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RESEARCH ARTICLE

Evaluation of antiarthritic activity on *Luffa echinata Roxb.* fruits on rats

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ABSTRACT

Luffa Echinata Roxb. fruits popularly known, as 'Bindal' in Hindi is a slender herb belonging to the Cucurbitaceae which grows widely in India. In traditional system of medicine, *Luffa echinata Roxb. f*ruits were used for various ailments including inflammation, diabetes etc. To investigate the folkloric use of the fruits the present study was carried out on Freund's adjuvant induced arthritic rats.

The present study states the effect of the aqueous extract (LEFAE) and the alcoholic extract (LEFEE) of *Luffa echinata Roxb.* fruits on the Freund's complete adjuvant (FCA) induced arthritic rat paw edema, body weight changes and alterations in haematological and biochemical parameters in both developing and developed phases of arthritis. Histopathology of proximal interphalangeal joints and radiology of hind legs were studied.

In FCA induced arthritic rats, there was significant increase in rat paw volume and decrease in body weight increment, whereas LEFEE and LEFAE treated groups, showed significant reduction in paw volume and normal gain in body weight. The altered haematological parameters (Hb, RBC, WBC and ESR) in the arthritic rats were significantly brought back to near normal by the LEFEE and LEFAE treatment at the dose of 250 mg/kg/p.o in both developing and developed phases of arthritis. Further the histopathological and radiological studies revealed the antiarthritic activity of LEFEE and LEFAE by indicating fewer abnormalities in these groups when compared to the arthritic control group.

The results of the current investigation concluded, ethanol extract of *Luffa echinata Roxb.* Fruits, extract possess a significant anti-arthritic activity against adjuvant induced arthritis and justifying its therapeutic role in arthritic condition. The observed antiarthritic activity may be due to the presence of phytoconstituents such as irridiod glycosides, alkaloids, phenolic compounds and flavonoids.

Keywords: *Luffa echinata Roxb,* adjuvant induced arthritis, antiarthritic activity, Freund's complete adjuvant (FCA).

1. INTRODUCTION:

Rheumatoid arthritis (RA) is a systemic autoimmune disease of unknown aetiology. The disease is characterized by articular inflammation and 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-tumour necrosis factor (TNF)-a 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 (Barbier A *et al,*1984), and the sensitivity of this model to antiarthritic agents

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(Ward JR *et al,* 1996) support the view the adjuvant arthritis is the best available model of rheumatoid arthritis.

Adjuvant induced arthritis is a chronic crippling, skeleton-muscular disorder having nearest approximation to human rheumatoid arthritis for which there is currently no medicine available effecting a permanent cure. Even modern drugs used for the amelioration of the symptoms, offer only temporary relief and also produce severe side effects. In the indigenous system of medicine, Plant of *Luffa Echinata Roxb*., is reported to be useful in the treatment of arthritis. It is a large shrub, used against a wide variety of diseases. However, no systematic study has been reported regarding its anti-arthritic activity.

Luffa Echinata Roxb. Fruits popularly known, as 'Bindal' in Hindi is a slender herb belonging to the Cucurbitaceae which grows widely in India (Kirtikar and Basu, 1933). Practitioners of the indigenous system of medicine, affirm to obtain beneficial results with the fruits of their plant in the treatment of liver ailments (Nadkarni and Nadkarni, 1954; Chopra et al., 1956). Stems and leaves decoction in fever, in case of rheumatism leaves applied, root paste used in Leprosy, Hypoglycemic (aerial part), emetic and anthelmintic (plant); purgative (fruit). (RLS Sikarwar, *et al*, 2008). Digestive, Liver stimulant, Blood purifier, Anti-inflammatory, Cough expectorant, Diuretic, Antipyretic, Hepatoprotective etc. (kirtikar KR, *et al*, 1933).

