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Carrageenan-Induced Arthritic Joint Inflammation in Rats: Evaluating the Anti-Arthritic Potential of Phytosomal *Cissus quadrangularis* Ethanolic Extract.

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Abstract

This research synthesizes, characterizes, and applies *Cissus quadrangularis* phytosomes to transdermal patches for arthritis. Phytosomes were prepared from lipoid P 30 and crude *C. quadrangularis* ethanolic extract and characterized by optical microscopy, SPR, ATR-FTIR, XRD, FE-SEM, HRTEM, and Zetasizer Nano ZS. The evaporating solvent created transdermal patches with different polymer-phytosome ratios. To ensure the safety of the phytosomal formulation, its acute toxicity was investigated. In Wistar Albino rats, carrageenan intradermally caused arthritis was used to test the anti-arthritis activity of transdermal patches. Four groups of rats were tested: control, commercial transdermal patch, *C. quadrangularis* phytosome patch, and arthritic control. The *C. quadrangularis* phytosome transdermal patch reduced inflammation compared to that in the arthritic control group. Statistical analysis was performed using Prism 5. This study suggests *C. quadrangularis* phytosome transdermal patches may be used to treat arthritis, paving the way for future research. Microscopic investigation revealed the phytosome structural shape, content, and delivery system applications. UV spectroscopy revealed 15.044 OD220, indicating the presence of aromatic or conjugated structures. FT-IR spectroscopy revealed O-H stretching vibrations at 3842.20 cm^-1 in bioactive compounds. XRD analysis showed a phytosome crystallinity of 49.67 %. Particle analysis indicated good stability and dispersion with 86.29 an average particle size and -20.9 mV zeta potential. FESEM and HR-TEM exhibited nanoscale homogeneity and morphology. In acute toxicity testing of female rats, phytosomes were harmless. The phytosome transdermal patch reduced the paw volume and thickness of Wistar rats, similar to commercial anti-arthritis medications. Histopathological examination confirmed the anti-inflammatory effects of transdermal patches. Statistical analysis demonstrated significant improvements across the experimental groups, proving treatment efficacy. LC-MS revealed C. quadrangularis substances with anti-rheumatic properties. *C. quadrangularis* phytosomes may improve arthritis treatment via a holistic approach.

Keywords: *Cissus quadrangularis*, Ethanolic Extract, Phytosomes, FE-SEM and HR-TEM, Anti-Arthritic activity

Introduction

Joint arthritis is a prevalent and debilitating condition that encompasses a spectrum of disorders characterized by inflammation, pain, and impaired joint functionality [1-4]. The most common forms are osteoarthritis and rheumatoid arthritis, both of which pose significant challenges to the affected individuals [5]. Understanding the nature of joint arthritis and its potential complications is essential for its comprehensive management and improved quality of life [6]. Osteoarthritis, often referred to as "wear and tear" arthritis, primarily affects the cartilage and protective tissue covering the ends of bones within a joint [7-9]. Over time, cartilage undergoes degeneration, leading to pain, swelling, and stiffness in the affected joint. Risk factors for osteoarthritis include age, joint injuries, obesity, and genetic predisposition. As the condition progresses, joint movement becomes increasingly restricted and individuals may experience difficulty in performing daily activities, affecting their overall quality of life [10, 11].

Rheumatoid arthritis, on the other hand, is an autoimmune disorder characterized by the immune system mistakenly attacking the synovial lining of the membranes surrounding the joints [12]. This results in inflammation that can eventually lead to joint deformities and erosion of both bone and cartilage. Unlike osteoarthritis, rheumatoid arthritis can affect multiple joints simultaneously and is associated with systemic symptoms such as fatigue and fever [13]. The exact cause of rheumatoid arthritis is still not fully understood, and early detection and intervention are critical for managing its progression. Complications arising from joint arthritis can extend beyond the immediate discomfort and pain [14]. One notable consequence of this is its impact on mental health. Living with chronic pain and the physical limitations imposed by arthritis can contribute to anxiety, depression, and a reduced overall sense of well-being. The constant battle with pain management and the unpredictability of flare-ups add a significant emotional burden, affecting the mental resilience of those with arthritis.

Additionally, there is an increased risk of cardiovascular complications associated with certain forms of arthritis. Chronic inflammation, a hallmark of rheumatoid arthritis, can affect the cardiovascular system, potentially leading to an elevated risk of heart disease and stroke. Managing arthritis involves not only addressing joint-related symptoms but also considering the broader impact on overall health [15, 16].

Phytochemicals have emerged as promising agents for the management of joint arthritis, offering a natural and holistic approach to alleviating symptoms and potentially modifying the course of the disease. Plant-derived compounds found in various herbs and

botanicals such as *Boswellia serrata* and *Curcuma longa* have demonstrated anti-inflammatory, antioxidant, and analgesic properties [16, 17]. These phytochemicals target key pathways involved in arthritis pathogenesis, effectively reducing inflammation and oxidative stress, while promoting joint health [18]. Studies suggest that a diverse array of bioactive constituents in these plants, including flavonoids, alkaloids, and polyphenols, may contribute to the modulation of immune responses and inhibition of proinflammatory mediators [19, 20]. The exploration of phytochemicals as complementary or alternative therapies for joint arthritis underscores the potential of harnessing the therapeutic arsenal to enhance the well-being of individuals experiencing this challenging condition. Current Investigation: Assessment of the anti-arthritic capacity of phytosomal *Cissus quadrangularis* ethanolic extract in a rat model of carrageenan-induced arthritic joint inflammation.

Material and Methods

Formulation of Phytosomes

Phytosomes were synthesized following the method described by Susilawati et al. [21], with minor modifications. One gram of Lipoid P 30 (lecithin) was diluted with dichloromethane, and 1 g of crude *Cissus quadrangularis* ethanolic extract was diluted with 90% ethanol. Dissolved phospholipids and *Cissus quadrangularis* ethanolic extracts were added to a conical flask. The dichloromethane was evaporated using a magnetic stirrer at 37°C at a constant speed ranging from 25 to 150 rpm until an evenly formed thin-film layer was achieved. The resulting layers were then refrigerated for 24 h. The thin coating was hydrated with a phosphate buffer pH 5.5 while shaking at 40°C. After the phytosomal suspension was formed, the phytosome formulation was developed via ultrasonication for 5 min and stored for further biological activity.

