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EXPLORING THE ANTI RHEUMATOID ACTIVITY OF ETHANOLIC EXTRACT FROM RHIZOME Alpinia calcarata USING A FORMALDEHYDE INDUCED RAT MODEL

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Abstract:
Aim: To evaluate the anti-rheumatoid activity of ethanolic extract from Alpinia calcarata rhizomes using a formaldehyde-induced rat model. Objectives: Collection and authentication of plant material. Assessment of the in-vitro anti-inflammatory and anti-arthritic potential of the ethanolic extract. Induction of rheumatoid arthritis using formaldehyde in rodent models. Comparison of pharmacological effects with a standard drug. Evaluation of oxidative stress parameters. Examination of the histopathological changes induced by the ethanolic extract in rheumatoid arthritis. Materials and Methods: Rats weighing 250-300 grams were randomly divided into six groups, each containing 6 animals, and received the following treatments. The first group served as a control and remained untreated. The second group received formaldehyde (0.1 ml, 2% v/v) via sub-plantar injection to induce disease. The third group was treated with diclofenac sodium at a dose of 10 mg/kg daily for 14 days as a standard drug. Results: Data are presented as mean ± SEM, with n=6 animals per group. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. **P < 0.01, ***P < 0.001 compared to the standard drug. Conclusion: The study demonstrated that serum CRP levels were significantly elevated in formaldehyde-induced arthritic rats compared to normal rats. Treatment with the ethanolic extract of Alpinia calcarata rhizomes resulted in a notable reduction in paw volume and other biochemical markers associated with rheumatoid arthritis.

Keywords: Rheumatoid Arthritis, paw, inflammation, Alpinia Calcarata.

I. INTRODUCTION

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder and autoimmune disease where the body's immune system mistakenly attacks its own tissues and joints, leading to inflammatory synovitis. This inflammation often progresses to joint destruction, ankylosis, and damage to articular cartilage [1]. RA predominantly affects females more than males and is commonly observed in the elderly. The prevalence of RA in 2002 ranged from 0.5% to 1% of the population, showing regional variations.

An autoimmune disease results from an abnormal immune response where the immune system, typically designed to protect the body by targeting and eliminating invaders, instead attacks healthy tissues[2]. The synovium, a thin lining inside joints that nourishes cartilage, thickens during RA, causing inflammation and pain around the joints [3]. RA primarily affects the synovial joints' lining and can lead to progressive disability, premature death, and significant socioeconomic burdens. Synovial cells produce joint lubricants such as collagens, fibronectin, and hyaluronic acid, which are crucial for joint mobility and form the structural framework of the synovial interstitial [3].

Several factors contribute to the risk of developing RA, including gender, age, environmental factors, and reproductive status. Genetic factors also play a significant role in susceptibility to RA [4]. The disease manifests with periods of flare-ups and remissions. Chronic inflammation in RA can permanently damage joints and cause deformities [5]. Symptoms include warm, swollen, painful, and stiff joints that worsen with rest. Typically, RA affects multiple joints symmetrically, including those in the fingers, hands, wrists, feet, and knees. It can also affect other organs, potentially leading to conditions such as anemia, lung inflammation, and heart complications [6].

Clinical manifestations of symmetric joint involvement include redness, swelling, and potentially limited range of motion, alongside joint pain (arthralgia). Effective management focuses on achieving the best possible outcomes[7].

Joint Destruction and Early Diagnosis

Effective management of rheumatoid arthritis (RA), aiming for outcomes like reduced joint destruction, minimal radiologic progression, absence of functional disability, and achieving DMARD-free remission, hinges significantly on early diagnosis. The first 12 weeks after symptom onset are considered the optimal therapeutic window [8]. However, early diagnosis remains challenging, relying predominantly on clinical evaluation, medical history, imaging studies, and laboratory tests for confirmation [9]. Delayed diagnosis and initiation of disease-modifying antirheumatic drugs (DMARDs) vary widely across healthcare systems, influenced by both physician and patient factors.

