FORMULATION AND EVALUATION OF HERBAL OIL BY USING *LYCHEE CHINENSIS* LEAVES EXTRACT FOR ANTI-ARTHRITIC ACTIVITY

Asiya Bano[1*], Dr. Umesh Pratap Singh[2], Miss.PurnimaTiwari[3],Poonam Yadav, Shaikh Asra

[1]*Research Schlor, Department of Pharmacy, Rameshwaram Institute of Technology and Management, Sitapur Road,Lucknow-227202 (UP)*

[2]*Director, Department of Pharmacy, Rameshwaram Institute of Technology and Management, Sitapur Road,Lucknow-227202 (UP)*

[3]*Associate Professor, Department of Pharmacy, Rameshwaram Institute of Technology and Management, SitapurRoad, Lucknow-227202 (UP)*

Abstract

This study investigates the formulation and evaluation of a herbal oil derived from Lychee chinensis leaves for potential anti-arthritic activity. A comprehensive phytochemical analysis identified significant bioactive compounds with anti-inflammatory and antioxidant properties. The formulated herbal oil underwent rigorous physicochemical evaluations, including pH, saponification value, acid value, viscosity, refractive index, and specific gravity, ensuring its quality and stability. The anti-arthritic efficacy was tested using the Complete Freund's Adjuvant (CFA) induced arthritis model in rats, demonstrating significant reductions in paw edema, inflammation, and other arthritis-related symptoms. Histopathological analysis confirmed these therapeutic effects, comparable to standard treatments. Safety was confirmed through acute dermal toxicity studies, showing no adverse effects, supporting its suitability for topical use. The study concludes that the herbal oil from Lychee chinensis leaves is a promising natural remedy for arthritis, contributing to the evidence for plant-based treatments in managing chronic inflammatory conditions.

Key words: *Lychee chinensis, herbal oil, anti-arthritic activity, phytochemical analysis, Complete Freund's Adjuvant, natural remedy*

Introduction

Arthritis is a prevalent chronic health condition that significantly impacts millions of people worldwide, with two primary forms being osteoarthritis (OA) and rheumatoid arthritis (RA). According to the World Health Organization (WHO, 2021), arthritis, particularly OA and RA, is a major cause of disability globally. The Centers for Disease Control and Prevention (CDC, 2018) estimates that over 54.4 million individuals in the United States alone have been diagnosed with some form of arthritis. With anticipated demographic changes, this number is expected to rise substantially in the coming years. The condition encompasses around 100 different types of rheumatic diseases and disorders that affect joints, surrounding tissues, and other connective tissues.Rheumatoid arthritis (RA)is an autoimmune disease where the immune system erroneously attacks the body's tissues, particularly the synovium—the lining of the membranes surrounding the joints. This immune response leads to inflammation, which can cause joint damage and affect other areas of the body. RA is more prevalent in women and individuals of middle age (Smolen et al., 2016).

Conventional treatment for arthritis aims primarily to alleviate symptoms and improve the patient's quality of life. Treatment options include medications, physical therapy, lifestyle modifications, and, in severe cases, surgical interventions. Nonsteroidal anti-inflammatory drugs (NSAIDs) like ibuprofen and naproxen are commonly used to relieve pain and reduce inflammation. However, long-term use of NSAIDs may lead to gastrointestinal, cardiovascular, and renal complications (Moore et al., 2015). Corticosteroids, another class of potent anti-inflammatory drugs, can be administered orally or through direct joint injections. While effective for short-term relief, corticosteroids are unsuitable for long-term use due to risks such as osteoporosis, weight gain, and increased susceptibility to infections (Gossec et al., 2016).Disease-modifying antirheumatic drugs (DMARDs), like methotrexate, are often prescribed to slow disease progression and prevent joint damage, particularly in RA. Despite their efficacy, DMARDs can cause significant side effects, including liver damage and bone marrow suppression (Smolen et al., 2016). Biologics, a newer class of DMARDs, target specific components of the immune system and can be highly effective in reducing inflammation and preventing joint damage. However, they are costly and may increase the risk of infections (Singh et al., 2015).

Despite the benefits of conventional treatments, their limitations—including side effects and the inability to halt disease progression—necessitate ongoing research into alternative therapies, including herbal treatments. Herbal medicines have gained renewed interest as potential treatments for arthritis due to their lower incidence of side effects and holistic approach to health. Formulating herbal extracts into oils offers several advantages, enhancing their efficacy, stability, and user acceptability. Oils improve the absorption and bioavailability of fat-soluble phytochemicals, leading to higher concentrations in the bloodstream and enhanced therapeutic effects (Manabe et al., 2011). Herbal oils also provide sustained release of active compounds, ensuring prolonged therapeutic effects, particularly beneficial for chronic conditions like arthritis (Sengupta et al., 2008). Moreover, oils protect sensitive phytochemicals from degradation, thereby extending their shelf life and maintaining potency (Surh, 2003).

