International Journal of Multidisciplinary Research and Development

International Journal of Multidisciplinary Research and Development

Volume :2, Issue :4, 548-554
April 2015
www.allsubjectjournal.com
e-ISSN: 2349-4182
p-ISSN: 2349-5979
Impact Factor: 3.762

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Evaluation of Anti-arthritic potential of *Adansonia digitata* seed extract

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Abstract
Adansonia digitata (Baobab) is a large deciduous tree up to 25m in height and may live for more than hundred years. It is a tree species native to Africa. The common English names for the Baobab include Dead rat tree, monkey bread tree. Upside-down tree and cream of tartar tree. In traditional system of medicine *Adansonia digitata* the seeds are used for various aliments including anti-inflammatory, analgesic, anti-pyretic activity, anti-oxidant, anti-diarrhoeal and anti-dysentry and trypanosmal activity. The present study evaluates the effect of the ethanolic extract of "*Adansonia digitata*" seeds on the Fruend's Complete Adjuvant (CFA) induced arthritis. The parameters assessed were rat paw edema, body weight changes, hematological parameters histopathology of proximal interphalangeal joints and radiology of hind legs. In CFA induced arthritic control rats, there was significant increase in rat paw volume and decrease in body weight, where as *Adansonia digitata* seed extract treated groups showed a significant reduction of paw volume and gain in body weight. The altered haematological parameters (Hb, RBC, WBC and ESR) in the arthritic control rats were significantly brought back to near normal by the *Adansonia digitata* seed treatment at the dose level of 200 mg/kg and 400 mg/kg in both developing and developed phases of arthritis. Further the histopathological and radiological studies revealed the significant anti-arthritic activity of *Adansonia digitata* seed as indicated by fewer abnormalities in these groups when compared to the arthritic control group. In conclusion, *Adansonia digitata* seed extract at the specified dose evel of 200 mg/kg and 400 mg/kg showed reduction in rat paw volume and it could significantly normalize the haematological abnormalities in adjuvant induced arthritis rats in both developing and developed phases of CFA induced arthritis. Further the histopathological and radiological studies confirmed the anti-arthritic activity of "*Adansonia digitata*".

Keywords: Rheumatoid Arthritis, Adansonia digitata Seed, Complete Fruend's Adjuvant, Diclofenac Sodium

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune systemic disease with chronic inflammation of the synovial joints and progressive destruction of cartilage and bone[1]. RA is a chronic systemic inflammatory disorder that affects many tissues and organs, skin, blood vessels, heart, limbs and muscles, but principally attacks the joints, producing nonsuperlative proliferative and inflammatory synovitis that leads to destruction of the articular cartilage and ankylosis of the joints. RA is characterized by the infiltration of a variety of inflammatory cells into the joints[2]. The synovial membrane becomes highly vascularized, synovial fibroblasts proliferate and inflammatory cells release numerous cytokines and growth factors into the joint. These agents subsequently cause synovial cells to release photolytic enzymes which results in destruction of bone and cartilage[3].

Anti-inflammatory drugs and analgesics including steroids are used to suppress the symptoms, while disease modifying anti-rheumatic drugs (DMARD's) newer therapics such as anti-tumor necrosis factor (TNF) – α therapy cetanercept infliximab and adalimumab are often required to inhibit or halt the underlying immune process. However, all of these agents are associated with numerous side effects.

In recent years, research is directed towards traditional system of medicine for the discovery of drugs that are having long acting anti-inflammatory effect with minimum side effects.

Although there is no ideal animal model for RA at the present time, rat adjuvant arthritis shares many features of human RA and the sensitivity of this model to anti-arthritic agents support the view that the adjuvant arthritis is the best available model of RA[4].

Herbal medicines are being accepted and used increasingly by general populations in both eastern and western countries because of the ethnic acceptability and compatibility having fewer side effects.

Adasonia digitata is a native deciduous tree of African Savannas and belongs to the family Bombacaceae. It is called by several local names in central Africa as "bu-hibab" and dead rat tree. The tree can grow up to 25m in height, 28m in girth and can live for up to several hundred years[5].

The bark is smooth, greyish, often with purplish tinge or brown, Leaves digitate, leaflets three in young plants. Oblong or lanceolate, fruits are 20-30 cm x 10cm, woody, grey with soft yellowish felt outside. Seeds reniform, shining brown or blackish with thick testa.

The tree has been reported for various pharmacological activities such as Anti-inflammatory, antioxidant, analgesic, anti-pyretic, anti-diarrhoeal, anti-dysentry, hepato protective, anti-rheumatic and anti-tyrpano somal activity. This is the first study to evaluate the ethanolic extract of "Adansonia digitata" seed on CFA induced arthritis in rats6.

