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EVALUATION OF ANTI-ARTHRITIC POTENTIAL OF *ELEPHANTOPUS SCABER* IN COMPLETE FREUND'S ADJUVANT INDUCED ARTHRITIC RAT MODEL

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ABSTRACT

*Elephantopus scaber L.*is widely used folklore in the treatment of nephritis, dampness, pain in the chest, fever and cough, scabies, edema and arthralgia. Rheumatoid arthritis (RA) is a multifactoral systemic autoimmune disease with unknown causes. The associated side effects of the anti arthritic agents motivated the author to carry out this work. The present study aims to evaluate the anti-arthritic potential of *Elephantopus scaber* leaves using complete freund's adjuvant induced arthritic rat model. Coarsely powdered leaves of *Elephantopus Scaber* Linn. were extracted with methanol and distilled water to get the test extracts. Arthritis was induced by single sub cutaneous injection 0.1 ml of Complete Freund's Adjuvant emulsion (CFA) into the sub plantar surface of the left hind paw. Treatment with test extracts and standard were continued up to 28 days after induction. The test extracts significantly $p<0.01$decreases the paw swelling and rectify the deranged hematological and biochemical parameters induced by complete freund's adjuvant. It can be concluded from the experiment that *Elephantopus scaber* extracts possesses significant anti-arthritic potential which justify its traditional use.

Key words: *Elephantopus scaber L.*, Rheumatoid arthritis, Anti-arthritic potential.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease of unknown etiology. The disease is characterized by articular inflammation by the formation of an inflammatory and invasive tissue, rheumatoid pannus that eventually leads to the destruction of joints. Analgesia (painkillers) and anti-inflammatory drugs, including steroids are used to suppress the symptoms, while disease-modifying antirheumatic drugs (DMARDs), newer therapies such as anti-tumor necrosis factor (TNF)-α therapy (Etanercept, infliximab and adalimumab), anti-CD20 therapy (Rituximab) and Abatacept are often required to inhibit or halt the underlying immune process. However, all of these agents are associated with numerous side effects. In recent days, researchers are directed towards traditional system of medicine for the discovery of drugs that are long acting anti-inflammatory with minimum side effects. Although there is no ideal animal model for RA at this time, rat adjuvant arthritis shares many features of human RA [1], and the adjuvant arthritis is the best available model of rheumatoid arthritis.

Elephantopus scaber L. is a member of *Asteraceae* family. The whole plant of *Elephantopus scaber L.* is a perennial herb and is well known as a Chinese folk medicine which is widely used in the treatment of nephritis, edema, dampness, pain in the chest, fever and cough of pneumonia, scabies, and arthralgia due to wounding [2,3]. It is also commonly used as a remedy for the treatment of gastropathy, hepatitis, nephritis, edema, chest pain, fever and cough of pneumonia, bronchitis, arthritis, and carbuncle. *Elephantopus scaber L.* is known to contain a large number of bioactive compounds such as ethyl hexadecanoate, ethyl-9, 12-octadecadienoate, ethyl-(*Z*)-9-

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octadecenoate, ethyl octadecanoate, lupeol, stigmasterol, stigmasterol glucoside, deoxyelephantopin and two new germacranolide sesquiterpene lactones named 17, 19-dihydrodeoxyelephantopin and *iso*-17, 19-dihydrodeoxyelephantopin [4]. The leaves are reported to have various activities like anticancer, anti bacterial, anti viral, hepatoprotective and anti microbial activity respectively. Therefore, we undertook the study to evaluate the anti-arthritic potential of *Elephantopus scaber* leaves using complete freunds adjuvant induced arthritic rat model.

MATERIALS AND METHODS

Collection of Plant material

The leaves were collected in west Godavari district. It was authenticated by Dr.D.V.Swami asst professor, Dr.Y.S.R.Horticulture University, Venkata ramannagudem-534101, W.G.Dt, A.P. The leaves were air-dried at room temperature. The dried leaves were crushed to powder with the help of mixer grinder. The resultant dried powder was used for extraction process.

Preparation of extract

The leaves of Elephantopus Scaber Linn. were dried under shade and then coarsely powdered. The powder was passed through sieve no.40 and stored in an air tight container for further use. The powder was then extracted with methanol and distilled water using Soxhlet apparatus for 72 hrs. The extract was dried and stored in desiccator. The extracts were subjected for chemical analysis by the standard procedures for identification of various phytoconstituents.