Characterization of Phytosomes

Light microscopy was used to determine the morphological structure of phytosomes. The structural and morphological characteristics of the phytosomes were characterized using a Bio Spec Nano (Shimadzu), which measures the surface plasmon resonance of the nanoparticles. ATR-FTIR spectrometry was used to determine the functional groups of the extracts responsible for biological activity (Shimadzu IR affinity). Phytosomes were covered with an ATR (ZnSe) substrate and an X-ray diffractometer (XPERT-PRO) was used to study the crystalline nature of the phytosomes. Field-emission scanning electron microscopy (FE-SEM, TESCAN) and high-resolution transmission electron microscopy (HRTEM, JEOL Ltd., Japan) were used to identify the microscopic nature and surface morphology of the

phytosomes. A Zetasizer Nano ZS (Malvern Instruments) was used to evaluate the particle size and zeta potential of the liquid suspensions [22,23].

Transdermal patch preparation

Four batches of *C. quadrangularis* phytosome transdermal patches were prepared using the solvent-evaporation method. Drugs with different concentrations of polymer in four different ratios (1:1,1:2, 1:3, and 1:4). The weighed quantity of polymer was dissolved in the calculated quantity of water. A calculated amount of phytosomes was added to the mixture and stirred until a homogenous mixture was formed. The calculated amount of permeation enhancer was then added. The number of phytosomes was the same in all four batches. The resultant mixture was poured into a petri dish and air-dried at room temperature for 24 h. The patches were then peeled off from the Petri dish using a knife and kept in a desiccator (Table 1).

Table 1: Formula for TDDS

Ingredients	Formulation code			
	F1	F2	F3	F4
Drug	100	100	100	100
HPMC K15M	100	200	300	400
Polyethylene glycol	0.1	0.1	0.1	0.1
Dimethyl sulfoxide	0.1	0.1	0.1	0.1
Methanol	5	5	5	5
Distilled water	5	5	5	5

***C. quadrangularis* phytosomes transdermal patch (F2)- Experimental Animals**

Healthy Wistar Albino female rats, weighing approximately 150-200 g were procured from an animal house at the Cape Bio Lab & Research Centre in Marthandam, Kanyakumari District, Tamil Nadu. This study was approved by the Institutional Animal Ethical Committee (IAEC Number: CBLRC/IAEC/10/02-2022), which was certified by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The animals were housed in well-ventilated animal houses with a 12-hour light/12-hour dark cycle and kept in clean, dry polycarbonate cages. The animals were fed a standard pellet diet, and water was provided ad libitum. For experimental purposes, the animals were fasted overnight, but allowed free access to water.

Acute toxicity class method

Three Wistar Albino rats, weighing 150-200 g, were used in this study. Rats were provided with water ad libitum while fasting overnight. After fasting, the animals were administered an oral dose of *C. quadrangularis* phytosomes at a rate of 2000 mg/kg body weight. As most crude extracts have an LD_{50} value greater than 2000 mg/kg body weight, this dosage was utilized as the starting point. Following oral administration, the rats were monitored hourly for 24 h to assess mortality and to observe any alterations in their autonomic or behavioral responses, such as changes in alertness, spontaneous activity, salvation, respiration, urination, aggression, irritability, convulsions, and corneal reflex. For 14 days, the rats were routinely observed to record any deaths or harmful effects.

Evaluation of anti-arthritis activity of *C. quadrangularis* phytosomes transdermal patches using albino Wistar rats

Albino Wistar rats of both sex (200-250 gm) were obtained from an animal experimental laboratory and were used throughout the study. They were housed in micro nylon boxes in a controlled environment (temperature 25±2°C) and 12 hours dark/light cycle with a standard laboratory diet and water *ad libitum.* As per standard practice, the rats were segregated based on their gender and quarantined for 15 d before the commencement of the experiment. The animals were fed a healthy diet and were maintained in a hygienic environment in our animal house (Figure 1).

Figure 1: Animals for arthritic activity

Technique for inducing arthritis

The rats were divided into four groups for the study: G1 (control), G2 (Marketed Transdermal Patch), G3 (C. quadrangularis phytosome transdermal patch), and G4 (Arthritis Control). Arthritis was induced in male rats by intra-dermal injection of carrageenan (100 µL of 1%) into the foot pad of the left hind paw using a 1 ml glass syringe with locking hubs and a 26G needle [1]. To ease the injection process, the rats were anesthetized by ether inhalation both before and during carrageenan injection because of its challenging viscosity. Swelling

paws were periodically examined (for up to 15 days) in each paw from the ankle using a digital Vernier caliper. Increased volume of edema = final paw volume − initial paw volume. Values are expressed as the mean ± SEM, n = 6. $^{**}P< 0.01$, $^{***}P< 0.001$ Compared to arthritic control.

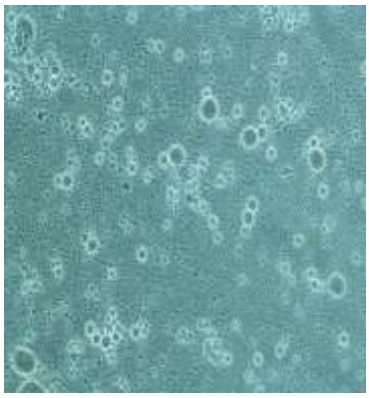
Statistical analysis

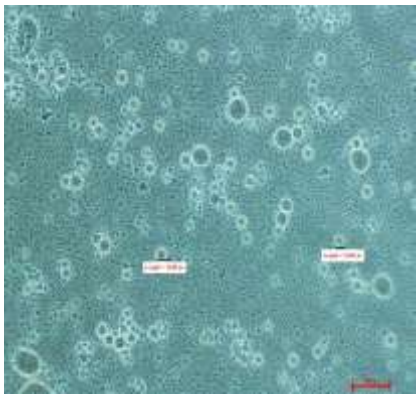
All assays were performed *in vitro* (n=6), and the results are expressed as the mean± standard deviation. An *In-vivo* anti-arthritis study was performed using GraphPad Prism version 5.

RESULTS AND DISCUSSION

Light microscope of phytosomes

Observation of phytosomes under a light microscope is important for understanding their structural characteristics and potential applications. Phytosomes, intricate complexes of plant-derived compounds, and phospholipids play crucial roles in enhancing the bioavailability and delivery of bioactive molecules. Through microscopic observations, researchers can unravel the morphology, size, and arrangement of phytosomes, shedding light on their stability and interactions. This information is pivotal for optimizing formulations for pharmaceuticals, nutraceuticals, and cosmeceuticals, as it aids in tailoring delivery systems for maximum efficacy. Additionally, microscopic analysis provides insights into the behavior of phytosomes in biological environments, thereby guiding advancements in drug delivery and therapeutic applications. Overall, studying phytosomes at the microscopic level is a key step towards harnessing their potential for improved health and wellness (Figure 1).