2. Materials and Methods

2.1 Drugs and Chemicals

Complete Freund's adjuvant (CFA) was procured from sigma Aldrich USA. Diclofenac sodium was purchased from Fourrts India Pvt. Ltd., Chennai, India and all other chemicals used in this study were obtained commercially and were of analytical grade.

2.2 Plant Material

Fresh fruits of "*Adansonia digitata*" were collected from the Madras Medical College Campus, Chennai, India and authenticated by Dr.G.V.S Murthy, Scientist 'F' , Head Office, Botanical Survey of India, Coimbatore, India.

2.3 Preparation of Extract

The seeds after separation were shade dried and powdered using a mechanical grinder and passed through 40 Mesh sieve.

The powder (100g) was defatted using 1.5 L of petroleum ether (bp:60-80c) and subjected to extraction in a soxhlet apparatus using ethanol for 18 hrs. The Ethanolic extract of *Adansonia digitata* (EEAD) fraction was concentrated under reduced pressure and controlled temperature (40-50°c) The yield of ethanolic extracts of *Adansonia digitata* was 6.1% w/w the extract was stored in tightly closed container in refrigerator and was screened for phyto constituents.

2.4 Animals

Female wistar rats (weighing around 150-200g) obtained from Madras Medical College, Animal house Chennai, were used in the study. They were maintained at 22±5°c relative humidity 55±5°c with free access to food and water *ad libitum* under a 12:12 light / dark cycle.

The experimental protocol for conducting the *in-vivo* study in the female adult albino wister rat was approved by institutional ethical committee (IEC) of the Madras Medical College, Chennai – 600003. Approval No. 17/243/CPCSEA. Dated 03.09.2014.

2.5 Acute toxicity studies [7-9]

The acute toxicity studies in rats were performed as per the OECD guidelines No:423 to evaluate the toxicity if any of EEAD extract. Three female albino wistar rats were selected. They were administered once orally with dose of 2000 mg/kg of EEAD extract. The rats were then critically observed for clinical signs of mood, motor activity muscle tone, gross behavioural changes and mortality after 30 mins, 1hr, 2 hrs, 3hrs and then after 24 hrs. These observations were continued for a period of 14 days.

2.6 Induction of Rheumatoid arthritis [10-13]

Arthritis was induced in rats by the intra plantar injection of 0.1ml of CFA in the left hind paw. The adjuvant contained heat killed mycobacterium tuberculosis in sterile paraffin oil (10 mg/ml). The paw volume of all the animals was measured by a plethysmograph at 0, 7th, 14th and 21st day after the injection of CFA.

2.7 Experimental design

A total of 30 adult female wistar rats were divided into 5 groups of 6 animals each.

Group I - Distilled water (DW) - 1 ml/kg/day, orally for 14 days as vehicle control – normal

Group II - DW 1ml/kg/day orally for 14 days as Arthritic control

Group III - Diclofenac Sodium 13.5 mg/kg/day orally for 14 days is standard.

Group IV- Ethanolic extract of *Adansonia digitata* (EEAD) 200 mg/kg/day orally for 14 days.

Group V - EEAD 400mg/kg/day orally for 14 days.

2.8 Body weight and Paw volume

Body weight and paw volume of all the animals were measured on day 0, 7th, 14th and 21st days. The paw volume of all the rats was recorded by means of a plethysmograph.

2.9 Blood Collection

At the end of day 21 the blood samples were collected by retro-orbital vein puncture of anaesthetized rats, to estimate various Haemotological parameter such as Haemoglobin content. Total WBC, RBC and Erythrocyte sedimentation rate (ESR) by using routine laboratory methods.

Statistical analysis

All the data obtained from the various parameters were statistically evaluated by one way analysis of variance test (ANOVA) followed by Dunnetis post test. P values less than 0.01 ($p<0.01$) were used as the significant level.

3. Results

In the acute toxicity studies at the dose of 2000mg/kg body weight of adansonia digitata no mortality was observed from the results test drug doses of 200 mg/kg and 400 mg/kg body weight were chosen for the efficacy studies.

3.1 Effect of EEAD on Rat Paw Volume

Treatment with CFA (0.1ml) shows development of paw volume which reached peak volume on 21st day of injection in control group ($p<0.001$) Diclofenac treated groups shaws significant inhibition of paw volume on 7th ($p<0.01$) 14th ($9<0.01$) 21st day. (Table No:1)

EEAD (200 mg/kg) treatment group shows significant inhibition of paw volume on day 7th ($p<0.01$), 14th ($p<0.01$) and day 21st day ($p<0.01$) table No. also rat treated with EEAD (400mg/kg) shows significant inhibition of paw volume on day 7 ($P<0.01$) and day 21 ($p<0.01$)

Paw volume increased upto 7th day of adjuvant injection and after that it slightly decreased.

