Research Article

Anti-Arthritic Activity of Calotropis Gigantean Root Bark Extract in Experimental Rats

Rudrapratap Khan*, Monika Pandey, Shubhasis Dan, Neelang Trivedi and Bhawana Chaudhary

Mahatma Gandhi College Of Pharmaceutical Sciences, ISI-A (15) RICCO Institutional Area, Sitapura, Tonk Road, Jaipur, Rajasthan, India.

INTRODUCTION

Rheumatoid arthritis (RA) is a common chronic and systemic autoimmune disorder characterized by inflammation of the synovial joints and concomitant destruction of cartilage and bone. It involves a complicated pathogenesis, with pathological changes in multiple targets. The proinflammatory cytokines, mainly tumor necrosis factor- α (TNF-α), interleukin-1β (IL-1β), and IL-6 produced by monocytes, macrophages, and synovial fibroblasts, are suggested to play an important role in the pathogenesis and disease progression of RA. TNF- α, IL-1 β, and IL-6 are present in highly increased amounts in synovial fluid and joint tissues of RA patients, while anti-TNF, anti-IL-1, and anti-IL-6 therapies have been reported to be effective in the treatment of RA[1]. A Joint consists of the ends of the articulating bones which are covered with cartilage. It is surrounded and kept in position by a capsule and special ligaments which are lined by a membrane called the synovial membrane[2].

The term arthritis is used to describe changes in the joints which may be either inflammatory or degenerative in character. If only one joint is affected the condition is referred to as monoarticular arthritis; if several joints are involved it is called polyarticular arthritis or polyarthritis[2].Rheumatoid arthritis can also cause inflammation of the tissue around the joints, as well as other organs in the body. Autoimmune diseases are illnesses which occur when the body tissues are mistakenly attacked by its own immune system. The immune system is a complex organization of cells and antibodies designed normally to "seek and destroy" invaders of the body, particularly infections. Patients with these diseases have antibodies in their blood which target their own body tissues, where they can be associated with inflammation. Because it can affect multiple other organs of the body, rheumatoid arthritis is referred to as a systemic illness and is sometimes called rheumatoid disease. In rheumatoid arthritis, multiple joints are usually inflamed in a symmetrical pattern (both sides of the body affected). The small joints of both the hands and wrists are often involved.

The inflammation of rheumatoid disease can also occur in tissues around the joints, such as the tendons, ligaments, and muscles[4].

Types of arthritis:

a) Rheumatic arthritis (Fibromyalgia)
b) Rheumatoid arthritis (Still's Disease)
c) Gout
d) Degenerative arthritis (Osteo-arthritis)
e) Psoriatic arthritis
f) Ankylosing Spondylitis[2]

Treatment of rheumatoid arthritis:

Rheumatoid arthritis is a chronic disorder for which there is no known cure. Fortunately in the last few years, a shift in strategy toward the earlier institution of disease modifying drugs and the availability of new classes of medications have greatly improved the outcomes that can be expected by most patients. The goal of treatment now aims toward achieving the lowest possible level of arthritis disease activity and remission if possible, the minimization of joint damage,

and enhancing physical function and quality of life. The optimal treatment of RA requires a comprehensive program that combines medical, social, and emotional support for the patient. It is essential that the patient and the patient's family be educated about the nature and course of the disease. Strategies are all aimed at reducing pain and discomfort, preventing deformities and loss of joint function, and maintaining a productive and active life. Inflammation must be suppressed and mechanical and structural abnormalities corrected or compensated by assistive devices. Treatment options include medications, reduction of joint stress, physical and occupational therapy, and surgical intervention.

a) Pharmacological Strategies
b) NSAIDs
c) Corticosteroids
d) Disease Modifying Anti-rheumatic Drugs (DMARDs)
e) Treatment during pregnancy
f) Reduction of joint stress
g) Surgical approaches

MATERIAL AND METHODS

Plant material

The Plant *Calotropis gigantea* was collected from the various places of and local area of jaipur city during the month of jan-feb. 2011. The plant was identified and authenticated by Mr. Vinod Sharma The herbarium was deposited in the department of botany, University Of Rajasthan, Jaipur (raj.). which has a specimen number **RUBL20918**. The root bark was separated from the fresh root of the plant growing in the wild. It was cleaned, and dried under shade at ambient temperature and crushed to get coarse Powder

Phytochemical evaluation

Solvent Extraction

Plant was collected from different area of Jaipur (Rajasthan) and bark was removed. The collected root bark pieces was dried for 7-10 days in sun and finally kept in an electric oven for 72 hours at 40^{o}C. After complete drying, the dried pieces were pulverized into a coarse powder[5].

The weighted amount of the powdered drug were first defatted with petroleum ether (ratio is 1:10, powdered drug and petroleum ether) for three days and it was filtered. The filtrate and powder (Residue) was separated and it was keep for drying for complete removal of petroleum ether. The dried powdered drug was then extracted with 90% hydroalcholic solvent (90:10, ethanol and water) for seven days with intermittent shaking. After the complete solvent extraction, extract was separate and solvent was removed by solvent evaporator in reduced pressure by rotary evaporator, until the solvent was completely removed and get dried extract with minimal solvent. After that weigh the accurate amount of dried extract and calculate the percentage yield of extract.

Animals

Male albino rats of wister strain weighing around 100-180g were procured from Animal house, Department of Pharmacology, Mahatma Gandhi College Of Pharmaceutical Sciences, Jaipur (Rajasthan), All animals were housed in polypropylene cages in a temperature - controlled animal house room at 24 ±1°C temperature, 60 ± 5 % relative humidity and 12 hour light and 12 hour dark cycle. The animals were fed with pelleted feed with standard rat diet and tap water throughout the experiment. These animals were used for anti inflammatory and locomotor activity. The experiment were designed and conducted in accordance with the ethical norms approved by Institutional Animal Ethical Committee Guidelines (reg. no. 1356/ac/10/CPCSEA/9/july /10) The animal experimentation was carried out in accordance to the guidelines mentioned in the CPCSEA.

Acute Toxicity studies

The preliminary pharmacological studies were conducted to assess the acute pharmacological effects and LD_{50} of the hydroalcholic drug extract.The acute toxicity study was carried out in adult female albino rats (100-180) by "fixed dose" method of OECD (Organization for Economic Co-operation and Development) guideline number-420. The animals were fasted overnight and next day extract (root bark, CGE) of plant *Calotropis gigantea*

were administered orally at dose level 5, 50, 200, 400, mg/kg (1% v/v tween 80) for sighting study then the animals were observed continuously for three hour for change in general Behavioral, Neurological, Autonomic, profile and then every 30 minutes for next three hour and finally for mortality (death) after 24 hour. The observation was tabulated according to 'Irwin's table'. For the assessment of anti-arthritic activity, dose level were choosen in such a way that, the dose was approximately one tenth of the maximum dose (200mg/kg), during acute toxicity study, and the high dose is twice of the one tenth dose (200 mg/kg and 400 mg/kg) of extract of *Calotropis gigantea*.

Experimental protocol

Arthritis was induced by a single intra-dermal injection of 0.1 ml of Freund's complete adjuvant (FCA) containing 1.0 mg dry heat-killed Mycobacterium tuberculosis per milliliter sterile paraffin oil. The rats were inoculated intradermally into planter surface of right hind paw. A glass syringe (1 ml) with the locking hubs and a 26G needle was used for injection. The rats were anesthetized with ether inhalation prior to and during adjuvant injection, as the very viscous nature of the adjuvant exerts difficulty while injecting. The swelling in hind paws were periodically examined in each paw from the ankle using plethysmometer[7].Before any treatment rats were divided into five groups, each group contains six animals as followed for analysis of histological and biochemical parameters[8].

Group I: Normal control, rats were injected intradermally saline 0.1 ml.

Group II: Arthritic control, AIA rats.

[Injected 0.1 ml Freund complete adjuvant (FCA, containing 10 mg of heat-killed *Mycobacterium tuberculosis* into right hind paw of the rats on day 0]

Group III: Treatment group I, 200mg/kg CGE.

