

Protective Effects of *Lantana camara (aculeata)* in Rheumatoid arthritis using *in vitro* and *in vivo* Complete Freund's Adjuvant induced arthritis in rats

Rupali Dhikale^{1*}, Vishal Gulecha², Amar Zalte³

ABSTRACT

The main objective of the present work is to evaluate the potential of *Lantana camara (aculeata)* for anti-arthritic activity using *in vitro* and *in vivo* study. *L. camara* is an important medicinal, ornamental, and essential oil-producing herb from family Verbenaceae. Different parts of *L. camara* have been used for the treatment of various human ailments such as itches, cuts, ulcers, swellings, bilious fever, eczema, tetanus, malaria, tumor, rheumatism, and headache. The anti-arthritic activities of pet ether, ethyl acetate, ethanol, and aqueous extracts of *L. camara* were initially evaluated using *in vitro* model of arthritis. The ethanolic and aqueous extracts were found to be better among all the extract in *in vitro* study and hence subjected for further *in vivo* anti-arthritic study using complete Freund's adjuvant (CFA) model in rats for 28 days. Both the extracts at 200 and 400 mg/kg were evaluated in rats for different parameters. The paw volume displacement, changes in body weight, arthritic index, alteration in serum biomarker, alteration in hematological parameters, secondary lesions changes as noticed from the radiograph, and histopathology study were measured as a mark of antiarthritic activity. The results indicate that *L. camara* protects rats remarkably against the primary and secondary arthritic lesions, body weight changes, serum biomarker changes, radiological, histopathological, and hematological perturbations induced by CFA in dose-dependent manner. However, the effect of the ethanolic extract at the dose of 400 mg/kg was more significant, superior, and pronounced than aqueous extract and also it is comparable to the standard drug.

Keywords: CFA, Histopathology, *Lantana camara (aculeata)*, Radiology, Rheumatoid arthritis

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INTRODUCTION

Lantana is the genus of the family *Verbenaceae* with 150 species. *Lantana* is mostly native to subtropical and tropical America, but a few taxa are indigenous to tropical Asia and Africa.^[1]

The plant *Lantana camara (aculeata)* Linn. Family-Verbenaceae is commonly known as wild sage or red sage and lantana weed.^[2] *L. camara (aculeata)* is an important medicinal plant with several medicinal uses in traditional medication system. It is been used to cure many health problems in different parts of the World. Leaves are used to treat cuts, rheumatism, ulcers, catarrhal infection, tetanus, rheumatism, malaria, cancer, chicken pox, asthma, ulcer, swelling, eczema, tumor, high blood pressure, bilious fever, ataxia of abdominal viscera, sores, measles, fevers, cold, and high blood pressure.^[3-5] It contains major phytochemical constituents such as Mono and sesquiterpenes, triterpenes, furanonaphthaloquinones, and flavonoids.^[6]

The word arthritis means inflammation of the joint ("artho" meaning joint and "itis" meaning inflammation). Rheumatoid arthritis (RA) is a ravaging inflammatory and autoimmune illness that affects the joints, although its cause is still unknown. With RA, inflammation manifests in the lining of the joints causing pain, swelling, joint damage, and deformity. It can occasionally involve other internal organs, such as the nerves, eyes, lungs, or heart.^[7] RA is a chronic systemic autoimmune disease that arises more frequently in females than males, being predominantly observed in the elderly. The prevalence rate reported in 2002 ranged from 0.5% to 1% of the population and had regional variation. RA primarily affects the lining of the synovial joints and can cause progressive disability, premature death, and socioeconomic burdens. The clinical manifestations of symmetrical joint involvement include arthralgia, swelling, redness, and even

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limiting the range of motion.^[8] Presently for the treatment of RA, strategies have changed from traditionally used nonsteroidal anti-inflammatory drugs (NSAIDs) or disease modifying ant rheumatic drugs to novel biological agents such as TNF monoclonal antibody and finally immunosuppressive or cytotoxic agents.^[9-11] The above-mentioned therapeutic agents reduce the inflammation and joint destruction but their long-term risks are still unknown. However, long-term risks of drugs include gastrointestinal ulcers, cardiovascular complications, hematologic toxicity, nephrotoxicity, pulmonary toxicity, myelosuppression, hepatic fibrosis, stomatitis, cirrhosis, diarrhea, immune reactions, and local injection-site reactions. Moreover, higher costs and side effects which include high risks of infections and malignancies require continuous

monitoring.^[12] As a result, alternative treatments based on natural plant products and herbal mixtures belonging to the realm of polyherbal formulation, complementary and alternative medicine (CAM) are becoming increasingly popular.