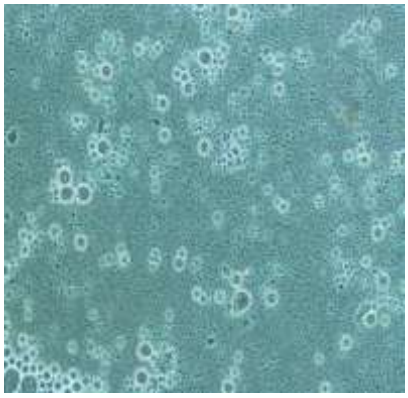


Figure 1: Light microscope images of *cissus quadrangularis* phytosomes

UV-Spectroscopy analysis of phytosomes

The UV spectroscopy data of *C. quadrangularis* phytosomes provide valuable insights into their optical density (OD) at different wavelengths. The OD values recorded across a range of wavelengths revealed the absorption patterns of the phytosomes. At higher wavelengths (e.g., 800 nm), the OD was relatively low, indicating minimal absorption in the near-infrared region. As the wavelength decreased, the OD values increased, suggesting increased absorption in the visible and ultraviolet regions. Notably, the OD values spiked significantly at 220 nm, indicating strong absorption in the ultraviolet range. The highest OD value at 220 nm (OD220:15.044) suggested that *C. quadrangularis* phytosomes exhibit pronounced absorption in the UV region, possibly due to the presence of compounds with aromatic or conjugated structures. This absorption pattern is crucial for understanding the electronic transitions occurring within phytosomes, providing insights into their composition and potential bioactive components (Figure 2).

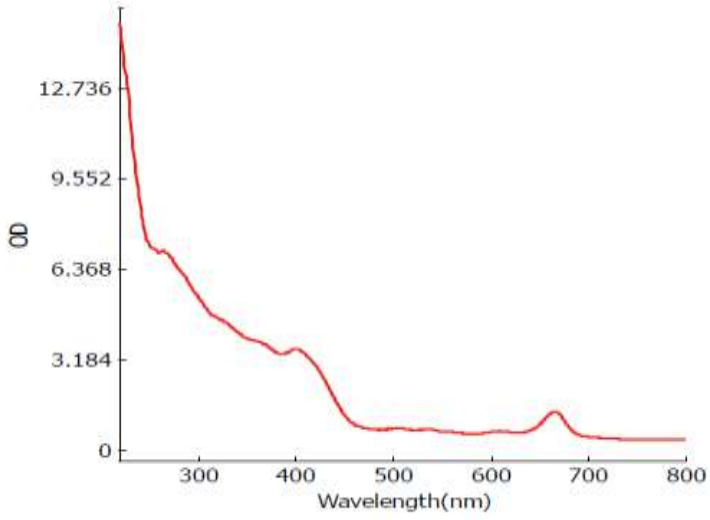


Figure 2: UV-Spectroscopy analysis of phytosomes

FT-IR-Spectroscopy analysis of phytosomes

FT-IR spectroscopy of the phytosomes revealed distinct absorption peaks at specific wavenumbers, offering valuable insights into their molecular composition. The spectrum exhibits prominent peaks at 3842.20, 3718.76, and 3286.70 cm^-1, indicative of stretching vibrations associated with O-H groups and hydrogen bonding. The presence of characteristic bands at 1635.64 and 1527.62 cm^-1 suggests the involvement of C=O and C-O stretching vibrations, potentially originating from flavonoids or other polyphenolic compounds. The peaks at 1072.42, 686.66, and 594.08 cm^-1 may be attributed to C-O-C stretching and C-H

bending vibrations, respectively, highlighting the presence of diverse functional groups. Additionally, peaks at 555.50, 501.49, 462.92, and 424.34 cm^-1 provide further information on the molecular structure of the phytosomes. Overall, FT-IR analysis offers a comprehensive overview of the phytosome composition, aiding in the understanding of its potential pharmacological and therapeutic applications.

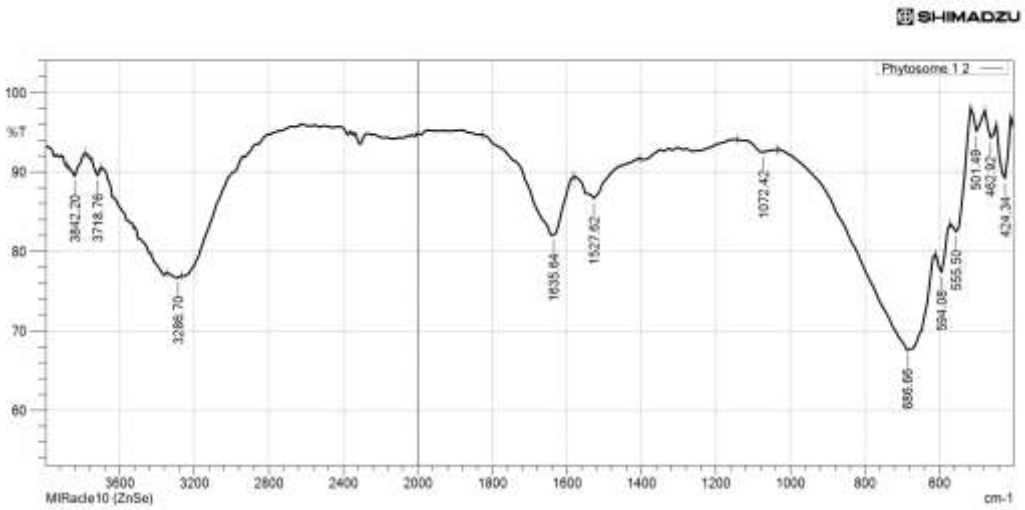
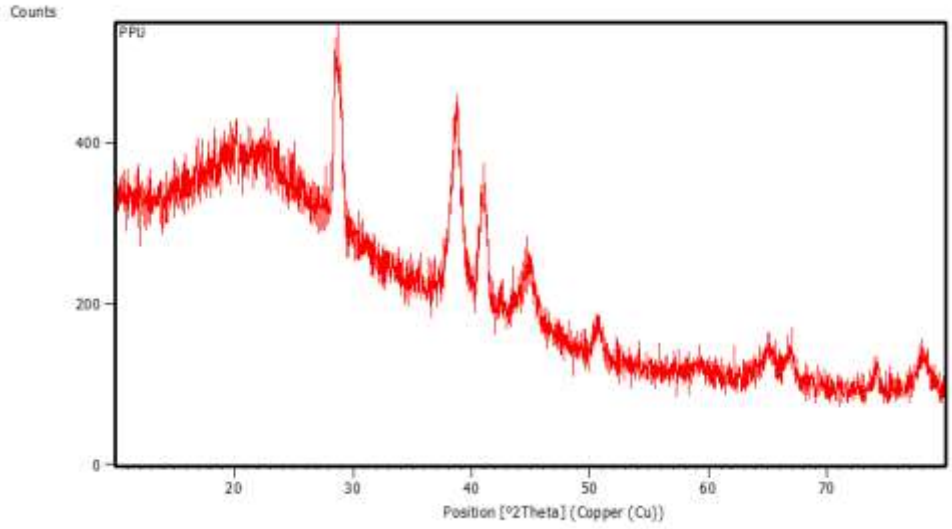