Lychee chinensis, commonly known as lychee, is an evergreen tree native to Southeast Asia. The leaves of *Lychee chinensis* contain various bioactive compounds, including procyanidin A2, procyanidin B2, and (–)-epicatechin, along with terpenoids, flavonoids, phenols, tannins, and

saponins. These compounds are known for their anti-inflammatory and antioxidant properties, making *Lychee chinensis* a promising candidate for developing natural therapeutic agents for arthritis.

MATERIALS AND METHODS

Chemicals and reagents

Lychee chinensis leaves were procured from local market. All other chemicals and reagents used were of analytical grade.

Preparation of extract

The fresh leaves of *Lychee chinensis* were harvested, thoroughly cleaned, and shade-dried before being ground into powder for extraction. Approximately 400 grams of the powdered leaves were soaked in methanol and kept at room temperature for seven days. After this period, the mixture was filtered using Whatman No. 1 filter paper to remove any solid residues. The filtrate was then concentrated using a rotary vacuum evaporator at a temperature below 50˚C. The resulting extract was air-dried to ensure complete evaporation of methanol and stored at 4˚C for future use and analysis (Wen et al., 2014).

Preparation of herbal oil

To prepare the herbal oil, 50 milliliters of castor oil were warmed in a water bath in two separate flasks. Methanol was added to each flask to dissolve the oil. Then, *Lychee chinensis* leaf extract was mixed into the oil at concentrations of 10 mg/ml (Test 1) and 20 mg/ml (Test 2) by adding 1 gram and 2 grams of the extract, respectively. These preparations were then used for studying anti-arthritic activity (Babu et al., 2019).

Preliminary Phytochemical Investigation

Phytochemical tests were conducted on the plant extracts to identify the presence of various bioactive compounds, including proteins, glycosides, essential oils, alkaloids, tannins, carbohydrates, flavonoids, and other chemicals responsible for biological activity (Shukla et al., 2012).

Physicochemical Investigations

Physicochemical investigations were conducted to evaluate the quality and purity of the herbal drug. These included determining the ash value for inorganic mineral content, water-soluble ash for water-soluble compounds, and acid-insoluble ash for siliceous materials like sand. The extractive value was measured to assess the concentration of active constituents, indicating the drug's potency. The loss on drying (LOD) test was performed to determine moisture content, which is important for assessing the drug's stability and shelf life. These analyses provide essential data on the drug's overall quality and therapeutic potential (Yuniarti et al., 2020).

UV analysis of extract

The *Lychee chinensis* leaves extract underwent UV spectroscopy analysis to assess its absorbance across wavelengths ranging from 200 to 800 nm. This analysis provided crucial information about the presence and quantity of phytochemicals, such as flavonoids and phenolic acids, which exhibit characteristic absorbance in the UV-Vis region. The data was collected at a

fine resolution of 0.5 nm, allowing for precise identification of absorption peaks that correspond to specific bioactive compounds. The resulting UV spectrum offers insights into the extract's purity, concentration of active components, and potential therapeutic efficacy.

IR analysis of extract

Infrared (IR) spectroscopy was performed on the *Lychee chinensis* leaf extract to identify the functional groups present within the compounds. This technique involves passing infrared radiation through the extract and measuring the specific wavelengths absorbed by different chemical bonds. The resulting IR spectrum displays characteristic peaks corresponding to various functional groups, providing detailed information about the molecular structures of the bioactive components in the extract.

Evaluation of herbal oil

The herbal oil formulated from *Lychee chinensis* leaves extract was evaluated for its physicochemical and organoleptic properties to ensure quality, stability, and suitability for therapeutic use. The assessments included measuring pH, saponification value, acid value, viscosity, refractive index, and specific gravity. These tests provided insights into the oil's stability, potential for soap formation, fatty acid content, and overall purity. Additionally, organoleptic properties such as color, odor, and potential skin irritation were evaluated to determine user acceptability (Shah et al., 2018).

Animal experiment

The antiarthritic activity of *Lychee chinensis* leaves extract was evaluated using a Complete Freund's Adjuvant (CFA) model in albino rats. Arthritis was induced by injecting CFA into the left foot pad of the rats, which were then divided into five groups: a control group, a disease control group, a conventional treatment group, and two groups treated with different doses of the herbal oil formulation. Paw size and body weight were monitored over five weeks to assess inflammation and overall health. Hematological analysis was conducted to measure key blood parameters, and radiological analysis was performed to evaluate joint damage. Finally, histopathological examination of the ankle joints was conducted to observe tissue changes, providing a comprehensive assessment of the antiarthritic efficacy of the herbal oil compared to conventional treatment.