[Adjuvant induced arthritic rats were administered with hydroalcholic extract of *C. gigantea* root bark extract of dose 200mg/kg body weight/rat/day from day 15-24 after adjuvant injection by oral administration]

Group IV: Treatment group II, 400mg/kg CGE.

[Adjuvant induced arthritic rats were administered with hydroalcholic extract of *C. gigantea* root bark extract of dose 400mg/kg body weight/rat/day from day 15-24 after adjuvant injection by oral route administration]

Group V: Standard group, treated with 0.7 mg/kg body weight/rat/day Dexamethasone from day 15-24 after adjuvant injection by i.p. administration

In the normal control group no treatment was given. In the every treated group, drugs were administered orally as suspension in normal saline. Adjuvant was given to each animals of each group other than normal control and treatments starts from day 14 which is immunization period called as onset of arthritis treatment was continued till day 24[9].The standard drug, Dexamethasone i. p. used in the experiment, was purchased from the market having the dose of 2mg/ml in the form of liquid injection. At the end of the experimental period, rats were fasted over night and anesthetized with xylazine/ ketamine (10/50 mg/kg, i. p.) the anesthetized rats were sacrificed by cervical decapitation and the blood was collected into tubes by cardiac puncture prior to sacrifice. Blood samples were immediately centrifuged at 3000 rpm for 10 min. and plasma or serum samples were stored under freezer until assayed[7].

Clinical assessment of adjuvant arthritis

Arthritic score (polyarthritic index)[10]

For clinical evaluation of AA, the polyarthritis severity was graded on a 0-4 scale, each paw was graded, and 4 grades were summed to a maximum possible score of 16. Rats of each group were evaluated daily for arthritis using a macroscopic scoring system describe as follows:

0 = Normal or no sign of arthritis or no swelling.
1 = Swelling and/or redness in one joint.
2 = Swelling and/or redness in more than one joint.
3 = Swelling and/ or redness in the entire paw.
4 = Deformity and/ or ankylosis.

Inflammation parameter

Measurement of paw volume indicates the effect of drugs on inflammation. Anti-inflammatory effect of the drugs was evaluated by measurement of physical changes in right hind paw of rats. It was evaluated by two different ways as follows:

Paw volume (Plethysmometer)[11,12,13]

Paw volume were examined after every 3-4 days. The right hind paw volume was measured with plethysmometer (basic value, day 0) and repeated on days 5, 10, 14, 20 and 24.Intensity of oedema in paw (in joint and soft tissue) was determined by measuring the paw volume of entire inflamed paw (right hind paw) with the help of mercury plethysmometer, equipped for accurate measurement of the rats paw swelling through dislocation of fluid volume. The change in volume of the affected paw was evaluated on before the induction of inflammation or arthritis ($V_{b.i.}$), 14 days after induction (V_{14}) and 24 days after induction (V_{24}) and paw volume index was calculated by following formula.

$$\text{Paw volume index (\%)} = \frac{V_{24} - V_{14}}{V_{b.i.} - V_{14}} \times 100$$

$$\text{Paw volume inhibition (\%)} = 100 - \frac{V_{24} - V_{14}}{V_{b.i.} - V_{14}} \times 100$$

Here $V_{b.i.}$ = Paw volume before arthritis
V_{14} = Paw volume after 14 days
V_{24} = Paw volume after 24 days

Assessment of pain behaviour

Hot Plate Paw Withdrawal Latency method

In the arthritic rats hind paw has inflamed so the heat sensitivity of the right hind paw has been changed. Heat sensitivity of the paw has been measured in arthritic rats by using the hot-plate test and paw withdrawal latency. As the inflammation progresses in the paw the sensitivity to heat of the rat paw decreases. Thus the paw withdrawal latency decreases. The rat was kept on the hot plate having temperature maintained at 55^0C, and the maximum exposure time to hot plate was 15 sec. For the evaluation of heat sensitivity reaction time (paw licking or paw withdrawal) were recorded for each animals of each group[14].

Radiographic analysis (Evaluation of bone Destruction by X- rays) [15,9,10]

At the end of experiment on the day 25 after adjuvant injection, rats were anesthetized by inhalation of anaesthetic ether and imaged on Fuji HR-Fast film (Fuji photo film), using a siemens X-rays tube assembly (Siemens AG, Munich, Germany). Whole bodies were X-rayed using a 90^0 projection from the dorsal ventral aspect. Radiographs of each rat were evaluated for soft tissue swelling, Bone matrix resorption, Periosteal new bone formation and bone erosion, and were scored in a blind fashion by two independent observers graded as follows:

0 = Normal, No change
1 = Slight change
2 = moderate change
3 = severe change

The total radiological scores were calculated from the sum of both hind paws, with a maximum possible score of 6 for each radiological parameter per rat.

Biochemical assay [7]

i) Aspartate transaminase (AST)

One milliliter of buffered substrate was incubated for 10 min at 37^0C. Then 0.2 ml of the enzyme was added and incubation was continued for an hour. To the control tubes, the enzyme was added after arresting the reaction with 1.0 ml of DNPH and the tubes were kept at room temperature for 20 min. Then 5.0 ml of 0.4 N NaOH was added. A set of standard pyruvate were also treated in a similar manner. The colour developed was read at 540 nm. The enzyme activity was expressed as micromoles of pyruvate liberated per milligram of protein per minute.

ii) Alanine transaminase (ALT)

One milliliter of the buffered substrate was incubated for 10 min at 37^0C. Then 0.2 ml of enzyme was added. The tubes were incubated at 37^0C for 30 min. To the control tubes, enzymes was added after arresting the reaction with 1.0 ml of DNPH and the tubes were kept at room temperature for 20 min. Then 5.0 ml of 0.4 N NaOH was added. A set of standard pyruvate were also treated in similar manner. The colour developed was read at 540 nm. The enzyme activity was expressed as moles of pyruvate liberated per milligram of protein per minute

Measurement of inflammation mediators:

i) Lipid Peroxidation in Erythrocytes (TBARS)

Lipid peroxidation in erythrocytes was estimated by measuring thiobarbituric acid reacting substances (TBARS). The method is based on spectrometric measurement of purple colour generated by the reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA). 2.5 ml of TCA solution (10% w/v) was added to 0.5 % supernatant of the tissue preparation in each centrifuge tube and tubes were placed in a boiling water bath for 15 min. After cooling to room temperature, the tubes were centrifuged at 1000X g for 10 min. and 2 ml of each sample supernatant was transferred to a test tube containing 1 ml of TBA solution (0.67 % w/v). Each tube was placed in a boiling water bath for 15 min. after cooling to room temperature; the absorbance was measured at 532 nm. The concentration of MDA was calculated based on the absorbance coefficient of TBA-MDA complex ($\varepsilon = 1.56 \times 10^5$/cm/M) and it was expressed as nmol/mg protein[16]

ii) Determination of Reduced Glutathione (GSH)

The liver was quickly removed from sacrificed rat, and was homogenized in 5 ml of distilled water. The homogenate centrifuged at 6000 rpm for 10 min. 0.5 ml of above solution was mixed with 0.5 ml of 10 % TCA and a protein free supernatant was obtained by centrifugation. 0.5 ml of TCA was mixed with 1 ml of 0.6 M Na_2HSO_4 and 0.5 ml of DTNB reagent. The absorbance of this solution was measured at 410 nm. The absorbance was reading from Concentration Vs Absorbance standard graph of pure glutathione[17].

Body weight measurement[18]

Every rats which was used in experiment weighed at the starting (Baseline) and at the end of experiment which is final day after injection of adjuvant (day 25).

BODY WEIGHT – Based on pre-study values

0 = < 5 % decrease
1 = 6 -10 % decrease
2 = 11-20 % decrease
3 = 21-25 % decrease
4 = > 25 % decrease

Index of immune organ (spleen)

In the course of experiment, the body weight of rats was measured. At day 28 after immunization, the animals were killed, and the spleen was promptly removed and weighed. The index of spleen was expressed as the percentage (%) of spleen wet weight Vs body weight, respectively[19].

$$\text{Index} = \frac{\text{Organ weight of AIA rat}}{\text{Body weight of AIA rat}} \times 100$$

Or

$$\text{Index} = \frac{\text{Organ weight of control rat}}{\text{Body weight of control rat}} \times 100$$

Balance beam activity

The unit feature of balance beam designed for the adult rat, constructed of solid oak, and mounted on each height pole stand. It contains 45 cm long open field and dark black cloured box are present at the opposite side of the beam.For determining the balance beam activity of rats, trained animals (training for three days) of each group one by one placed at the one side of the beam and length of time for which animal remained on the beam was recorded with a maximum time limit being 3 min.

