Anti inflammatory effect of *Calotropis procera* root bark extract.

Gaurav Parihar*, Ankur Sharma, Santosh Ghule, Praveen Sharma, Pradeep Deshmukh, DN Srivastava

1. College of Pharmacy IPS Academy, Rajendra Nagar, A.B. Road, Indore – 452012 (M.P.), INDIA
2. B.R.Nahata College of Pharmacy, Mhow-Neemuch Road, Mandsaur 458001 (M.P.), INDIA

**Abstract**

The ethanolic extract of root bark of *Calotropis procera* R.br. (Family: Asclepiadaceae) was investigated for its anti-inflammatory activity at different dose in the different animal models. The experimental paradigms used were Complete Freund's Adjuvant (CFA) induced arthritis (chronic inflammation), acetic acid induced vascular permeability model in mice for anti-inflammatory activity. Toxicity study was also performed in rats. The extract of *Calotropis procera* (CPE) exhibited significant anti-inflammatory effect at the dose 100 and 200 mg/kg. The extract showed 21.6 & 71.6% inhibition against CFA induced arthritis at the dose of 100 & 200 mg/kg after drug treatment, as compared to standard drug dexamethasone produced 99% of inhibition. The extract also exhibited significant inhibition in polyarthritic index in rats caused by CFA induced arthritic inflammation. In the acetic acid induced vascular permeability the CPE (100 and 200 mg/kg), significantly reduced dye leaking by 45.4% and 61.5% (p<0.001) as compared to standard drug dexamethasone and ibuprofen 23.7% and 67.4% respectively. The effect of CPE was assessed by the change in the lipid peroxidation, glutathione content (GSH), and total plasma thiols activities were measured from hepatic tissues. Thus, the present study revealed that the ethanol extract of *Calotropis procera* exhibited significant anti-inflammatory activity in the tested models.

**Key words:** *Calotropis procera*, root bark, acute and chronic inflammation, Complete Freunds Adjuvant (CFA).
Introduction

*Calotropis procera* (Ait) R Br, a plant of family Asclepiadaceae, is well known for its medicinal as well as toxic properties. *Calotropis procera* is a shrub or small tree up to 2.5 m (max. 6m) high, stem usually simple, rarely branched, woody at base and covered with a fissured, corky bark; branches some what succulent and densely white to mentose, early glabrescent. It is known by various names like swallow wort, dead sea apple, sodom apple or milk weed. Commonly used and known as arka or madar. In the traditional Indian medicinal system, it has been used for pain, asthma, bronchitis, and dyspepsia, leprosy, ulcers, tumors, piles, spleen, liver and abdomen. The root bark in the form of paste with rice vinegar is applied to elephantiasis of the legs and scrotum. The plant is also known for its toxic properties that include iridocyclitis, dermatitis and acts like a poison and produces lethal effects. Compounds derived from the plant have been found to have emeto-cathartic and digitalic properties. The principal active medicinals are asclepin and mudarin. As mentioned above, almost all traditional uses of the plant are concerned with anti-inflammatory activity. In order to prove the traditional utilization of *Calotropis procera*, this paper was intended to investigate the effect of *Calotropis procera* ethanolic root bark extract on inflammation using different animal models.

Inflammation or phlogosis is a pathophysiological response of living tissue to injuries that lead to local accumulation of plasmatic fluid and blood cells. The inflammatory process is invariably characterized by a production of prostaglandins, leukotrienes, histamine, bradykinin, platelet-activating factor (PAF) and by a release of chemicals from tissues and migrating cells. Anti-inflammatory drugs, presently available for the treatment of joint inflammation of various kinds, have undesirable side effects such as causing peptic ulcers. Therefore plant remedies have become increasingly popular and are often preferred to synthetically derived pharmaceuticals. In the screening of new anti-inflammatory compounds, carageenan induced oedema in the rat hind paw and Freund’s adjuvant induced arthritis are widely employed. In the present work, the anti-inflammatory and anti-arthritic activities of the ethanol extract of the root bark have been performed using these models.

Materials and Methods.