Figure 3: FT-IR-Spectroscopy analysis of phytosomes

X-RD analysis of *C. quadrangularis* phytosomes

X-ray diffraction (XRD) analysis of *C. quadrangularis* phytosomes provides crucial insights into the crystalline structure of the formulation. This technique helps to determine the arrangement of atoms within phytosomes, offering valuable information about their physical properties. By studying the diffraction patterns, one can identify the presence of crystalline phases, assess the degree of crystallinity, and understand the overall solid-state characteristics of phytosomes. This knowledge is vital for pharmaceutical and biomedical applications because it influences factors such as bioavailability, stability, and drug release kinetics. Thus, X-RD analysis of *C. quadrangularis* phytosomes plays a pivotal role in optimizing formulation parameters to enhance the efficacy and performance of these bioactive compounds in various therapeutic contexts. In Figure 3, The X-ray diffractogram of the optimized C. quadrangularis phytosomes is depicted, revealing distinct crystalline peaks at 2θ angles of 28.12°, 38.2°, and 30.95°. These peaks, characterized by their intensity and sharpness, signify the crystalline nature of the phytosome formulation. Notably, the calculated crystallinity of the optimized phytosome was 49.67%. This finding suggested that a significant portion of the extract was molecularly dispersed within the phospholipid matrix, indicating an amorphous form. The observed crystalline peaks and moderate crystallinity

contribute valuable insights into the solid-state characteristics of phytosomes, influencing factors such as stability and drug release kinetics for potential pharmaceutical applications.



***C. quadrangularis* phytosomes Particle analysis (particle size, distribution and Zetapotential)**

The average size distribution of *C. quadrangularis* phytosomes is an important parameter affecting the overall performance of the product. Particle size is a major factor affecting drug absorption and distribution. Drug Development in dosage form, content uniformity, and stability depends on particle size formation and its distribution. In many cases, for both drugs and additives, particle size reduction is required to achieve desired physicochemical characteristics. Based on these criteria, the prepared phytosome formulation was subjected to particle-size analysis (Figure 1).

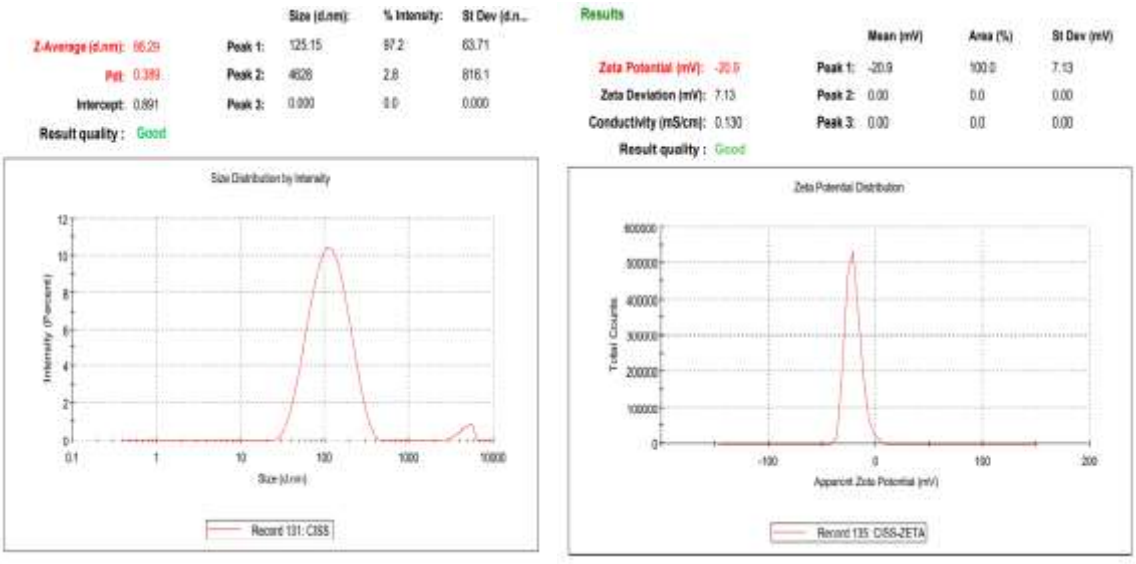


Figure 1: Zeta size and Zeta potential distribution of the *cissus quadrangularis* phytosomes

Particle analysis of the *C. quadrangularis* phytosomes revealed a favourable profile for pharmaceutical applications. With an average particle size of 86.29, phytosomes exhibited a size conducive to enhanced bioavailability and cellular uptake. Additionally, the measured zeta potential of -20.9 suggests good stability and dispersion owing to the presence of charged particles, minimizing the risk of aggregation. This combination of particle size and zeta potential signifies an optimized formulation, promising improved drug delivery efficiency, and potential therapeutic effectiveness in various biomedical applications.

HR-TEM

Cissus quadrangularis were studied using HR-TEM. High-resolution transmission electron microscopy (HR-TEM) of *C. quadrangularis* phytosomes revealed that they are morphologically nanoscale in size. The HR-TEM images showed well-defined and uniform structures, confirming the successful formation of phytosomes (Figure 3). The nano-sized particles, presumably encapsulating the bioactive components of *C. quadrangularis*, exhibited a distinct and homogeneous distribution.

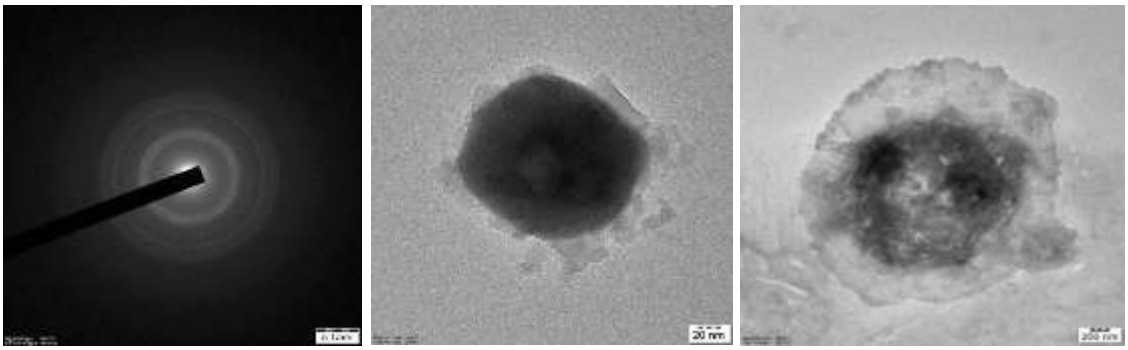


Figure 3: HR-TEM microscope observation of *cissus quadrangularis* phytosomes

FE-SEM

Examination of *Cissus quadrangularis* phytosomes using a Field Emission Scanning Electron Microscope (FE-SEM) provides detailed insights into their nanoscale morphology. The FE-SEM images illustrate well-defined and uniform structures, confirming the successful formation of phytosomes, as depicted in Figure 4. These nano-sized particles, likely encapsulating the bioactive constituents of *C. quadrangularis*, demonstrated a consistent and homogeneous distribution. This microscopic analysis enhances our understanding of the structural characteristics of phytosomes, particularly at the nanoscale, contributing valuable information for potential applications in drug delivery systems and supporting the overall efficacy of the formulation.