Epidemiology

Globally, RA affects approximately 3 out of every 10,000 individuals annually, with a prevalence rate around 1%. The disease typically worsens with age, peaking between 35 and 50 years. RA affects all populations, but prevalence rates vary, with some groups such as certain Native American populations having higher prevalence rates (5-6%) compared to others. First-degree relatives of RA patients have a two- to three-fold higher risk of developing the disease, indicating a significant genetic component. However, environmental factors also play a crucial role, as evidenced by the 15-20% disease concordance rate in monozygotic twins. The stable global prevalence of RA suggests a potential role for a ubiquitous infectious agent in disease causation [10].

RA is more prevalent in women, affecting approximately three times as many women as men, though this discrepancy diminishes with age [11]. Research investigating reproductive risk factors found that women who had given birth to one child had a higher RA risk compared to

those who had two or three children. However, nulliparous women or those with a history of miscarriages did not show an increased risk [12].

Signs and Symptoms

The onset of rheumatoid arthritis is typically insidious, often starting with symptoms like fever, malaise, arthralgias, and muscle weakness before progressing to joint inflammation and swelling. Common symptoms include joint pain, stiffness, and swelling, often worse in the morning. Affected joints may appear red and feel warm, persisting for at least six weeks. Severe cases can lead to joint deformities if untreated.

RA usually begins in smaller joints like those in the hands and feet, progressing to involve wrists, knees, ankles, elbows, hips, and shoulders as the disease advances. Symptoms typically affect joints bilaterally (both sides of the body). Symptoms may vary in severity and can alternate between periods of increased disease activity (flares) and periods of relative remission. Over time, RA can cause joints to deform and shift out of place.

RESULTS

1. Phytochemical Constituents of the Crude Extract

The crude extract yielded 17.10% by weight. Qualitative phytochemical screening indicated the presence of carbohydrates and proteins in the *Alpinia calcarata* rhizome extract. Steroids were detected, while flavonoids were absent in both extracts.

2. Pharmacological Investigations

2.1. Protein Denaturation Method

In the assessment of anti-inflammatory activity, the effect of the plant extract on protein denaturation was investigated. The extract demonstrated significant inhibition of formaldehyde-induced protein denaturation. Diclofenac sodium, used as a standard anti-inflammatory agent, exhibited the highest percentage inhibition at 92.40%. The ethanolic extract of *Alpinia calcarata* rhizome showed substantial percentage inhibition values of 81.08%, 85.46%, and 90.74% at concentrations of 400 µg/ml, 800 µg/ml, and 1000 µg/ml, respectively, indicating potent anti-inflammatory activity comparable to the standard.

Effect of ethanolic extract of *Alpinia calcarata* on protein denaturation.

Concentration (µg/ml)	Absorbance [A]	% inhibition
100	0.395 ± 0.002	70.88
200	0.225 ± 0.01	75.55
400	0.185 ± 0.01**	81.08**
800	0.172 ± 0.02***	85.46***
1000	0.162 ± 0.01***	90.74***
Diclofenac sodium (10mg/ml)	0.158 ± 0.02	92.40

3. Histopathological Study

Histopathological analysis revealed distinct differences between the joints of rats with adjuvant-induced arthritis and those of normal rats. In the hind paw joints of the arthritic control group, histological examination showed significant abnormalities such as breakdown

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of bone marrow and extensive cell infiltration on the articular surface. Cellular infiltrations were prominently observed at the synovial lining in arthritic control rats. In contrast, rats treated with EREAC (Ethanolic Extract of Alpinia calcarata) and Diclofenac showed no evidence of cell infiltration or bone marrow damage. Throughout the 14-day treatment period with EREAC at 250 mg/ml, some cell infiltration persisted. EREAC at concentrations of 500 mg/ml and 1000 mg/ml demonstrated reduced cell infiltration and bone marrow damage in both early and established stages of arthritis.