Plant material

The plant *Calotropis procera* was collected from the mandsaur city growing in the wild. The Plant was identified and authenticated by Dr. S.N. Mishra, (Sr. Scientist, Medicinal and Aromatic Plant Project, K.N.K. College of Horticulture, Mandsaur). The herbarium was deposited in the Department of Pharmacognosy, B.R.N.C.P., Mandsaur, which has a voucher specimen number BRNCP/C/007/2008 *Calotropis procera* Ait./Gaurav.

The root bark was separated from the fresh root of the plant. It was cleaned, and dried under shade at ambient temperature and crushed to get coarse powder.

Extraction

Root bark was first defatted by petroleum ether for 3 days and was filtered. The residue were then dried and extracted by maceration in ethanol (90% v/v) for 7 days with intermittent shaking. The extract was filtered and evaporated to dryness under reduced pressure at low temperature in a rotary evaporator to get dried extract with minimal solvent. Finally percentage yield was calculated.
**Calculation of percentage yield:**

\[
\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}} \times 100
\]

**Phytochemical analysis**
The extract and fractions were subjected to phytochemical analysis for constituent identification using standard protocol\(^6\).

**Animals.**
Adult Swiss albino mice (20-25 g) and rats (200-250 g) of both sexes were used. Animals were procured from College of Veterinary Science & A.H., J.N.K.V.V., Mhow. Animals were housed in polypropylene cages under standard conditions and fed with standard pellets and water *ad libitum.*

**Acute toxicity test**
The method described by the OECD 425 was employed in the determination of the LD\(_{50}\). Groups of animals of a single sex, female were dosed in a stepwise procedure using the Acute Oral Toxicity – Up-and-Down Procedure\(^7\).

**Anti-inflammatory tests**

1. **Evaluation of Adjuvant Arthritis**
   
   **Induction of arthritis in rats**
   Arthritis was induced by a single intraplantar injection of 0.1 ml of Complete Freund’s Adjuvant (CFA) containing 1.0 mg dry heat killed *M. tuberculosis* per ml sterile paraffin oil. A glass syringe with 26G needle was used for injection. The rats were anaesthetized with ether inhalation prior to and during adjuvant injection\(^8\).

   **Experimental Setup**
   In the treatment group, drug was administered orally as suspension (1% v/v Tween 80). The rats with arthritis received freshly prepared *C. procera* root bark extract at 100 and 200 mg/kg o.d. by intragastric (i.g.) from day 14 to day 28 after immunization\(^9,10,11\).

   Before drug treatment rats were divided into different groups as shown in table I.

   From day 7 after immunization (onset of arthritis), rats were examined every 3–4 days for two parameters: Ankle diameter and arthritic score. The right hind paw ankle diameter was measured with digital vernier calliper (basic value, day 0) and repeated on days 7, 10, 14, 17, 21, 24 and 28. Paw swelling (Δml) was calculated by subtracting the ankle diameter at day 0 from the related one days\(^11\).

   **a. Arthritic score (Polyarthritic index)**
   For clinical evaluation of AA, the polyarthritis severity was graded on a scale of 0–4\(^12\): The maximum joint score was 3 of arthritis paws for each rat. Rats were evaluated daily for arthritis using a macroscopic scoring system described as in table II\(^10\).

   **b. Inflammation Parameter**
   **Ankle Diameter measurement:**
   Therapeutic evaluation\(^13\)
   The anti-inflammatory effect of the drugs was evaluated by the changes on the physical swelling of the right hind paw, which was evaluated through:
   
   \[
   \text{Average change in ankle diameter (Δ): Ankle diameter}_{(\text{day } x)} - \text{Ankle diameter}_{(\text{day } 0)}
   \]
   
   The regression of the oedema by measuring the ankle diameter was calculated according to the following formula:
   
   \[
   \text{Paw thickness index (\%) = } \frac{(\text{Ankle}\_f - \text{Ankle}\_14)}{(\text{Ankle}\_\text{bef. ind.} - \text{Ankle}\_14)} \times 100
   \]