RESULTS

Phytochemical Analysis

The extract of Lychee chinensis leaves was found to contain a number of different chemicals, as evidenced by the findings of the phytochemical study. The presence of alkaloids was verified by a number of different tests, including Wagner's Test, Mayer's Test, Hager's Test, and Dragendroff's Test. Terpenoids were found to be present. There was evidence of decreasing sugars, as shown by both the Fehling's Test and the Benedict's Test. The Foam Test confirmed the presence of saponins. Tannins were detected using the $FeCl_3$ Test, and their presence was further confirmed by the Ferric-chloride test and Lead-Acetate test. Proteins were present as indicated by Millon’s test, and the presence of steroids was confirmed by Salkowski’s test (Table 1).

Table 1: Phytochemical Analysis Results of *Lychee Chinensis* Leaves Extract

S. No	Test Performed	Results
1	Wagner's Test	Present
2	Mayer's Test	Present
3	Hager's Test	Present
4	Dragendroff's test	Present
5	Terpenoids	Present
6	Fehling's Test	Present
7	Benedict's Test	Present
8	Foam Test	Present
9	Tannins ($FeCl_3$ Test)	Present
10	Ferric-chloride test	Present
11	Lead-Acetate test	Present
12	Millon's test	Present
13	Salkowski's test	Present
14	Shinoda test	Present

Physicochemical analysis

The physicochemical analysis of *Lychee chinensis* leaves extract revealed the following results (Table 2).

Table 2: Physicochemical Analysis Results of Lychee Chinensis Leaves Extract

S. No	Name of Analysis	Result
1	Ash value	1.5%
2	Extractive value	7.5% w/w
3	Loss on drying	4%
4	Water soluble ash value	0.8%
5	Acid insoluble ash value	1.4%

UV analysis of extract

The UV spectrum of *Lychee chinensis* leaves extract revealed distinct absorption peaks, particularly between 200 nm and 400 nm, indicating the presence of various phytochemicals. These peaks suggest the presence of flavonoids, phenolics, and other compounds commonly found in plant extracts. Notable peaks were observed around 220 nm and 280 nm, which are characteristic of phenolic compounds and flavonoids, though specific values could not be determined without numerical data (Figure 1).

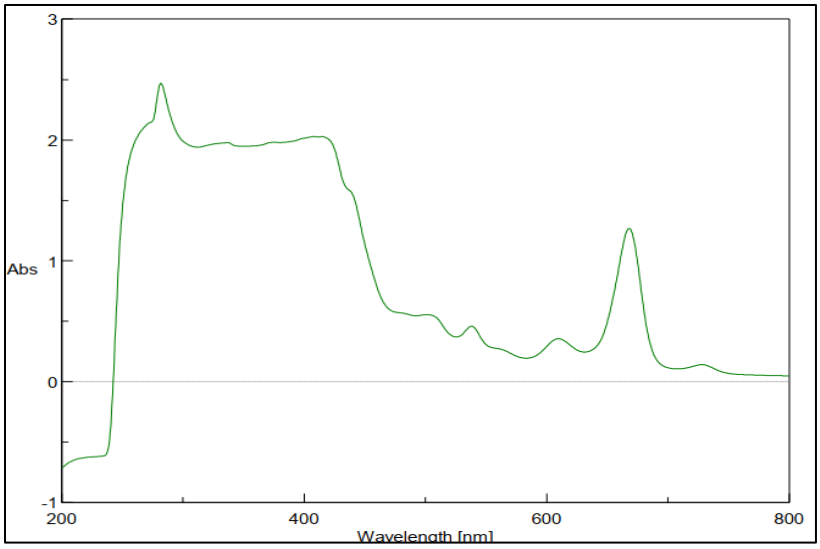


Figure 1: UV spectra of methanolic extract of leaves of *Lychee chinensis*

IR analysis of extract

The IR spectroscopy analysis of *Lychee chinensis* leaves extract revealed key functional groups. Peaks at 3923.17 cm^{-1} and 3775.46 cm^{-1} indicate free hydroxyl groups, while 3424.36 cm^{-1} suggests hydrogen-bonded O-H groups. Peaks at 2381.58 cm^{-1} and 2096.57 cm^{-1} suggest the presence of alkynes or nitriles, and the peak at 1634.73 cm^{-1} indicates C=C bonds in alkenes or aromatic rings. C-H bending at 1389.13 cm^{-1} and C-O stretching at 1269.45 cm^{-1} and 1071.27 cm^{-1} suggest aliphatic hydrocarbons and alcohols. Peaks at 766.43 cm^{-1} and 693.67 cm^{-1} correspond to aromatic compounds and alkenes.