There is a promising future of medicinal plants as there are about half million plants around the world, and most of them are not investigated yet for their medical activities and their hidden potential of medical activities could be decisive in the treatment of present and future studies.^[13] In ancient texts, more than 500 plants have been traditionally claimed for arthritis treatment; however, only some plants have been investigated scientifically. It is the need of time to explore the hidden potential inside these plants for the better, effective, and safe treatment for arthritis.^[14] Hence, considering all above aspects, the present study is designed to investigate the above-mentioned medicinal plants utilized traditionally in development of hepatoprotective polyherbal formulation using various models with quality control profile.

METHODS

Collection of Plant Material

Fresh herb of *L. camara (aculeata)* was obtained from Nashik district. The parts of the selected plants were cleaned thoroughly.

Authentication, Drying, and Processing of Selected Plants Materials

An aerial parts of *L. camara (aculeata)* were authenticated at K.L.E Society's Raja Lakhamagouda Science Institute, Belgaum, India. The selected parts of all the three plants were cleaned and dried at room temperature. The size reduction of plant materials was done to get a coarse powder. The powdered drug was oven dried at 110°C for an hour and packed in air tight bottles until further use.

Extraction

The extraction of aerial parts of *L. camara (aculeata)* was carried out using continuous successive solvent extraction scheme. The powder of the air dried drugs was loaded in thimble of Soxhlet apparatus and was extracted with petroleum ether, ethyl acetate, ethanol, and water using successive method. The extract was filtered and concentrated extract was air-dried.^[15]

PHARMACOLOGICAL SCREENING

In vitro Anti-Arthritic Study^[16]

Inhibition of Bovine serum albumin (BSA) denaturation

Test solution: 0.5 ml of test solution consists of 0.45 mL of BSA (5% w/v) and 0.05 mL of extracts in various concentrations (100, 250, 500, 750, and 1000 µg/mL).

Test control solution: 0.5 mL of test control solution consists of 0.45 mL of BSA (5% w/v) and 0.05 mL of distilled water.

Standard solution: 0.5 mL of standard solution consists of 0.45 mL of BSA (5% w/v) and 0.05 mL of Diclofenac sodium solution (100 µg/mL).

The pH of the above solutions was adjusted to 6.3 using a small amount of 1N HCL. The samples were incubated at 37°C for

20 min and heated at 57°C for 3 min which were cooled and 2.5 mL of phosphate buffer (pH 6.3) was added to it. Control represents 100% proteins. After cooling, their absorbance was measured at 660 nm. The absorbance of Diclofenac sodium which was used as reference drug is measured. The percentage inhibition of protein denaturation was calculated as follows:

$$\text{Percentage inhibition} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}}$$

Inhibition of egg albumin denaturation

Test solution: 5 ml of test solution consists of 0.2 mL of egg albumin and 2.8 mL of phosphate buffer saline and 2 mL of in various concentrations of extracts. (100, 250, 500, 750, and 1000 µg/mL).

Test control solution: 5 ml of test control solution consists of 0.2 ml of egg albumin and 2.8 ml of phosphate buffered saline and 2 ml of distilled water.

Standard solution: 5 ml of standard solution consists of 0.2 mL of egg albumin and 2.8 mL of phosphate buffer saline and diclofenac 100 µg/mL.

The pH of the above solutions was adjusted to 6.4 using a small amount of 1N HCl. The samples were incubated at 37°C for 20 min and heated at 70°C for 5 min denaturations and the results were compared with standard diclofenac sodium.

After cooling, their absorbance was measured at 660 nm. The absorbance of Diclofenac sodium which was used as reference drug is measured. The percentage inhibition of protein denaturation was calculated as follows:

$$\text{Percentage inhibition} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}}$$

Acute toxicity study: The acute toxicity studies were carried out as per the Organization for Economic Co-operation and Development guideline (OECD) no. 423 (Acute Toxic Class Method). The limit dose of 5000 mg/kg (p.o.) was administered and they were observed for behavior and other signs of toxicity such as twitches, respiratory changes, righting reflex, and motor coordination for 4 h and monitored up to 14 days.^[17]

In vivo Anti-Arthritic Study

Experiment animals

Sprague-Dawley rats of either sex weighing from 200 to 300 g were used. The rats were housed under standard conditions of temperature (23–25°C), relative humidity (55%) with 12 h light and 12 h dark cycle. The study has got the approval from the Institutional Animal Ethical Committee (IAEC) of Committee for the Purpose of Control and Supervision of Experiments on Animals and the approval No. is SIPS/IAEC/2020/10.