   \(\text{Ankle}_f\) – ankle diameter at final day
   \(\text{Ankle}_{14}\) – ankle diameter at 14th day
   \(\text{Ankle}_{\text{bef. ind.}}\) – ankle diameter 0th day

2. **Effect of CPE in Acetic Acid-Induced Vascular Permeability in Mice**
Mice were injected 0.2 ml Evans blue dye (0.25% w/v in normal saline) i.v. after 30 min of drug administration. 15 min later the mice were injected i.p. (1 ml/100 g) with freshly prepared acetic acid
(0.6% v/v) in normal saline. After 30 min of Acetic acid injection the peritoneal cavities were washed with 5 ml of heparinised sterile normal saline (500 IU/L) and centrifuged for 10 min. Finally absorbance was taken at 607 nm $\lambda_{\text{max}}$.14

3. Measurement of inflammatory mediators (Oxidative stress markers)

a. Lipid peroxidation in Liver (TBARS) (malondialdehyde, MDA),

Lipid peroxidation in Liver 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 tricarboxylic acid (TCA) solution (10% w/v) was added to 0.5 ml 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 1000 X 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 then 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$^{-1}$M$^{-1}$) and it was expressed as nmol/mg protein[15]

b. Determination of total plasma thiols

Total plasma sulphhydryl groups from animals were determined by measuring the colour complex formed by the reaction of sulphhydryl groups with dithiobisnitrobenzoic acid (DTNB). Briefly, a plasma sample of 0.2 ml was mixed with 0.6 ml of 0.2 M Tris buffer (pH 8.2, 20 mM EDTA) and then 40 $\mu$l of 10 mM DTNB was added. The mixture was brought to 4.0 ml with 3.16 ml of absolute methanol. Colour was developed for 15 min followed by centrifugation of the contents at 3000xg at room temperature for 15 min. The absorbance of the clear supernatants was read at 412 nm by setting zero with blank. Total quantity of thiols was estimated by using extinction coefficient of colour complex at 412 nm i.e. 13100 and the results are expressed as $\mu$mol –SH/ml plasma.16

c. 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 $\text{Na}_2\text{HPO}_4$ 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

Statistical analysis

Results were analyzed using one way analysis of variance (ANOVA) and expressed as Mean ± S.E.M. Data was further subjected to Dunnett post hoc test and differences between means were regarded significant at P<0.05.

Results

Phytochemical Investigation

1. Percentage Yield of Extracts (% w/w)

The extraction process yielded 10.91% of the ethanol extract (CPE), 3.2% of petroleum ether fraction (PF).

2. Qualitative chemical evaluation

The qualitative phytochemical tests showed that CPE tested positive for carbohydrates, reducing sugars, glycosides, flavonoids, terpenoids sterols...
and saponins. The petroleum fraction (PF) gave positive reaction for resins only.

**Pharmacological Investigation**

**Toxicity study**

The acute toxicity testing of the ethanolic extract in rats gave an i.p LD$_{50}$ of 1000 mg/kg.

**Evaluation of Adjuvant Arthritis**

1. **Arthritic Score (Polyarthritic Index)**

   The Arthritic score was found to be significantly decreased on treatment with the drug as shown in table III.

2. **Effect of ethanolic extract (CPE) of *Calotropis procera* on Inflammation parameter**

   a. **Measurement of Ankle Diameter**

   Injection of CFA in right hind paw of rat produced an increase in joint diameter that was maximum at day 10 (7.91 ± 0.42mm), and there after it gradually declined table IV, V. The inhibitory effect of CPE was evaluated on the day of peak inflammation that is day 14. Oral administration of CPE produced a dose dependent decrease in joint diameter 6.28 ± 0.07 mm (21.6% inhibition) and 6.20 ± 0.4 mm (71.6% inhibition, P<0.05) in test extract CPE 100 and 200 mg/kg respectively. The immunosuppressant, Dexamethasone was more effective in inhibiting joint inflammation as compared to test extracts. The decrease in joint diameter in standard drug dexamethasone treated rats was 5.82 ± 0.35 mm (99% inhibition, P<0.01)