Histopathological analysis

At the end of experiment Rats paw were collected on day 25, after the injection of adjuvant, by first sacrificing animals and the hind paws were excised from the femur bone then the following process was done:

1. Fixation: The tissue was fixed in 10 % buffered formalin for 5 days.

2. Decalcification: The decalcification was done by 5 % nitric acid for 3- 14 days. Fresh solution of nitric acid were prepared daily and changed. The end point was determined by either chemical mean or direct mechanical method. The bone was pierced with sharp pin if it was properly decalcified it would easily penetrate the bone.

3. Processing: the fragments were then dehydrated by successive solvent treatment (Acetone 50 %, 70 %, 90 %, Absolute, Benzene) cleared and embedded in paraffin. Serial sagittal sections of the whole paw were cut (5 µm thick), stained with hematoxylin and eosin (H&E) and examined for the degree of synovitis and bone destruction in the blinded manner by the pathologist.

Statistical analysis

The experimental results were expressed as Mean ± S.E.M. of n = 6 rats per group. Their 95% Confidence *intervals* (95% CI) were calculated by linear regression analysis. Software Graph Pad Prism 5.01.336 was used for data analysis. Statistical analysis was evaluated by independent Student's t-test or one-way ANOVA followed by Dunnett's test, with the level of significance chosen at $P < 0.05$.

RESULTS

The various result obtained from different experiments carried out are compiled.

Acute toxicity study

The hydroalcholic extract of root bark (CGE) of Calotropis gigantea was found to be toxic at 2000 mg/kg and non-toxic safe up to 400 mg/kg body weight by oral route. After 24 h animal were found well tolerated. We have fixed 1000 mg/kg as cutoff LD_{50}. So dose level i.e. 200 mg/kg and 400 mg/kg were selected for present study **(table no 1).** The toxicity data were represented by 'Irwin table for acute toxicity' **(table no 2).**

Evaluation of adjuvant arthritis

Clinical assessment of AIA

Arthritic score (Polyarthritic index)

The arthritic score was found to be significantly decreased on treatment with the drug as shown in table **(Table no. 3 & Figure no. 1).**

these changes were normalized **(Figure no. 2)**

EFFECT ON INFLAMMATION PARAMETER

Paw Volume

Injection of FCA in right hind paw of rat produced an increase in paw volume that was maximum at day 14 **(1.13 ± 0.27 mm),** and there after it gradually declined **(Table no. 4).** The inhibitory effect of CGE was evaluated on the day of peak inflammation that is day 14. Oral administration of test extract produced a dose dependent decrease in paw volume it was **0.94 ± 0.26 mm** and **0.76 ± 0.17 mm** in test extract CGE 200/400 mg/kg respectively as compared with day of peak inflammation**.** The immunosuppressant, Dexamethasone was more effective in inhibiting joint inflammation as compared to test extracts. The decrease in paw volume in standard drug Dexamethasone treated rats was **0.70 ± 0.11 mm** as compared with day of peak inflammation as shown in **Figure no. 2**

Assessment of pain parameter:

Hot Plate Paw Withdrawal Latency method

As the pain progress the latency of bearing heat by arthritic rats reduced as shown in **Figure no. 3,** when compared with the normal rats. Increment of latency by treatment as dose dependent manner in extract treated rats as comparable with Dexamethasone treated rats. Better result was shown by Dexamethasone as increase in heat latency. Result is shown in **Table no. 5**

Radiographic analysis (Evaluation of bone Destruction by X- rays)

The radiographic pictures of the joints of arthritic animals, which shows the narrowing of a number of joint space, severe soft tissue swelling, pronounced decrease in bone density, marked destruction of bones, and abnormal ossification in the tarsal, metatarsal, and interphalangeal regions, marginal erosion of joint can be seen in drug treated group (CGE and dexamethasone), in which

Biochemical assays

i) Aspartate transaminase (AST)

A marked increase in the activity of AST (membrane marker enzymes) were observed in the blood homogenate **(Table no. 6),** liver tissues homogenate **(Table no. 7)**, and spleen tissue homogenate **(Table no. 8)** of arthritic rats (group II) when compared to normal control rats (Group I). Arthritic rats treated with *C. gigantea* root bark extract showed a significant ($p < 0.05$) decrease in the activity of AST enzymes.

ii) Alanine transaminase (ALT)

A marked increase in the activity of ALT (membrane marker enzymes) were observed in the blood homogenate **(Table no. 9),** liver tissues homogenate **(Table no. 10)**, and spleen tissue homogenate **(Table no. 11)** of arthritic rats (group II) when compared to normal control rats (Group I). Arthritic rats treated with C. gigantea root bark extract showed a significant ($p < 0.05$) decrease in the activity of ALT enzymes**.**

Measurement of inflammatory mediators:

i) Lipid Peroxidation in Erythrocytes (TBARS) on Liver tissue

FCA injection produced an increased in level of tissue TBARS (Thiobarbituric acid reacting substances) expressed as nmol of MDA normal in control rats to increase in arthritic rats tissue supernatant. **Table no. 12** shows the level of lipid peroxides in liver. Lipid peroxide MDA level was found to be significantly increased ($p<0/001$). After drug treatment for 14 days, the level was found to be significantly reduced in CGE-I (200 mg/kg) and CGE –II (400mg/kg) shows dose dependent reduction in MDA level. Standard drug dexamethasone shows significantly better reduction of tissue peroxidase than CGE group.

ii) Determination of Reduced Glutathione (GSH) on Liver tissue

Oxidative stress associated with FCA-induced polyarthritis was evaluated by measuring level of GSH in the inflamed liver tissues**.** FCA injection into right hind paws decreased the tissue GSH, which is naturally occurring antioxidant in body. In normal control rats tissue supernatant level changes from 11.38 ± 1.16 **mg/ml** to 3.26 ± 2.32 **mg/ml** in arthritic rats. Both the extract produces an increase in the level of GSH **Table no. 13**

Body weight measurement

During experimental period significant changes in body weight are shown in **Table no. 14.** It was observed that the normal rats gain body weight during experiment, where as arthritic rats reduce their body weight. Extract treated rats significantly improved the body weight and in standard group rats, dexamethasone shows more weight increment than extract treated rats**.**

Index of immune organ (spleen)

The index of spleen of adjuvant arthritic rats was determined at day 24 after immunization **(Table no. 15)**. It was found that there was a decrease of spleen in arthritic rats. The administration of CGE (200 and 400 mg/kg) evidently increased the weight of spleen of adjuvant arthritic rats, but increased better in higher dose (400 mg/kg) of extract. Significantly increases the body weight of dexamethasone treated group.

Balance beam activity

Balance beam performances of each animals of each group were observed which was showed that the arthritic control rats were spending more time in the balance beam as compared to the other animals. Normal control rats were taken very less time in balance beam. Drug treated animals also shows more length of time in balance beam than normal control rats **(Table no. 16).**

Histopathological analysis

Histopathological study was carried out after completion of 24 days of the activity. It shows some changes like leukocytes infiltration cartilage and bone destruction in treated animals. CGE treated animals had a lower degree of sub-synovial infiltration that was cure, found in non arthritic animals.A histopathological study produced marked infiltration of leukocytes, edema, fibrosis, bone and cartilaginous erosion, cellular inflammatory changes in the nearer tissues, and also shows joint space narrowing, vascular proliferation, and congetion. Figure no. 3.1 to Figure no. 3.5 shows the different types of changes occurs in hind paw of treated and non treated animals.

