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Antirheumatoid Activity of Ethanolic Extract of Rhizome Alpinia Calcarata by Formaldehyde Induce Rat Model

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ABSTRACT:

Aim: Antirheumatoid activity of ethanolic extract of rhizome Alpinia calcarata by formaldehyde induce rat model.

Objective: Collection and Authentication of plants. To study the in-vitro anti-inflammatory or anti-arthritic activity of the ethanolic extract of Alpinia calcarata rhizomes. To develop formaldehyde-induced rheumatoid arthritis in a rodent model.To compare the pharmacological actions with standard drug. To evaluate oxidative stress.To examine the effect of ethanolic extract of Alpinia calcarata rhizomes on histopathological alteration in rheumatoid arthritis.

Material and methods: Rats (250-300 gm) were randomly divided into six groups with each group having 6 animals and received the following treatments. The first group served as control ground was untreated, second group served disease group on which Formaldehyde 0.1 ml 2% v/v by Sub-plantar region. The third group served as standard group administered diclofenac sodium daily at a dose of 10 mg/kg for 14 days.

Result:All values are represented as mean ± SEM, n=6 animals in each group. Data were analyzed by one-way ANOVA, followed by Tukey's multiple comparison test. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with standard drug.

Conclusion: The results indicated that serum CRP levels in formaldehyde induced arthritic rats were higher than in normal rats and showed a significant reduction in paw volume and other biochemical markers or rheumatoid arthritis.

Keywords: Rheumatoid Arthritis, paw, inflammation, Alpinia Calcarata

I. INTRODUCTION

Rheumatoid Arthritis

Rheumatoid arthritis is a chronic, systemic inflammatory disorder or a long term auto immune multisystem illness in which the body's immune system attacks the body's tissues and joints mistakenly causing an inflammatory synovitis which often progresses the destruction of joint ankylosis and articular cartilage [1] It arises more frequently in females than males, being predominantly observed in the elderly. The prevalence rate reported in 2002 ranged from 0.5% to 1% of the population and had regional variation. An autoimmune disease is a condition which arises from an abnormal response to our normal immune system. The immune system is a host defense mechanism comprising complex organization of cells and antibodies designed normally to "seek and destroy" invaders of the body. The synovium (inside of joints) is a thin delicate lining serves as an important source of nutrients for cartilage which thickens during RA resulting in inflammation and pain in and around the joints[2]. RA primarily affects the lining of the synovial joints and can cause progressive disability, premature death, and socioeconomic burdens. Additionally, synovial cells synthesize joint lubricants and helps them move smoothly such as collagens, as well as fibronectin and hyaluronic acid that constitute the structural framework of the synovial interstitial [3]

The following factors can affect a person's risk of developing rheumatoid arthritis (RA): gender, age, environment, and reproductive status. Several studies have shown that genetic factors also have a significant impact on an individual's risk of developing RA. The disease is characterized by flare-ups and remissions. The chronic inflammation of RA can cause permanent joint destruction and deformity. It causes warm, swollen, painful, and stiff joints that worsen with rest (Kinne et al., 2007). Usually, multiple joints of the fingers and hands, wrists, feet, and knees get affected in a symmetrical distribution (affecting both sides of the body). It may also affect other parts of the body, which can result in low red blood cell count, inflammation around the lungs, and a heart.[4]

Clinical signs of symmetric joint involvement can include redness, edema, and possibly limited range of motion in addition to arthralgia. The most desirable outcomes (i.e., less

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joint destruction, less radiologic progression, no functional disability, and DMARD-free remission) and cost effectiveness are thought to be largely dependent on early diagnosis, with the first 12 weeks following the onset of symptoms being thought to be the optimal therapeutic window.

Early diagnosis is still difficult, though, since it depends mostly on clinical data from the patient's physical examination and medical history, which is further confirmed by imaging analysis and blood testing.[5] The causes for a postponed diagnosis varies significantly throughout nations with different healthcare systems, and the reasons for a postponed DMARD medication start tend to be physician- and patient-dependent.

Epidemiology

Globally, there are roughly 3 cases of RA annually per 10,000 people, and the prevalence rate is roughly 1%. The disease worsens with age and peaks between the ages of 35 and 50. All populations are affected by RA, however some are affected more than others (e.g., certain Native American groups have a 5-6% prevalence) while others are affected less than others (e.g., black folks from the Caribbean region)

First-degree relatives of RA patients have a two- to three-fold increased risk of developing the illness. About 15-20% of monozygotic twins have disease concordance, indicating a significant contribution from nongenetic variables. Owing to the comparatively stable global prevalence of RA, an omnipresent infectious agent has been proposed as the causative factor.[6]

About three times as many women as men are afflicted with RA, while the differences in sex disappear as people get older. A Danish study that looked into whether certain reproductive risk factors might be associated with women's higher rate of RA discovered that women who had given birth to just one child had a higher likelihood of RA than women who had given birth to two or three children. Nonetheless, women who were nulliparous or had a history of miscarried pregnancies did not see an increase in the rate.