Animals

Wistar albino rats weighing between 140-200gm were selected. The animals were acclimatized to the standard laboratory conditions (temperature 25 ± 2◦C) and maintained on 12 hr light, 12 hr dark cycle. The animals were fed with standard diet and water ad libitum. The animals were maintained as per the norms of CPCSEA.

Treatment groups

Animals were divided into five groups of six animals each.

Normal Control Group : Normal saline 5ml/kg, and diet
Disease Control Group : Normal saline 5ml/kg, and diet
Standard Group: Methotrexate 0.6 mg/kg/week and diet
Test group-1 : Methanol extract 200mg/kg and diet
Test group-11 : Aqueous extract 200mg/kg and diet

Anti-arthritic activity

Arthritis was induced by single sub cutaneous injection 0.1 ml of Complete Freund's Adjuvant emulsion (CFA) into the sub plantar surface of the left hind paw of the all animal except those in normal control group [5]. CFA consists of Mycobacterium tuberculosis (H 37 RA, ATCC 25177) Heat killed and dried, 0.85 ml sterile liquid paraffin oil and 0.15 ml mannide monooleate to a final concentration of 1mg/ml. Two different extracts of test drugs prepared by triturating with 3% gum acacia (200mg/kg) preparations were administered to two different test groups comprising six animals each. Methotrexate is given as standard drug at a dose of 0.6mg/kg/week [6] .The doses were administered daily orally at the same time. The doses were revised by body weight changes every week of CFA and continued up to 14 th post CFA challenge day. The change in the inflammatory reaction was measured by using plethysmometer at 1 hr, 3, 6, 8,12,16,18,24, after CFA induced day. Again paw volume measured on 2, 4, 8, 10, 14, 18, 22, 24, 26 days .At the end of last day animals were anaesthetized with ketamine. Blood was withdrawn by retro-orbital vein puncture for assessment of hematological and biochemical parameters. Arthritis induced leg was sent for radiology. Bone tissues were removed for histology assessment.

RESULTS

FCA induced rat paw edema

There is a significant increase in rat paw volume in FCA injected arthritic control rats when compared to the normal control rats. Treatment with standard and test extracts showed significant reduction in rat paw volume when compared with the arthritic control group.

Body weight changes

The arthritic rats showed marked weight loss on 4th day after adjuvant injection. Whereas the standard and *Elephantopus scaber* test extracts significantly rectify body weight of the animal as compared to arthritic control.

Hematological parameters

Induction of adjuvant in rats leads to increase in the WBC, ESR, Eosinophils and lymphocyte count and decrease in the level of Hb and RBC, when compared with the normal control. Treatment with standard and the test extracts significantly rectify these haematological parameters (table: 1).

Bio-chemical parameters

Induction of freunds adjuvant leads to significant increase in the levels of SGOT, SGPT and ALP when compared with the normal control. Treatment with the standard and *Elephantopus scaber* extracts significantly rectify the deranged parameters. Alcoholic extracts found to be more effective than the aqueous extracts which can be seen in table 2.

CFA-induced arthritis in rats is associated with an increase in the plasma levels of C - reactive protein (CRP) and Rheumatoid Factor (RF).Treatment with the standard and extracts results in rectifying the RF factor to negative which is positive in induced group. However the aqueous extract does not have protective effect on the CRP induced by freunds adjuvant. Alcoholic extract renders the CRP level non-reactive. This indicates the significant protective effect of alcoholic extracts on Arthritis.

Table 1. Effect of Elephantopus scaber on different haematological parameters

Groups	Hb%	RBC	WBC	ESR	Eosinophils	Lymphocytes
Control	13.2±0.15	4.0±0.05	8975±278.4	12.2± 0.14	3.8±0.10	32.5±1.0
Disease control	7.5±0.12	3.3±0.11	2465±221.2	21.03± 0.16	10.2±0.14	60.3±1.4
Standard	8.7±0.14	4.2±0.08	8463±197.5	15.6 ± 0.08	4.3±0.14	35.0±1.0
Test-1	10.6±0.18	5.0±0.11	9403±229.9	17.5±0.18	6.0±0.08	39.33±0.8
Test-2	11.5±0.14	4.7±0.15	9700±158.1	19.1 ± 0.15	5.7±0.17	41.33±1.4

The observations are mean ± SEM of 6 animals. * P<0.01 Vs Disease control group by one way ANOVA.