DISCUSSION

The present study demonstrates that freund's complete adjuvant (FCA) containing killed *M. tuberculosis* induced AA (adjuvant arthritis) in rats. Treatment of AIA (adjuvant induced arthritis) in rats with *Calotropis gigantea* root bark extract shown decline in inflammation which was comparable to Dexamethasone treated groups (standard drug). Adjuvant induced arthritis (AIA) is thought to occur through cell mediated autoimmunity by structural mimicry between mycobacteria and cartilage proteoglycans in rats. Thus activated macrophages and lymphocytes by adjuvant inoculation or their product monokines, cytokines, chemokines, may be involved in abnormal lipid and protein metabolism. Lipid peroxide formed by auto oxidation of polyunsaturated fatty acids of cell membranes. In the damaged cells concentration is increased. In the present study lipid peroxide were significantly increased in blood, spleen and liver cells of arthritic rats. This may be due to less activity of antioxidants and inactivation of them. Inhibition of inflammation and accumulation of lipid peroxides may be due to presence of flavonoids in the *C. gigantea.* Flavonoids have antioxidation property by scavenging singlet O_2[18] Adjuvant induced arthritis (AIA) is a rather aggressive and monophasic form of arthritis, usually the disease is quite severe and finally leads to complete ankylosis and permanent joint mal-formations. Therefore AIA is most frequently used as a model for screening and testing anti-arthritic agents, especially NSAIDs, as the inflammation associated

with AIA is very dependent on prostaglandin E_2 (PGE_2) generated by cyclooxygenase (COX)[19,20].The FCA administered rats showed soft tissue swelling around the ankle joints during the development of arthritis, which was considered as edema of the particular tissues. As the disease progressed, a more diffused demineralization developed in the extremities. Secondary lesions of adjuvant arthritis occurred after a delay of approximately 10 days and were characterized by inflammation of non-injected rats (normal control rats) and further increase in the volume of the injected hind leg. Reduction of paw swelling in the *C. gigantea* root bark extract (CGE) treated rats from the third week onwards may be due to immunological protection rendered by the plant extract. Cellular enzymes, such as aspartate transaminase (AST), alanine transaminase (ALT), membrane bound indicator of type II cell secretary activity or the lysosomal enzyme b-glucuronidase, an indicator of phagocytic activity, can also be used as sensitive markers of cellular integrity and cellular toxicity induced by pathological conditions. Increased activities of these enzymes were observed in arthritic rats. This may be attributed towards persistent inflammation.The pathogenesis of RA is perpetuated by the activity of a complex network of cytokines. As a consequence of the inflammatory processes, a large number of cytokines and growth factors with overlapping biological effects are found in the synovium. Several cytokines such as TNF-α, IL-1 and IL-6 have been implicated in the pathological mechanism of synovial tissue proliferation, joint destruction and programmed cell death in rheumatoid joint. It was reported that the expression of inflammatory cytokines such as TNF- α and IL-1 β, and the tissue enzymes such as cathepsin and matrix metalloproteinases were observed to be increased in the subchondral bone region of the knee joint samples from human osteoarthritis or rheumatoid arthritic patients. Biological agents that specifically inhibit the effects of TNF-α or IL-1 represent a major advancement in the treatment of RA.In the histological studies of joints showed the destruction of inflamed joints are the continued migration into the synovium and joint fluid of polymorphonuclear leukocytes, lymphocytes and monocytes/macrophages, all of which produce inflammatory cytokines. Thus, pharmacological inhibition of this leukocyte migration and accumulation in arthritis may have beneficial effects for joint preservation. In our study, our histological observations supported that the administration of *C. gigantea* root bark extract may be protective by decreasing the leukocytic migration. It has also been reported that the flavonoids significantly inhibit the leukocyte migration in a dose dependant manner[7].The bones become thin and fragile of the arthritic rats on inspection and were easily crushed during cutting and homogenizing. Change in body weight is in response to the incidence and severity of arthritis and used to assess the onset of the disease. It was reported that Arthritis characterized by reduced body weight and loss of body weight is associated with increased production of pro-inflammatory cytokines such as TNF-α and IL-1. Treatment with *C. gigantea* and dexamethasone recovered the body weights, which support and confirms the above observations.[21]

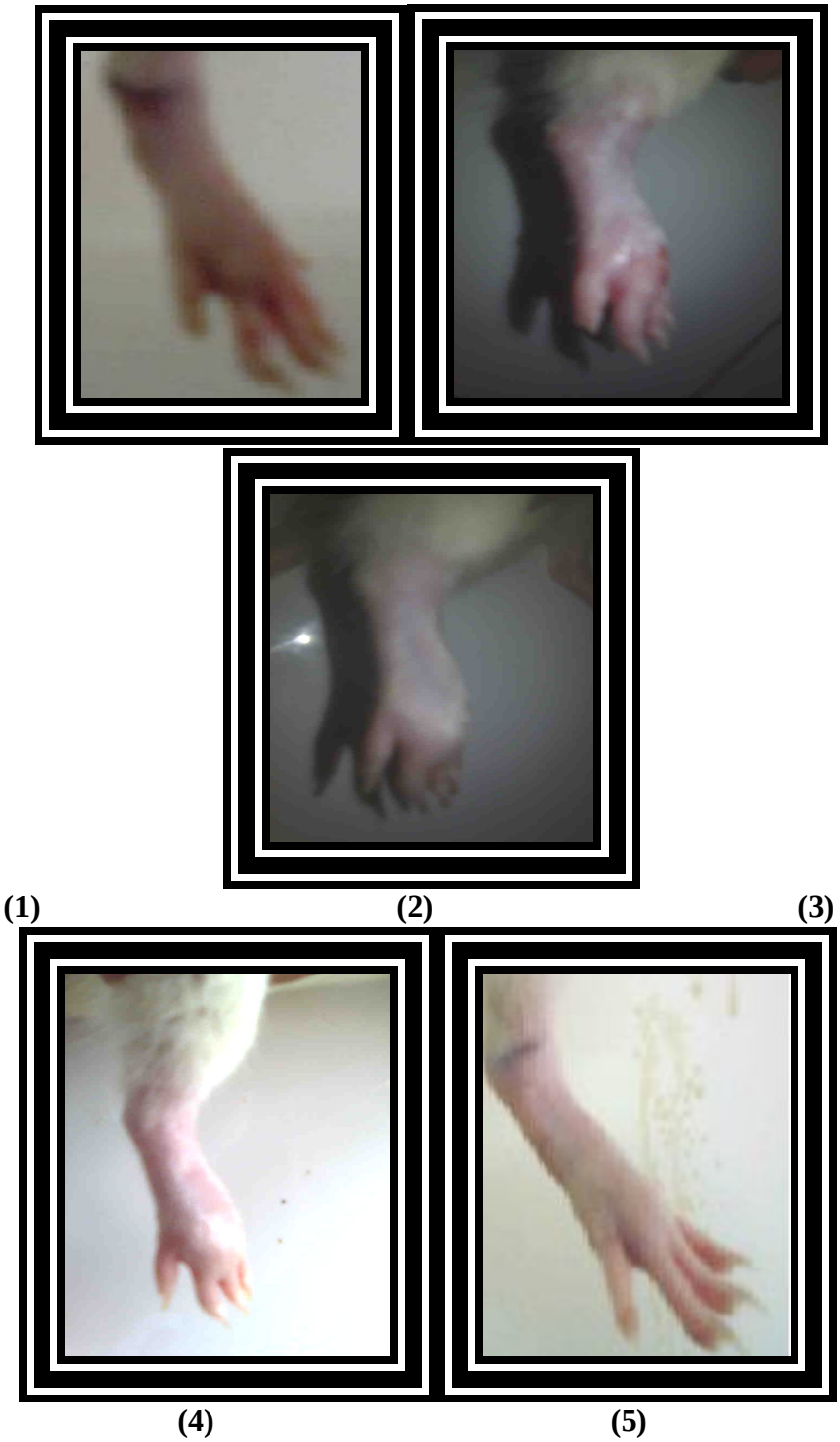

Fig. 1: Morphological features of the hind limb paw swelling of Control and Experimental animals

1) **Control:** Showing normal picture of the paw.
2) **Arthritic rat:** Showing the maximum swelling.
3) **Test-I [CPE -100 mg/kg]:** Showing the less swelling and less edema as compared to Arthritic rat.
4) **Test-II [CPE -200 mg/kg]:** Showing the minimal swelling.
5) **Standard: Dexamethasone (0.7 mg/kg):** Showing normal paw