3. **Effect of CPE on Acetic Acid-Induced Vascular Permeability in Mice**

   CPE produced a dose-related inhibitory effect on peritoneal capillary permeability produced by acetic acid in mice. The standard drug, Dexamethasone (10 gm/kg) and Ibuprofen (100 mg/kg), also markedly inhibited peritoneal capillary permeability 23.7% and 67.4% (4.31 ± 0.02 µg/mouse and 1.84 ± 0.27 µg/mouse, P<0.01) respectively. Meanwhile, CPE (100 and 200 mg/kg) significantly reduced dye leaking by 45.4% and 61.5% (3.08 ± 0.09 µg/mouse and 2.17 ± 0.26 µg/mouse, P<0.001, P<0.01) respectively. As compared to normal control mouse which was 5.65 ± 0.03 µg/mouse (table VI).

4. **Measurement of Inflammatory mediators**

   Effect of *Calotropis procera* hydroethanolic extract on liver tissue levels of reduced Glutathione (GSH), Thiobarbituric acid reacting substance (TBARS)

   a. **Measurement of reduced Glutathione (GSH) on liver tissues**

   The inflammatory meadiators associated with CFA-induced polyarthritis was evaluated by measuring levels of GSH in the inflamed liver tissues. CFA injection into right hind paw decreased the tissue GSH, which is naturally occurring antioxidant in body. Levels changes from 13.52 ± 1.14 mg/ml (P<0.001) tissues supernatant in normal control rats to 2 ± 0.06 mg/ml of tissue supernatant in Arthritic rats. Both extracts produces an increase in the level of GSH. However CPE (100 mg/kg) 4.28 ± 0.07 mg/ml produced better result than CPE (200 mg/kg) 4.22 ± 0.03 mg/ml (P<0.05). The effect of these drugs was comparable with standard Dexamethasone 4.51±0.03 mg/ml (P<0.01) on other hand produced better increase in GSH level, Fig. I.

   b. **Measurement of Thiobarbituric acid reacting substance (TBARS) on Liver tissues**

   CFA injection produced an increased in level of tissue TBARS expressed as nmol of MDA normal in control rats 0.20 ± 0.02 nmol/ml to increase in Arthritic rats 0.27 ± 0.01 nmol/ml (P<0.05) tissue supernatant. Fig. II shows the levels 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 CPE (100 mg/kg) 0.22 ± 0.01 nmol/ml. Treated rats with higher dose CPE (200 mg/kg) shows dose dependent reduction in MDA level 0.15±0.01 nmol/ml (P<0.001) comparable with standard drug dexamethasone 0.06 ± 0.007 nmol/ml (P<0.001).

c. Measurement of Total plasma Sulfhydryl (Thiol) Content

Plasma concentration of total sulfhydryl or thiol groups is presented in Fig. III. Vehicle treated arthritic rats had significantly lower total thiol content in plasma 17.4 ± 0.60 µmol/ml (P<0.01) as compared to normal control 40.6 ± 1.49 µmol/ml. However treatment groups with extract and standard drug tends to normalize it. CPE-I shows highly significant increase 61.9 ± 1.63 µmol/ml as compare to CPE-II 33.2.4 ± 0.65 µmol/ml and Dexamethasone 26.0 ± 0.99 µmol/ml (P<0.001).

Discussion

Folkloric treatment of inflammation of various etiologies, using medicinal plants, is well known to masters of the art of traditional medicine practice. Pharmacological screening of root bark extracts of *Calotropis procera* has revealed that the root bark possesses potent anti-inflammatory effect in models of acute and chronic inflammation. The extract may have inhibited the release of pro-inflammatory mediators of acute inflammation such as histamine and prostaglandin. Thus, these extracts may exert anti-inflammatory effect by inhibiting the synthesis of prostaglandin.