Signs And Symptoms

The onset of rheumatoid arthritis in most patients is insidious, often beginning with fever, malaise, arthralgias, and muscle weakness before progressing to inflammation and swelling of the joints. Joint pain, stiffness and swelling are the most common symptoms of arthritis. Redness and warm joint which is lasts for 6 weeks Sudden high fever. Rheumatoid factor presents and red blood cell decreased in blood testing Many people with arthritis notice their symptoms are worse in the morning. Severe rheumatoid arthritis can cause joint deformity if left untreated. Early rheumatoid arthritis tends to affect your smaller joints first — particularly the joints that attach your fingers to your hands and your toes to your feet. As the disease progresses, symptoms often spread to the wrists, knees, ankles, elbows, hips and shoulders. In most cases, symptoms occur in the same joints on both sides of your body[7]

Rheumatoid arthritis signs and symptoms may vary in severity and may even come and go. Periods of increased disease activity, called flares, alternate with periods of relative remission. when the swelling and pain fade or disappear. Over time, rheumatoid arthritis can cause joints to deform and shift out of place.[8]

II. EXPERIMENTAL WORK

1. Material

1.1. Plant selected

In the present study, Alpinia calcarata was selected because of its traditional uses. Alpinia calcarata rhizome was collected from local area of Ratnagiri district (India).

1.2. Drugs protocol

Diclofenac sodium was purchased from apex medical store, Nagpur.

1.3. Experimental Animals and IAEC approval

The animals were used as healthy male Wistar rats (250-300 g) were obtained Kusum life science A 3, MIDC, Wasmat, Hingoli. The animals were kept in sterilized cages in rooms at approximately 24±1℃ temperature and humidity of 55± 5% with a 12/12-hour light-dark cycle. Free access to food and water was allowed. Before starting the animal, study rats had been held under quarantine for 5 days. All the experiments were performed by the Institutional Animal Ethics Committee (IAEC) (Registration number-388/PO/Re/S/02/CPCSEA Dt. 19.10.2023; Protocol no./2023-24/05) constituted as per directions of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), Ministry of Animal Welfare Division, Government of India, New Delhi.

1.4. Instrument

U-V spectrophotometer, Centrifuge, weighing balance, volumetric flask, mortar and pestle.

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2. Methods

2.1. Collection and authentication of Alpinia calcarata

The dried rhizomes of the Alpinia calcarata were collected. The rhizomes were cleaned and shade dried and milled into coarse powder by a mechanical grinder.

2.2. Preparation of plant extracts

The powdered rhizomes were extracted using ethanol by Soxhlet extractor. In this process the powdered drug is placed into the extractor with ethanol as solvent. After extraction the extract was evaporated under reduced pressure at 40o C using a rotary evaporator to have ethanol extract [4]

2.3. Experimental protocol

Rats (250-300 gm) were randomly divided into six groups with each group having 6 animals and received the following treatments. The first group served as control ground was untreated, second group served disease group on which Formaldehyde 0.1 ml 2% v/v by Sub-plantar region. The third group served as standard group administered diclofenac sodium daily at a dose of 10 mg/kg for 14 days. The fourth, fifth and sixth groups, were treated as test groups and were administered ethanolic rhizome extract of Alpinia calcarata (EREAC) at a dose of 250, 500 and 1000 mg/kg, respectively, for 14 days. All treatments were dissolved in distilled water and given orally; 30 min after drug administration. The treatments were administered to rats, 1 day ahead of formaldehyde injection and daily treatment continued for 14 days. The experimental design and exposure protocol are shown in Table 1.

Expiremental animal group

Sr. No	Animal Groups	Treatment
1.	**Control**	0.9 % normal saline administered byp.o for 14 days
2.	**Disease Group**	Treatment of Formaldehyde 0.1 ml 2% v/v by Sub-plantar region on days 1 and 3 of the experiment.
3.	**Standard**	Treatment of 10 mg/kg of diclofenac sodium p.o for 14 days.
4.	**EREAC 250 mg/kg**	Treatment of 250 mg/kg EREAC p.o for 14 days
5.	**EREAC 500 mg/kg**	Treatment of 500 mg/kg EREAC p.o for 14 days
6.	**EREAC 1000 mg/kg**	Treatment of 1000 mg/kg EREAC p.o for 14 days

III. RESULTS

1. Phytochemical Constituents of the Crude Extract

The percentage yield value of the crude extract was 17.10%. According to the qualitative phytochemical screening study, the crude extract of the of Alpinia calcarata rhizomes extract was found to be positive for the presence of Carbohydrate, Proteins, Steroids, and flavonoids were absent in both extracts.