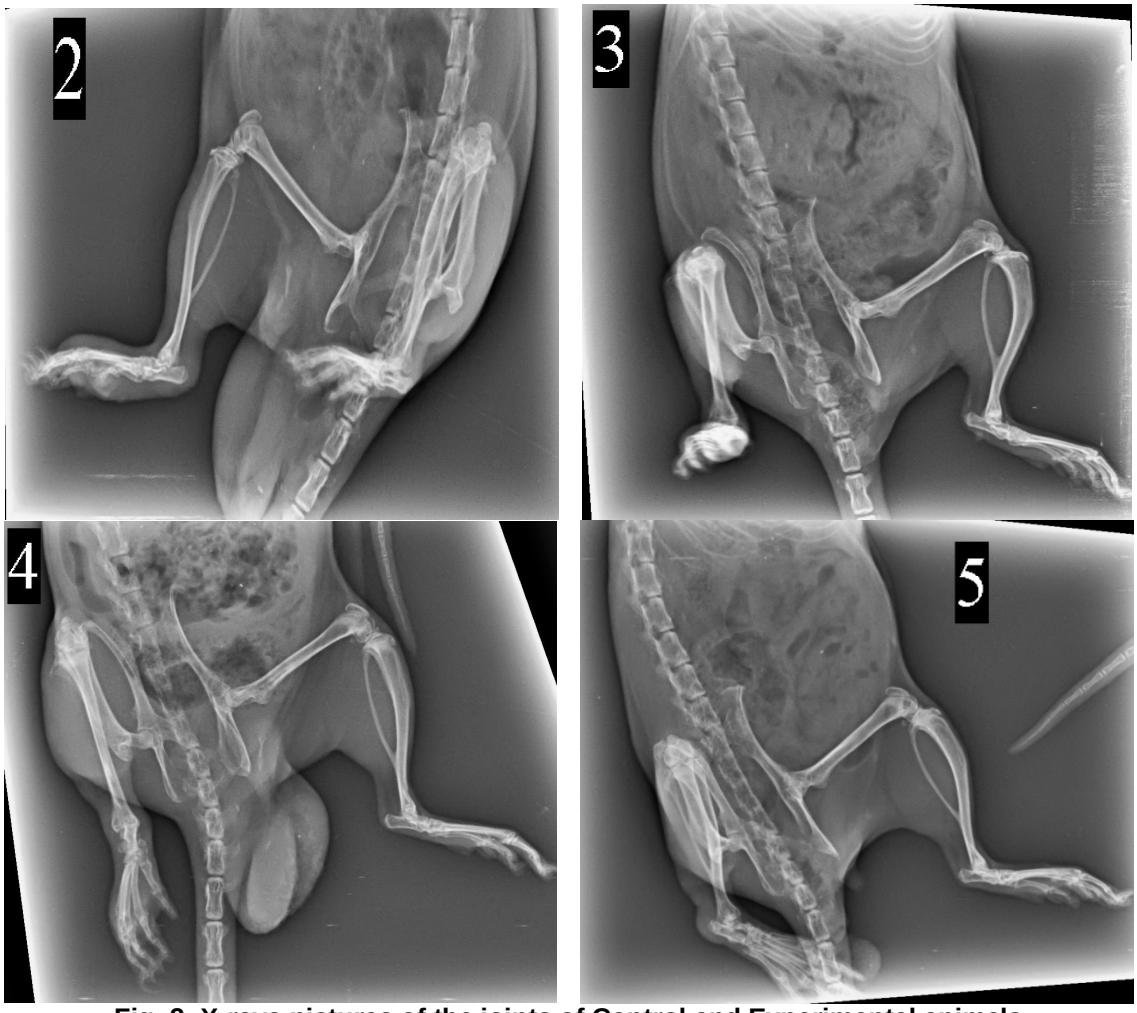

Fig. 2: X-rays pictures of the joints of Control and Experimental animals

2) **Arthritic rat:** Showing the diffused joint and narrowing of joint space.
3) **Treatment group [CGE-I] (200 mg/kg):** Showing clear and minimal narrowing of joint space.
4) **Treatment group [CGE-II] (400 mg/kg):** Showing clear and minimal narrowing of joint space.
5) **Treatment group Standard [Dexamethasone] (0.7 mg/kg):** Showing clear and minimal narrowing of joint space.

Fig. 3: Histopathological changes in Adjuvant Induced Arthritic rats

After the 14 days of arthritis induction treatment were started daily by oral treatment with different doses of CGE (200 and 400 mg/kg) and standard drug (Dexamethasone) and this treatment will continued till 24 days. After this treatment will stop and taking the samples of arthritic rat hind paw (Tibiotarsal joints) and go for H and E stained (x10, x40) histopathological studies which shows following types of changes:

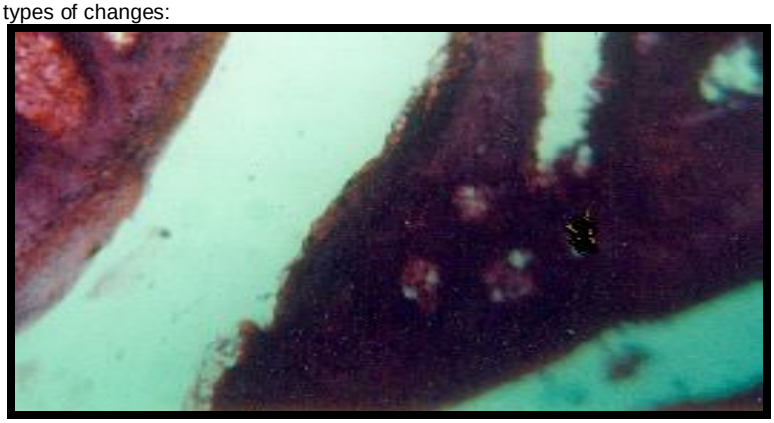

Fig. 3.1(a): Normal control: Histopathology of normal control rat showing normal structure of joint and normal synovial cavity

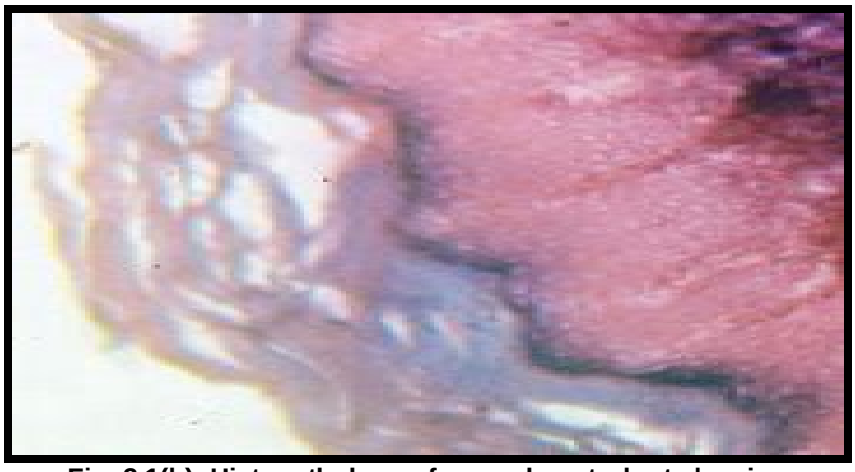

Fig. 3.1(b): Histopathology of normal control rat showing normal structure of bones and cartilages

Fig. 3.2(a): Arthritic control: Histopathology or microphotograph of arthritic rats showing cartilage destruction

