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ORIGINAL ARTICLE **OPEN ACCESS**

In Vivo Anti-Arthritic Effect of Ethanol Extract of *Plunchea Lanceolata* on Complete Freund's Adjuvant (CFA)-Induced Arthritis in Rats

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ABSTRACT
Herbal medicines are in substantial demand in the developed country for primary healthcare because of their high efficacy, safety and few side effects. Plunchea lanceolata is an ancient plant belongs to family astreaceae. Traditionally it is used for the treatment of various disorders such as diabetes, anxiety, hepatotoxicity, ulcer and anti-inflammatory activity. Therefore the present investigation was undertaken to investigate the anti-arthritic potential of P. lanceolata extract against CFA induced arthritis in experimental animals. The Ethanol extract of P. lanceolata were prepared by successive extraction procedure and phytochemical analysis of extract was done by different chemical test. Treatment of ethanol extract and standard drug for twenty one days nine after CFA administration significantly decrease the paw volume, hepatic biomarkers as well compare to diseased group. From the result of the current investigation it can concluded that the ethanol extract of P. lanceolata possess potent antiarthritic activity.
Key Word: P. Lanceolata, CFA, Arthritis, Hepatic, antioxident

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INTRODUCTION

Arthritis is an acute or chronic joint inflammation of polyarticular tissue. It has a multiple causes and portrayed by synovitis, destruction of bone and cartilage, and ankyloses [1]. Epidemiological studies suggest that RA affect approximately 23% population in united state and 15%in India and the prevalence is found to be higher in females than male [2-3]. The specific etiology of this staggering disease is unknown. Moreover, diverse flagging signaling molecules such as TNF-alpha, IL-1, IL-6, and IL-8 etc are involved in the pathophysiology of RA [4]. Biological response modifiers, disease-modifying antirheumatic drugs and non steroidal anti-inflammatory drugs (NSAIDS) are currently used for the treatment of arthritis and prolong use of these medications produces various unwanted harmful effects on various organ system [5]. Therefore there is a need to search an alternative drug treatment which have minimal side effects and effectively cure the disease.

Pluchea lanceolata, is a fast growing small perennial shrub that grow up to 30-100 cm hight and belongs to family Asteraceae [6, 7]. Traditionally it is also known as "Rasna", Gandhamula Rasya and Yuktarasa. Traditionally it is used for the treatment of inflammation, bronchitis, psoriasis, cough and piles, antipyretic, analgesic, and dyspepsia [8-10].

Based on the literature review on *Pluchea lanceolata* which yielded positive anti-inflammatory results the present study has been designed to investigate the anti-arthritic effect of *Pluchea lanceolata* leaves extract on CFA-induced arthritis in spargue dwely rats.

MATERIAL AND METHODS

Pluchea lanceolata were collected from the local Mathura U.P. India and authentified by Chief Scientist, CSIR-NISCAIR, New Delhi. The dried leaves of *Pluchea lanceolata* were dried and powdered in the grinder. The coarse powder was subjected to extraction in the soxhlet apparatus and successively extracted with pet. ether, chloroform, and ethanol. Individual extract of *Pluchea lanceolata* was concentrated in rotary

evaporator and excess of solvent was recovered and dried ethanol extract of *Pluchea lanceolata* leaves was chosen for the investigation of anti-arthritic potential in experimental animals [11-12].

Preliminary phytochemical screening- The Preliminary phytochemical examination of various extracts of *Pluchea lanceolata* was subjected to standard protocols of identification tests for alkaloids, tannins, glycosides, flavonoids, and phenols [13].

Bovine serum albumin

The inhibitory effect of *P. lanceolata* extract on protein denaturation was assessed according to the procedure illustrated by Mizushima & Kobayashi, 1968. The sample of the resultant solution has pH 6.3, adjusted with 1N Hcl, and incubated at 37^0C, heated at 57^0C for 20 minutes. The resultant sample was than cooled, followed by addition of phosphate buffer and absorbance was measured using Spectrophotometer at 660 nm [14].