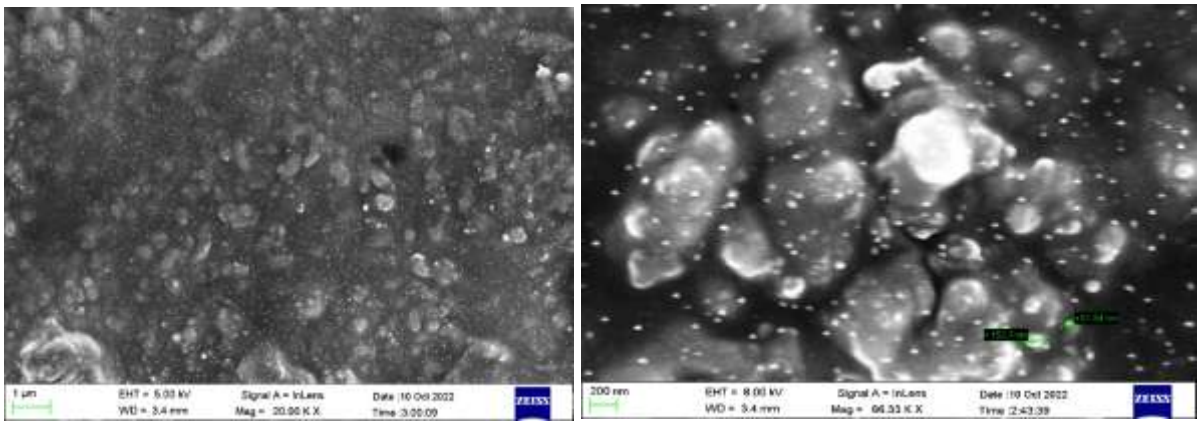


Figure 4: FE-SEM microscope observation of *cissus quadrangularis* phytosomes

EDAX

Energy-dispersive X-ray analysis (EDAX) of *Cissus quadrangularis* phytosomes provides elemental composition information, shedding light on the chemical constituents present in the formulation. This analysis revealed the presence and distribution of elements within the phytosomes, offering insights into their overall composition. By identifying specific elements such as carbon, oxygen, and phosphorus, EDAX contributes to our understanding of the elemental makeup of phytosomes, which is crucial for assessing their potential in pharmaceutical and biomedical applications. This analytical technique serves as a valuable tool for characterizing the elemental profile of *C. quadrangularis* phytosomes, aiding in the optimization and fine-tuning of their formulations for desired therapeutic outcomes.

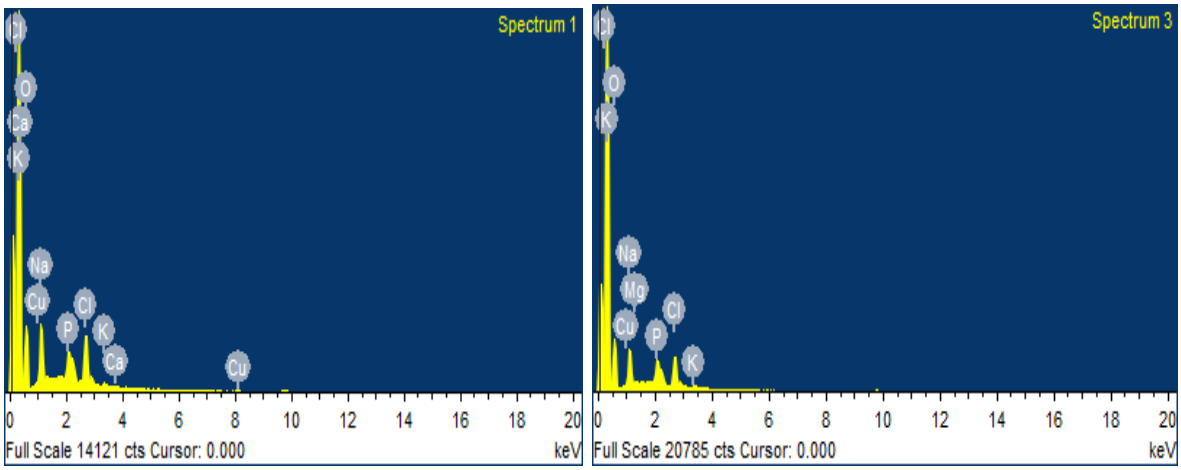


Figure 5: EDAX observation of *cissus quadrangularis* ethanolic extract phytosomes

***C. quadrangularis* ethanolic extract phytosomal transdermal patch preparation**

Transdermal drug delivery system (TDDS) formulations, denoted as F1, F2, F3, and F4, were meticulously crafted considering various essential ingredients. Of the four formulations, the F2 formulation for in vivo anti-arthritis activity was based on an assessment of the drug release kinetics.

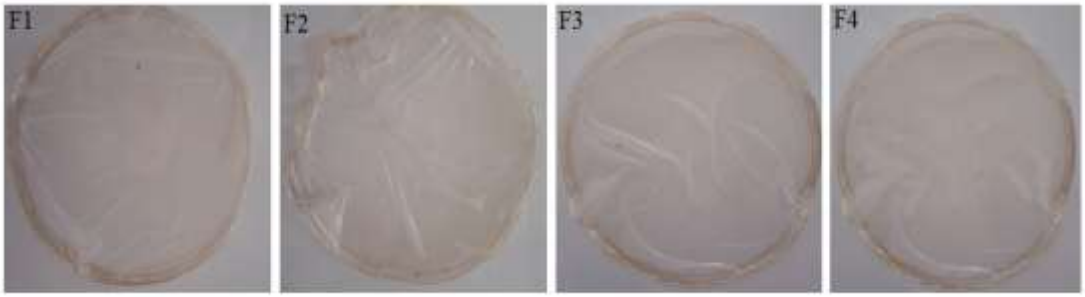


Figure 4: C*issus quadrangularis* ethanolic extract transdermal patches

Acute toxicity *C. quadrangularis* phytosomes

The present study evaluated the effects of *C. quadrangulari*s phytosomes on acute toxicity in female rats, and a comprehensive examination of various physiological parameters revealed a consistent pattern of normalcy. From alertness and grooming to touch and torch responses, as well as indicators such as pain response, convulsions, and righting reflex, phytosomes demonstrated no discernible impact. Grip strength, reflexes such as pinna and corneal reflexes, and other vital signs including pupil, urination, and skin color remained within the expected ranges. The absence of changes in parameters such as salivation, lacrimation, and writhing further supports the conclusion that *C. quadrangularis* phytosomes do not induce acute toxicity in female rats. This comprehensive assessment underscores the potential safety profile of *C. quadrangularis* phytosomes in the context of acute toxicity.

