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# Phyto-Therapeutic Potential Of Flower Of The Wonder Tree, Prosopis Cineraria In Complete Freund's Adjuvant Model: An Anti-Arthritis Study

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***Abstract***
*Numerous distinguished researchers have thoroughly investigated Prosopis cineraria's therapeutic potential against a range of major illnesses, although its efficacy against CFA (Complete freund's adjuvant. Consequently, the current experimental study was carried out to identify and evaluate the anti-inflammatory capacity of Prosopis cineraria ethanol extract to prevent inflammation caused by CFA in Wistar albino rats. This research aimed to evaluate the effect of ethanol extract flower of prosopis cineraria on parameter limb circumfarence, change body weight, paw volume and hematology profile in rats induced by complete freund's adjuvant. Thirty rats were divided into five groups each group contain 6 rats. Ethanol extract flower of prosopis cineraria doses selected of 250 mg/kg and 500 mg/kg orally. Each group was given the test preparation orally for 21 days. After 1 h, CFA induced at a dose 10 mg/mL hind paw of animal under mild anesthesia with diethyl ether. On the 21th day, to measure all the parametersand blood samples were collected. Finding was shows ethanol extract flower of prosopis cineraria at a dose of 500 mg/kg orally good effect of limb circumference, body weight, paw volume and haematological parameters as compare with normal group (p>0.05)..*

***Keywords:*** *Hematological, Prosopis Cineraria, Erythrocytes Sedimentation Rate, Limb Circumference, Paw Volume.*

## INTRODUCTION

Plants' nutritional and medicinal properties have been known for a long time, but their disease-preventing and health-promoting elements have just recently been recognized. Plant foods, including such as fruits and vegetable, are essential for human health as they contain crabs, lipids, proteins, vitamins, and minerals. Medicinal herbs have been used for centuries to cure illnesses and alleviate symptoms[1]. It has had a profound impact on health-care systems. Traditional medicine is based on the therapeutic experience of generations of indigenous physicians. Traditional remedies include therapeutic herbs, minerals, and organic substances. Herbal pharmaceuticals are traditional medicines that use medicinal plant extracts for therapeutic purposes. Natural plant products are believed to be healthier than manufactured medicines[2]. Adverse effects of conventional pharmaceuticals are more commonly reported in the lay press compared to herbal toxicities. This is due to the existence of systems to track adverse effects for conventional prescriptions. The body uses inflammation as an immune system response in reaction to burns, infection from bacteria, physiological injuries, and other harmful stimuli that could endanger the host's health.According to chronic inflammation is the leading cause of death globally. Inflammation is a pathological process that causes pain, redness, loss of tissue function edema and heat[3-5].

Rheumatoid arthritis, also known as RA, is a condition that affects around 1% of the global population. Across the world, researchers are working hard to improve ways for treating rheumatoid arthritis[6]. This condition affects women three times more than males[7]. As a chronic, inflammatory condition that affects organs and organ system, it primarily targets synovial joints[8].Arthritis induction in animals can be carried out in several methods, one of which is immunization with emulsified adjuvant[9-10].The complete Freund's adjuvant (CFA)-induced arthritic animal model is the most widely used model for RA for chronic inflammation.

CFA-induced arthritis is a model of chronic osteoarthritis with similar characteristics to RA[11].This study analyzes and compares their antioxidant and anti-inflammatory properties. The study also conducts in-vivo assessments to potentially aid in the identification of new medications for rheumatoid arthritis. Further research on the potential of P. Cineraria is dependent on this study[12].

## MATERIAL AND METHOD

### Collection of Plant Material

The P. Cineraria (Fabaceae or Leguminosae) plant material, referred to the plants were collected from the surroundings of near Jabalpur Madhya Pradesh. P. Cineraria floweridentified by Dr.UdayHomkar senior research officer, of (SFRI)State Forest Research Institute Jabalpur, Madhya Pradesh, India.