Luffa echinata Roxb. is reported to contain: Echinatin, Saponins (Bhatt and Khurana, 1957), Hentriacontane, Gypsogenin (Khorana and Raisinghani, 1961), Amariin (Chaudhary et al., 1951) Cucurbitacin-B & -E, Sapogenin, _-Sitosterol, Echinatol-A & -B, Oleanolic acid (Bhakuni et al., 1961a,b,c), Elaterin-2-O-_-D-Glucopyranoside, Isocucu & bitacin-B, Elaterin glucoside, Chrysoeriol-7-glucoside, Graviobioside-B, Sitosterol glucoside (Seshadri and Vydeeswaran, 1971), Datiscacin, 2-O-_-D-glucopyranosyl cucurbitacin-B & 2-O-_- D-glucopyranosyl cucurbitacin-S (Mesbah et al., 1994).

2. Material and methods:

Plant material:

The fruits of *L. echinata Roxb.* were procured from the Mool chand and phool chand Jain shope of local market, Bhopal Madhya Pradesh India, and collected in the month of Dec 2011. It was authenticated by Dr. Ziaul hasan, Department of Botany, Saifia Science College, Bhopal (M.P.) the voucher specimen was (No. 344 /bot / saifia /2012). The fruits have been stored for future reference.

Preparation of Extracts:

Shade dried powdered material of *Luffa Echinata Roxb. Fruits* were used. Fruits exhaustively extracted with distilled water and Ethanol alcohol (95%) using (40-60C^{o}) Soxhlet's apparatus. The extracts were concentrated under vacuum pressure and semisolid mass were obtained and were placed in desiccator for further study. The percentage yield of the extract (LEFEE) was 9.3% w/w and (LEFAE) was 8.9% w/w from the starting crude material. For the experimental study, both the extract were triturated and suspended (using gum acacia 2%) with distilled water and administered immediately.

Animals used:

Wistar albino rats (130 ± 20 g) procured from authorised animal house of Truba Institute of Pharmacy Bhopal (MP) was used for the study. The animals were kept in polypropylene cages and maintained at a temperature of 22 ± 2°C. They were fed with standard pellet feed and water ad libitum. The study has got the approval from the Institutional Animal Ethical Committee (IAEC) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Induction of arthritis:

Arthritis was induced in rats by the intraplantar injection of 0.1 ml of Complete Freund's Adjuvant (CFA) in the left hind paw. The adjuvant contained heat killed Mycobacterium tuberculosis (H37Rv strain, Tuberculosis Research Centre, ICMR, Chennai) in sterile paraffin oil (10 mg/ml). The paw volume of all the animal groups was measured by plethysmograph at 0, 7, 14, 21 and 28 days after the injection of complete Freund's adjuvant (Bhakuni *et al,* 1961).

Experimental set up: (Bhatt *et al,* 1957)

Wistar albino rats of either sex weighing 130 ± 20 g were used for the study. The animals were divided into nine groups of six animals each. The standard drug Diclofenac sodium is dissolved in water whereas LEFEE and LEFAE were triturated with water in a glass mortar, made as a suspension and administered immediately. Group I served as control (without treatment), Group II served as arthritic control (negative control), Group III is treated with Diclofenac sodium (positive control) the standard antiarthritic drug, Group IV & VII were treated with LEFEE and LEFAE for 28 days without inducing arthritis, Group V and VIII received LEFEE and LEFAE treatment for 28 days from the induction day (0-28 days treatment). Groups VI and IX received LEFEE and LEFAE for 14 days from the 14th day of induction (14th to 28th day treatment). The body weight changes were observed on every week. On 29th day, at the end of experiment, all animals were sacrificed by cervical decapitation and blood was collected in plain and EDTA containing tubes, respectively for plasma/serum separation.

For histopathology, the proximal interphalangeal joints were removed, washed with saline and stored in 10% formalin. The interphalangeal joint sections were obtained, stained with eosin-haematoxylin stain and viewed under 100 X magnifications. The hind (arthritis induced) legs of the experimental rats were taken X-ray,

and examined for the soft tissue swelling, bony erosions and narrowing of the spaces between joints (Ekambaram *et al,* 2005).