Complete Freund's Adjuvant (CFA) induced arthritis

CFA-induced arthritis is a scientifically justified standard experimental procedure for the induction of chronic immune-pathological RA in laboratory animals with similar cellular immunity response and pathological mechanism as in human.^[18]

Animals and experimental design

The animals were randomly assigned into different groups of six animals per group ($n = 6$): group as shown in Table 1.

Induction of arthritis

All groups, except normal group, were made arthritic by injecting 0.1 mL Complete Freund's adjuvant intradermally into the sub plantar region of left hind paw on day "0." This adjuvant consists of dead mycobacterium tuberculosis bacteria suspended in heavy paraffin oil to give final concentration of 0.5 mg/mL. Saline or extracts or prednisolone were administered orally once daily from the 8th day of arthritis induction and continued till 28th day. Following parameters were evaluated.^[19,20]

Hind paw volume (HPV) measurement^[18]

The HPV of all animal groups was measured by plethysmometer at 0, 7, 14, 21, and 28th days after the injection of CFA emulsion. The percentage inhibition of paw edema in treated groups was then calculated.

Body weight examination^[18]

Animal's body weight of all groups was measured on day 0, 7, 14, 21, and 28 day after immunization. The percent weight change on day 28 was calculated.

Arthritic index^[21]

Primary lesions and secondary lesions are measured and arthritic index calculated as the sum of scores according to the method of Schorlemmer et al., 1999; Vogel. 2002, as shown in Table 2.

Arthritic score was evaluated on 28th day using macroscopic scoring which is carried out by independent observers using scale from 0 (no sign of arthritis), 1 (mild swelling and redness of the paw), 2 (moderate swelling and redness of the paw), and 3 (severe swelling and redness of the paw). The arthritic index was calculated by adding the scores for each individual paw.^[22]

Hematological parameters

On day 28, hematological parameters such as Red blood cell (RBC) count and hemoglobin (Hb) are estimated.^[22]

Erythrocyte sedimentation rate (ESR) estimation

ESR was measured on blood sample obtained from retro orbital plexus using Westergren's method.^[23]

Table 1: Treatment of different groups of animals in CFA-induced anti-arthritic study

S. No	Groups	Treatment
1.	Normal Control	No CFA injection.
2.	Disease Control	Only vehicle after CFA
3.	Standard/Positive control	Prednisolone 10 (day 8 to 28 day by oral route)
4.	LC Ethanol High	An ethanolic extract of LC at 400 mg/kg (day 8 till 28 day orally)
5.	LC Ethanol Low	An ethanolic extract of LC at 200 mg/kg (day 8 till 28 day orally)
6.	LC Aqueous High	An ethanolic extract of LC at 400 mg/kg (day 8 till 28 day orally)
7.	LC Aqueous Low	An ethanolic extract of LC at 200 mg/kg (day 8 till 28 day orally)

CFA: Complete Freund's adjuvant, LC: *Lantana camara*

Serum rheumatoid factor (RF)^[24] and C-reactive protein (CRP) estimation^[25]

Serum RF factor estimation and CRP were done by turbidimetry method.

Biochemical parameters

On day 28, blood of the rats was withdrawn by retro-orbital puncture and serum was used for the estimation of serum aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP).^[26]

Radiological analysis

For radiological studies, the affected paws of experimental rats were radiographed and checked for the soft swelling, bony erosions, and narrowing of the spaces between joints. The animals X-rays were taken at the joints of the hind paw of the animals for evaluating the bone damage using X-ray apparatus.^[22]

Histopathological analysis of ankle joints

On day 28, ankle joints were separated from the hind paw and immersed in 10% buffered formalin for 24 h followed by decalcification in 5% formic acid, processed for paraffin embedding sectioned at 5 μ m thickness. The sections were stained with hematoxylin-eosin and evaluated under light microscope with $\times 10$ magnifications for the presence of inflammatory cells, hyperplasia of synovium, pannus formation, and destruction of joint space.^[27]

Statistical analysis

The data were expressed as mean \pm SEM (Standard error mean). The significance of the difference was evaluated by one-way and two-way ANOVA followed by Bonferroni and Dunnett's test.