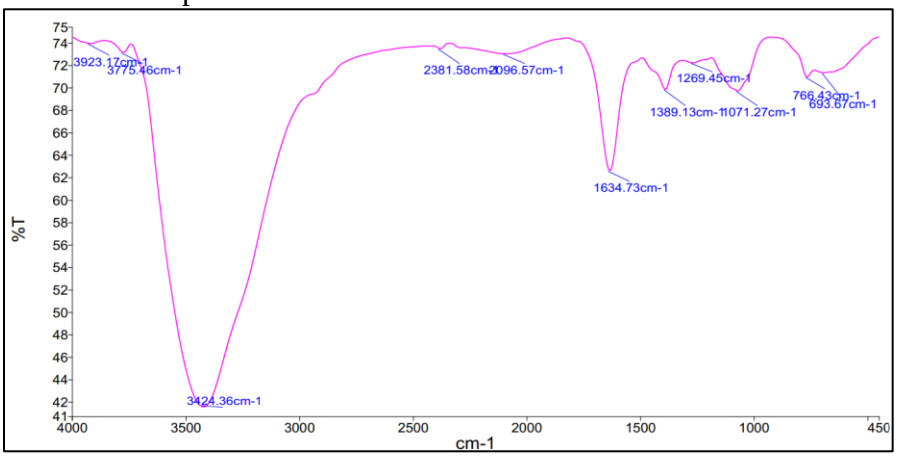


Figure 2: IR spectra of methanolic extract of leaves of *Lychee chinensis*

Assessment of Herbal Oil

The following factors were used to assess the hair growth oil that was made, and the findings are given in table 3.

Table 3.Assessment of herbal oil

S. No.	Parameters	Observations
1	pH	6.3
2	Acid value	4.8
3	Viscosity	0.912 poise
4	Refractive index	1.415
5	Specific gravity	1.079
6	Colour	Greenish
7	Odour	Characteristic
8	Irritation test	Non irritant

Evaluation of Anti-Arthritic Activity Using Complete Freund's Adjuvant (CFA) in Albino Rats

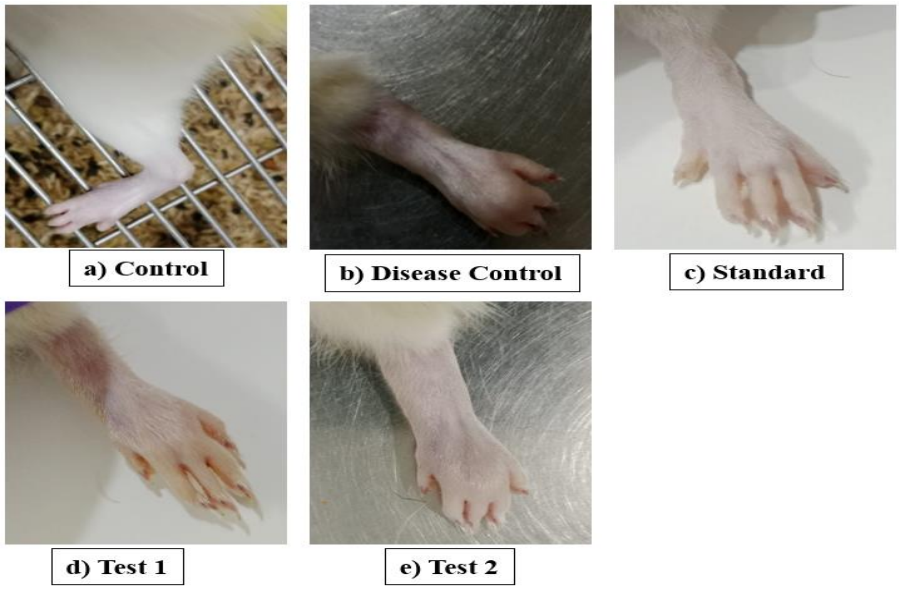


Figure 3: Figure of left paw of animals from each group

Table 4. Comparative Analysis of Anti-Arthritic Activity

Group	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
Control	4.22 ± 0.09 **	4.22 ± 0.07 **	4.20 ± 0.07 **	4.60 ± 0.07 **	4.66 ± 0.05 **	4.30 ± 0.07 **
Disease Control	4.76 ± 0.05	7.38 ± 0.31	8.70 ± 0.07	9.00 ± 0.07	9.00 ± 0.07	9.70 ± 0.07
Standard	4.28 ± 0.14	6.94 ± 0.09 **	6.50 ± 0.11 **	6.76 ± 0.11 **	6.70 ± 0.39 **	6.24 ± 0.32 **
Test 1	4.10 ± 0.16 **	6.82 ± 0.07 **	6.64 ± 0.11 **	6.60 ± 0.20 **	5.66 ± 0.21 **	6.10 ± 0.22 **
Test 2	4.18 ± 0.11 **	6.44 ± 0.17 **	6.54 ± 0.13 **	5.94 ± 0.14 **	5.66 ± 0.47 **	5.64 ± 0.48 **

On Day 0, there is a significant difference in paw diameter between the groups, indicating initial differences before the disease induction and treatment.From Day 7 onwards, the differences between the groups become highly significant ($p < 0.001$), demonstrating the impact of the treatments and the progression of the disease.The significant p-values suggest that the differences in mean paw diameters between the groups are not due to chance, particularly as the disease progresses and treatment effects manifest.Since all the comparisons yielded p-values below 0.01, only the ****** symbol is present in the table.

