzyme levels while reducing MDA concentrations. The phytosomal formulation produced the strongest antioxidant effect, indicating enhanced scavenging of reactive oxygen species and protection against oxidative tissue damage. These findings are consistent with previous reports demonstrating the potent antioxidant properties of *A. Gangetica* due to its rich polyphenolic content.³⁴

Histopathological examination further supported the biochemical and pharmacological findings. The disease control group showed severe synovial hyperplasia, inflammatory cell infiltration, pannus formation, cartilage degradation, and bone erosion, which are hallmark features of rheumatoid arthritis. Treatment with *A. Gangetica* extract markedly reduced these pathological changes, while the phytosomal formulation restored nearly normal joint architecture with minimal inflammatory lesions. The superior histological protection observed in the phytosome-treated group may be attributed to enhanced delivery and sustained release of bioactive phytoconstituents at the site of inflammation.³⁵

Overall, the results of this study demonstrate that *Asystasia Gangetica* possesses significant anti-arthritic, anti-inflammatory, antioxidant, and immunomodulatory activities. Furthermore, incorporation of the extract into a phytosomal delivery system substantially enhanced its therapeutic efficacy, likely through improved stability, absorption, and bioavailability of active phytoconstituents. The phytosomal formulation exhibited effects comparable to the standard anti-inflammatory drug, suggesting its potential as a promising herbal nanocarrier-based therapeutic strategy for the management of rheumatoid arthritis.

6. CONCLUSION

Asystasia Gangetica phytosomes effectively attenuated FCA-induced arthritis by reducing inflammation, oxidative stress, immune activation, and joint destruction. These findings provide



scientific evidence supporting the development of *A. Gangetica* phytosomes as a potential alternative or adjunctive therapy for rheumatoid arthritis. Future studies involving cytokine profiling, molecular mechanism investigations, and clinical evaluation are warranted to further validate its therapeutic potential.³⁶

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