RESULTS

Extraction

The characterization of different extracts of *L. camara* (aculeata) is as mentioned in following Table 3:

In vitro Anti-Arthritic Assay

Assay: Inhibition of egg albumin

The percentage inhibition of egg albumin denaturation by the different extracts of *Lantana camara* at different concentrations and

Table 2: Nature of lesions and its associated scores in arthritic index calculation

Lesion Site	Nature of lesion	Score
Ear	a. Absence of nodules and redness	0
	b. Presence of nodules and redness	1
Nose	a. No swelling of connective tissue	0
	b. Intense swelling of connective tissue	1
Tail	a. Absence of nodules	0
	b. Presence of nodules	1
Forepaw	a. Absence of inflammation	0
	b. Inflammation of at least one joint	1
Hind paw	a. Absence of inflammation	0
	b. Slight inflammation	1
	c. Moderate inflammation	2
	d. Marked inflammation	3

their comparison with the standard are shown in Table 4 and Figure 1 respectively. Inhibition of heat induced egg albumin denaturation is prominently observed in ethanolic and aqueous extracts of plant.

Assay: Inhibition of BSA

The percentage inhibition of BSA denaturation by the different extracts of *Lantana camara* at different concentrations and their comparison with the standard are shown in Table 5 and Figure 2. Inhibition of heat induced BSA denaturation is prominently observed in ethanolic and aqueous extracts of plant.

Acute Toxicity Studies

The acute toxicity of *L. camara (aculeata)* was determined as per the OECD guidelines no. 425 (acute toxicity class method). This study showed the non-toxic nature of extracts of *L. camara (aculeata)* even at the highest dose of 5000 mg/kg body weight of the animal (OECD, 2001). Hence, 5000 mg/kg is considered as cutoff LD50. Hence, 200 mg/kg and 400 mg/kg of *L. camara (aculeata)* were selected for the further study.

In vivo Anti-Arthritic Studies

Based on the results of *in vitro* anti-arthritis protein denaturation assay, only those extracts showing significant and promising outcome were evaluated further for *in vivo* anti-arthritis activity. Thus, aqueous and ethanolic extracts at their high (400 mg/kg) and low dose (200 mg/kg) were selected for evaluation of *in vivo* anti-arthritis activity of *L. camara (aculeata)*.

Table 3: The characterization of different extracts of *Lantana camara (aculeata)*

S. No	Extract	Color	Nature	Yield (%w/w)
1.	Pet. Ether	Green	Semisolid	4.9
2.	Ethyl Acetate	Green	Semisolid	11.2
3.	Ethanol	Brown	Semisolid and sticky	28.3
4.	Aqueous	Reddish brown	Semisolid	24.2

Table 4: The percentage Inhibition of egg albumin denaturation by different extracts of *Lantana camara (aculeata)*

S. No.	Concentration (µg/mL)	% Inhibition of egg albumin denaturation by different extracts of <i>L. camara (aculeata)</i>				
		100	250	500	750	1000
1.	Pet ether	14.67±2.47	35.50±2.13	37.34±2.41	40.72±1.89	48.120±1.76
2.	Ethyl acetate	69.56±2.16	70.57±1.95	74.10±1.62	75.68±1.27	76.75±1.09
3.	Ethanol	82.53±0.88	91.96±1.78	92.28±1.05	96.36±1.37	98.25±1.28
4.	Aqueous	56.67±0.69	67.06±1.15	75.63±1.67	84.81±1.87	93.66±1.48
5.	Diclofenac-100 (Standard)	91.39±0.56	93.44±0.27	94.62±0.91	95.27±0.26	97.62±0.82

Table 5: The percentage Inhibition of BSA denaturation by different extracts of *Lantana camara (aculeata)*

S. No.	Concentration (µg/ml)	% Inhibition of BSA denaturation by different extracts of <i>L. camara (aculeata)</i>				
		100	250	500	750	1000
1.	Pet ether	12.04±2.20	39.68±1.74	56.61±1.14	65.61±0.94	74.21±0.27
2.	Ethyl acetate	44.57±2.13	54.86±1.84	65.85±1.58	78.69±0.68	86.21±0.91
3.	Ethanol	58.09±2.46	68.70±1.92	79.65±0.97	85.99±0.83	95.39±0.84
4.	Aqueous	47.65±2.57	59.98±1.58	73.81±1.09	82.98±0.76	93.76±0.69
5.	Diclofenac-100 (standard)	92.11±1.16	93.49±1.56	94.06±2.72	94.98±1.89	96.28±2.26

BSA: Bovine serum albumin

Following parameters were studied during *in vivo* anti-arthritis activity of *L. camara (aculeata)*.