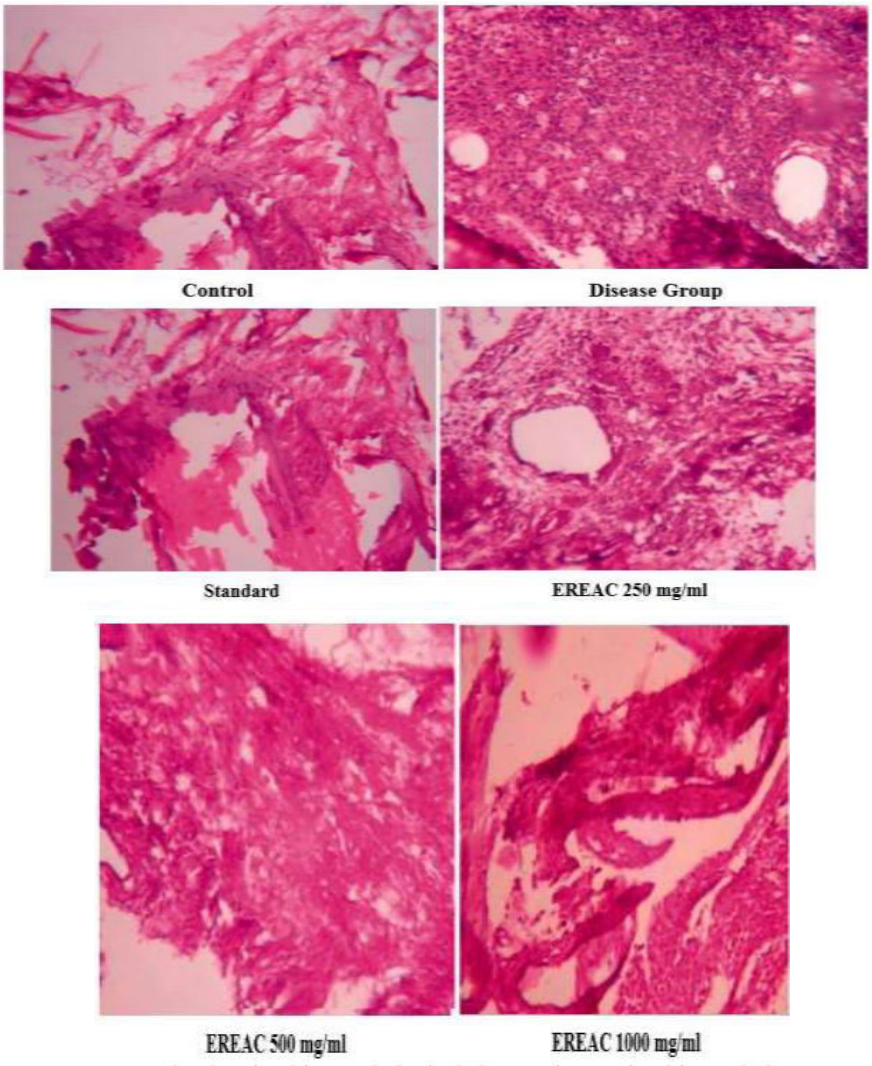

Figure 1: Photomicrographs showing histopathological changes in proximal interphalangeal joints tissue using H & E (40X) on formaldehyde-induced arthritic.

CONCLUSION

The investigation demonstrated that the ethanolic extract of *Alpinia calcarata* rhizomes possesses anti-arthritic activity. This activity is likely attributed to its antioxidant and anti-inflammatory properties, supported by the presence of Carbohydrate, Proteins, Steroids, and flavonoids identified through phytochemical analysis. The observed anti-arthritic effects could be linked to these chemical constituents. The study results validate the plant's traditional use and emphasize the need for further research to isolate and characterize the active principles responsible for its anti-arthritic activity. Furthermore, the study indicated that serum CRP levels in formaldehyde-induced arthritic rats were significantly higher

compared to normal rats, but treatment with the ethanolic extract showed a substantial reduction in paw volume and other biochemical markers of rheumatoid arthritis. Administering the ethanolic extract of *Alpinia calcarata* rhizomes alone improved the RA therapeutic score, particularly evident in the reduction of serum CRP levels. The findings suggest that EREAC at doses of 500 mg/kg and 1000 mg/kg exhibits promising anti-arthritic activity, effectively controlling inflammation in a formaldehyde-induced arthritis model in rats.