Complete freund’s adjuvant-induced arthritis has been used as a model of sub-chronic or chronic inflammation in rats and is of considerable relevance after the study of pathophysiological and pharmacological control of inflammatory processes, as well as the evaluation of analgesic potential or anti-inflammatory effects of drugs18,19. One of the reasons for the wide utilization of this model is due to the strong correlation between the efficiency of therapeutic agents in this model and in rheumatoid arthritis in humans. Rat adjuvant-induced arthritis is a model of chronic polyarthritis with features that resemble inflammation in RA20. Therefore, therapeutic efficacy was mainly investigated in the rat adjuvant arthritis model in present study. All animals tolerated the experimental procedures well and no death up to the study termination by day 28 was observed. Dosage selection for CPE (100 and 200 mg/kg) was based on acute toxicity studies. CPE significantly inhibited the development of chronic joint swelling induced by CFA in rats for 12 days whereas the standard dexamethasone produced anti-arthritic effect till day 21. The effect of CPE was dose-dependent and linear, but for a short period compared to the standard.
mediators of inflammation increase vascular permeability at various times after injury. Chemical-induced vascular permeability (such as is seen with acetic acid) causes an immediate sustained reaction that is prolonged over 24 hours and its inhibition suggests that the extract may effectively suppress the exudative phase of acute inflammation.

The present study demonstrates that complete freund’s adjuvant (CFA) containing killed \textit{M. tuberculosis} induced AA in rats. Treatment of the AA rats with \textit{Calotropis procera} shown decline in inflammation which was comparable to Dexamethasone-treated groups.

Adjuvant induced arthritis (AA) is thought to occur through cell-mediated autoimmune 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 peroxides are formed by auto-oxidation of polyunsaturated fatty acids found primarily in cell membranes. Lipid peroxidation is a very important process in free radical pathology as it is damaging to cells. The liver of rat was used as a source of polyunsaturated fatty acids for determining the extent of lipid peroxidation. Malondialdehyde, a lipid peroxidation product, is an indicator of reactive oxygen species (ROS) generation in the tissue.

An increase in the level of lipid peroxides in tissues, therefore, reflects membrane damage. In many diseases, especially rheumatoid arthritis, membrane damage often occurs in some organ or tissue, which provokes lipid peroxidation in the membrane and accelerates the disorder in structure and function of these membranes. The lack of an antioxidant defense leads to an increase in lipid peroxidation and subsequent deleterious effects. In the present research, lipid peroxides in the organs were significantly increased in arthritic condition. The increased level of lipid peroxides under arthritic condition might be due to poor antioxidant defense as well as inactivation of antioxidant systems. Increased lipid peroxidation in body tissue has been observed in a wide range of tissue injury such as CCl\textsubscript{4} toxicity, atherosclerosis and carrageenan-induced inflammation. Results of this present investigation elucidate that the drug inhibit both inflammation as well as accumulation of lipid peroxides in tissues.

This effect may be due to the presence of flavonoids in the \textit{Calotropis procera}. Flavonoids are a group of ubiquitously distributed plant polyphenols which exhibit a wide pharmacological spectrum of effects. The inhibition of lipid peroxidation may be due to the free radical scavenging property of flavonoids, which can scavenge singlet O\textsubscript{2} which is evident from the results of this study, terminating peroxides by their low redox potential due to presence of a hydroxyl group. They are also proved to reduce the lipid peroxidation through reducing the levels of malondialdehyde and conjugated dienes.

Although the mechanism underlying the decline in GSH content in the drug-treated animal is not well understood, recent reports suggest that the flavonoids, the major component of the drug, are found to be the free radical scavengers. As adequate concentrations of GSH are required for a variety of immune functions, it has been suggested that oxidative stress and deficiency of thiol compounds may play an important pathogenic role in the development of immune deficiency. The present study reports a decline in GSH content in organ i.e, liver, from...
normal rats as compared to arthritic rats. There is a decline in plasma, liver, kidney, and heart GSH. Plasma thiols (sulfhydryl groups) are among endogenous defence mechanisms identified as protectors against the damaging effects of free radicals.\(^\text{37}\) Depletion of plasma thiols has been observed in adjuvant-induced arthritis and has been attributed to reduction in plasma albumin levels.\(^\text{38}\)