### Preparation of Plant Extract

The freshly picked flowers were collected, shade-dried, and ground into a fine powder. The extract was made by combining 500 g of powder withethanol separately for 72 hours Afterward,the solutions were centrifuged at a speed of 3000 rpm for 15 min. The resulting extract was filtered and then allowed to evaporate. Finally, the extract was stored at a temperature of 4°C for future use

### Qualitative analyses

The extracts underwent a preliminary phytochemical testing. Active compounds include carbohydrates (Molish test), proteins (Ninhydrin test), starch (Iodine test), and alkaloids (Mayer's, Dragendroff Test), Resin ( Turbidity test). The presence of phenolics (Ferric chloride test), flavonoids (Alkaline Reagent test, Shinoda test), Tannin (Lead acetate test and Feeric chloride test) and saponin (Foam test)[13-14].

## IN VIVO ASSAY

### Experimental animals

Nair et al.'s description of the induction of arthritis in experimental immunological arthritis, with minor modifications[15]. 30 male and female Wistar albino rats, weighing between 120 and 150 grams, were used in this study.The animals were kept in plastic cages and allowed to become acquainted to their new environment. Five groups of six rats each group were kept. Under light diethyl ether anesthesia, a CFA containing 10 mg/mL of heat-killed M. tuberculosis was injected into the animal's hind paw subplantar. Day 0 was the time of the adjuvant injection. Beginning on day 0 and continuing until day 21 after injection, the daily oral dosages of vehicle, P. Cineraria extract, and indomethacin were administered[16-17].

Group-I (Negative Control) :- 0.2 ml CFA (Complete freund'sadjuvant ) injection
Group-II (Control) :- Normal saline(0.9%)
Group-III (Standard) :- 0.2ml CFA injection + Indomethacin (15 mg/kg)
Group-IV (Extract treated (250 mg/kg) :- 0.2ml CFA injection + ethanolic extract of Prosopis Cineraria (250mg/kg).
Group-V (Extract treated (500mg/kg ):-0.2ml CFA injection +ethanolic extract of Prosopis Cineraria (500mg/kg) daily.

### Biochemical and hematological analysis

The rats was assessed daily by measuring the limb circumference using a suitable calliper, and on day 7, under chloroform anesthesia, rats were subjected to radiological imaging, after which rats were sacrificed and histopathological (Hb, RBC, and ESR) examination by using automatic blood cell counter.(Sysmex KX 21)[18-19].RA is a varied clinical illness along with elevation of particular pro-inflammatory and inflammatory mediators such as interleukins and TNF-a, which, if not blocked, may accelerate infiltration of macrophages into the inflamed site with excessive manufacture of auto-antibodies[20-22].

### Statistical analysis

We employed the least significant difference test and one-way ANOVA to evaluate parameters between groups. A significant p-value was defined as less than 0.05.

## RESULTS AND DISCUSSION

### Phytochemical screening

The ethanolic extract of P. cineraria flower contained many chemical groups, including alkaloids, resins, protiens, flavonoids, tannins, saponins, starch and glycosides etc.(Table 1)

### Biochemical and Hematological analysis

Rats in the negative control group were given 0.2 milliliters of CFA (Complete Freund's Adjuvant) once on the hind paw on day zero, while the control group was given 0.9% normal saline orally once a day for 21 days. The third group, which was the standard, was given 15 mg/kg of Indomethacin once daily for 21 days. The fourth and fifth groups were given 250 and 500 mg/kg of P. cineraria ethanolic extract orally once daily for 21 days, respectively, along with 0.2 ml of CFA on day zero. It is well known that CFA induction in rats causes an inflammatory response in humans that results in rheumatoid arthritis. P. cineraria flower ethanol extract at 250 mg/kg and 500 mg/kg (Group IV and V) shown a statistically

significant (p>0.05) reduction in inflammation (paw volume, body weight, and leg circumference).(Table 2, Table 3 Table 4)

The negative control group's hematological values were significantly lower (p<0.05) than those of the normal group. When compared to the normal group, the levels of RBC, WBC, ESR, and Hb were statistically significantly different after treatment with 250 and 500 mg/kg of P. Cineraria extract.(Table 5)