Percent inhibition of protein denaturation was calculated as follows:

$$\text{Percentage inhibition} = 100 - \frac{(\text{Abs test solution} - \text{Abs of control}) \times 100}{\text{Abs test control}}$$

Egg albumin

The inhibitory effect of *P. lanceolata* extract on protein denaturation was assessed according to the procedure illustrated by Hasan *et al,* 2015. Briefly, 5 ml of sample solution contained egg albumin 0.2 ml, phosphate buffer 2.8 ml and 2ml of *P. lanceolata* extract and diclofenac sodium at different concentrations from (12.5 to 800 µg/ml) respectively. The test tubes of each sample incubated at 37°C for 15 min and then warmed at 70 °C for 5 min and the absorbance of test and standard were measured using a spectrophotometer at 660 nm [15].

$$\text{Percentage inhibition} = \frac{(\text{Abs of control} - \text{Abs of test solution}) \times 100}{\text{Abs of test control}}$$

Absorbance- (Abs)

Animals and experimental design

Healthy Wistar rats of both sex weighing 150-200g, housed in the polypropylene cages under standard condition12 h light and 12 h dark cycle and allowed to free access to food (Aishrwad) and water. Prior approval was taken from the Institutional animal ethical committee (IAEC) to carry out the experimental work. The whole experimental protocol was designed for 21 days, and animals were divided into different groups containing five animals in individual group, namely control, CFA, CFA+Indomethacin (10 mg/kg) [16], CFA+ *P. lanceolata* 200 and CFA+ *P. lanceolata* 400.

Induction of arthritis

The experimental protocol used in the current study by the method portrayed by Voon et al.; [17]. Briefly, 0.1 mL of CFA was injected into the sub plantar region of the left hind paw of each animal under light anesthesia (Thiopental sodium). The time of adjuvant injection was referred to as day 0. Three days after induction of arthritis, daily oral treatments were started for 21 days and on the last day, one hour after the treatment the body weight and paw volume were measured, and then animals were sacrificed and blood samples were collected via cardiac puncture.

Assessment of Arthritis-

The paw volume of each animal was measured before the administration of CFA injection using a digital plethysmometer (Rolex India). Further, after administration of CFA body weight and paw volume of each animal were measured at different day's intervals 0, 5,10,15,20, 25and 30. [18]

Biochemical and hematological analysis

On the last day of the experiment blood sample of each animal was collected in test tubes by cardiac puncture and allowed to stand for 30 min to separate the serum. The serum was then centrifuged at 4000 rpm, after which total RBC, WBC was determined by hemocytometer, ESR was measured by Westergren and SGOT, SGPT and Total protein were examined by a commercially available kit (Span diagnostic Pvt. ltd).

Statistical Analysis

The results were expressed as mean ± S.E.M. Statistical analysis was performed using Graph Pad Prism 5.2® software. Two-way analyses of variances (ANOVA) were performed on percentage inhibition of paw volume and effect on weight variation in arthritic rats followed by Turkey's post hoc test. All other data were analyzed by one-way ANOVA followed by Newman keuls test.

RESULT

Effect of *P. lanceolata* extracts on inhibition of protein denaturation using BSA and egg albumin