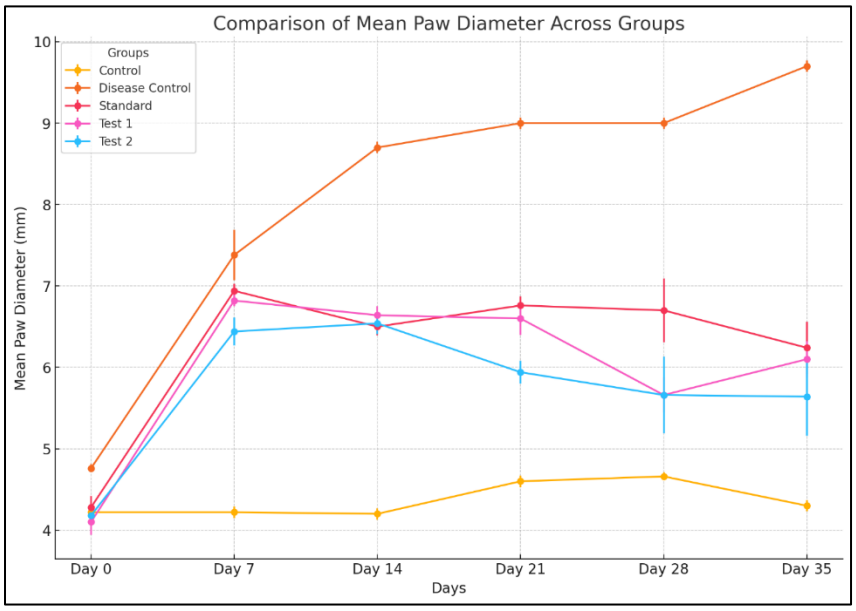
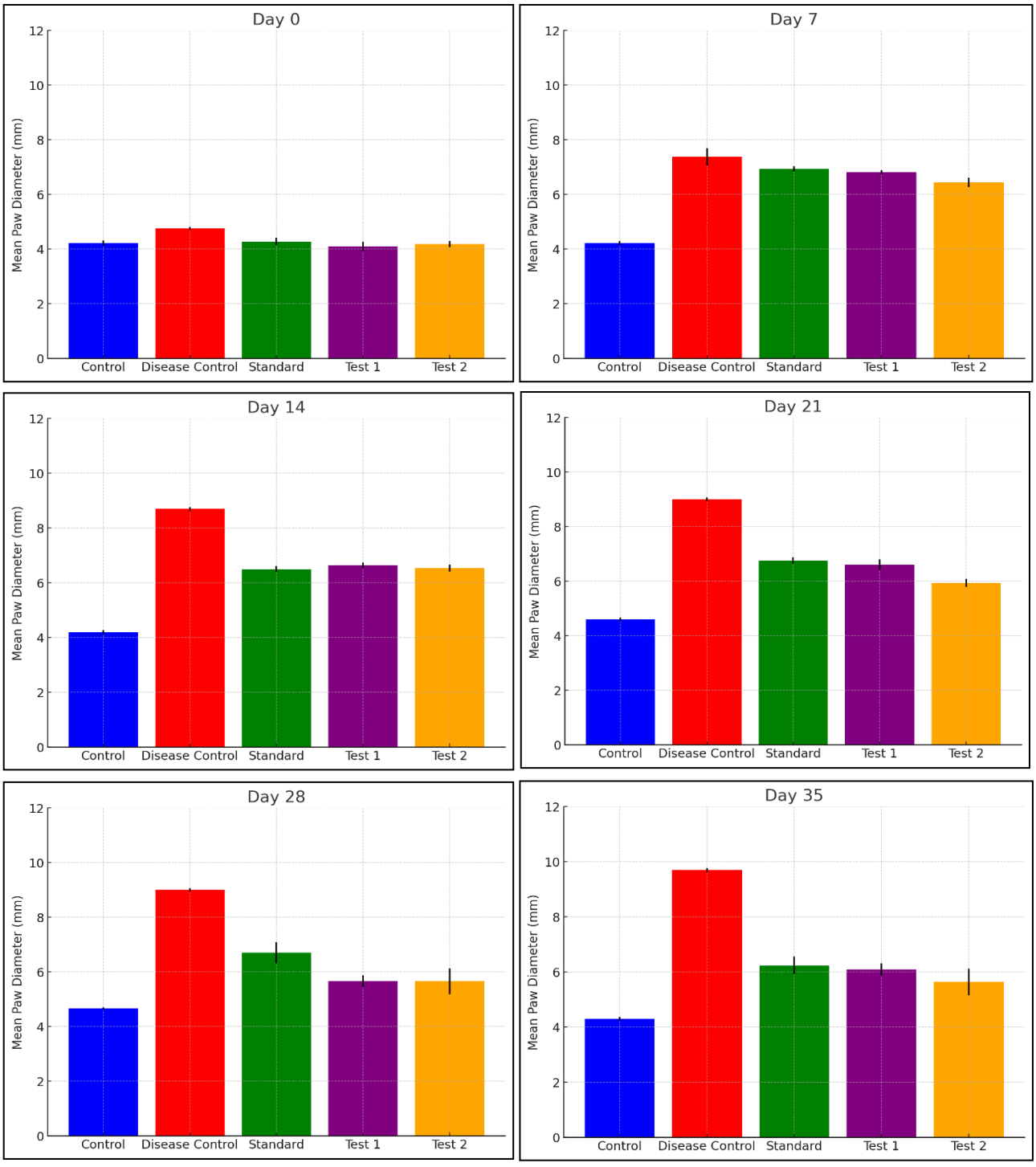


Figure 4: The mean paw diameter across different groups (Control, Disease Control, Standard, Test 1, and Test 2) over time.