Diclofenac treated group shows significant inhibition of paw volume on day 7 ($p<0.01$) 14 ($p<0.01$) and 21 ($p<0.01$) EEAD (200mg/kg) shows significant inhibition of paw volume on day 7th to 21st with ($p<0.01$). Also rats treated with EEAD (400 mg/kg) shows significant inhibition of paw volume on day 7, 14 and 21 day with $p<0.01$. (Table No: 2)

3.2 Effect on EEAD on Body weight

Body weight of all the animals in the normal control group increased till day 21 whereas body weight of all the animals in the arthritic control group significantly decreased till day 21 as compared to the normal control group.

Further body weight of all the animals in EEAD and Diclofenac sodium treated groups increased significantly ($p<0.01$) as compared to the arthritic control group (Table No: 3)

Intra plantar administration of CFA in rats induced severe inflammation and redness over a period of 24 hrs.

Oral administration of EEAD (200mg/kg and 400 mg/kg) ameliorated the alteration in paw volume significantly ($p<0.01$, $PZ<0.001$) respectively on day 21 when compared with arthritic control.

3.3 Hematological Parameters

The changes in hematological parameters in adjuvant induced arthritic rats are shown in (Table No: 4) The EEAD 200mg/kg treated group reduces the WBC, ESR and increases the Hb ($P<0.01$) and RBC ($P<0.05$) significantly when compared with arthritic control. In addition, EEAD 400mg/kg treated group reduces the WBC ($P<0.05$), ESR ($P<0.01$) and increase the Hb ($P<0.01$) significantly when compared with arthritic control group

Table 1: Mean of Paw volume using plethysmograph in Adjuvant-induced Arthritis in Rats

Treatment	0 day	7th day	14th day	21th day
Group I (Control)	2.56 ± 0.172^{a1}	2.75 ± 0.43^{b}	$2.99\pm.174^{b}$	2.69 ± 0.22^{b}
Group II (Arthritic control)	2.98 ± 0.251^{b1}	18.22 ± 0.15^{a}	19.79 ± 0.52^{a}	21.32± .114a
Group III Diclofenac Sodium	2.12±0.410ab	13.72 ± 0.107^{b}	10.23 ± 0.214^{b}	6.96 ± 0.147^{ab}
Group IV (200 mg/ kg b.w)	2.54 ± 0.117^{ab}	15.56 ± 0.521^{b}	12.69 ± 0.06^{b}	11.54 ± 0.039^{b}
Group V (400mg/kg b.w)	2.69 ± 0.22^{b}	21.32± .114a	13.98 ± 0.147^{ab}	9.89 ± 0.118^{a1b}

The values are expressed as mean ±SD, (n=6). $^{a}P<0.01$ as compared to control ; $^{a1}P<0.05$ as compared to control. $^{b}P<0.01$ as compare to arthritic control ; $^{b1}P<0.05$ as compare to arthritic control. The data was analyzed using one way analysis of variance (ANOVA) followed by Dunnett's test. P values <0.01 were considered as Significant

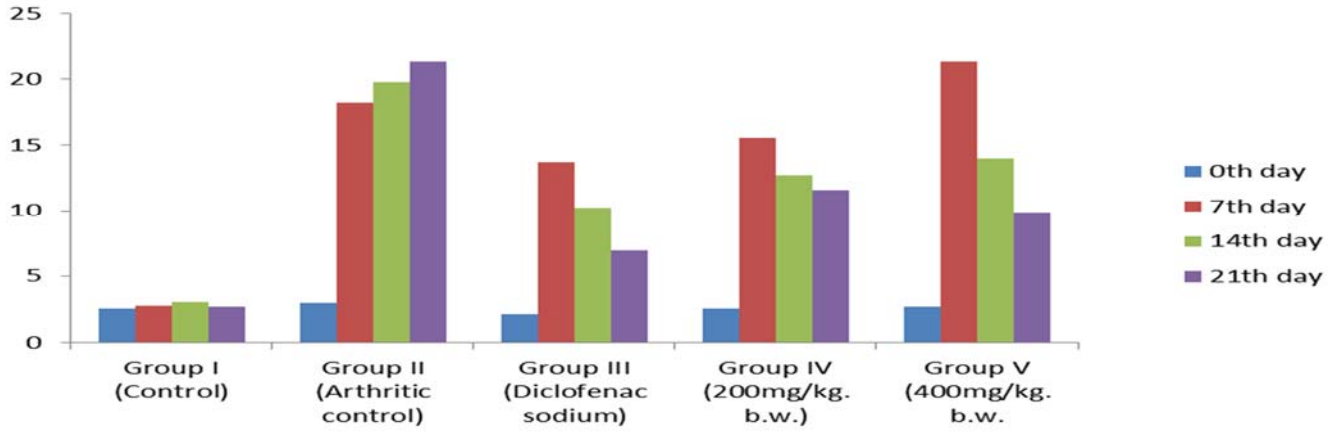


Table 2: Percentage inhibition of Paw value in Adjuvant-induced Arthritis in Rats.