Body weight of the experimental animals

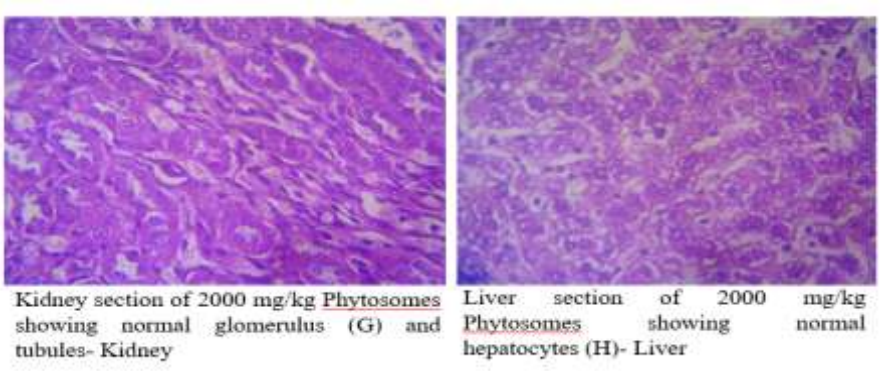
The body weight dynamics of the experimental animals over a 15-day period showed noteworthy trends. While control animals exhibited a modest increase, the marketed transdermal patch group displayed consistent growth, suggesting compatibility with normal physiological conditions. In contrast, the *C. quadrangularis* phytosome transdermal patch group experienced a reduction in body weight, indicative of potential pharmacological effects on metabolism or inflammation. The arthritis control group showed a significant decrease in body weight, underscoring the impact of arthritic conditions on overall health. The distinct weight changes observed between the marketed and *C. quadrangularis* phytosome transdermal patch groups highlight the need for a comprehensive understanding of both the intended therapeutic effects and systemic influences in transdermal formulations. Further investigations are warranted to elucidate the mechanisms underlying these observations and to assess the safety and efficacy of transdermal patches in the context of arthritis treatment (Table).

Table 3: Body weight of the experimental animals

Days	Control animals	Marketed transdermal patch	*C. quadrangularis* phytosomes transdermal patch	Arthritis control
00	221 ± 2.20	239 ± 2.60	242 ± 3.64	227 ± 3.12
07	222 ± 2.13	221 ± 2.36	221 ± 3.12	211 ± 2.60
15	225 ± 2.01	212 ± 2.55	213 ± 2.84	204 ± 2.18

Histopathology observation of metabolic organs (Kidney and Liver)

Kidney sections from subjects treated with 2000 mg/kg phytosomes revealed normal glomeruli (G) and tubules. Similarly, liver sections from subjects administered 2000 mg/kg phytosomes exhibited typical hepatocytes (H).



Kidney section of 2000 mg/kg Phytosomes showing normal glomerulus (G) and tubules- Kidney

Liver section of 2000 mg/kg Phytosomes showing normal hepatocytes (H)- Liver

Figure 1: Acute toxicity of *C. quadrangularis* phytosomes

Evaluation of anti-arthritis activity of *C. quadrangularis* phytosomes transdermal patches using albino Wistar rats

Table 1 shows the change in paw volume before and after the administration of carrageenan and marketed trans-derma patch-treated rats. The first 7 days of marketed prednisolone treatment reduced paw volume in a static manner, and the following week, a change in paw volume was found in all rats. Paw volume was significantly reduced $^{**}P< 0.01$. *C. quadrangularis* phytosome transdermal patch showed $^{***}P< 0.001$ highly significant effect on the reduction of paw volume from the day of study to the end, which showed similar results to that of the marketed transdermal patch.

Evaluation of anti-arthritis activity of *C. quadrangularis* phytosomes transdermal patch

Albino Wistar rats were divided into four groups of six each. All animals in the three groups were injected (100 µL of 1%) of carrageenan to induce arthritis, and one group was used as the control (Table 2 and Figure 3).

Table 2: Evaluation of anti-arthritis activity of *C. quadrangularis* phytosomes transdermal patch (ml).

Days	Control animals	Marketed transdermal patch	*C. quadrangularis* phytosomes transdermal patch	Arthritis control (Carrageenan)
00	3.44 ± 1.12	8.22 ± 1.21	7.16 ± 1.17	7.26 ± 1.02
03	3.72 ± 1.73	7.91 ± 1.20	7.51 ± 1.31	8.77 ± 1.03
05	3.16 ± 1.54	6.30 ± 1.22	6.41 ± 1.72	7.76 ± 1.11
07	3.52 ± 1.85	5.73 ± 1.42	5.83 ± 1.40	7.25 ± 1.12
09	3.79 ± 1.43	4.74 ± 1.24	5.12± 1.54**	6.87 ± 1.81
11	3.56 ± 1.20	4.15 ± 1.26	5.16 ± 1.22**	6.66 ± 1.01
13	3.33 ± 1.36	3.52 ± 1.26	4.52 ± 1.21**	5.81 ± 1.51
15	3.12 ± 1.46	3.17 ± 1.33	3.35 ± 1.01**	5.69 ± 1.14

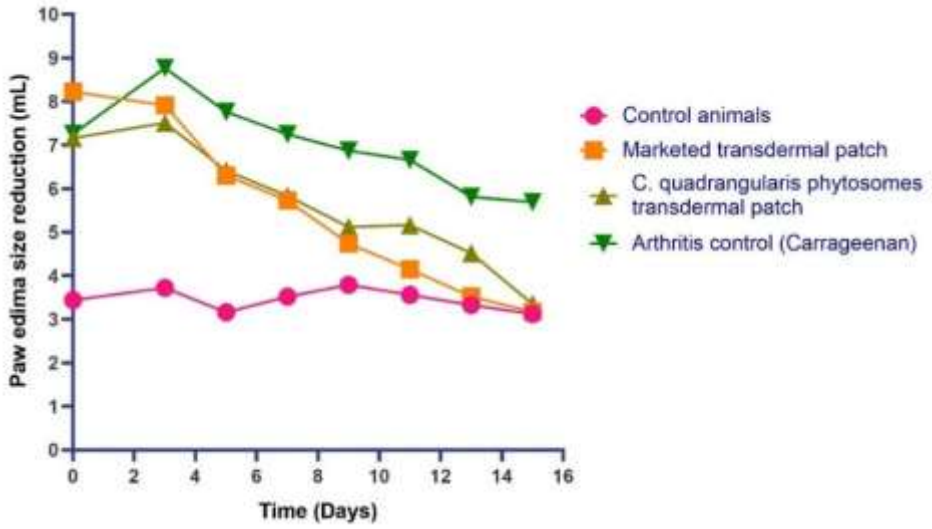


Figure 3: Evaluation of anti-arthritis activity of *C. quadrangularis* phytosomes transdermal patch.