Figure 5.: The individual bar graphs for each day (Day 0, 7, 14, 21, 28, and 35) showing the mean paw diameter for each group with the standard error of the mean (SEM).



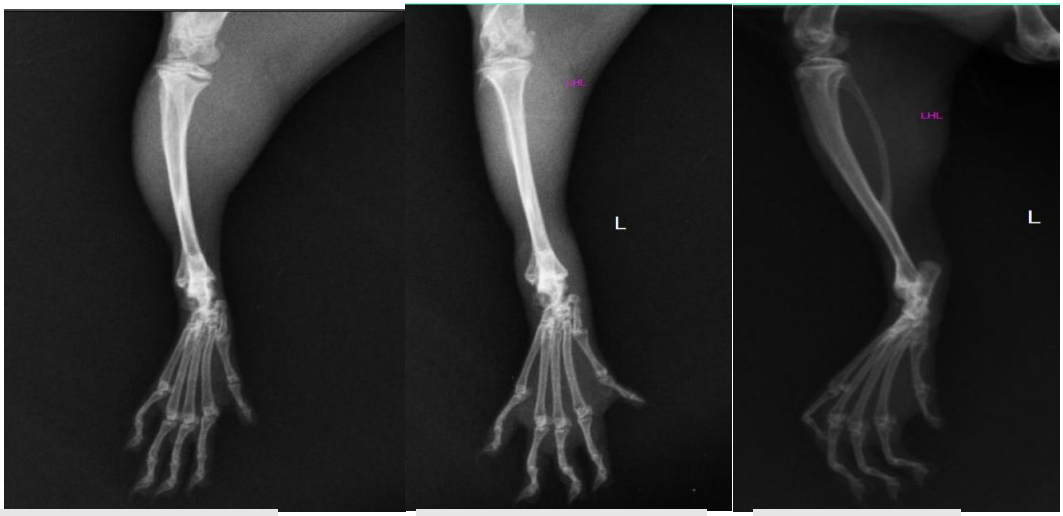
Hematological Analysis

Table 5 Hematological Parameters with Mean ± SEM

Parameter	Control	Disease Control	Standard	Test 1	Test 2
Hemoglobin (g/dL)	12.40 ± 0.08	11.86 ± 0.06	13.50 ± 0.06	10.56 ± 0.05	12.34 ± 0.07
Total Leucocyte Count (Cells/mm³)	5586.0 ± 104.3	10320.6 ± 124.5	10620.4 ± 90.4	7509.6 ± 48.7	8514.4 ± 67.3
Platelet Counts (Lac cells/mm³)	451861.0 ± 2138.1	458749.6 ± 2048.7	510670.6 ± 2178.1	462767.6 ± 1723.4	568063.6 ± 1642.7
RBC Count (Million cells/µL)	6.87 ± 0.03	3.89 ± 0.06	3.60 ± 0.04	5.68 ± 0.02	6.75 ± 0.03
Serum Urea (mg/dL)	49.12 ± 0.20	42.13 ± 0.11	47.04 ± 0.17	55.11 ± 0.18	46.09 ± 0.16
Rheumatoid Factor (IU/mL)	11.80 ± 0.07	24.26 ± 0.18	13.60 ± 0.07	18.38 ± 0.09	14.90 ± 0.09
C-Reactive Protein Assay (mg/L)	2.77 ± 0.01	3.75 ± 0.02	3.81 ± 0.02	3.66 ± 0.03	4.44 ± 0.03

Results are expressed as mean ± Sem (n=5)