Treatment	7th day	14th day	21st day
Group III Diclofenac sodium	27.8	48.4	67.35
Group IV (200 mg/ kg b.w)	19.04	35.84	54.8
Group V (400mg/kg b.w)	26.01	42.7	58.5

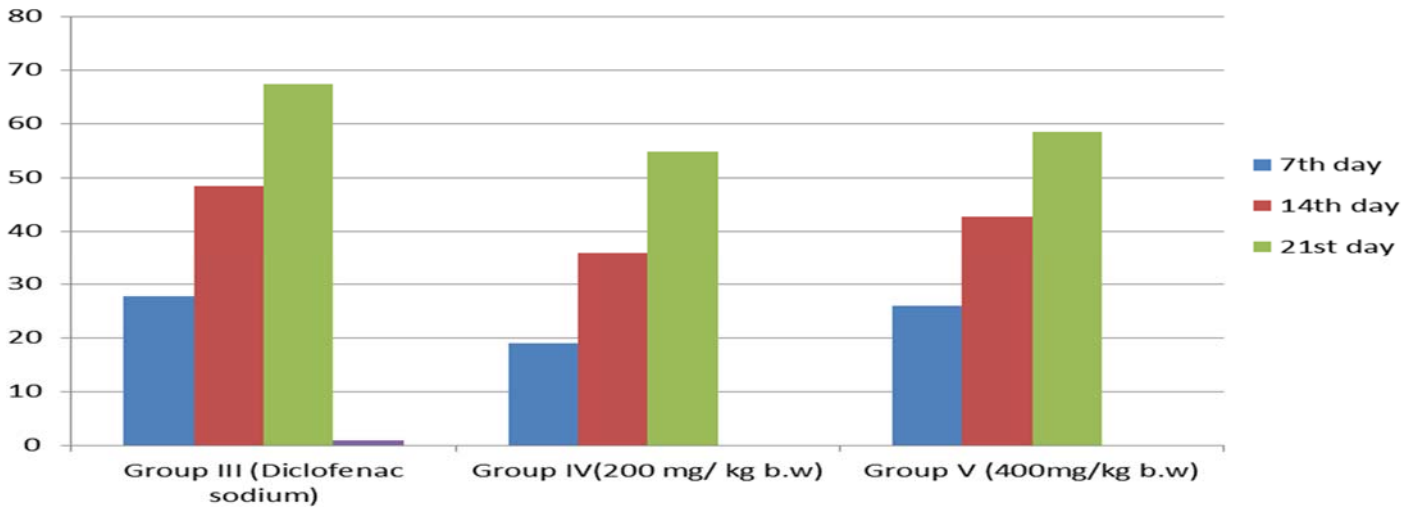


Fig 2. Percentage inhibition of Paw value in Adjuvant-induced Arthritis in Rats

Table 3: Changes in the body weight (gm) in Adjuvant-induced Arthritis in Rats

Treatment	0 day	7th day	14th day	21th day
Group I	159 ± 9.9b	161 ± 9.58b	163 ± 9.3380b	167 ±10.30b
Group II(Arthritic control)	162 ± 6.408a	157 ± 6.91a	154.± 56.64a	151 ± 5.78a
Group IIIDiclofenac sodium	174 ± 8.846b	177 ± 8.189	180 ± 6.31b1	182 ± 8.252b
Group IV200 mg/ kg b.w	168 ± 4.49	171 ±5.09b	174 ±6.33ab	177 ± 4.816b
Group V400mg / kg b.w	173 ± 5.79b	176 ±2.81a	179 ±4.979a	182 ± 5.42a1

Values are expressed as mean ± SEM (n=6). P<0.05, P<0.01, P<0.001,
as compared with control (One way ANOVA followed by Dunnett' s test).