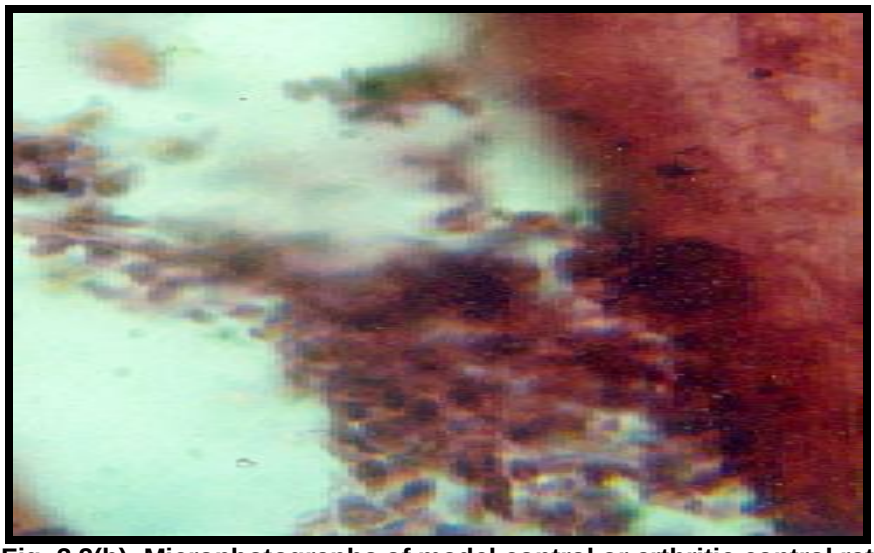

Fig. 3.2(b): Microphotographs of model control or arthritic control rat shows cellular infiltration in synovial cavity

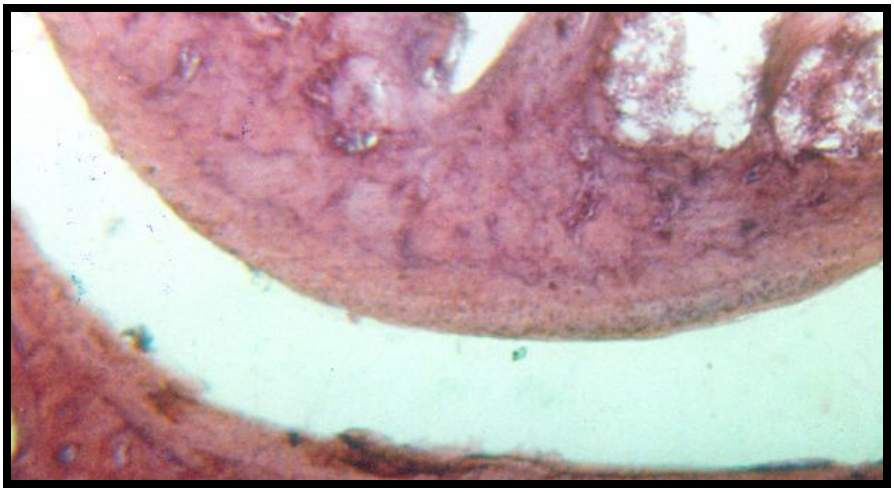

Fig. 3.3: Microphotograph of CGE-I (100mg/kg) treated rat showing normalization of joints and bones

Fig. 3.4: Microphotographs of CGE-2 (200 mg/kg) treated rats showing normal structure of joints and bones, less inflammation and normalization of joint space, synovial space

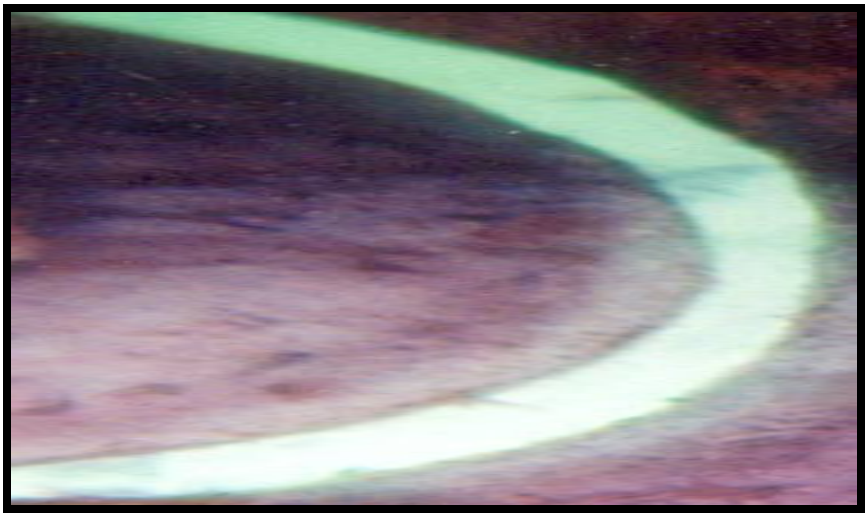

Fig. 3.5: Microphotographs of Dexamethasone (0.7 mg/kg) treated rats showing normal structure of joints and bones

Table 1: Acute toxicity study

S. No.	Treatment	Dose Mg/kg	Number of animals	Mortality			Toxicity profile
				After 3 hrs	After 6 hrs	After 24 hrs	
1.	Sighting Study	5	1	0	0	0	Safe
		50	1	0	0	0	Safe
		400	1	0	0	0	Safe
		2000	1	0	0	death	Toxic
2.	Main test	400	5	0	0	0	Safe
		2000	5	0	1	4	toxic

Table 2: Irwin Table for Acute Toxicity

S. No.	Treatment	PARAMETER OBSERVED																					
		Behavioural Response									Neurological Response							Autonomic Response						
		Alertness	Stero type	Irritabilitty	Fearfullness	Touch Response	Pain Response	Spontaneous	Grooming	Restlessness	Righting reflex	Limb Tone	Grip Strength	Pinna Reflex	Corneal Reflex	Straub Tail	Convulsions	Writhing	Defaecation	Urination	Piloerection	Heart Rate	Respiratory	Pupil Size	Skin Color
		1.	2.	3.	4.	5.	6.	7.	8.	9.	1.	2.	3.	4.	5.	6.	7.	1.	2.	3.	4.	5.	6.	7.	8.
1.	Hydro Alcoholic Extract	-	N	N	-	N	N	-	-	N	N	N	-	-	N	ab	ab	a b	-	N	+ +	+ +	+ +	+	N

N = Normal
- = Reduced / Decreased, + = Increased, ab = Absent

Table 3: Effect of *Calotropis gigantea* root bark extract (CGE) on Arthritic score (Polyarthritic score) in adjuvant induced arthritis in rats

Groups	Arthritic score (Poly Arthritic score)				
	Day 5	Day 10	Day 14	Day 20	Day 24
Normal Control	0	0	0	0	0
Arthritic control	1.40 ± 0.24	1.80 ± 0.36	2.40 ± 0.45	2.00 ± 0.27	2.20 ± 0.22
CGE-I	1.80 ± 0.45	2.00 ± 0.28	1.80 ± 0.23	2.20 ± 0.42	1.60 ± 0.48*
CGE-II	2.20 ± 0.35	2.20 ± 0.49	1.60 ± 0.29	2.20 ± 0.40	1.80 ± 0.28*
Standard	2.20 ± 0.29	2.40 ± 0.28	2.20 ± 0.35	1.60 ± 0.42	1.40 ± 0.35*

Values are expressed as mean ± SEM, n = 6 rats in each group.
ns = not significant
*p < 0.05 when compared with arthritic control One way ANOVA followed by Dunnet's test

Table 4: Effect of *Calotropis gigantea* root bark extract (CGE) on change in paw volume (ml)

Groups	Paw volume of rats on different days in ml					
	Day 0	Day 5	Day 10	Day 14	Day 20	Day 24
Normal Control	0.53 ± 0.03	0.59 ± 0.08	0.60 ± 0.02	0.58 ± 0.17	0.52 ± 0.13	0.51 ± 0.16
Arthritic control	0.62 ± 0.02	0.86 ± 0.04	0.98 ± 0.16	1.13 ± 0.27	1.15 ± 0.18	1.16 ± 0.27
CGE-I	0.61 ± 0.04	0.95 ± 0.17	0.85 ± 0.23	0.87 ± 0.27	0.89 ± 0.23	0.94 ± 0.26
CGE-II	0.65 ± 0.06	0.92 ± 0.28	0.73 ± 0.31	0.82 ± 0.24	0.79 ± 0.14	0.76 ± 0.17
Standard	0.60 ± 0.03	0.96 ± 0.32	0.72 ± 0.7	0.79 ± 0.18	0.72 ± 0.24	0.70 ± 0.11