Edema and pain are characteristic signs of an inflammatory response where the role of prostaglandins and histamine is well established.\(^\text{39}\) Prostaglandins are endogenous mediators of inflammation and are generated from arachidonic acid by the action of cyclooxygenase (COX) that exists in two isoforms, COX-1 and COX-2. COX-1 is constitutive whereas COX-2 is the cytokine inducible form of the enzyme. There was a clinically significant reduction in swelling of inflamed area in *Calotropis procera* and Dexamethasone-treated animals. In the investigation of paw edema, arthritic rats showed the soft tissue swelling that was noticeable around ankle joints and was considered to be due to edema of peri-articular tissues such as ligament and joint capsule. The initial reduction of edema and soft tissue thickening at the depot site is probably due to the effect of adjuvant, whereas the late occurring disseminated arthritis and flare in the injected foot are presumably immunological events.\(^\text{40}\) The ability of the drug to reduce edema formation may thus be related to its inhibitory action on prostaglandin synthesis. Investigation of arthritic score further evidenced the antiarthritic effect of the drug. The paw thickness and arthritic scores were very much increased in arthritic rats, which were significantly reduced on treatment with the drug. These effects can be attributed to the presence of flavonoids and other synergistic components in the drug.

Its apparent safety over long-term is encouraging enough to warrant further studies to explore its possible therapeutic role in clinical practice.

**Table I: Schematic representation of drug treatment**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of animals</th>
<th>Treatment Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Saline 1 ml/kg.</td>
<td>6</td>
<td>Rats were administered with normal saline upto 28 days</td>
</tr>
<tr>
<td>Model control</td>
<td>CFA 0.1 ml/rat intraplantar injection</td>
<td>6</td>
<td>Arthritic rats were administered with normal saline upto 28 days</td>
</tr>
<tr>
<td>CPE-I</td>
<td>CFA+ Extract 100 mg/kg p.o.</td>
<td>6</td>
<td>Arthritis induced rats were administered with ethanolic extract of <em>C. procera</em> root bark (100 mg/kg body weight/rat/day upto 28 days by gavage starting 14 days after adjuvant injection).</td>
</tr>
<tr>
<td>CPE-II</td>
<td>CFA+ Extract 200 mg/kg p.o.</td>
<td>6</td>
<td>Arthritis induced rats were administered with ethanolic extract of <em>C. procera</em> root bark (100 mg/kg body weight/rat/day upto 28 days by gavage starting 14 days after adjuvant injection).</td>
</tr>
<tr>
<td>Standard</td>
<td>CFA+ Dexamethasone, 0.7 mg/kg</td>
<td>6</td>
<td>Arthritis induced rats were administered with dexamethasone (0.7 mg/kg body weight/rat/day upto 28 days i.p. starting 14 days after adjuvant injection).</td>
</tr>
</tbody>
</table>
**Fig. 1: Reduced Glutathione.** Effect of root bark extract CPE on reduced glutathione in liver of control and experimental animal’s mg/ml of tissue homogenates.

*P < 0.05, **P < 0.01 when compared with Adjuvant arthritic group, ***P < 0.001 when compared with normal control. Values are given in mean ± S.E.M. (n=5). The statistical difference was evaluated by One-way ANOVA followed by Dunnett test.

**Table II: Macroscopic scoring system in arthritic rats**

<table>
<thead>
<tr>
<th>Arthritic score</th>
<th>Evaluation parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>normal or no signs of arthritis or no swelling.</td>
</tr>
<tr>
<td>1</td>
<td>swelling and / or redness of the paw (mild erythema) or one digit or isolated phalanx joint involvement or limb deformity.</td>
</tr>
<tr>
<td>2</td>
<td>moderate erythema, mild swelling, no limb deformity or two joints involved or involvement of phalanx joint and digits.</td>
</tr>
<tr>
<td>3</td>
<td>severe erythema, severe swelling, moderate to severe limb deformity severe arthritis of the entire paw and digits including ankle.</td>
</tr>
</tbody>
</table>
Fig. II: Thiobarbituric Acid Reacting Substances (TBARS). Effect of root bark extract CPE on lipid peroxides in liver of control and experimental animals nmol/ml of tissue homogenates.