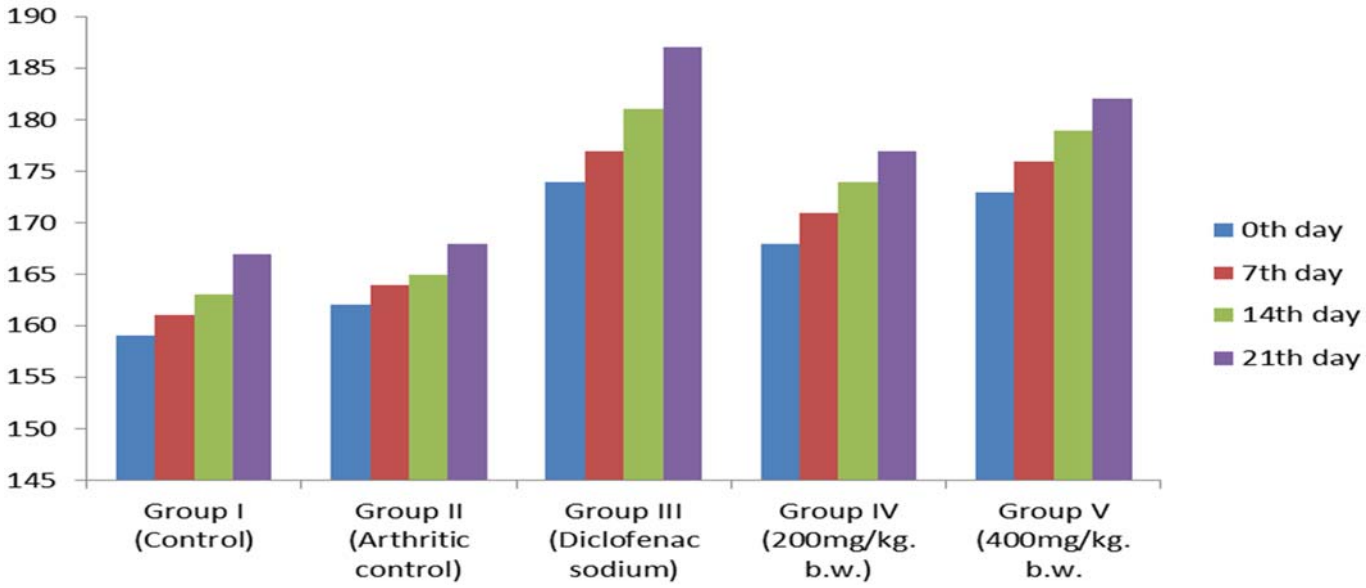


Fig 3: Changes in the body weight in Adjuvant-induced Arthritis in Rats

Table 4: Effect of Haematological parameters in Adjuvant-induced Arthritis in Rats after 21 days treatment

PARAMETERS	RBC count (cells/cu.mm)	Total WBC count (cells/cu.mm)	Hb (gm %)	ESR (mm/hr)
Group I(Control)	4.9±0.183b	6.642± 1.158b	14.64±0.364b	3.66±0.350b
Group II(Arthritic control)	4.6±0.174a	10.987±1.119a	11.08±0.277a	10.9±0.894a
Group III(Diclofenac sodium)	5.4± 0.284b	8.545±7.512	13.16±0.151b1	8.3± 1.48 b
Group IV(200 mg/ kg b.w)	4.7±0.255	9.232±8.216b	12.02±0.258ab	10±1.274b
Group V(400mg/kg b.w)	5.4±0.102b	8.250±7.755a	12.64±0.207a	9.48±0.843a1

Values are expressed as mean ± SEM (n=6). P<0.05, P<0.01, P<0. 001,
as compared with control (One way ANOVA followed by Dunnett' s test).