**Table 1 : Phytochemical Screening of Ethanol extract of flower Prosopis cineraria**

| S.NO | Type of Activity | Observation | Result |
|---|---|---|---|
| 1 | **Carbohydrate Test** | | |
| | Anthrone Test | Green colour observed | - |
| | Molish Test | Blue white ring observed | + |
| | Fehling | Brick-Red colour a observed | + |
| 2 | **Alkaloid Test** | | |
| | Mayers Test | Pale Yellow colour observed | + |
| | Dragendroff Test | Light Yellow colour observed | + |
| 3 | **Resin Test** | Turbidity is present | + |
| 4 | **Protein Test** | | |
| | Ninhydrin Test | Purple colour observed | + |
| | Xanthoproteic Test | Yellow colour observed | + |
| 5 | **Tannin Test** | | |
| | Lead acetate test | Yellow precipitate observed | + |
| | Feeric chloride test | Dark Green colour observed | + |
| 6 | **Saponin test** | | |
| | Foam test | Foam is present | + |
| 7 | **Flavonoid test** | | |
| | Alkaline regent test | Yellow colour observed | + |
| | Shinoda test | White Foam observed | - |
| 8 | **Starch Test** | Greenish colour observed | - |

**Table 2 Limb circumference measurement**

| GROUP | DAY | | | |
|---|---|---|---|---|
| | **0 Day** | **Day 7** | **Day 14** | **Day 21** |
| **GROUP I** Negative Control | 9.6±0.52 | 13.2±0.25 | 12.8±0.11 | 12.6±0.6 |
| **GROUP II** Control | 9.9±0.7 | 9.77±0.9 | 9.7±0.41 | 9.8±0.04 |
| **GROUP III** Standard | 10±0.04 | 11.1±0.4 | 10.4±0.87 | 10.3±0.8 |
| **GROUP IV** Extract treated (250mg/kg) | 9.8±0.8 | 11.88±0.6 | 11.4±0.84 | 10.5±0.2 |
| **GROUP V** Extract treated (500mg/kg) | 9.5±0.3 | 11.2±0.18 | 10.6±0.67 | 10.9±0.08 |

No. of animals in each group = 6; All values in ml; value are mean±SEM; ** p<0.01, * p<0.05. Data is analyzed by one way ANOVA followed by Bonferroni tests

**Table 3Change in Body Weight**

| Group | I | II | III | IV | V |
|---|---|---|---|---|---|
| **0 Day** | 138.1 ± 4.5 | 136.3±1.2 | 134.5 ± 4.5 | 138.7 ± 5.0 | 134.1 ± 4.5 |
| **7 Day** | 131.8 ± 5.5 | 136.9±2.3 | 130.3 ± 4.0 | 132.1 ± 5.5 | 132.6 ± 4.5 |
| **14 Day** | 126.7 ± 6.5 | 137.7±1.8 | 133.8 ± 4.5 | 131.8 ± 4.0 | 131.0 ± 5.5 |
| **21 Day** | 119.1 ± 4.5 | 137.9±1.6 | 129.1 ± 4.5 | 128.8 ± 4.5 | 129.6 ± 4.5 |

No. of animals in each group = 6; All values in ml; value are mean±SEM; ** p<0.01, * p<0.05. Data is analyzed by one way ANOVA followed by Bonferroni tests