*P < 0.05 when compared with normal control, ***P < 0.001 when compared with AA group, P < ns Represent non significant. Values are given in mean ± S.E.M. (n=5). The statistical difference was evaluated by One-way ANOVA followed by Dunnett test.
Fig. III: Effect of *Calotropis procera* root bark extract (CPE) on Total Plasma Thiols

![Bar chart showing effect of CPE on plasma thiols](chart.png)

#P<0.001 when compared with normal group, ***P < 0.001 when compared with Adjuvant arthritic group. Values are given in mean ± S.E.M. (n=5). The statistical difference was evaluated by One-way ANOVA followed by Dunnett test.

Table III: Effect of *Calotropis procera* root bark extract (CPE) on Arthritic Score (Polyarthritic index) in aduvant induced arthritis in rats.

<table>
<thead>
<tr>
<th>Day</th>
<th>7</th>
<th>10</th>
<th>14</th>
<th>17</th>
<th>21</th>
<th>24</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Model Control</td>
<td>2.00 ± 0.00</td>
<td>1.60 ± 0.24</td>
<td>2.20 ± 0.37</td>
<td>2.70 ± 0.25</td>
<td>2.20 ± 0.48</td>
<td>2.60 ± 0.24</td>
<td>2.70 ± 0.25</td>
</tr>
<tr>
<td>CPE-I</td>
<td>2.20 ± 0.37</td>
<td>2.20 ± 0.20</td>
<td>2.00 ± 0.31</td>
<td>2.40 ± 0.40</td>
<td>2.40 ± 0.40</td>
<td>2.40 ± 0.24</td>
<td>1.50 ± 0.28*</td>
</tr>
<tr>
<td>CPE-II</td>
<td>2.60 ± 0.33</td>
<td>2.00 ± 0.40</td>
<td>2.00 ± 0.40</td>
<td>2.00 ± 0.57</td>
<td>1.60 ± 0.33</td>
<td>2.30 ± 0.66</td>
<td>1.60 ± 0.33*</td>
</tr>
<tr>
<td>Standard</td>
<td>1.80 ± 0.37</td>
<td>2.40 ± 0.24</td>
<td>2.20 ± 0.37</td>
<td>1.40 ± 0.24</td>
<td>1.20 ± 0.48</td>
<td>1.20 ± 0.20*</td>
<td>1.20 ± 0.25*</td>
</tr>
</tbody>
</table>

*P<0.05 when compared with arthritic control. Values are given in mean ± S.E.M. (n=5). The statistical difference was evaluated by one-way ANOVA followed by Dunnett test.
Table IV: Effect of *Calotropis procera* root bark extract (CPE) on Average Change in Ankle diameter (Δ) in mm

<table>
<thead>
<tr>
<th>Day</th>
<th>0</th>
<th>7</th>
<th>10</th>
<th>14</th>
<th>17</th>
<th>21</th>
<th>24</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.43 ± 0.09</td>
<td>5.42 ± 0.08</td>
<td>5.45 ± 0.09</td>
<td>5.45 ± 0.07</td>
<td>5.42 ± 0.25</td>
<td>5.41 ± 0.23</td>
<td>5.41 ± 0.28</td>
<td>5.46 ± 0.12</td>
</tr>
<tr>
<td>Model Control</td>
<td>5.75 ± 0.34</td>
<td>7.07 ± 0.15</td>
<td>7.91 ± 0.42</td>
<td>6.91 ± 0.36**</td>
<td>6.81 ± 0.25**</td>
<td>6.97 ± 0.09#</td>
<td>6.95 ± 0.25#</td>
<td>6.95 ± 0.24</td>
</tr>
<tr>
<td>CPE-I</td>
<td>5.89 ± 0.12</td>
<td>7.34 ± 0.35</td>
<td>7.32 ± 0.06</td>
<td>6.68 ± 0.2</td>
<td>6.04 ± 0.24</td>
<td>6.44 ± 0.06</td>
<td>6.30 ± 0.11</td>
<td>6.28 ± 0.07</td>
</tr>
<tr>
<td>CPE-II</td>
<td>6.02 ± 0.25</td>
<td>7.95 ± 0.19</td>
<td>6.95 ± 0.24</td>
<td>6.57 ± 0.28</td>
<td>6.56 ± 0.30</td>
<td>6.55 ± 0.41</td>
<td>6.35 ± 0.29</td>
<td>6.20 ± 0.40</td>
</tr>
<tr>
<td>Standard</td>
<td>5.79 ± 0.19</td>
<td>7.74 ± 0.45</td>
<td>6.32 ± 0.34</td>
<td>6.54 ± 0.20</td>
<td>5.88 ± 0.17</td>
<td>5.56 ± 0.17</td>
<td>5.44 ± 0.12*</td>
<td>5.82 ± 0.35</td>
</tr>
</tbody>
</table>