Paw volume

Subplantar administration of CFA intradermally in the rat paw resulted in the progressive increase in the volume of the injected paw as well as non-injected paw which indicates primary and secondary arthritic lesions. Maximum Paw volume was observed on day 14, after which there was a gradual decrease except in the disease controlled group.

The *L. camara (aculeata)* also has shown more promising results in suppressing paw edema volume in ethanolic as well as aqueous extracts. Although the efficacy of ethanolic and aqueous extracts is comparable to standard drug Prednisolone, ethanolic extract maintained significantly inhibitory effect after Day 14 in greater extent as shown in Table 6 and Figure 3.

Body weight

The incidence and severity of arthritis increased the changes in the body weight of the rats induced with CFA models.^[28] The changes in the body weight were monitored as apparent indicator of arthritic symptoms and the loss of body weight usually began to appear at the onset stage of arthritis.^[29] As shown in Table 7, arthritic control animals showed a marked weight loss (weight change is -4.78%) after 7 days of CFA injection till the end of study. Rats of normal group showed progressive weight gain (weight change is 7.69%) from 0 day to 28 days as they were not administered CFA injection. In standard group, Prednisolone 10 treated rats significantly improved the body weight (weight change is 6.97%). In *L. camara* treated groups, it is found that the groups which received ethanolic extract 400 mg/kg showed highest improvement in weight gain (weight change is 10.48%) which shows its superior efficacy compared to other groups.

Effect of the ethanolic and aqueous extract of *L. camara (aculeata)* at high (400 mg/kg) and low dose (200 mg/kg) on body weight of CFA-induced arthritic rats is shown in Figure 4.

Table 6: Effect of ethanolic and aqueous extract of *Lantana camara* (aculeata) at high (400 mg/kg) and low dose (200 mg/kg) on paw volume of CFA-induced arthritic rats

Groups	Day 0	Day 7	Day 14 (Mean±SEM)	Day 21	Day 28	PV % Inhibition
Normal	0.22±0.012	0.22±0.02	0.22±0.013	0.22±0.014	0.22±0.015	72.5
Control	0.23±0.022	0.31±0.013	0.49±0.016	0.61±0.017	0.80±0.024	NA
Prednisolone 10	0.21±0.023	0.3±0.013	0.34±0.015	0.3±0.024	0.28±0.026	65
LC-Eth-High	0.21±0.012	0.31±0.013	0.38±0.021	0.35±0.023	0.32±0.016	60
LC-Eth-Low	0.23±0.012	0.34±0.026	0.45±0.023	0.42±0.015	0.39±0.014	51.25
LC-Aq-High	0.22±0.018	0.32±0.025	0.4±0.012	0.37±0.031	0.34±0.021	57.5
LC-Aq-Low	0.26±0.013	0.35±0.021	0.47±0.013	0.44±0.028	0.42±0.012	47.5

CFA: Complete Freund's adjuvant, LC: *Lantana camara*

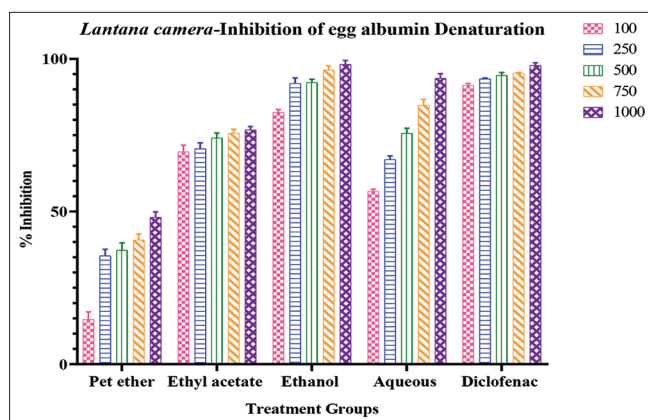


Figure 1: The percentage inhibition of egg albumin denaturation by different extracts of *L. camara* (aculeata). Values are expressed as mean ± SEM for $n = 3$ and analysis by two-way ANOVA followed by Bonferroni's multiple comparison test using GraphPad Software; * $P < 0.0001$ compared to Standard Diclofenac