Table: Phytochemical constituent of Alpinia Calcarata rhizomes extract

Secondary metabolite	Test results
Carbohydrate	+
Proteins	+

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Amino acids	-
Steroids	+
Flavonoids	+
Alkaloids	-
Fats and oils	-

+: Present, -: Absent

2. Pharmacological investigations

2.1. Protein Denaturation Method

As part of the evaluation of anti-inflammatory activity, ability of plant extract on protein denaturation was studied. It was effective in inhibiting formaldehyde induced protein denaturation. Diclofenac sodium a standard anti-inflammatory agent possesses maximum % inhibition. Percentage inhibition of Diclofenac sodium (Standard) was found to be 92.40. The results indicated that ethanolic extract showed the maximum percentage inhibition which is compared with the standard. The percentage inhibition of protein denaturation assay by ethanolic extract of the plant Alpinia calcarata rhizome was found to be 81.08, 85.46 and 90.74 possess significant % inhibition activity at concentration 400 µg/ml, 800 µg/ml and 1000 µg/ml.

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

An analysis of the histopathology reveals the distinctions between the rat joint with adjuvant-induced arthritis and the normal ankle joint. The arthritic control group's hind paw joints' histological analyses revealed notable abnormalities such as bone marrow breakdown and widespread cell infiltration on the articular surface. In the arthritic control rats, there were numerous cellular infiltrations at the synovial lining. There was no evidence of cell infiltration or bone marrow damage in the EREAC and Diclofenac-treated groups. Cell infiltration was observed for the course of the 14-day EREAC 250 mg/ml treatment. In both the nascent and established stages of arthritis, EREAC 500 mg/ml and EREAC 1000 mg/ml demonstrated reduced cell infiltration and bone marrow damage.

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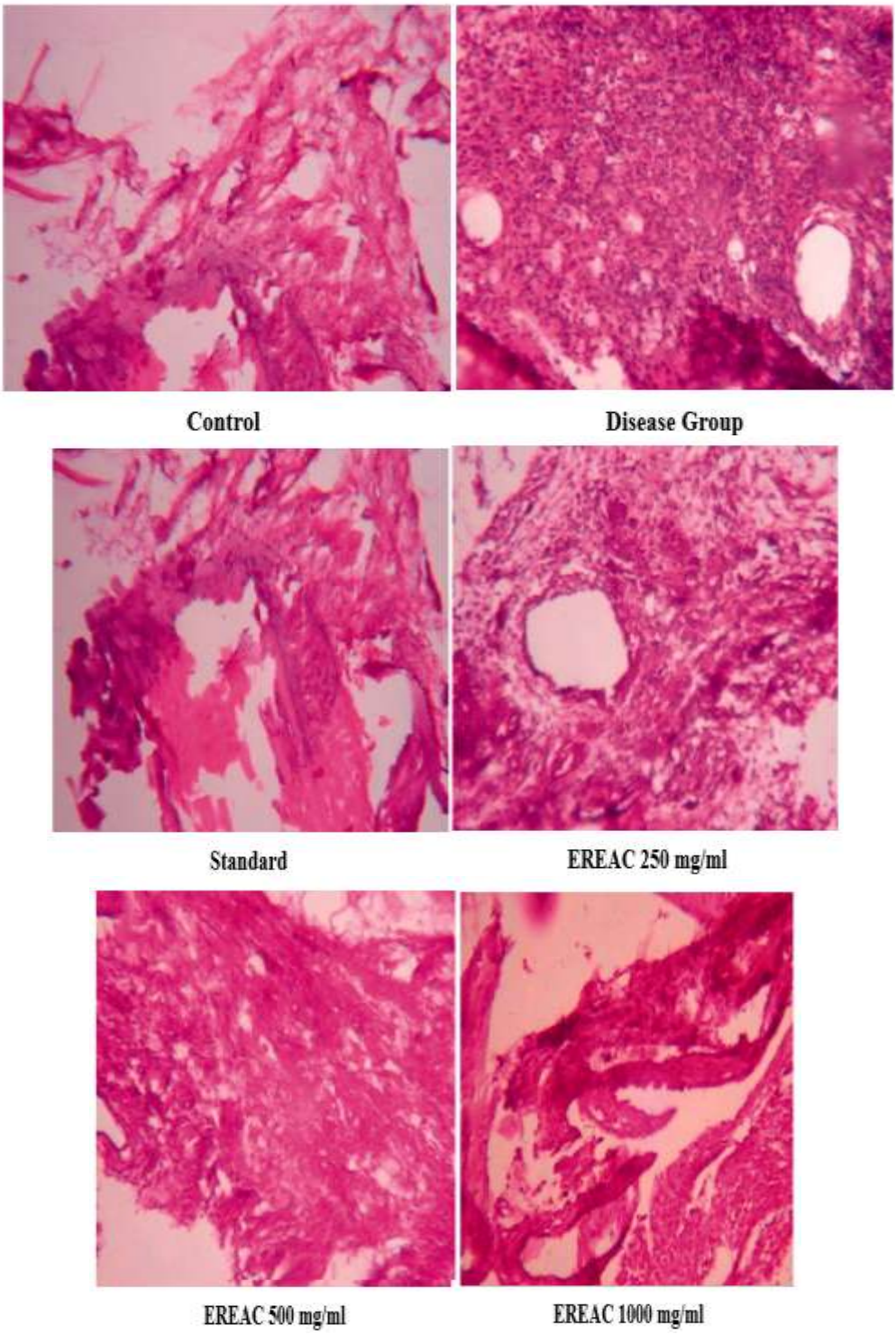