Radiological Analysis



a) Control Group **b) Disease Control** **c) Standard**

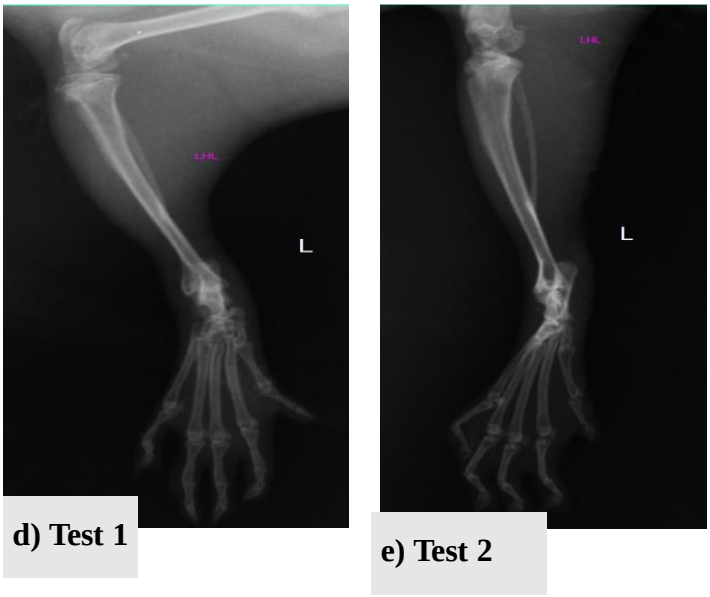


Figure 6: Radiographical images of hind paws of rats.

The radiographic images of Wistar rats' left hind paws illustrate various conditions. The control image shows normal bone structure with no signs of inflammation or bony changes, serving as a baseline. The disease control image, likely from a model induced by Complete Freund's Adjuvant, shows subtle soft tissue changes and possible joint space narrowing. The standard image reflects mild bone or joint changes typical of a standard experimental response. Test 1 shows increased soft tissue swelling and early bone changes, while Test 2 reveals more pronounced joint space narrowing, swelling, and advanced bone erosions, indicating a more severe pathology.

Histopathological Assessment

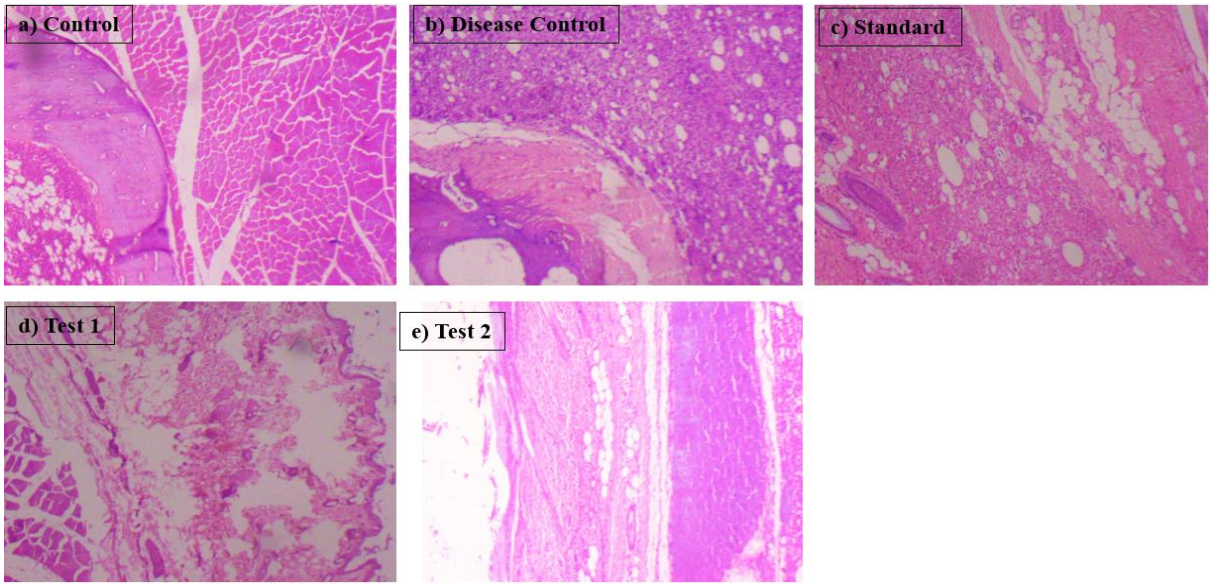


Figure 7: Histopathological evaluation

The histopathological evaluation using Hematoxylin and Eosin (H&E) staining revealed distinct tissue responses across the groups. The control group showed normal histology with no pathological changes. In contrast, the disease control group exhibited significant pathological alterations, including granuloma formation and marked inflammation. The standard treatment group showed reduced edema and inflammation, indicating effective disease mitigation. The Test 1 group displayed decreased granuloma formation and mild inflammation, suggesting a positive but less pronounced response. The Test 2 group showed no granuloma and only mild edema, indicating significant efficacy, comparable to the standard treatment.

DISCUSSION

The phytochemical analysis of *Lychee chinensis* leaves extract confirmed the presence of various bioactive compounds, including alkaloids, terpenoids, saponins, tannins, phenolic compounds, proteins, and steroids. These compounds are known for their anti-inflammatory, antioxidant, and analgesic properties, crucial for managing arthritis. The physicochemical analysis indicated the extract's quality and stability, with acceptable ash value, substantial extractive value, and low moisture content, ensuring its purity, potency, and shelf-life. UV and IR spectroscopic analyses further confirmed the presence of phenolic compounds and flavonoids, as well as functional groups like hydroxyl and carbonyl, which contribute to the extract's anti-inflammatory and antioxidant activities.