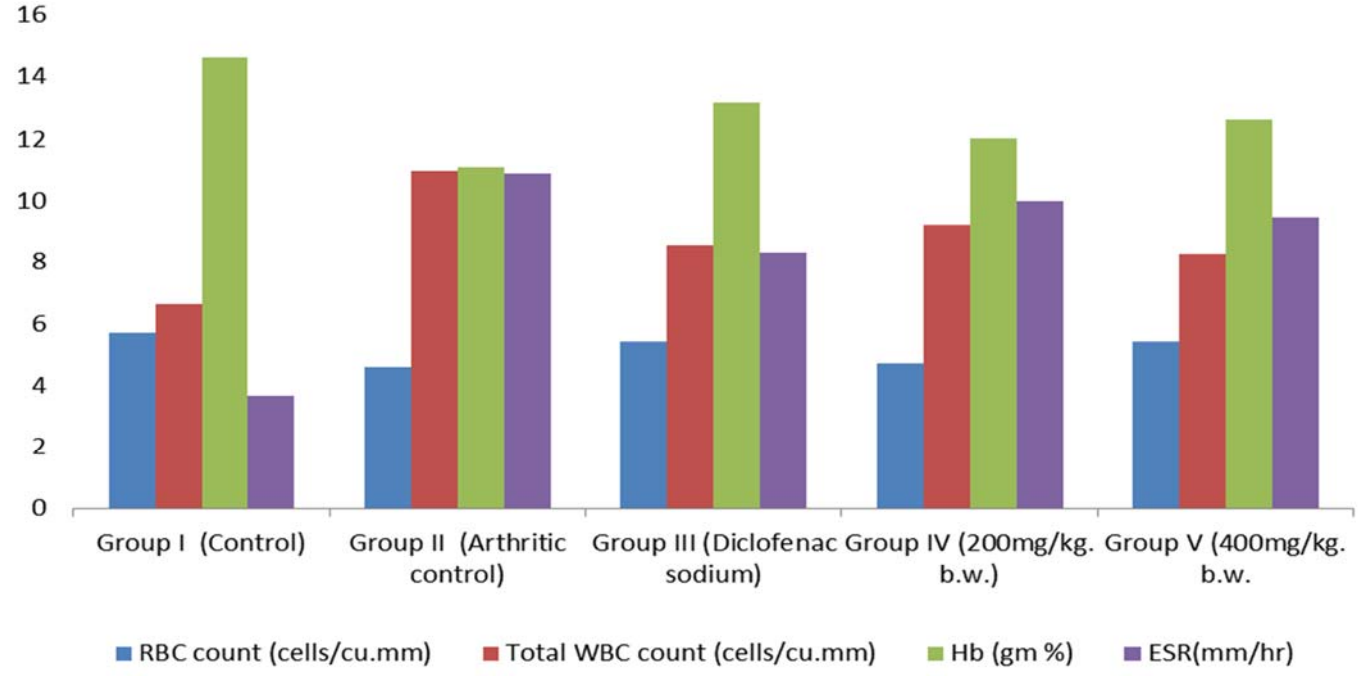


Fig 4: Effect of Hematological parameters in Adjuvant-induced Arthritis in Rats after 21 days treatment

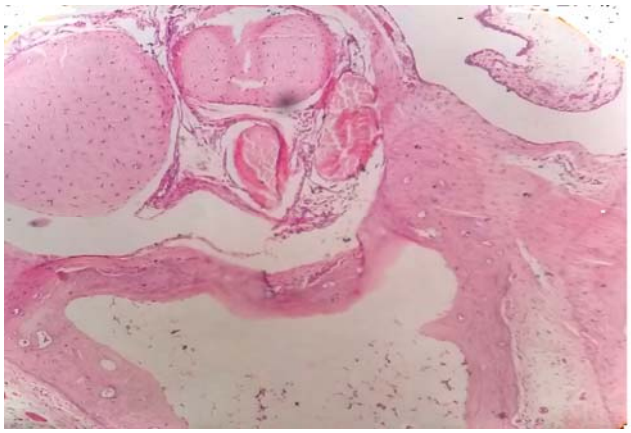
4. Histopathology

The pronimal interphalangeal joints were removed, washed with saline and stored in 10% formalin. The interphalangeal joint sections were obtained, stained with eosin-haemotoxylin stain and viewed under 100 magnifications. Histopathology of ankle joints in the normal control group showed normal joint structure, no cartilage destruction and no signs of inflammation or other destruction. However, arthritic control animals showed soft tissue swelling, severe erosion of cartilage and joint space narrowing. Treatment with EEAD and Diclofenac sodium group showed significant improvement in soft tissue swelling and reduction erosion of cartilage as compared to the disease control group. (Figure-5)

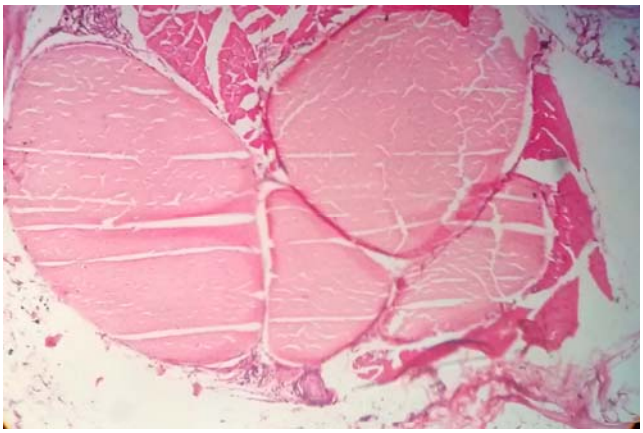
5. Radiographic analysis

At the end of study, the animals were anaesthetized using pentobaritone sodium and digital x-rays were taken for radiographic analysis of the joints. X-rays were taken at the knee joints for confirmation and evaluation of the severity of arthritis in CFA induced rats.