Values are expressed as mean ± SEM, n = 6 rats in each group.
ns = not significant
*p < 0.05 when compared with arthritic control
One way ANOVA followed by Dunnet's test

Table 5: Effect of *Calotropis gigantea* root bark extract (CGE) on Hot Plate Paw Withdrawal Latency method (in seconds)

Groups	Paw withdrawal time in second					
	Day 0	Day 5	Day 10	Day 14	Day 20	Day 24
Normal Control	8.61 ± 0.37	9.14 ± 0.24	9.53 ± 0.27	8.73 ± 0.38	9.94 ± 0.74	10.78 ± 0.42
Arthritic control	5.43 ± 0.26	3.52 ± 0.42	3.28 ± 0.59	3.65 ± 0.63	4.34 ± 0.26	4.24 ± 0.48
CGE-I	5.56 ± 0.28	2.47 ± 0.68	2.16 ± 0.45	3.44 ± 0.65	4.15 ± 0.29	5.64 ± 0.63*
CGE-II	4.91 ± 0.36	2.42 ± 0.59	2.15 ± 0.38	3.46 ± 0.31	5.46 ± 0.42*	5.94 ± 0.52*
Standard	4.86 ± 0.34	1.17 ± 0.73	1.18 ± 0.26	3.35 ± 0.27	5.63 ± 0.52**	6.47 ± 0.76**

Values are expressed as mean ± SEM, n = 6 rats in each group.
ns = not significant
*p < 0.05, **p<0.01 when compared with adjuvant arthritic control One way ANOVA followed by Dunnet's test

Table 6: Effect of *Calotropis gigantea* root bark extract (CGE) on blood homogenate for membrane markers (Aspartate transaminase, AST)

Groups	Treatment	Dose (mg/kg)	Aspartate transaminase (AST)
Normal Control	ethanol	-	2.86 ± 0.24***
Arthritic control	ethanol	-	6.44 ± 0.07
CGE-I	Extract	200 mg/kg	3.63 ± 0.24*
CGE-II	Extract	400 mg/kg	3.43 ± 0.04*
Standard	Dexamethasone	0.7 mg/kg	3.32 ± 0.14**

Values are expressed as mean ± SEM, One way ANOVA followed by Dunnet's test, n = 6 rats in each group.
*p < 0.05 when compared with normal control, ***p<0.001 when compared with arthritic control,
p<ns = not significant

Table 7: Effect of *Calotropis gigantea* root bark extract (CGE) on liver homogenate for membrane markers (Aspartate transaminase, AST)

Groups	Treatment	Dose (mg/kg)	Aspartate transaminase (AST)
Normal Control	ethanol	-	2.25 ± 0.17***
Arthritic control	ethanol	-	6.72 ± 0.06
CGE-I	Extract	200 mg/kg	3.53 ± 0.07*
CGE-II	Extract	400 mg/kg	3.41 ± 0.16*
Standard	Dexamethasone	0.7 mg/kg	3.12 ± 0.14**

Values are expressed as mean ± SEM, One way ANOVA followed by Dunnet's test, n = 6 rats in each group.
*p < 0.05 when compared with normal control, ***p<0.001 when compared with arthritic control,
p<ns = not significant

Table 8: Effect of *Calotropis gigantea* root bark extract (CGE) on spleen tissue homogenate for membrane markers (Aspartate transaminase, AST)

Groups	Treatment	Dose (mg/kg)	Aspartate transaminase (AST)
Normal Control	ethanol	-	3.18 ± 0.29***
Arthritic control	ethanol	-	7.25 ± 0.06
CGE-I	Extract	200 mg/kg	3.37 ± 0.03*
CGE-II	Extract	400 mg/kg	3.62 ± 0.26*
Standard	Dexamethasone	0.7 mg/kg	3.21 ± 0.15**

Values are expressed as mean ± SEM, One way ANOVA followed by Dunnet's test,
n = 6 rats in each group. *p < 0.05 when compared with normal control, ***p<0.001
when compared with arthritic control, p<ns = not significant

Table 9: Effect of *Calotropis gigantea* root bark extract (CGE) on blood homogenate for membrane markers (Alanine transaminase, ALT)

Groups	Treatment	Dose (mg/kg)	Alanine transaminase (ALT)
Normal Control	ethanol	-	2.26 ± 0.15***
Arthritic control	ethanol	-	5.54 ± 0.02
CGE-I	Extract	200 mg/kg	3.53 ± 0.06*
CGE-II	Extract	400 mg/kg	3.71 ± 0.18*
Standard	Dexamethasone	0.7 mg/kg	3.62 ± 0.1**

Values are expressed as mean ± SEM, One way ANOVA followed by Dunnet's test, n = 6 rats
in each group. *p < 0.05 when compared with normal control, ***p<0.001 when compared
with arthritic control, p<ns = not significant

Table 10: Effect of *Calotropis gigantea* root bark extract (CGE) on liver tissue homogenate for membrane markers (Alanine transaminase, ALT)

Groups	Treatment	Dose (mg/kg)	Alanine transaminase (ALT)
Normal Control	ethanol	-	3.29 ± 0.16***
Arthritic control	ethanol	-	6.82 ± 0.03
CGE-I	Extract	200 mg/kg	3.83 ± 0.05*
CGE-II	Extract	400 mg/kg	3.91 ± 0.18*
Standard	Dexamethasone	0.7 mg/kg	3.62 ± 0.1**

Values are expressed as mean ± SEM, One way ANOVA followed by Dunnet's test, (n=6)
***p < 0.05, **p<0.01 when compared with arthritic control, ***p<0.001 when compared with normal control**

Table 11: Effect of *Calotropis gigantea* root bark extract (CGE) on spleen tissue homogenate for membrane markers (Alanine transaminase, ALT)

Groups	Treatment	Dose (mg/kg)	Alanine transaminase (ALT)
Normal Control	ethanol	-	2.88 ± 0.29***
Arthritic control	ethanol	-	8.25 ± 0.06
CGE-I	Extract	200 mg/kg	4.37 ± 0.05*
CGE-II	Extract	400 mg/kg	4.62 ± 0.16*
Standard	Dexamethasone	0.7 mg/kg	4.21 ± 0.19**

Values are expressed as mean ± SEM, One way ANOVA followed by Dunnet's test, (n=6).
***p < 0.05, **p < 0.01 when compared with arthritic control, ***p<0.001 when compared with normal control**

Table 12: Effect of *Calotropis gigantea* root bark extract (CGE) on lipid peroxides (TBARS) of liver tissue homogenate (in nmol/ml)

Groups	Treatment	Dose (mg/kg)	TBARS (nmol/ml tissue supernatant)
Normal Control	ethanol	-	0.23 ± 0.01*
Arthritic control	ethanol	-	0.34 ± 0.03
CGE-I	Extract	200 mg/kg	0.31 ± 0.02^{ns}
CGE-II	Extract	400 mg/kg	0.20 ± 0.01***
Standard	Dexamethasone	0.7 mg/kg	0.14 ± 0.01***

Values are expressed as mean ± SEM, The statistical difference was evaluated by One way ANOVA followed by Dunnet's test, *p < 0.05 when compared with normal control, ***p<0.001 when compared with arthritic control, p<ns = not significant

Table 13: Effect of *Calotropis gigantea* root bark extract (CGE) on reduced Glutathione (GSH) in Liver tissue (in mg/ml)

Groups	Treatment	Dose (mg/kg)	GSH (mg/ml)
Normal Control	ethanol	-	11.38 ± 1.16***
Arthritic control	ethanol	-	3.26 ± 2.32
CGE-I	Extract	200 mg/kg	4.82 ± 0.72*
CGE-II	Extract	400 mg/kg	3.98 ± 0.37*
Standard	Dexamethasone	0.7 mg/kg	5.28 ± 0.69**

Values are expressed as mean ± SEM, The statistical difference was evaluated by One way ANOVA followed by Dunnet's test, *p<0.05, **p<0.01 when compared with adjuvant arthritic group, ***p<0.001 when compared with control group, n=6 animals

Table 14: Effect of *Calotropis gigantea* root bark extract (CGE) on changes in body weight in grams