Table 2. Effect of Elephantopus scaber on different biochemical parameters

Groups	SGOT	SGPT	ALP
Control	31.6±1.2	38.2±1.2	114.0±1.807
Disease control	52.2±1.1	58.6±1.1	172.2±1.493
Standard	37.4±1.4 (28.3%)	41.5±1.4 (29.1%)	120.0±0.816 (30.0%)
Test-1	39.8±1.2 (23.7%)	29.2±1.2 (50.1%)	145.8±1.662 (15.3%)
Test-2	42.7±1.8 (18.1%)	35.4±1.8 (39.5%)	139.3±1.542 (19.1%)

The observations are mean ± SEM of 6 animals. * P<0.01 Vs Disease control group by one way ANOVA. Each value in parenthesis indicates the percentage protection as compared to control

Table 3. Effect of Elephantopus scaber methanol and aqueous extracts and standard drug on Rheumatoid factor (RF) and CRP of CFA induced rats

Groups	Rheumatoid factor	CRP
Control	Non-reactive	Non-reactive
Disease control	Reactive	Reactive
Standard	Non-reactive	Non-reactive
Test-1	Non-reactive	Non-reactive
Test-2	Non-reactive	Reactive

Fig 1. Effect of *Elephantopus scaber* methanol and aqueous extracts on paw volume. (All values are Mean ± SEM of 6 animals)

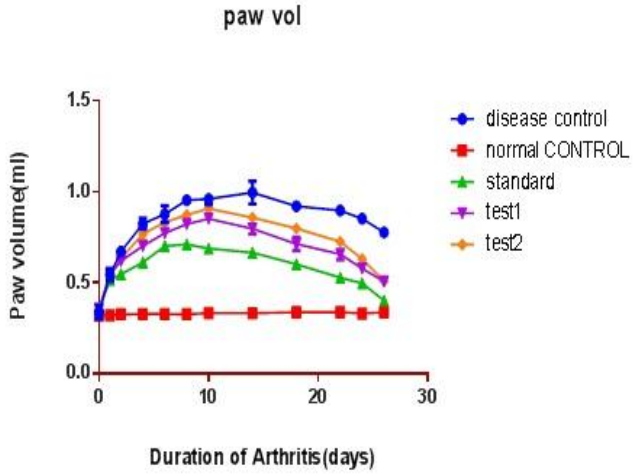
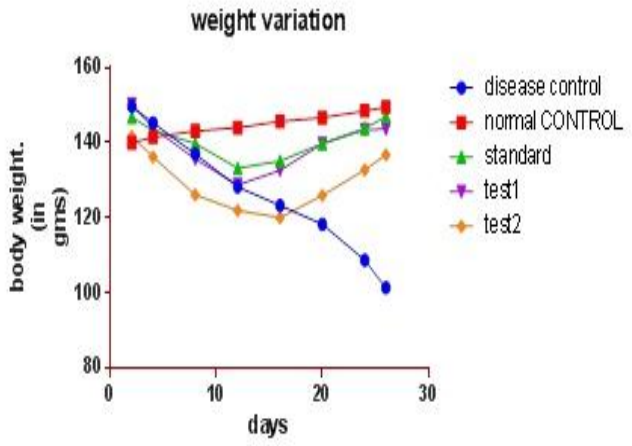


Fig 2. Effect of *Elephantopus scaber* methanol and aqueous extracts on weight variation. (All values are Mean ± SEM of 6 animals)



DISCUSSION

In the present study, Wistar albino rats were selected to induce arthritis because rats develop a chronic swelling in multiple joints with the influence of inflammatory cells, erosion of joint cartilage and bone destruction. It has close similarities to human rheumatoid arthritis [7].

CFA-induced polyarthritis is associated with an immune-mediated inflammatory reaction [8]. The initial reaction of edema and soft-tissue thickening at the depot site in this model is caused by the irritant effect of the adjuvant, whereas the late-phase arthritis and flare in the injected foot are presumed to be immunologic events [9]. The appearance of secondary lesions, that is, non-injected paw swelling is a manifestation of cell-mediated immunity.

The results of the present study shown that the test drug *Elephantopus scaber* leaf extracts possess significant anti-inflammatory and anti-arthritic activities in all the tested experimental models indicating inhibition

of all phases of inflammation.