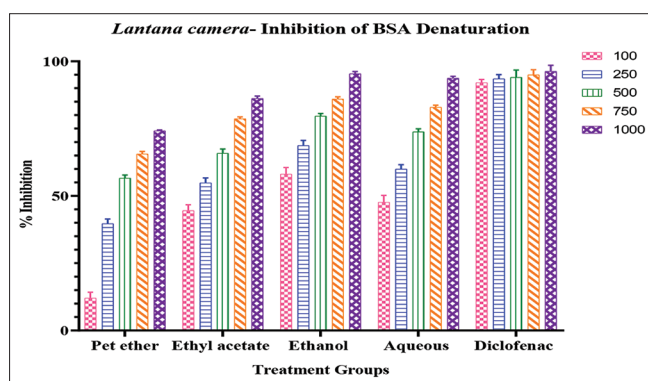


Figure 2: The percentage inhibition of BSA denaturation by different extracts of *L. camara* (aculeata). Values are expressed as mean ± SEM for $n = 3$ and analysis by two-way ANOVA followed by Bonferroni's multiple comparison test using GraphPad Software; * $P < 0.0001$ compared to Standard Diclofenac

Arthritic index

CFA induced arthritis model is associated with destruction of the joints. Paw swelling and arthritic index are indicative measures to determine antiarthritic activity of any drug.^[30] Arthritic scoring is a benchmark of puffiness of joints succeeding to introduction in a CFA-induced arthritic study.^[31]

Arthritic index is not evident in animals from normal

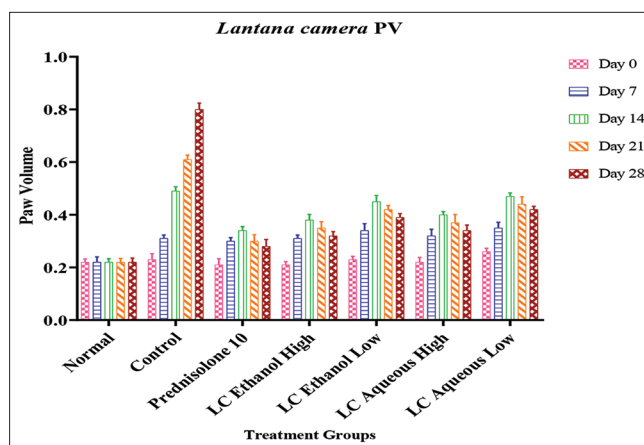


Figure 3: Effect of ethanolic and aqueous extracts of *Lantana camara* (aculeata) at high (400 mg/kg) and low dose (200 mg/kg) on paw volume of CFA-induced arthritic rats. Values are plotted as the mean ± SEM, $n = 6$ in each group; significant reduction in paw volume was analyzed by two-way analysis of variance followed by Dunnett's multiple comparison test using GraphPad Software; * $P < 0.0001$ compared to CFA control

groups as they were not administered CFA injection. Arthritic control groups are more vulnerable to develop the signs of inflammation, swelling, redness after CFA injection and no reduction in arthritic index was observed until the 28 Days. As shown in Table 8 and Figure 5, animals treated with standard drug prednisolone showed a marked decrease in arthritic index on day 28. Furthermore, the rats treated with ethanolic and aqueous extracts of *L. camara* caused significant ($P < 0.0001$) and dose-dependent reduction in arthritic index on day 28 as compared to CFA control animals. However, ethanolic extracts of *L. camara* (aculeata) at 400 mg/kg demonstrated suppressive effect (1.47) which is closer to standard (1.41).

Hematological parameters (Hb, RBC and ESR) estimation

The CFA-induced hematological perturbations, such as an increase in the WBC count, a decreased RBC count, a decreased Hb count and an increased ESR.^[32] Hb and RBCs decreases in RA due to reduced bone marrow erythropoietin response and demolition of premature RBCs.^[33] The ESR is an indirect method for the measurement of inflammation in the body and it is key biomarkers that is regulated during inflammation, stress and cell necrosis.^[34,35] Increase in ESR reflects the chronicity of the disease.^[36]

As shown in Table 9 induction of arthritis by CFA resulted in significant decrease in RBC count, Hb and increase in ESR

Table 7: Effect of ethanolic and aqueous extract of *Lantana camara* (*aculeata*) at high (400 mg/kg) and low dose (200 mg/kg) on body weight of CFA-induced arthritic rats