Groups	Treatment	Dose (mg/kg)	Body weight			
			Initial	Final	% wt. change	Score
Control	ethanol	-	108.00±8.68	130.50±3.75	20.83 ↑	0
Arthritic control	ethanol	-	136.7±14.06	80.8±4.64	40.8 ↓	4
CGE-I	Extract	200 mg/kg	136.7±10.85	108.7±5.75*	20.48 ↓	3
CGE-II	Extract	400 mg/kg	136.7±9.45	101.8±4.27*	25.53 ↓	4
Standard	Dexamethasone	0.7 mg/kg	126.7±8.43	105.00±6.73*	17.12 ↓	3

Values are expressed as mean ± SEM, The statistical difference was evaluated by One way ANOVA followed by Dunnet's test, *p<0.05, when compared with adjuvant arthritic group, n=6 animals

Table 15: Effect of *Calotropis gigantea* root bark extract (CGE) on weight measurement index of immune organ (spleen)

Groups	Treatment	Dose (mg/kg)	Index (100%)
Normal Control	ethanol	-	0.53 ± 0.14
Arthritic control	ethanol	-	0.29 ± 0.02
CGE-I	Extract	200 mg/kg	0.37 ± 0.17*
CGE-II	Extract	400 mg/kg	0.31 ± 0.15
Standard	Dexamethasone	0.7 mg/kg	0.42 ± 0.09**

Values are expressed as mean ± SEM, The statistical difference was evaluated by One way ANOVA followed by Dunnet's test, *p<0.05, **p<0.01 when compared with adjuvant arthritic group, n=6 animals

Table 16: Effect of *Calotropis gigantea* root bark extract (CGE) on balance beam activity

Groups	Treatment	Dose (mg/kg)	Time (sec.)
Normal Control	ethanol	-	35.66 ± 4.27
Arthritic control	ethanol	-	91.67 ± 2.261
CGE-I	Extract	200 mg/kg	80.83 ± 3.146*
CGE-II	Extract	400 mg/kg	76.50 ± 3.16**
Standard	Dexamethasone	0.7 mg/kg	71.00 ± 3.19***

Values are expressed as mean ± SEM, The statistical difference was evaluated by One way ANOVA followed by Dunnet's test, *p<0.05, **p<0.01. ***p<0.001 when compared with adjuvant arthritic group, n=6 animals

ACKNOWLEDGEMENT

Every mature individual in professional life is keenly aware of his sense of indebtness to many people who have stimulated & influenced his intellectual development. Ordinarily, this feeling is expressed in customary gesture of acknowledgement. Therefore, it seems as a right to acknowledge our gratitude with sense of veneration to the Almighty God and various people who helped us during the course of the project work. Their valuable guidance and wise direction have enabled us to complete our project in systematic and smooth manner.

We are highly thankful to our guide **Mr.Rudrapratap Khan**, who took a lot of pain to supervise this research work. His expert guidance, advice, timely suggestions, explicit decision, deep personal interest and attention had been privilege for us. we have no words to express our heavy debt of gratitude to him, for his encouragement and relevant criticism without which this work could not have seen this present day.

We owe gratitude and thankfulness to **Mr. Ratan Lal (lab. assistant) , Mr. Pooran Singh** .

We are thankfull to **Nand Kumar Sharma (technologist)** of **Reliable Diagnostic Centre** B-20 , Hari Marg , Malviya Nagar , Jaipur-302017 .

REFERENCES

1. Cai X., Zhou H., Wong Y. F., Xie Y., Liu Z. Q., Jiang Z. H., Bian Z. X., Xu H., Liu L., Suppression of the onset and progression of collagen-induced arthritis in rats by QFGJS, a preparation from an anti-arthritic Chinese herbal formula, Journal of Ethnopharmacology 110 2007 39–48.
2. Chochberg Marc, Silman Alanj , Smolen Josef, Seinblatt e Michael, Weisman h Michael, Pathological Basis Of Disease , 3rd edition ,page no. 69-74.
3. Chochberg Marc, Silman Alanj , Smolen Josef, Seinblatt e Michael, Weisman h Michael, Pathological Basis Of Disease , 3rd edition ,page no. 1611-1621.
4. Chochberg Marc, Silman Alanj , Smolen Josef, Seinblatt e Michael, Weisman h Michael, Pathological Basis Of Disease , 3rd edition ,page no. 57-68.
5. Alam M. Ashraful, Habib M. Rowshanul, Nikkon Farjana, Rahman Matiar, and Karim M. Rezaul, Antimicrobial Activity of Akanda (*Calotropis gigantea* L.) on Some Pathogenic Bacteria., Bangladesh J. Sci. Ind. Res. 43(3), 2008, 397-404.

6. Mukherjee PK., Quality Control of Herbal Drugs, Business Horizon Pharmaceutical Publishers, New Delhi, 1st Edition, 2002, 398-401.
7. Nanrendhirakannan RT., Subramanian S., and Kandaswamy M., Anti-inflammatory and lysosomal stability actions of *Cleome gynandra* L. studied in adjuvant induced arthritic rats, Food and Chemical Toxicology, 2007, 45, 1001–1012.
8. Nazir N., Koul S., Qurishi M.A., Taneja S. C., Ahmad S. F., Bani S., Qazi G. N., Immunomodulatory effect of bergenin and norbergenin against adjuvant induced arthritis-A flow cytometric study.
9. Noguchu M., Kimoto a., Kobayashi S., Yoshino T., Miyata K., Sasamata M., effect of celecoxib, a cyclooxygenase-2 inhibitor, on the pathophysiology of adjuvant arthritis in rat, European journal of pharmacology 513, 2005, 229-235.
10. Kang S. S., Choi S. H., Prophylactic effect of plaster and cataplasm contained ketoprofen in rats with adjuvant arthritis, journal of veterinary science, 2001, 2(1), 65-70.
11. Li H., Zhang Y. Y., Tan H. W., Jia y. F., Li D., Therapeutic effect of tripterine on adjuvant arthritis in rats, Journal of Ethnopharmacology 118, 2008 479–484.
12. Tsai C. C., Lin C. C., Anti-inflammatory effects of Taiwan folk medicine 'Teng-Khia-U' on Carrageenan- and adjuvant-induced paw edema in rats, Journal of Ethnopharmacology 64 1999, 85–89.
13. Popp P. T., Peddinghaus R. M., Anti-rheumatic drug profile evaluated in the adjuvant arthritis of rats by multiparameter analysis, agents action 42: 50-55 (1994).
14. Kulkarni S. K., Hand book of experimental pharmacology, Vallabh prakashan, Third revised and enlarged edition, 2005, Page no.117, 125.
15. Cai X., Wong Y. F., Zhou H., Xie Y., Liu Z.Q., Jiang Z. H., Bian Z. X., Xu H. X., Liu L., The comparative study of Sprague-dawley and lewis rats in adjuvant-induced arthritis, Naunyn-Schmiedeberg's arch pharmacol, 2006, 373: 140-147.
16. Yazdanparast R., Ardestani A. and Jamshidi S., experimental diabetes treated with achillae santolin: Effect on pancreatic oxidative parameters, Journals of Ethnopharmacology, 2007, 112, 13-18.
17. Elman G., L., Archives of biochemistry and biophysics, 1959, 82, 70-79.
18. Van Eden WJ, Holoshitz Z, Nevo A, Frenkel A and Cohen IR, Arthritis induced by a T-lymphocytes clone that responds to mycobacterium tuberculosis and to cartilage proteoglycans, proc. Natl. Acad. Sci. U. S. A., 1985, 82, 5117-5120.
19. Anderson GD, Hauser SD, Mcgarity KL, Bremer ME, Isakson PC, Gregory SA, selective inhibition of cyclooxygenase (COX)-2 reverses inflammation and expression of COX-2 and interleukin 6 in rats adjuvant arthritis. J Clin Invest 97, 1996, 2672-2679.
20. Billingham ME Model of arthritis and the search for antiarthritic drugs. Pharmacol Ther 21, 1983, 389-428.
21. Roubenoff R, Freeman LM, Smith DE, Adad LW, Dinarello CA and Kehayias JJ, Adjuvant arthritis as a model of inflammatory cahexia, Arth. Rheum, 1997, 40, 534-539.