Initially the injection of CFA into the left hind-foot produces an inflamed swelling in the paw which reaches to its maximum during the first 3 days. Thereafter, the swelling is slow until the 8th day when the foot begins to swell again. As the disease progresses, on day 10th more severe edema develops in the injected paw and inflamed lesions termed as secondary lesions are detected in the non-injected paw. The secondary lesions then begin to appear and increase in thickness, in the fore-paws, ears and tail. After the 13th day further swelling of the feet or joints occurs and by the 26th day the inflammation starts to subside leaving pale granulomatous swellings around the joints [8,9].

The standard methotrexate suppresses the inflammation from 4^{th} day onwards where as the methanol extract does it after 14^{th} day and aqueous extract from 24^{th} day onwards. This indicates the effect of extracts on 2^{nd} phase of the inflammation mostly. However, *Elephantopus scaber* extracts significantly ($p<0.001$) reduce the paw swelling which indicate the anti-arthritic potential of the extracts.

Changes in the body weight have also been used to assess the course of disease and the response to therapy of anti-inflammatory drugs [10], as the incidence and severity of arthritis increased, the changes in the body weights of the rats also occurred during the course of the experimental period. Earlier findings suggest that absorption of 14C-glucose and 14C-leucine in rats intestine was reduced in the case of inflamed rats [11]. The arthritic rats showed marked weight loss on 4th day after adjuvant injection, where as the standard and *Elephantopus scaber* test extracts significantly rectify body weight of the animal as compared to arthritic control.

From the results it is clear that the decrease in RBC count and hemoglobin level represents the anemic condition in arthritic rats. The more important causes are the abnormal storage of iron in the reticuloendothelial system and synovial tissue and the failure of bone marrow to respond to anemia [12]. Treatment with standard and the test extracts significantly rectify these parameters. In arthritic condition, there is a mild to moderate rise in WBC count due to the release of IL-IB increases the production of both granulocyte and macrophages colony stimulating factors [13,14]. In the present study, the *Elephantopus scaber* test extracts and standard showed significant reduction in WBC count. ESR is influenced by several factors such as the plasma concentration of fibrinogen, immunoglobulin's, RF and Hb, the increased level of ESR in arthritic rats adds information reflecting the chronicity and severity of the disease [15]. The above-mentioned changes were brought back to near normal levels upon *Elephantopus scaber* methanolic extract treatment, which emphasizes the beneficial effect of the methanolic extract on adjuvant induced arthritis rats. When compared to aqueous extract, methanolic extract showed increased effect.

Induction of freunds adjuvant leads to significant increase the levels of SGPT, SGOT and ALP in rats. Arthritic control rats found to have SGPT, SGOT and ALP in range of 58.6, 52.2 and 172.2 units/l. Treatment with the *Elephantopus scaber* extracts significantly rectify the deranged parameters. Alcoholic extracts found to be more effective than the aqueous extracts. It is evidence that lysosomal enzymes play an important role in the development of acute and chronic inflammation [16]. Most of the anti-inflammatory drugs exert their beneficial effects by inhibiting either release of these enzymes or by stabilizing lysosomal membrane, which is one of the major events responsible for the inflammatory process (16). So, we can assume that our drug extract might be acting by either inhibiting the lysosomal enzymes or stabilizing the membrane.

CFA-induced arthritis in rats is associated with an increase in the plasma levels of C - reactive protein (CRP) and Rheumatoid Factor (RF). The serum CRP concentration directly reflects the intensity of the pathological process in RA. Significant reduction in the level of the test extracts indicates for their inhibitory effects on inflammatory cascade in the progression of arthritis.

Rheumatoid factor is useful as a measure for assessing the severity of Rheumatoid Arthritis. RF is an antibody against the Fc portion of IgG, which is itself an antibody[18]. These auto antibodies can belong to any of the three main Ig classes, G, A or M, but the classical rheumatoid factor is pentameric IgM. Rheumatoid factors react against IgG molecules that are abnormal in their

carbohydrate moieties, a feature that probably renders them immunogenic.

Treatment with the standard and extracts results in rectifying the RF factor to negative which is positive in induced group. However the aqueous extract does not have protective effect on the CRP induced by freund's adjuvant. Alcoholic extract renders the CRP level non-reactive. This indicates the significant protective effect of alcoholic extracts on Arthritis.

CONCLUSION:

From the above studies it is quite apparent that the *Elephantopus scaber* extracts possesses significant anti-arthritic activity. The study justifies its use in inflammatory conditions as suggested in the folklore medicines.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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