3. Results:

Statistical analysis:

The data represents Mean ± S.E.M. Results were analyzed statistically by One-Way ANOVA followed by Tukey's multiple comparison using graphpad Instat software student's version. The difference was considered significant when $p < 0.001$.

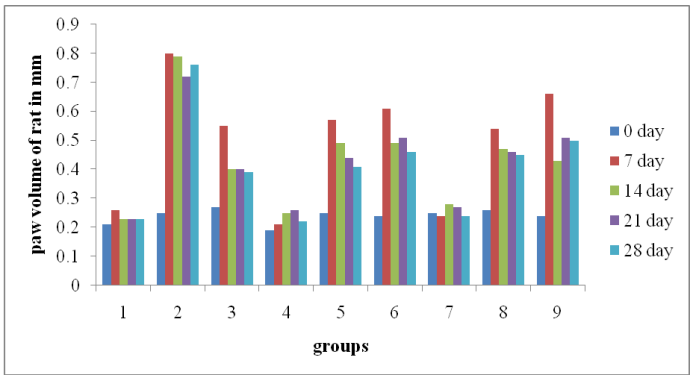
CFA induced rat paw edema:

There is a significant increase in rat paw volume in CFA injected arthritic control rats when compared to the normal control rats. LEFEE and LEFAE treatment at the dose of 250 mg/kg p.o. showed significant reduction in rat paw edema volume when compared with the arthritic group (table 1).

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
Group I	0.21 ± 0.02	0.26 ± 0.01	0.23 ± 0.01	0.23 ± 0.01	0.23 ± 0.01
Group II	0.25 ± 0.01	0.80 ± 0.01 a***	0.79 ± 0.02 a***	0.72 ± 0.02 a***	0.76 ± 0.02 a***
Group III	0.27 ± 0.01	0.55 ± 0.01 a***,b***	0.40 ± 0.02a a** b***	0.40 ± 0.02 a***, b***	0.39 ± 0.02 a***,b***
Group IV	0.19 ± 0.01	0.21 ± 0.01 b***,c***	0.25 ± 0.01 b***,c**	0.26 ± 0.01 b***,c***	0.22 ± 0.01b b***,c***
Group V	0.25 ± 0.08	0.57 ± 0.01 a*** b***, c***	0.49 ± 0.02 a***b***, d***	0.44 ± 0.02 a***, b***,d***	0.41 ± 0.03 a***,b***, d***
Group VI	0.24 ± 0.007	0.61 ± 0.02 a*** b***	0.49 ± 0.05 a***, b*** d***	0.51 ± 0.02 a***, b***,c*,d***	0.46 ± 0.02 a***,b***, d***
Group VII	0.25 ± 0.007	0.24 ± 0.01 a***, b***	0.28 ± 0.01 b***,c* ,e***,f***	0.27 ± 0.01 b***,c**,e***, f***	0.24 ± 0.01 b***,c**, e***,f***
Group VIII	0.26 ± 0.008	0.54 ± 0.01 b***, c***	0.47 ± 0.04 a***, b***, d***	0.46 ± 0.02 a***, b***,d***,g***	0.45 ± 0.02 a***,b***, d***,g***
Group IX	0.24 ± 0.01	0.66 ± 0.01 a***,b***	0.53 ± 0.01 a***, b*** c*, d***	0.51 ± 0.01 a***,b***, c**,d***,g***	0.50 ± 0.01 a***,b***, c*,d***, g***

Values are Mean ± S.E.M, n = 6 animals in each group. Comparisons were made between: a- Group I vs II, III, IV, V, VI, VII, VIII and IX. b- Group II vs III, IV, V, VI, VII, VIII and IX. c- Group III vs V, VI, VIII and IX. Symbols represent statistical significance: *** - $p < 0.001$, ** - $p < 0.01$, * - $p < 0.05$.