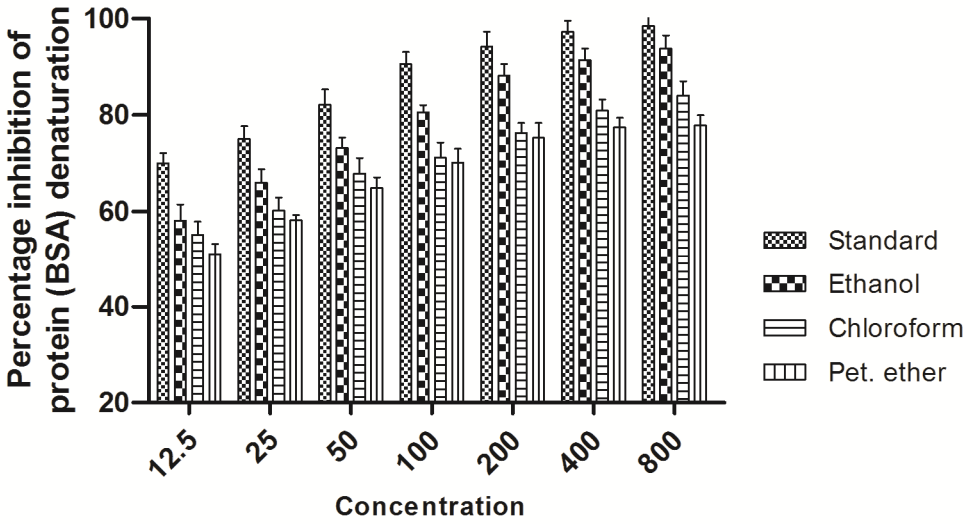

Fig 1: The inhibitory effect of *Brassica rapa* on denaturation of protein [BSA]

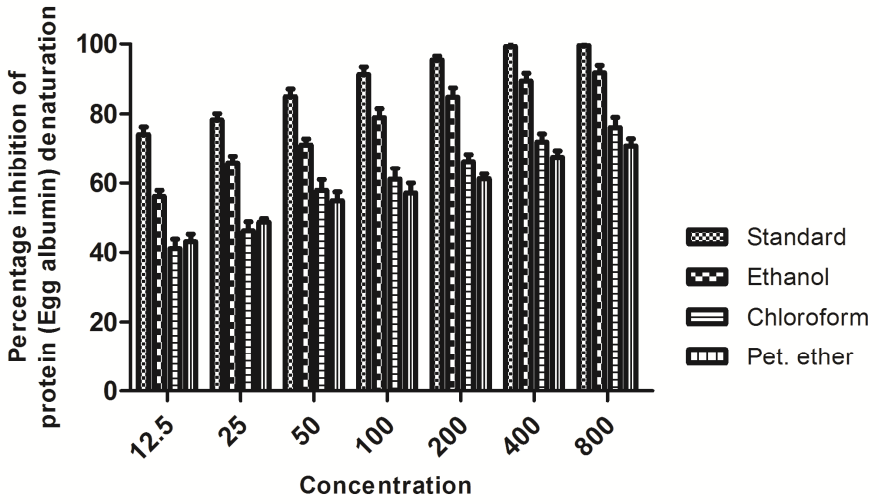

Fig 2: The inhibitory effect of *Brassica rapa* on denaturation of protein [BSA]

The inhibitory effect of *Brassica rapa* on denaturation of protein is depicted in fig-1 (BSA) and Fig-2 (Egg albumin). The outcome of the current investigation showed that ethanol extract of *P. lanceolata* exhibited the maximum 92.10% and 88.75% protection against the BSA and Egg albumin denaturation of protein at 800 µg/ml compared to chloroform and petroleum ether extract, which is near to standard drug aspirin 95.20%.

Bodyweight

The changes in body weight of control, CFA, indomethacin (10 mg/kg), and *P. lanceolata* (200 and 400mg/kg), treated arthritic rats are depicted in fig 3. There was a continuous loss of body weight was observed in all the CFA treated experimental animals. Oral treatment of *P. lanceolata* (200 and 400mg/kg) showed a dose-dependent remarkable improvement on the loss of body weight against the CFA induced inflammation.