Groups	Day 0	Day 7	Day 14	Day 21	Day 28	% change in body weight
Normal	208±0.36	212±0.25	215±1.06	220±1.29	224±1.59	7.69
Control	230±0.63	232±0.93	225±0.36	222±0.44	219±0.57	-4.78
Prednisolone 10	244±0.93	248±0.73	251±0.63	255±1.23	261±1.09	6.97
LC-Eth-High	210±0.61	215±0.39	220±0.26	226±0.16	232±0.02	10.48
LC-Eth-Low	205±0.65	208±0.43	211±0.38	214±0.51	217±0.52	5.85
LC-Aq-High	200±0.47	203±0.63	205±0.52	208±0.92	212±0.23	6.00
LC-Aq-Low	205±0.52	208±0.53	211±0.32	214±0.35	217±0.52	5.85

CFA: Complete Freund’s adjuvant, LC: *Lantana camara*

Table 8: Effect of ethanolic and aqueous extract of *Lantana camara* (*aculeata*) at high (400 mg/kg) and low dose (200 mg/kg) on body weight of CFA-induced arthritic rats

AI on Day 28		
S. No.	Treatment Group	AI (Mean±SEM)
1.	Normal	0±0
2.	Control	5.12±0.26
3.	Prednisolone 10	1.41±0.18
4.	LC Ethanol High	1.47±0.12
5.	LC Ethanol Low	1.59±0.21
6.	LC Aqueous High	1.55±0.23
7.	LC Aqueous Low	1.68±0.25

CFA: Complete Freund’s adjuvant, LC: *Lantana camara*, AI: Arthritic index

compared to Normal rats. The effects of ethanolic and aqueous extracts of *L. camara* are promising in exerting their anti-anemic action by stabilizing the altered hematological changes effectively. Among all the groups the group which is treated with ethanolic extract of *L. camara* at a dose of 400 mg/kg prevented alterations in hematological parameters to a greater extent than any other group and it is comparable to standard (Prednisolone). The ethanolic extracts of *L. camara* significantly restored ESR, blood Hb content, and hence it supports the involvement of antioxidant and anti-anemic properties in maintenance.

Effect of the ethanolic and aqueous extract of *L. camara* at high (400 mg/kg) and low dose (200 mg/kg) on hematological parameters (Hb, RBC, and ESR) of CFA-induced arthritic rats is shown in Figure 6a-c, respectively.

Estimation of RF and CRP

RFs contribute actively to disease severity and chronicity by enhancing immune complex formation and complement fixation; therefore, RF-producing B cells and their activation mechanisms, including Toll-like receptor ligation may be important targets for RA treatment.^[37]

Predominant CRP check is followed as an excellent lab technique for scrutinizing RA and other inflammatory diseases arising from inflammation.^[38] The increase in the level of CRP is due to the rise in the plasma concentration of IL-6, which is produced by the macrophages and the adipocytes.^[39] In general, CRP plays an important role in host defense mechanisms against infectious agents and in the inflammatory response. CRP is a valuable marker and regulator of systemic inflammation in RA that also appears to play a direct role in bone destruction and radiographic progression.^[40]

As shown in Table 10, the serum RF (49.44 ± 0.35 IU/mL) and CRP (12.8 ± 0.63 mg/L) which are markers of systemic inflammation

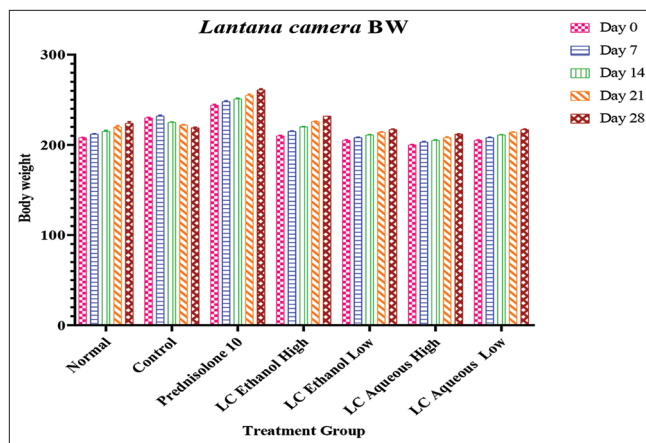


Figure 4: Effect of ethanolic and aqueous extracts of *Lantana camara* at high (400 mg/kg) and low dose (200 mg/kg) on body weight of CFA-induced arthritic rats. Values are plotted as the mean ± SEM, n = 6 in each group; significant reduction in paw volume was analyzed by two-way analysis of variance followed by Dunnett’s multiple comparison test using GraphPad Software; *P < 0.0001 compared to CFA control