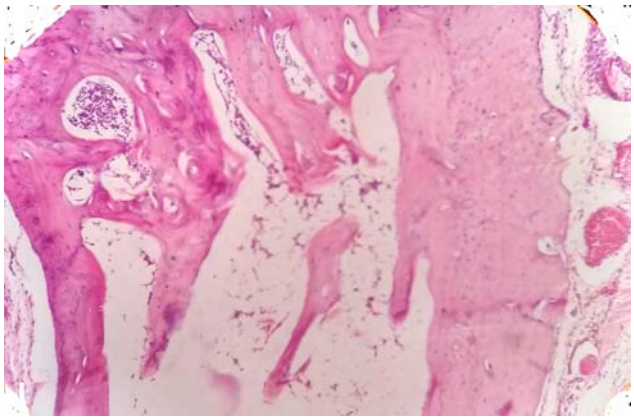
Soft tissue swelling is the earlier radiographic sign, where as prominent radiographic changes like bony erosions and narrowing of joint spaces can be observed only in the final stages of arthritis. (Figure-6)



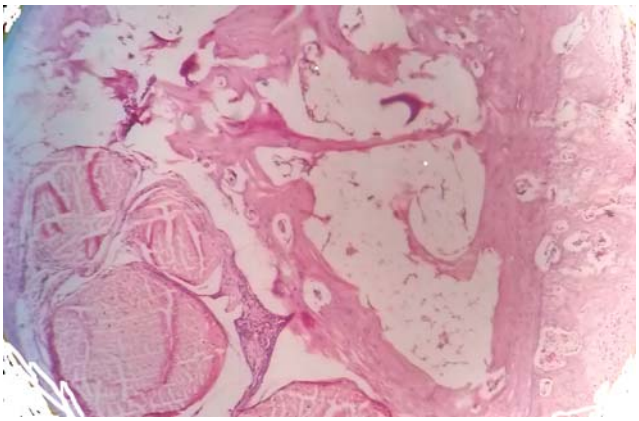
Control group



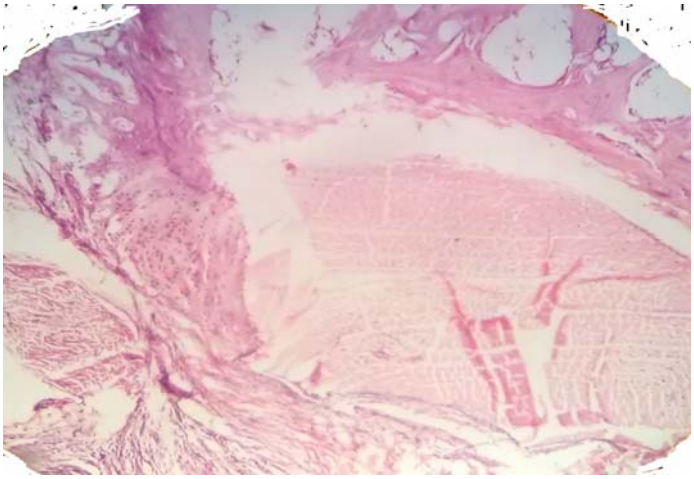
Arthritic control group



Diclofenac Sodium (Standard) treated group

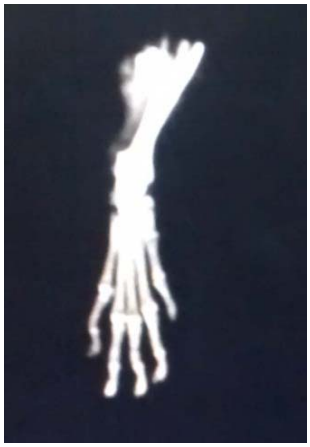


Test drug I group (200mg/kg)



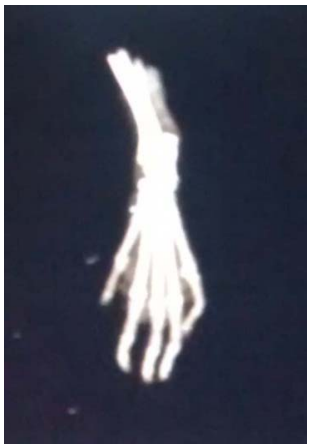
Test drug II group (400mg/kg)

Fig 5: histopathaological examination of bone

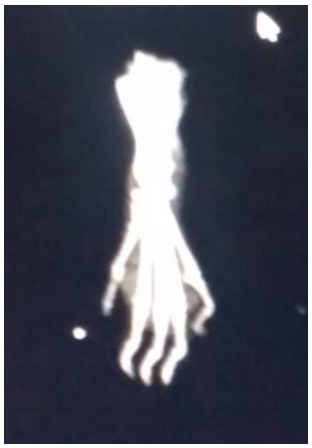


Radiographic hind leg image of control group rat

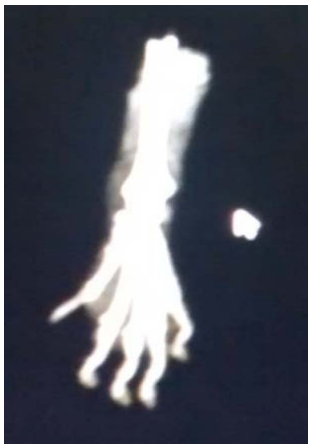
Radiographic hind leg image of Arthritic control group rat