Measurement of paw thickness of rat (mm)

The investigation of the effect of transdermal patches on paw thickness in rats with carrageenan-induced arthritis revealed significant findings over the 15-day study period. Paw thickness, measured using a digital Vernier caliper on days 5, 9, and 15, exhibited notable trends. Control animals maintained consistent paw thickness levels, whereas those treated with the marketed transdermal patch and *C. quadrangularis* phytosome transdermal patch demonstrated a reduction in paw thickness. Specifically, on day 15, the control group showed

a paw thickness of 7.15 ± 1.23 mm, whereas the marketed patch and *C. quadrangularis* phytosomes patch groups exhibited 5.82 ± 1.26 mm and 5.84 ± 1.01 mm, respectively. This reduction implies an anti-arthritic activity effect, with the *C. quadrangularis* phytosome transdermal patch displaying a comparable impact to the marketed counterpart. The observed values underscore the potential therapeutic efficacy of transdermal patches, particularly those containing *C. quadrangularis* phytosomes, in alleviating paw thickness associated with carrageenan-induced arthritis in rats, thus emphasizing the need for further research and clinical validation.

Table 3: Effect of transdermal patches on paw thickness (mm) of rats with carrageenan induced arthritis

Days	Control animals	Marketed transdermal patch	*C. quadrangularis* phytosomes transdermal patch	Arthritis control (Carrageenan)
05	3.22 ± 1.23	6.10 ± 1.12	6.11 ± 1.14	7.15 ± 1.23
09	3.23 ± 1.35	6.02 ± 1.12	6.04 ± 1.54	6.86 ± 1.12
15	3.24 ± 1.53	5.82 ± 1.26	5.84 ± 1.01	6.79 ± 1.22

Statistical analysis

In the present study, we evaluated the anti-arthritis activity of a transdermal patch containing *C. quadrangularis* phytosomes by methodically dividing Albino Wistar rats into four distinct groups, each comprising six individuals. Statistical analysis revealed a remarkably low p-value of <0.0001, signifying a highly significant difference among the groups. These results strongly support the notion that the transdermal patch enriched with *C. quadrangularis* phytosomes exerts a noteworthy impact on mitigating arthritis in the tested rat population. The meticulous grouping of rats and compelling statistical significance underscore the robustness and reliability of the study, suggesting a promising avenue for further exploration of the anti-arthritis potential of the investigated transdermal patch formulation.

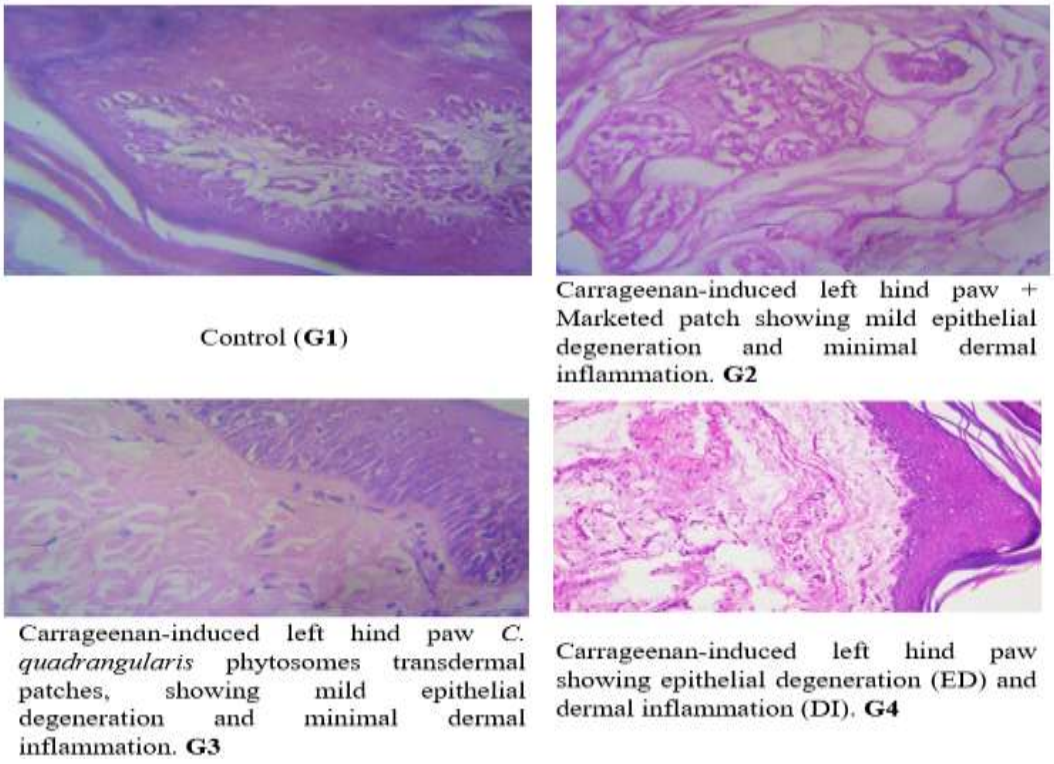
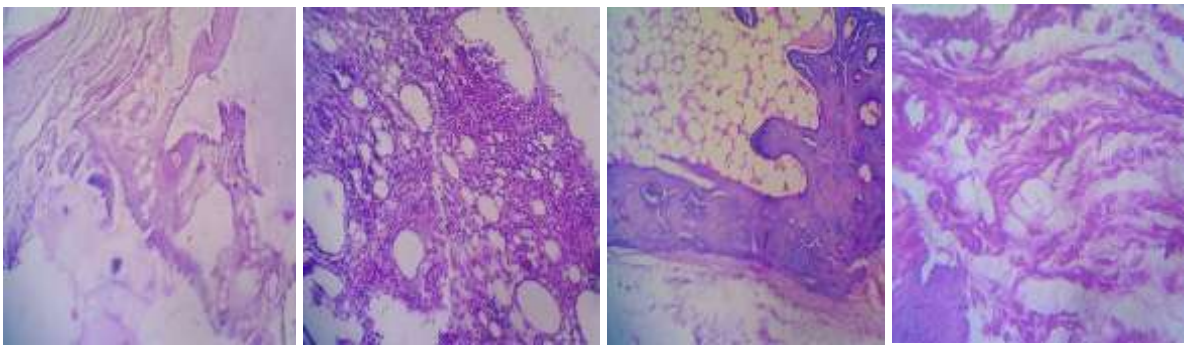