REFERENCES

1. A. B. Gokhale, A. S. D. K. R. K. and M. N. S. (2002). the form of a multicomponent oral formulations, Rumalaya ® and Geriforte®.Adeyeye, C. M., &Li, P. K. (1990). Diclofenac Sodium. Analytical Profiles of Drug Substances and Excipients, 19(C), 123–144. https://doi.org/10.1016/S0099-5428(08)60366-4
2. Mechling, C. et al. ,. (2020). Rheumatoid Arthritis: A Literature Review and Comprehensive Rheumatoid Arthritis: A Literature Review and Comprehensive Treatment Analysis Treatment Analysis. https://red.library.usd.edu/honorsthesis/81
3. Mcinnes, I. B., &Schett, G. (2011). The Pathogenesis of Rheumatoid Arthritis.Perveen, R., Islam, F., Khanum, J., & Yeasmin, T. (2012). Preventive effect of ethanol extract of Alpinia calcarataRosc on Ehrlich ' s ascitic carcinoma cell induced malignant ascites in mice Asian Pacific Journal of Tropical Medicine. In Asian Pacific Journal of Tropical Medicine. www.elsevier.com/locate/apjtm
4. Gabriel, S. E., & Michaud, K. (2009). Epidemiological studies in incidence, prevalence, mortality, and comorbidity of the rheumatic diseases. In Arthritis Research and Therapy (Vol. 11, Issue 3). https://doi.org/10.1186/ar2669.
5. Chandrakanthan, M., Handunnetti, S. M., Premakumara, G. S. A., &Kathirgamanathar, S. (2020). Topical Anti-Inflammatory Activity of Essential Oils of Alpinia calcarataRosc., Its Main Constituents, and Possible Mechanism of Action. Evidence-Based Complementary and Alternative Medicine, 2020. https://doi.org/10.1155/2020/2035671.
6. Radhika Chikatipalli, Saravanakumar K, Chandra Sekhar Kothapalli Bannoth, Pharmacognostic evaluation and free radical scavenging activity of Bombax ceiba leaf extracts, International Journal of Green Pharmacy 2021, 15 (1), 59-65.
7. Choudhary, M., Kumar, V., Gupta, P. K., & Singh, S. (2014). Anti-arthritic activity of Barleriaprionitis Linn. leaves in acute and chronic models in Sprague Dawley rats. Bulletin of Faculty of Pharmacy, Cairo University, 52(2), 199–209. https://doi.org/10.1016/j.bfopcu.2014.07.002
8. Felson, D. T., Smolen, J. S., Wells, G., Zhang, B., Van Tuyl, L. H. D., Funovits, J., Aletaha, D., Allaart, C. F., Bathon, J., Bombardieri, S., Brooks, P., Brown, A., Matucci-Cerinic, M., Choi, H., Combe, B., De Wit, M., Dougados, M., Emery, P., Furst, D., … Boers, M. (2011). American college of rheumatology/European league against rheumatism provisional definition of remission in rheumatoid arthritis for clinical trials. Arthritis and Rheumatism, 63(3), 573–586. https://doi.org/10.1002/art.30129.

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 12, Iss 1, 2023

9. Premrajan, P., Jayananadan, A., Chelankara Suresh, S., & Jayadevi Variyar, E. (2021). Protective effects of phytocompounds from Alpinia calcarata (Haw.) Roscoe in Freund's adjuvant induced arthritis in rats. In Indian Journal of Experimental Biology (Vol. 58).
10. Saravanakumar K, Chandra Sekhar KB, Jayachandra Reddy P, Grace Rathanam. Elucidation of pharmacognostic profiles and pharmacological activity of Tephrosia purpurea. International Journal of Research in Pharmaceutical Sciences 2011, 2(4), 1-4.
11. Ramadan, G., Al-Kahtani, M. A., & ElSayed, W. M. (2011). Anti-inflammatory and anti-oxidant properties of curcuma longa (turmeric) versus Zingiber officinale (ginger) rhizomes in rat adjuvant-induced arthritis. Inflammation, 34(4), 291–301. https://doi.org/10.1007/s10753-010-9278-0.
12. Trier, N., Izarzugaza, J., Chailyan, A., Marcatili, P., & Houen, G. (2018). Human MHC-II with shared epitope motifs are optimal Epstein-Barr virus glycoprotein 42 ligands—relation to rheumatoid arthritis. In International Journal of Molecular Sciences (Vol. 19, Issue 1). MDPI AG. https://doi.org/10.3390/ijms19010317.