Table No. 1 Effect of LEFEE and LEFAE on rat paw edema in Freund's adjuvant induced arthritic rats. Rat paws volume in ml ± S.E.M



Graph No. 1 Relationship between the extents of joint inflammation with groups.

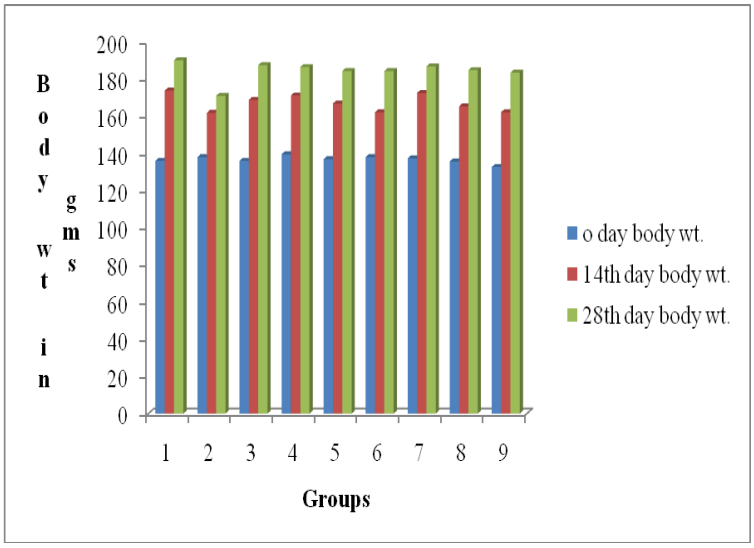
Body weight changes:

In the present study, it is clear from the data obtained that there is a close relationship between the extent of joint inflammation and the degree of weight loss. In the first week after adjuvant injection, the arthritic rats showed marked weight loss, followed by normal weight gain in the subsequent weeks, whereas the LEFEE, LEFAE and standard drug treated groups did not show any weight loss. **(**Table No. 2)

Groups	Day 0 Body wt in (gm)	Day 14 Body wt in (gm)	Day 28 Body wt in (gm)	Mean changes in the body weight 0 day- 28 days	Mean changes in the body weight 14th days- 28 days
Group I	136.16±1.6	174±1.23	190.33±0.88	54.17±0.72	16.33±0.51
Group II	138.16±1.7	162±1.23 a***	171.16±1.07 a***	33.00±0.00	9.16±0.16
Group III	136.16±1.3	169±0.73 a*, b***	187.66±0.71 b***	51.5 ±0.55	18.66±0.02
Group IV	139.66±1.66	171.33±0.91 b***	186.66±0.84 b***	47.00±0.00	15.33±0.07
Group V	137±1.36	167±1.06 a***,b* ,c**	184.5±0.99 a**,b***	47.5±0.37	17.5±0.07
Group VI	138.16±1.75	162.33±1.30 a***	184.00±0.9 a** ,b***	45.84±0.85	21.67±0.4
Group VII	137.5±0.95	172.66±0.95 b***	187±1.34 b***	49.5±0.39	14.34±0.39
Group VIII	135.83±0.6	165.5±0.88 a***,	188±0.63 a** , b***	52.17±0.03	22.5±0.25
Group IX	132.83±1.04	162.33±0.88 a*** ,c**	183.66±0.76 a*** , b***	50.83±0.28	21.33±0.12

Values are expressed as mean ±SEM, $p<0.05$ - significant, a*$p<0.01$ - more significant when compared to the control.

Table No. 2 Changes in the body weight in adjuvant-induced arthritis in rats.



Graph No. 2 Relationship between the days and body weight with groups.