The anti-arthritic potential of the herbal oil formulated from *Lychee chinensis* extract was assessed using the Complete Freund's Adjuvant (CFA) model in albino rats. The results showed significant reductions in paw edema and inflammation, with the efficacy of the test formulations comparable to standard treatment. Hematological analysis supported these findings, showing a marked decrease in inflammatory markers such as total leucocyte count and C-reactive protein, along with normalized hemoglobin and platelet counts, indicating the extract's ability to reduce inflammation and maintain overall health in arthritic conditions.

Histopathological evaluation provided further evidence of the extract's therapeutic efficacy, with test groups showing reduced granuloma formation, mild inflammation, and absence of edema. These results highlight the protective effects of *Lychee chinensis* extract against pathological changes induced by CFA, consistent with the known benefits of anti-inflammatory phytochemicals in reducing tissue damage and promoting healing in inflammatory conditions. Overall, these findings suggest that *Lychee chinensis* extract has strong potential as a natural therapeutic agent for treating arthritis.

CONCLUSION

In conclusion, the study indicates that herbal oil from *Lychee chinensis* leaves extract shows significant anti-arthritic activity, likely due to bioactive compounds like alkaloids, terpenoids, and phenolics. The extract's physicochemical stability and therapeutic potential support its use as a natural remedy for arthritis. Future research should focus on identifying specific bioactive constituents and their mechanisms, alongside clinical trials to validate the formulation's efficacy and safety in humans as a safer alternative to conventional treatments.

References

- Babu D, Vinaya J, Kumar S, Akhila CR. Herbal Oil and its Anti-arthritic Activity. International Journal of Novel Trends in Pharmaceutical Sciences. 2019 Sep 29;9(3):47-50.
- Centers for Disease Control and Prevention (CDC). (2018). Arthritis prevalence and activity limitations—United States, 2013–2015. Morbidity and Mortality Weekly Report, 67(9), 245-250.
- Gossec, L., Smolen, J. S., Ramiro, S., de Wit, M., Cutolo, M., Dougados, M., ... &Landewé, R. (2016). European League Against Rheumatism (EULAR) recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. Annals of the Rheumatic Diseases, 75(3), 499-510.
- Manabe, Y., Yang, J., Mori, R., Wada, M., Miyamoto, K., & Nagaoka, S. (2011). Formulation and characterization of a novel lipid-based oil-in-water emulsion containing CoQ10 and resveratrol. Journal of Nutritional Science and Vitaminology, 57(2), 123-129.
- Moore, R. A., Derry, S., McQuay, H. J., & Moore, R. (2015). Single dose oral analgesics for acute postoperative pain in adults. Cochrane Database of Systematic Reviews, (9).
- Sengupta, K., Sharma, M., & Pandey, R. (2008). A double blind, placebo controlled study of the efficacy and safety of 5-loxin for treatment of osteoarthritis of the knee. Arthritis Research & Therapy, 10(4), R85.
- Shah, R. R., Mohite, S. A., & Patel, N. R. (2018). Preparation and evaluation of polyherbal hair oil-an effective cosmetic. Asian Journal of Pharmaceutical Research, 8(1), 36-38.
- Shukla RK, Painuly D, Porval A, Shukla A. Proximate analysis, nutritive value, total phenolic content and antioxidant activity of Litchi chinensis Sonn. Nat Prod Indian J. 2012;8(9):361-9.
- Singh, J. A., Saag, K. G., Bridges, S. L., Akl, E. A., Bannuru, R. R., Sullivan, M. C., ... & Drazin, T. (2015). 2015 American College of Rheumatology guideline for the treatment of rheumatoid arthritis. Arthritis Care & Research, 67(1), 1-25.
- Smolen, J. S., Aletaha, D., McInnes, I. B. (2016). Rheumatoid arthritis. The Lancet, 388(10055), 2023-2038.
- Surh, Y. J. (2003). Cancer chemoprevention with dietary phytochemicals. Nature Reviews Cancer, 3(10), 768-780.
- Wen L, Wu D, Jiang Y, Prasad KN, Lin S, Jiang G, He J, Zhao M, Luo W, Yang B. Identification of flavonoids in litchi (Litchi chinensis Sonn.) leaf and evaluation of anticancer activities. Journal of functional foods. 2014 Jan 1;6:555-63.
- World Health Organization (WHO). (2021). Musculoskeletal conditions. Retrieved from https://www.who.int/news-room/fact-sheets/detail/musculoskeletal-conditions
- Yuniarti, R., Nadia, S., Alamanda, A., Zubir, M., Syahputra, R. A., & Nizam, M. (2020, February). Characterization, phytochemical screenings and antioxidant activity test of kratom leaf ethanol extract (Mitragyna speciosa Korth) using DPPH method. In *Journal of Physics: Conference Series* (Vol. 1462, No. 1, p. 012026). IOP Publishing.