Figure :Photomicrographs showing histopathological changes in proximal interphalangeal joints tissue using H & E (40X) on formaldehyde-induced arthritic.

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IV. DISCUSSION

Rheumatoid arthritis is an inflammatory, autoimmune disorder which destroys its own immune system. The immunologically mediated formaldehyde induced arthritic model of chronic inflammation is considered as the best available experimental model of rheumatoid arthritis. Formaldehyde -induced arthritis is a model of chronic polyarthritis with features that resemble rheumatoid arthritis.

The ethanolic extract of Alpinia calcarata rhizomes were subjected to phytochemical screening. The result indicated that, rhizome extract shows the presence of carbohydrate, proteins, Steroids and flavonoid. The knowledge of chemical constituents of plant is desirable because such information will be valuable for synthesis of complex chemical substances and to screen for biological activities. Denaturation of protein is a well-documented cause of inflammation. Production of auto antigens may be due to the denaturation of tissue protein.[9] Agents that can prevent protein denaturation therefore would be worthwhile for anti-inflammatory drug development. The mechanism of denaturation probably involves the alteration electrostatic hydrogen, hydrophobic and disulphide bonding. It has been reported that one of the features of several non-steroidal anti-inflammatory drugs in their ability to stabilize heat treated protein at physiological PH. The ethanolic extract of the Alpinia calcarata rhizomes exhibited concentration dependent inhibition of protein denaturation. Therefore, from the study it can be concluded that the rhizomes of the plant extract possess marked in vitro anti-inflammatory effect. Histopathological study shows the differences in the normal ankle joint and formaldehyde-induced arthritic rat joint. In general, histopathological studies on arthritic joint shows the prominent abnormalities from the normal joint like oedema formation, degeneration with partial erosion of the cartilage, destruction of bone marrow and extensive infiltration of inflammatory exudates in the articular surface. In the present study, the histopathological studies of hind paw joints in arthritic control rats showed the prominent abnormalities like destruction of the bone marrow and extensive infiltration of the cells in the articular surface.[10] EREAC 500 mg/ml and EREAC 1000 mg/ml treatment have shown marked reduction in all the above-mentioned pathological conditions, indicating its effective antiarthritic activity by protecting the bone from degeneration.

V. CONCLUSION

The result of the investigation showed that the ethanolic extract of Alpinia calcarata rhizomes possess anti arthritic activity. The antioxidant and anti-inflammatory property of the plant also supports its anti- arthritic property. Phytochemical analysis showed presence of Carbohydrate, Proteins, Steroids, and flavonoids. The anti-arthritic property showed by the plant may be because of these chemical moieties. The results obtained in the study supports the traditional and also demands further research and to isolate and characterize active principles responsible for anti-arthritic activity. The results indicated that serum CRP levels in formaldehyde induced arthritic rats were higher than in normal rats and showed a significant reduction in paw volume and other biochemical markers or rheumatoid arthritis. The RA therapeutic score may be improved by administering the ethanolic extract of Alpinia calcarata rhizomes alone based on the principal excursion of serum CRP.[11] it is concluded that at the doses of 500mg/kg and 1000 mg/kg EREAC possesses potentially useful anti-arthritic activity since it gives a positive result in controlling inflammation in formaldehyde induced arthritis model in rats.

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