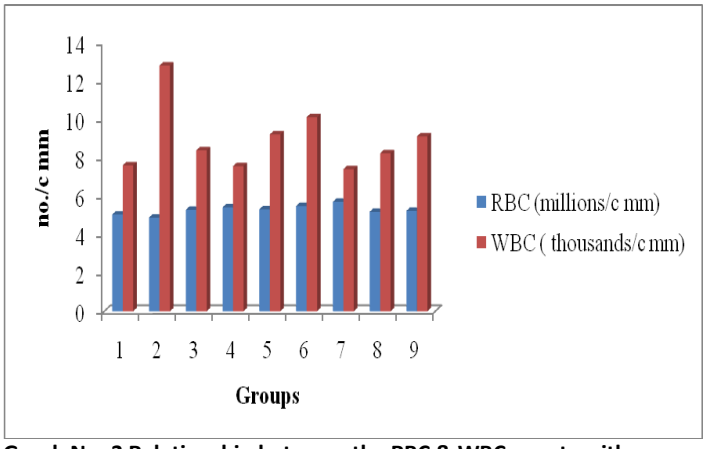
Haematological parameters: The changes in hematological parameters in adjuvant induced arthritic rats are shown in table 3. There was a significant, i) decrease in RBC count and haemoglobin, ii) increase in WBC count and ESR of arthritic rats, when compared with the control rats. The drug treatment has significantly brought back the altered

haematological changes in both developing and developed phases of adjuvant induced arthritis.

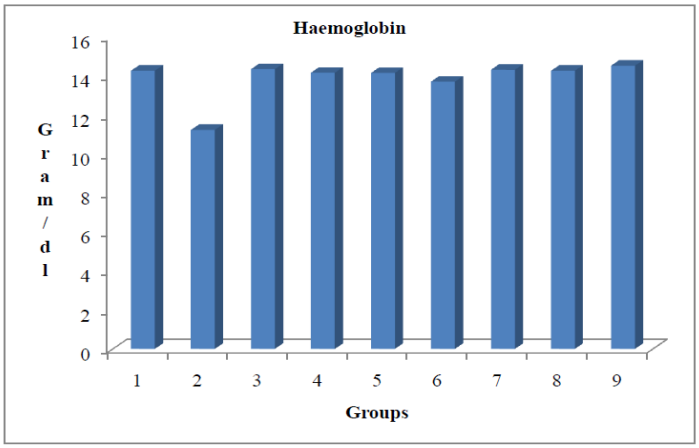
Groups	RBC in million/c.mm	WBC in thousand/C.mm	Hb in gram/100 ml	ESR mm/hr
Group I	5.04 ± 0.15	7.60 ± 0.28	14.25 ± 0.55	3.55 ± 0.24
Group II	4.88 ± 0.12	12.80 ± 0.18 a***	11.22 ± 0.57 a***	10.21 ± 0.43 a***
Group III	5.28 ± 0.17	8.38 ± 0.29 b***	14.33 ± 0.45 b***	5.06 ± 0.17 a***, b***
Group IV	5.41 ± 0.18	7.56 ± 0.26 b***	14.15 ± 0.38 b***	3.78 ± 0.21 b***,c**
Group V	5.31 ± 0.17	9.21 ± 0.29 a*, b***,d*	14.14 ± 0.16 b***	6.13 ± 0.21 a***, b***,c*,d***
Group VI	5.48 ± 0.16	10.11 ± 0.53 a***, b***,c*,d***	13.69 ± 0.37 b**	6.31 ± 0.12 a***, b***,c*,d***
Group VII	5.70 ± 0.08 b*	7.40 ± 0.37 b***,e*,f***	14.30 ± 0.20 b***	2.91 ± 0.11 b***,c***,e***,f***
Group VIII	5.18 ± 0.14	8.23 ± 0.40 b***,f*	14.25 ± 0.48 b***	5.08 ± 0.23 a***, b***,d**,h*,g***
Group IX	5.23 ± 0.18	9.11 ± 0.35 b***,g*	14.51 ± 0.33 b***	5.63 ± 0.14 a***, b***,d***,g***