Radiographic hind leg image of Diclofenac sodium (Std) group rat



Radiographic hind leg image of Test drug I (200mg/kg)treated group rat



Radiographic hind leg image of Test drug II(400mg/kg) treated group rat

Fig 6: Radiography study

6. Discussion

In complete Fruend's adjuvant arthritic rat model treatment with Adansonia digitata seed extract showed significant inhibitory effect on injected hind paw edema and maximum inhibition was observed on 21st day. In the present study, the increased lymphocyte count and migration of leucocytes into the inflamed area of arthritic rats were significantly prevented with the treatment of the herbal product and the standard drug as reflected from the significant decrease in total WBC count.

The erythrocyte sedimentation rate (ESR) level which was markedly elevated in arthritic control group of rats was decreased significantly with herbal product at different doses (200mg/kg and 400mg/kg) and the effect was comparable to standard drug.

The chronic inflammation involves the release of various inflammatory mediators like cytokines (IL-1α and TNF- α) granulocyte monocytes colony stimulating factor (GM-CSF). Platelet derived growth factor (PGDF) and others. These mediators are responsible for the pain, destruction of cartilage and leads to severe disability. Paw swelling is one of the major factor in assessing the degree of inflammation and efficacy of the drugs. Adjuvant induced arthritis is non-

specific immune response within the joint and can also result in inflammatory and erosion disease.

Paw swelling is an index of measuring the anti-arthritic activity of various drugs and it is employed here to determine the activity of EEAD, Reference standard Diclofenac Sodium, EEAD administered group showed marked reduction in paw volume when compared with the arthritic control group by inhibiting the release of inflammatory mediators. As inflammation is progressed, a more diffused demineralization is developed in the extremities. In the present study the significant role of the EEAD is due to decreased demineralization in the joint extremities.

The acute stage of arthritis is characterized by signs of hyperalgesia, lack of mobility and pause in the body weight gain. During the acute period hind paw and fore paw joint diameter increases. In later acute stages of the disease (day 7) rats with adjuvant arthritis are often relatively immobile due to the severity of paw swelling, body weight, food intake and metabolism are affected by immunity and inflammation and they are regulated by a cytokine – like hormone known as Leptin. In CFA induced arthritis, within 24 hrs of administration of CFA the plasma leptin level were rapidly increased which led to anorexia and body weight loss.

Decrease in RBC count and haemoglobin level represents the anemic condition in arthritic rats. The more important causes are the abnormal storage of iron in the reticulo endothelial system and synovial tissue and failure of bone marrow to respond to anemia.

It has been reported that the moderate rise in the WBC count occurs in arthritic conditions due to an IL-1B mediated rise in the respective colony stimulating factor.

In the present study; EEAD exhibited significant increase in the ESR which is attributed to the accelerated formation of endogenous protein such as fibrinogen and α, β globulin, and such a rise in the ESR indicates an active but obscure disease process. In the present study Ethanolic extract of *Adansonia digitata* seed treatment restored the altered haematological profile by decreasing the ESR provides support for its anti-arthritic effect.

Histopathological evaluations of ankle joint in the normal control group showed normal joint structure, no cartilage destruction, and no signs of inflammation or other distortion.

However, in arthritis control groups showed soft tissue swelling, severe erosion of cartilage and joint space narrowing seen. Treatment with EEAD and Diclofenac sodium group showed significant improvement in soft tissue swelling, cartilage erosin as compared to the disease control group, which showed the protective effect of EEAD of soft tissue swelling and cartilage erosion, which supports its anti-arthritic effect.

7. Conclusion

The present study was carried out to evaluate the activity of "*Adansonia digitata*" seed extract.

Diclofenac sodium (13.5 mg/kg) was used as a standard drug.

In conclusion, Adansonia digitata at the specified dose level of 400mg/kg b.w. produced significant reduction in inflammation and redness of paw edema on the 21 day treatment when compared to control group rats. It could normalize the body weight, hematological abnormalities in adjuvant induced arthritic rats in both developing and developed phases of CFA induced arthritis. Further histopathological and radiological studies confirmed the anti-arthritic activity of Adansonia digitata in CFA induced arthritis.

It exerts potent anti-arthritic activity by significantly altering the pathogernesis during arthritis without exerting any side effects in CFA induced arthritis in rats.

The standard drug and ethanol extract reduced the paw volume by 67.3% and 58.5% respectively on 21st day, which may be due to the suppression of inflammatory mediator released due to inductions of Fruend's adjuvant.

The result showed that ethanol extract showed high percentage inhibition in paw edema on 21 day treatment. Therefore we can conclude that the flavonoids present in the ethanolic extract may be responsible for the Anti-arthritic activity.

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