Figure 4: Evaluation of anti-arthritis activity of *C. quadrangularis* phytosomes transdermal patch

Evaluation of anti-arthritis activity of *C. quadrangularis* phytosomes transdermal patch (Histopathology of bone at the joints)

The assessment of the anti-arthritis activity of the *C. quadrangularis* phytosome transdermal patch involved a histopathological examination of bone samples from the joints. In Group 1 (G1), representing the normal condition, mature bone structures were observed at 40X magnification, showing a typical and healthy appearance. In Group 2 (G2), which received the transdermal patch, mature bone with moderate stroma and inflammatory infiltrates was noted at 40X magnification, indicating a potential impact on the inflammatory response in arthritic conditions. Group 3 (G3), treated with the phytosome transdermal patch, displayed mature bone structures similar to the normal state at 40X magnification, suggesting a potential protective effect against arthritis-induced changes. Group 4 (G4), observed at 45X magnification, exhibited a bone surrounded by fibrocollagenous stroma, indicating a potential reparative or protective response. These histopathological findings provide valuable insights into the potential anti-arthritis activity of the *C. quadrangularis* phytosome transdermal patch, suggesting its influence on bone morphology and inflammatory infiltrates within the joints. Further studies are warranted to comprehensively understand the mechanisms underlying these histological changes (Figure 5).



G1 G2 G3 G4

Figure 5: Histopathology of bone at the joints

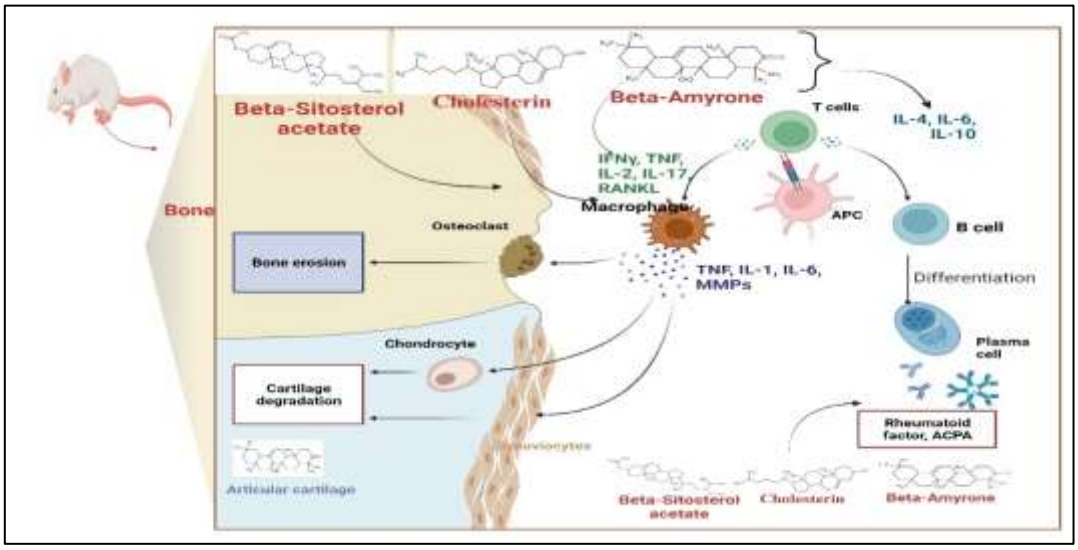


Figure 6: Possible anti-arthritis activity of *C. quadrangularis* phytosomes transdermal patches.

Cissus quadrangularis, a plant with a rich history in Ayurvedic medicine, contains compounds such as Beta-Amyrone, Beta-Sitosterol acetate, and cholesterol, as confirmed by LC-MS analysis [24]. Notably, in a rat model, steroid compounds such as beta-amyrone exhibited promising antirheumatic activity, suggesting its potential role in managing rheumatoid arthritis [25]. Additionally, the presence of β-sitosterol acetate, known for its anti-inflammatory properties, and cholesterol in the plant extract raises intriguing possibilities for therapeutic applications. While these findings offer exciting prospects, the translation of animal model results to human outcomes necessitates further research, including clinical trials, to validate the safety and efficacy of these compounds for human rheumatoid arthritis treatment (Figure 6).

CONCLUSION

A comprehensive analysis of *C. quadrangularis* phytosomes, spanning from their microscopic characteristics to their therapeutic potential, provides valuable insights into their structural features and pharmacological attributes. Light microscopy observations shed light on the intricate structure of phytosomes, emphasizing their importance in enhancing bioavailability and facilitating bioactive compound delivery. UV spectroscopy analysis revealed a distinctive absorption pattern, especially in the ultraviolet region, suggesting the presence of aromatic or conjugated structures within the phytosomes. FT-IR spectroscopy provides a detailed molecular composition, highlighting the functional groups associated with potential bioactive components. X-ray diffraction analysis offers insights into the crystalline nature of phytosomes and influencing factors such as stability and drug release kinetics. Particle size analysis demonstrated favorable characteristics of C. quadrangularis phytosomes for pharmaceutical applications, indicating enhanced bioavailability and cellular uptake. Microscopic observations using HR-TEM and FE-SEM further affirmed the nanoscale morphology of the phytosomes, which is essential for understanding their potential in drug delivery systems. This acute toxicity study underscores the safety profile of C. quadrangularis phytosomes, as evidenced by the absence of adverse effects on various physiological parameters in female rats. Moreover, the evaluation of anti-arthritis activity using transdermal patches demonstrated promising outcomes, with the C. quadrangularis phytosome group showing a significant reduction in paw volume, thickness, and potential protective effects on bone morphology in a rat model of arthritis. Statistical analysis further supported the robustness of the study and indicated a highly significant difference among the experimental groups.

In-depth exploration of the chemical constituents of *C. quadrangularis* through LC-MS analysis identified compounds with potential anti-inflammatory and antirheumatic properties, suggesting a promising avenue for further research and clinical trials. Overall, this multifaceted investigation provides a solid foundation for harnessing the therapeutic potential of *C. quadrangularis* phytosomes, opening new avenues for their application in pharmaceuticals, nutraceuticals, and cosmeceuticals. However, further research, including clinical studies, are imperative to validate their safety, efficacy, and translational potential in human health and wellness.

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CONFLICT OF INTEREST: Nil

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