Values are Mean ± S.E.M, n = 6 animals in each group. Comparisons were made between: a- Group I *vs* II, III, IV, V, VI, VII, VIII and IX. b- Group II *vs* III, IV, V, VI, VII, VIII and IX. c- Group III *vs* V, VI, VIII and IX. Symbols represent statistical significance: * - p < 0.001, # - p < 0.01, @ - p < 0.05

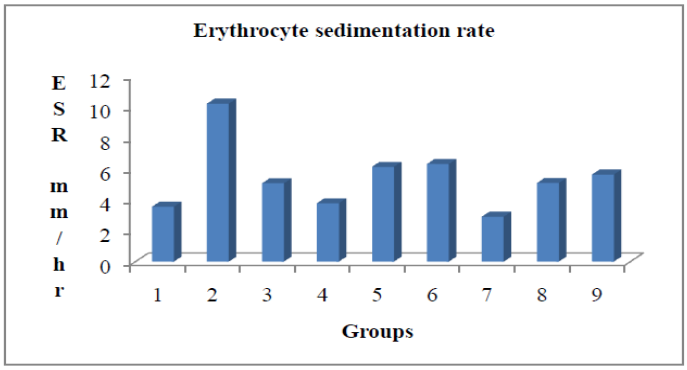
Table No. 3 Effects of LEFEE and LEFAE on haematological parameters of control and adjuvant induced arthritic rats



Graph No. 3 Relationship between the RBC & WBC counts with groups



Graph No. 4 Relationship between the groups with Haemoglobins.



Graph No. 5 Relationship between the groups with ESR.