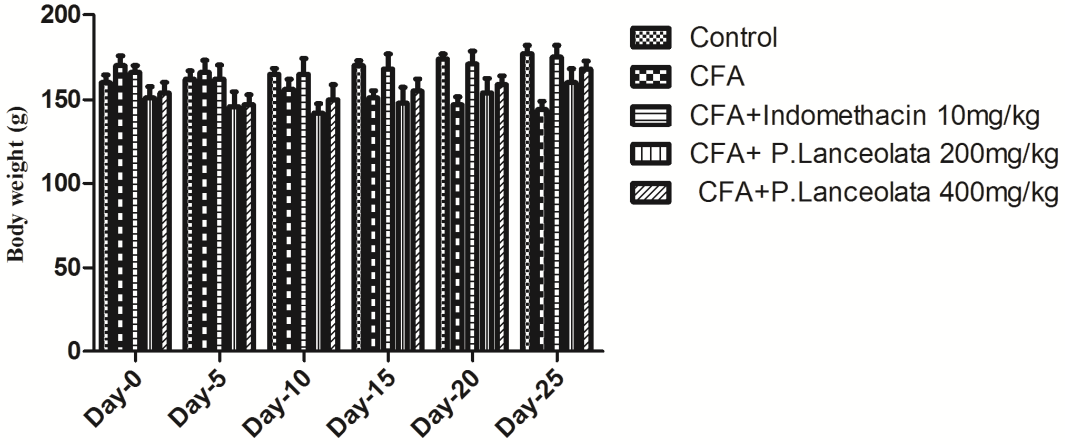

Fig 3: Changes in body weight of control, CFA, indomethacin (10 mg/kg), and *P. lanceolata* (200 and 400mg/kg), treated arthritic rats are depicted in fig 3

Effect on paw swelling

The effect of EEBR on CFA induced paw swelling is depicted in Fig.4 Sub plantar injection of CFA significantly ($p<0.001$) increased the swelling of the paw in experimental animals. Daily treatment of P . lanceolata (200 and 400mg/kg) and indomethacin(10mg/kg) for twenty-one days after nine day of CFA injection, attenuated the CFA induced paw volume ($p<0.05$) in Wistar rats compared to CFA treated group

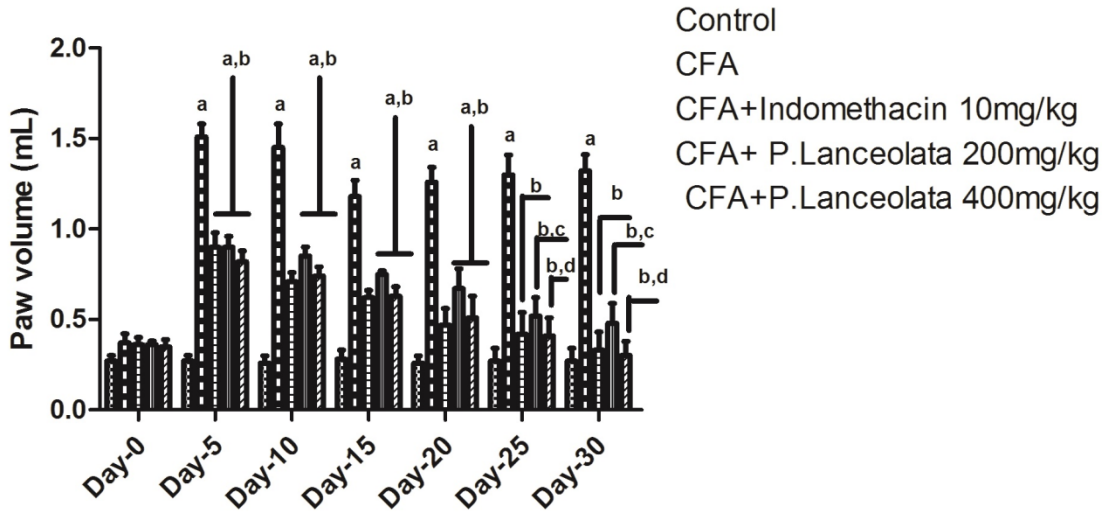

Fig 4: The effect of EEBR on CFA induced paw swelling

Estimation of Biochemical parameters:

Effect on AST and ALT

Fig.5 and 6 illustrate the effect of P. lanceolata on CFA induced changes in the level of serum AST and ALT in Wistar rats. Sub plantar injection of CFA significantly ($p<0.001$) increased the serum AST and ALT levels in all the groups of animals compared to control. Repeated treatment of *P. lanceolata* extract showed a remarkable ($p<0.05$) reduction in elevated levels of blood AST and ALT against the CFA induced arthritic group. Moreover, the treatment of a higher dose of 400mg/kg of *P. lanceolata* treatment showed a remarkable reduction in hepatic biomarker compared to indomethacin treated group.