*P < 0.05, when compared with adjuvant arthritic group.  †P<0.05, ‡P < 0.01 when compare with normal control. Values are given in mean ± S.E.M. (n=5). The statistical difference was evaluated by One-way ANOVA followed by Dunnett test.

Table V: Effect of *Calotropis procera* root bark extract (CPE) on Inhibition of joint inflammation (Ankle diameter) by various drugs in FCA induced polyarthritis.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose (in mg / Kg)</th>
<th>Increase in Ankle diameter (in mm)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Vehicle</td>
<td>----</td>
<td>0.03 ± 0.09</td>
<td>---</td>
</tr>
<tr>
<td>Model Control</td>
<td>Vehicle</td>
<td>----</td>
<td>0.60 ± 0.24*</td>
<td>---</td>
</tr>
<tr>
<td>CPE-I</td>
<td>Extract</td>
<td>100 p.o.</td>
<td>0.47 ± 0.07**</td>
<td>21.6</td>
</tr>
<tr>
<td>CPE-II</td>
<td>Extract</td>
<td>200 p.o.</td>
<td>0.17 ± 0.40**</td>
<td>71.6</td>
</tr>
<tr>
<td>Standard</td>
<td>Dexamethasone</td>
<td>0.7 i.p.</td>
<td>0.01 ± 0.35***</td>
<td>99</td>
</tr>
</tbody>
</table>

*P < 0.05 when compared with normal control, **P < 0.01, ***P < 0.001 when compared with adjuvant arthritic group. Values are given in mean ± S.E.M. (n=5). The statistical difference was evaluated by One-way ANOVA followed by Dunnett test.
Table VI: Effects of CPE on acetic acid-induced vascular permeability in mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Concentration of dye (µg / mouse)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1 ml normal saline</td>
<td>5.65 ± 0.03</td>
<td>---</td>
</tr>
<tr>
<td>CPE-I</td>
<td>100</td>
<td>3.08 ± 0.09*</td>
<td>45.4*</td>
</tr>
<tr>
<td>CPE-II</td>
<td>200</td>
<td>2.17 ± 0.26*</td>
<td>61.5*</td>
</tr>
<tr>
<td>Standard (Dexamethasone)</td>
<td>10</td>
<td>4.31 ± 0.02*</td>
<td>23.7*</td>
</tr>
<tr>
<td>Standard (Ibuprofen)</td>
<td>100</td>
<td>1.84 ± 0.27*</td>
<td>67.4*</td>
</tr>
</tbody>
</table>

Data represent mean ± S.E.M. (n=6) *P < 0.01 compared with control group; **P < 0.001 compared with Standard. The statistical difference was evaluated by One-way ANOVA followed by Dunnett test.

Conclusion
Thus, it can be concluded that the ethanolic extract of root bark of *Calotropis procera* possess anti-inflammatory activity. Further studies involving the purification of the chemical constituents of the plant and the investigations in the biochemical pathways may result in the development of a potent anti-inflammatory agent with low toxicity and better therapeutic index.

References
2. Mahmoud O.M., Adam S.E., Tartour G. The effects of *Calotropis procera* on small ruminants II. Effects of administration of the latex to sheep and goats. J Comp Pathol. 1979;89(2):251–263.


36. Aukrust P., Svaral A.M., Muller F., Lunden B., Bergc R.K. and Froland S.S. Decreased levels of total and reduced glutathione in CD4+ lymphocytes in common variable immunodeficiency are