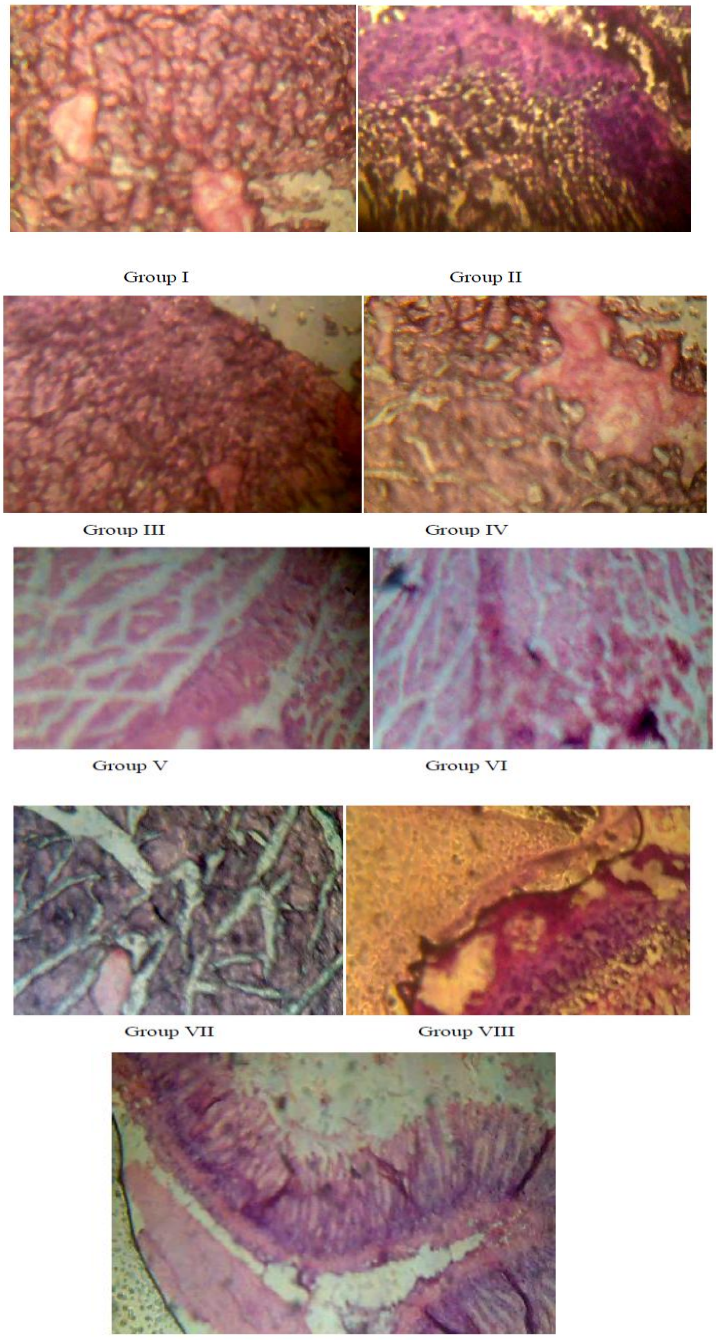


Figure 2 Histopathology of proximal interphalangeal joints on adjuvant induced arthritic rats.

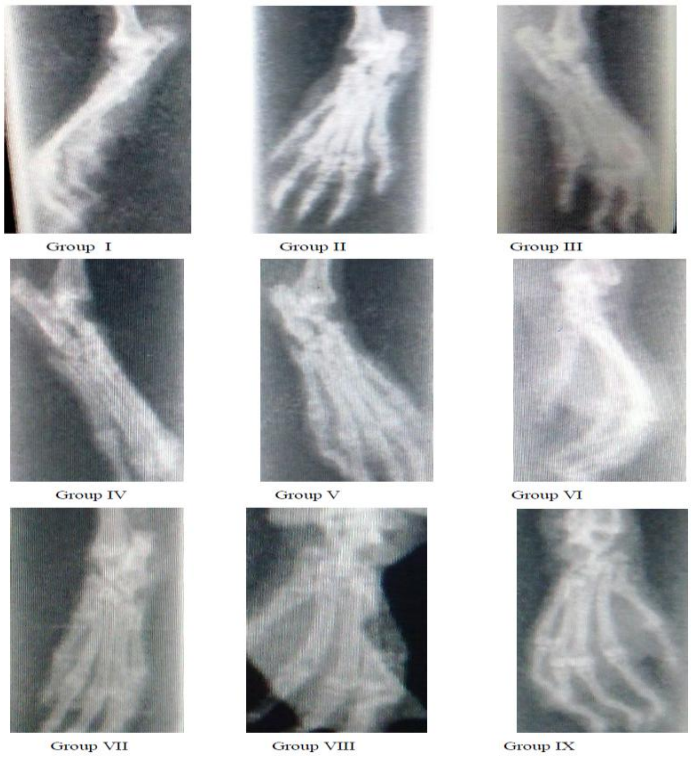


Figure 3 Radiology of hind legs in adjuvant induced arthritic rats.

4. Discussion

In the present study, the 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 disease (Singh S *et al,* 1996).The determination of rat paw swelling is apparently simple, sensitive and one of the quick procedures for evaluating the degree of inflammation and the therapeutic effects of drugs. The chronic inflammation involves the release of number of mediators like cytokines, GM-CSF, interferons and PGDF. These mediators are responsible for the pain, destruction of bone and cartilage that can leads to severe disability (Eric GB *et al,* 1996). However, the standard drug, Diclofenac sodium and the extract significantly suppressed the swelling of the rat paws. In arthritic condition, there is a mild to moderate rise in WBC count due to the release of IL-IB inflammatory response, IL-IB increases the production of both granulocyte and macrophages colony stimulating factors (Wilian JK *et al,* 1996).

In the present study, the migration of leucocytes into the inflamed area is significantly suppressed by extract when compared to standard drug Diclofenac sodium, as seen from the significant reduction in the total WBC count. Erythrocyte Sedimentation Rate (ESR) is an estimate of the suspension stability of RBC's in plasma. It is related to the number and size of the red cells and to the relative concentration of plasma proteins, especially fibrinogen, alpha and beta globulins. The ESR count significantly increased in arthritic control group, whereas these counts were remarkably counteracted in the standard, Diclofenac sodium and extract groups and thus justifying its significant role in the arthritic conditions (Wilian JK *et al,* 1996).