**Table 4Change in Paw volume**

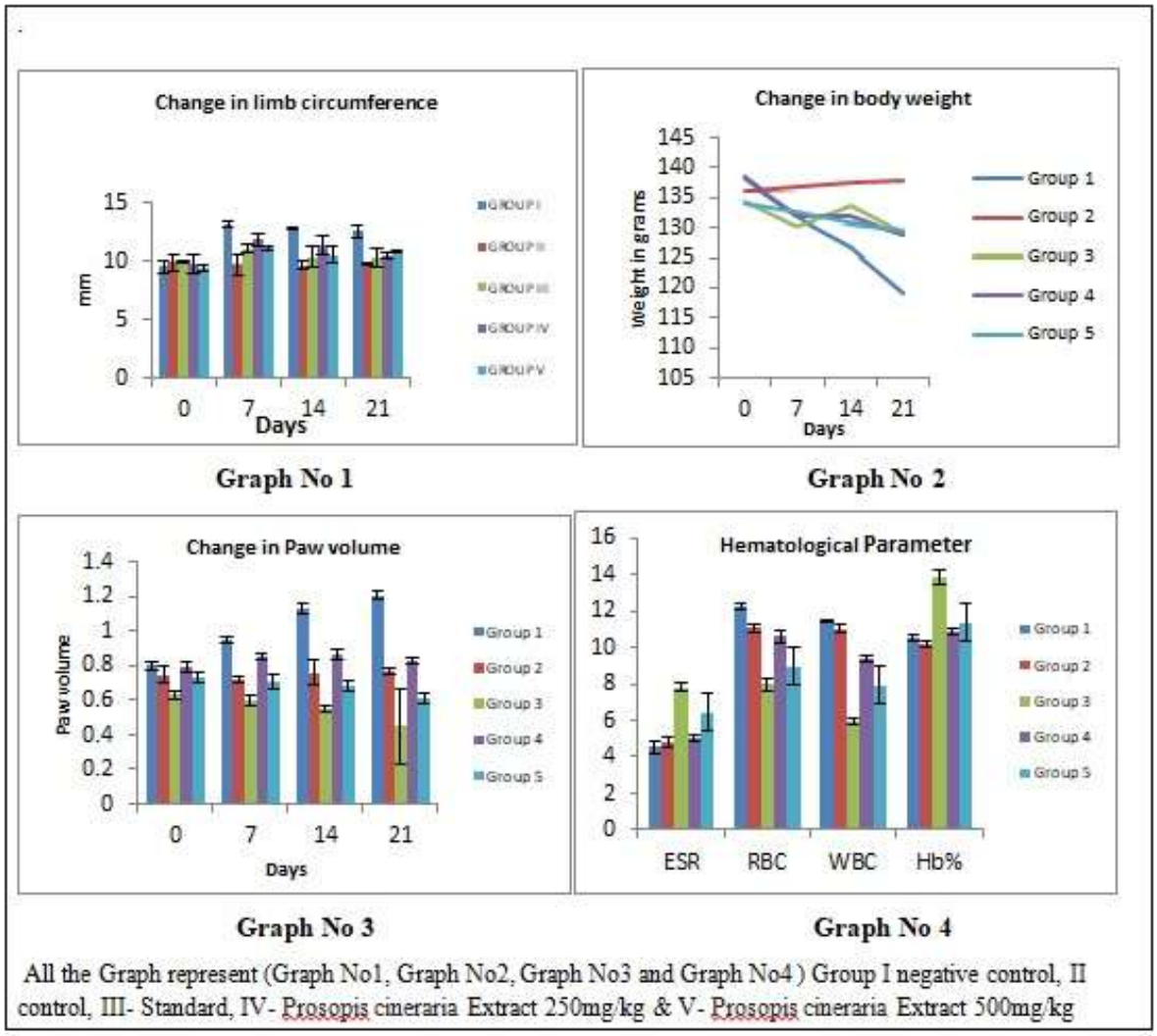
| Group | I | II | III | IV | V |
|---|---|---|---|---|---|
| **0 Day** | 0.8 ± 0.025 | 0.75±0.05 | 0.63 ± 0.02** | 0.79 ± 0.03 | 0.73 ± 0.03** |
| **% inh.** | | | **20.8 %** | **5 %** | **8.75 %** |
| **7 Day** | 0.95 ± 0.02 | 0.72±0.02 | 0.6 ± 0.03** | 0.85 ± 0.02 | 0.71 ± 0.04** |
| **% inh.** | | | **36.8 %** | **10.5 %** | **24.6 %** |
| **14 Day** | 1.13 ± 0.03 | 0.76±0.07 | 0.55 ± 0.02** | 0.86 ± 0.03** | 0.68 ± 0.03** |
| **% inh.** | | | **51.3 %** | **23.3 %** | **39.8 %** |
| **21 Day** | 1.21 ± 0.03 | 0.77±0.02 | 0.45 ± 0.22** | 0.83 ± 0.02** | 0.61 ± 0.03** |
| **% inh.** | | | **62.8 %** | **31.1 %** | **49.5 %** |

No. of animals in each group = 6; All values in ml; value are mean±SEM; ** p<0.01, * p<0.05 Data is analyzed by one way ANOVA followed by Bonferroni tests