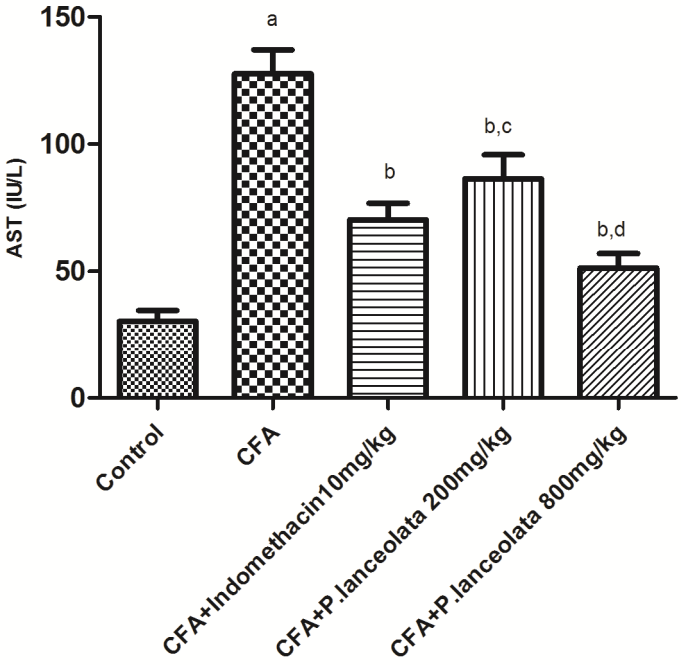

Fig.5 : The effect of *P. lanceolata* on CFA induced changes in the level of serum AST

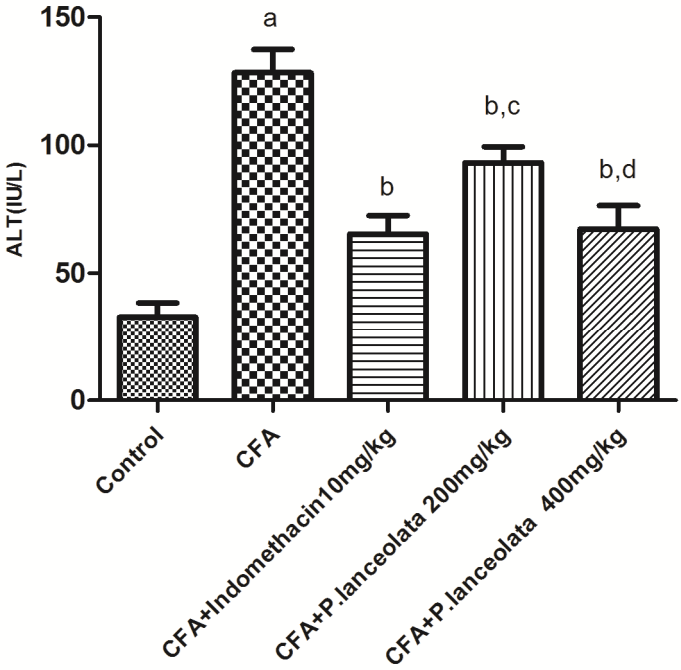

Fig.6 : The effect of *P. lanceolata* on CFA induced changes in the level of serum ALT

Effect on Serum total Protein:

Fig-7 demonstrates the effect of *P. lanceolata* on CFA induced change in serum total protein concentration in experimental animals. There was a substantial reduction in the level of serum total protein that was observed in all the experimental animals compared to the control group of rodents. Statistical analysis revealed that repeated treatment of EEBR and indomethacin treated rats showed a remarkable ($p<0.05$) increment in their serum total protein compared to the CFA group.