Changes in body weight have also been used to assess the course of the disease and the response to therapy of anti-inflammatory drugs(Winder CV *et al,* 2005). 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. The loss of the body weight during arthritic condition was also supported by earlier observations (Walz DT *et al,* 1971), on alterations in the metabolic activities of diseased rats. Earlier findings suggest that absorption of 14C-glucose and 14C-leucine in rat's intestine was reduced in the case of inflamed rats (Somsundaram *et al,* 1983).

Treatment with anti-inflammatory drugs, the decrease in absorption was nullified and it shows that the anti-inflammatory drugs have corrected the decreased absorption capacity of intestine during inflammation (Elmali N *et al,* 2005). The increased body weight during the treatment of standard drug and the extract may be due to the restoration of the absorption capacity of the intestine. From the results observed in the current investigation, it may be concluded that the extract of *Luffa Echinata Roxb.* Fruits., (Choi EJ *et al,* 2009) Extract at the dose of 250 *mg/kg p.o.*body weight displays a significant anti-arthritic activity which may due to the presence of phytoconstituents such as alkaloids, steroids, flavonoids, phenolic compounds and glycosides, specifically iridoid glycosides. Several studies indicate that aforementioned phytoconstituents possess significant anti-arthritic activity (Amresh G *et al,* 2007).

The observed histopathological changes of proximal interphalangeal joints of the experimental Group I showed the histopathology of normal joint. Group II the negative control arthritic rat joint showed prominent abnormalities from the normal joint like edema formation, degeneration with partial erosion of the cartilage, destruction of bonemarrow and extensive infiltration of inflammatory exudatesin the articular surface. The standard drug treated rat joint showed normal bone marrow with less cellular infiltrates. LEFEE and LEFAE treatment for 28 days showed less inflammatory signs like scanty cellular infiltrate, absence of edema formation and normal bone marrow, whereas the LEFEE and LEFAE treatment for 14 days showed cellular infiltrates on the articular surface with less cartilage destruction. The overall prevention of the inflammatory signs of the rat joints was significant in 28 days drug treated group compared with 14 days drug treated group. Degeneration of the joint was not observed

in any of the drug treated groups when compared with the negative control.

Radiographic changes in RA conditions are useful diagnostic measures which indicate the severity of the disease. Soft tissue swelling is the earlier radiographic sign, whereas prominent radiographic changes like bony erosions and narrowing of joint spaces can be observed only in the developed stages (final stages) of arthritis. In adjuvant induced arthritic rat (group II), soft tissue swelling along with narrowing of the joint spaces were observed which implies the bony destruction in arthritic condition. The standard drug Diclofenac sodium treated groups have prevented this bony destruction and also there is no swelling of the joint. Similar to histopathological studies, LEFEE and LEFAE treatment for 28 days have shown significant prevention against bony destruction by showing less soft tissue swelling and narrowing of joint spaces when compared with 14 days LEFEE and LEFAE treated groups (Harris ED *et al,* 1990).

The study is further extended to identify and characterize the exact active phytoconstituents and to elucidate the exact mechanism of action, which is responsible for the observed significant anti-arthritic activity against adjuvant induced arthritis in rats.

5. Conclusion

In conclusion, both LEFEE and LEFAE at the specified dose level of 250 mg/kg, p.o. showed reduction in rat paw edema volume and it could normalize the haematological and biochemical abnormalities in adjuvant induced arthritic rats in both developing and developed phases of CFA induced arthritis. Further the histopathological and radiological studies confirmed the antiarthritic activity of LEFEE and LEFAE in CFA induced arthritis. The actual mechanism of action of LEFEE and LEFAE on adjuvant induced arthritis is not clear with these studies. The action of LEFEE and LEFAE on proinflammatory mediators like TNF- a, Interleukins and other relevant mediators will be carried out in future to study its mechanism.

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