**Table 5 Hematological Parameters**

| Groups | RBC (million/$mm^3$) | WBC (thousand/$mm^3$) | ESR (mm/hr) | Hb (g/dl) |
|---|---|---|---|---|
| **I** | 4.53 ± 0.31 | 12.24 ± 0.31 | 11.43 ± 0.27 | 10.5 ± 0.20 |
| **II** | 4.82±0.22 | 11.09±0.24 | 11.0±0.31 | 10.2±0.31 |
| **III** | 7.83 ± 0.07** | 7.96 ± 0.24** | 5.91 ± 0.19** | 13.83 ± 0.16** |
| **IV** | 5.05 ± 0.16* | 10.60 ± 0.17** | 9.38 ± 0.38** | 10.88 ± 0.21 |
| **V** | 6.46 ± 0.17** | 8.97 ± 0.25** | 7.95 ± 0.22** | 11.38 ± 0.14** |

No. of animals in each group = 6; All values in ml; value are mean±SEM; ** p<0.01, * p<0.05 Data is analyzed by one way ANOVA followed by Bonferroni tests.




All the Graph represent (Graph No1, Graph No2, Graph No3 and Graph No4) Group I negative control, II control, III- Standard, IV- Prosopis cineraria Extract 250mg/kg & V- Prosopis cineraria Extract 500mg/kg

## DISCUSSION

Present in-vitro investigations on flower of P. Cineraria extracts have shown strong anti-arthritic effect. This action could be attributed to the presence of active components such as flavonoids, glycosides, saponin, protein, resins,etc. Thus, P. Cineraria can be employed as a powerful anti-arthritic agent.

In-vivo studies flower of P. Cinerariaextract at a dose of 500mg/kg orally significantly reduced the inflammation of synovium joint destruction and joint erosion. The circumference of rat knee was measured daily and the group of rats which extracts showed lesser inflammation of knee joints which is caused by CFA.

Paw swelling is a metric used to assess the antiarthritic properties of different medications. In this case, it is used to assess the effectiveness of P. cineraria ethnolic extract at dose levels of 250 mg/kg and 500 mg/kg, respectively.Both P. Cineraria extract groups showed a significant decrease in paw volume as compared to the arthritic control group. Rats continued to grow normal weight following the adjuvant therapy, however there was a noticeable weight reduction the day after. The current study's findings also show a strong correlation between changes in hematological markers and the degree of inflammation.

The findings demonstrate that (Graph no1, Graph no2, Graph No 3 and Graph No4) the anemic state in arthritic rats is correlated with a drop in hemoglobin level and RBC count.In our study Group I (Control) showed normal RBC count, normal WBCs count, Hb level and normal ESR, while in Group II(Negative control) a decrease RBC count, decreaseHb level, increase WBCs and an increased erythrocyte sedimentation rate (ESR) was found. Group III is (StandardIndomethacin ) showed ESR which significantly decrease, Hb level incraese, RBC count incraese, WBCs count decrease. All these symptom shows an anemic symptoms as well as inflammatory condition, which is persisted in the Indomethacin treated group (Group IV and GroupV). The most prominent reasons include aberrant iron storage in the

reticuloendothelial system and synovial tissue, as well as a bone marrow's difficult to respond to anemia. The immune system's stimulation against the invasive antigens may be the cause of the significant increase in leukocyte count in rats with adjuvant-induced arthritis, while Prosopis Cineraria's modest immunomodulatory effect was evident in the corresponding drop in treated groups.In the arthritic control group, the standard medication Indomethacin and Prosopis Cineraria extract remarkably counteracted the declines in the ESR, Hb level, RBC count, and WBC count (normal condition) to return to nearly normal, thereby justifying its important role in arthritic conditions.

## CONCLUSION

According to the study's findings, P. cineraria flowers significantly reduce arthritis in rats by preventing the full effects of Freund's adjuvant.The ethanol extract of flowerP. cineraria at a dose of 500mg/kg orally significantly reduced the inflammation of synovium joint destruction and joint erosion.The flower of P. cineraria is a good source of active compounds, phenols, and antioxidants. Its extracts possess potential applications in various industries and traditional medicine. The study's conclusions help to clarify the phytochemical makeup of the plant and its therapeutic properties, opening the door for more investigation and advancement in the field of naturally occurring bioactive compounds.

**CONFLICTS OF INTEREST:** There are no conflicts of interest.

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