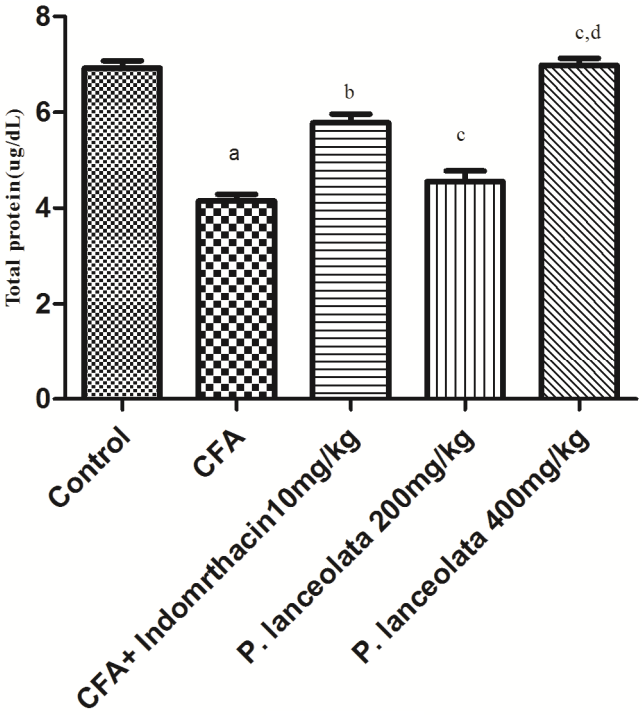

Fig-7 The effect of *P. lanceolata* on CFA induced change in serum total protein concentration in experimental animals

DISCUSSION

Medications have a vital role in the treatment of different illnesses of human beings since ancient times and home grown medications are habitually utilized as a conventional medication for the relief of pain and inflammation. These drugs can not only prevent the inflamed joint from pain and bone erosion but in comparison to allopathic medicine, these are safe, free from side effects, and suitable for patients [19]. The present investigations demonstrate that ethanol extract of *P. lanceolata* exhibited the inhibition of protein denaturation activity as well as attenuated the CFA induced inflammation in experimental animals. In the present investigation, the inflammation lowering potential of *P. lanceolata* extracts was investigated, using in vitro BSA and egg albumin method. Protein denaturation is one of the reasons for joint inflammation, which may occur due to loss of secondary and tertiary structure of proteins [20]. In the present study, the ethanol extract of *P. lanceolata* extracts successfully inhibited the denaturation of protein. Moreover, the ethanol extract of *P. lanceolata* showed the highest inhibitory percentage of protein denaturation, close to standard drug. Adjuvant induced inflammation model is one of the most accepted and validated screening methods for the assessment of anti- arthritic drugs because it elicits the similar symptoms of RA as occurs in humans such as pain, redness, and swelling in joints [21]. Therefore in the current study adjuvant-induced arthritis model was selected for the demonstration of the anti-arthritic activity of *P. lanceolata* leaves. In the present investigation oral administration of ethanol extract of *P. lanceolata* attenuated the CFA induced paw volume in experimental animals that shows that deterioration in the progression of the disease. Loss of body weight most frequently occurs in RA patients which maybe occur due to loss of appetite and inadequate absorption of nutrients from the gastrointestinal intestine [22]. The outcomes of the current investigation demonstrated that there may be a close connection between the loss of body weight and inflammation of joints. Treatment of ethanol extract of *P. lanceolata* attenuated the loss of body weight in adjuvant induced inflammation in experimental animals, which could be due to presence of vitamin B and omega 3 fatty acids [23]. Our findings were in agreement with the result of [24] which shows that supplementation of vitamin B improves the health status of the patient.

Various researchers have proposed that the estimation of liver biomarkers such as SGOT, SGPT, and total protein is an excellent approach to measure the anti-arthritic potential of test drugs [25]. In the current investigation, CFA caused a significant elevation in the level of SGOT and SGPT and reduction in total protein concentration similar to that of earlier findings which indicate that impairment in hepatic tissue [26, 27]. Treatment of *P. lanceolata* significantly diminished the activity of SGOT and SGPT and reverses the diminished concentration of protein to normal level in CFA induced arthritic rats and thus demonstrating its ability in protecting the liver against the CFA induced arthritis in experimental animals.

CONCLUSION

The present study revealed that repeated treatment of P. lanceolate extract attenuated the CFA induced symptoms of arthritis such as paw volume, bodyweight and hepatic markers in the experimental animals. The symptomatic relief in the present study might be due to the presence of flavonoid or any other phytoconstituents present in the extract. Further study is required to confirm the anti-arthritic potential of the extract.

SOURCE OF FUNDING

Nil

CONFLICT OF INTEREST

None

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