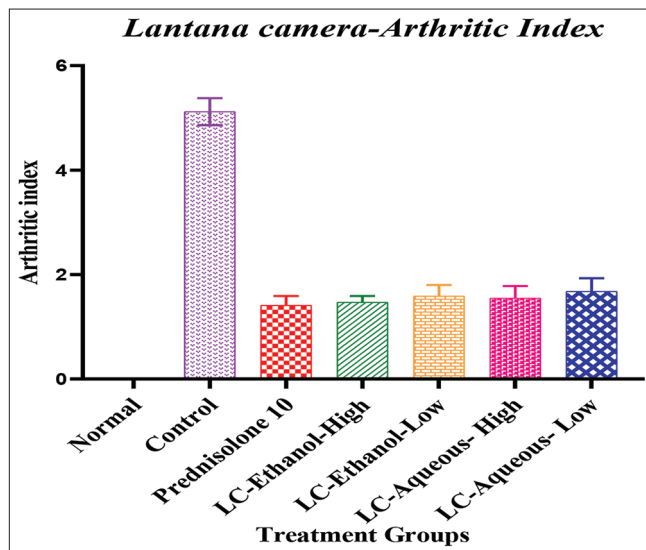


Figure 5: Effect of the ethanolic and aqueous extracts of *Lantana camara* (*aculeata*) at high (400 mg/kg) and low dose (200 mg/kg) on arthritic index of CFA-induced arthritic rats. Values are plotted as the mean ± SEM, n = 6 in each group; significant reduction in paw volume was analyzed by one-way analysis of variance followed by Dunnett’s multiple comparison test using GraphPad Software; *P < 0.0001 compared to CFA control

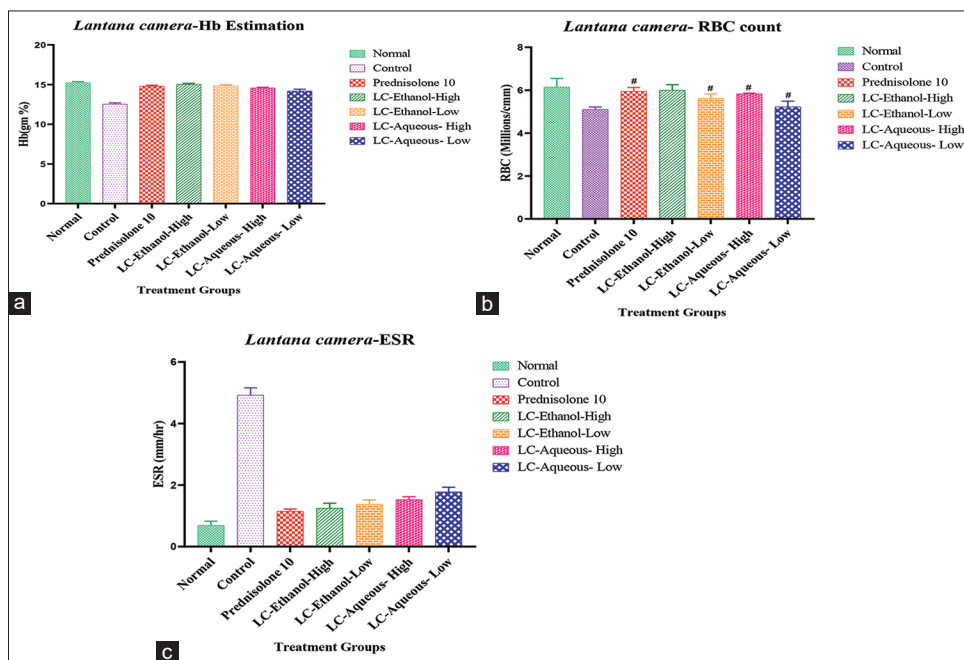


Figure 6: (a) Effect of ethanolic and aqueous extracts of *Lantana camara* (aculeata) at high (400 mg/kg) and low dose (200 mg/kg) on Hb of CFA-induced arthritic rats. Values are expressed as mean ± SEM for six animals and analysis by One-way ANOVA followed by Dunnett’s multiple comparison test using GraphPad Software; **P* < 0.0001 compared to CFA control. (b) Effect of the ethanolic and aqueous extracts of *L. camara* (aculeata) at high (400 mg/kg) and low dose (200 mg/kg) on RBC count of CFA-induced arthritic rats. Values are expressed as mean ± SEM for six animals and analysis by One-way ANOVA followed by Dunnett’s multiple comparison test using Graph pad Software; **P* < 0.0001 compared to CFA control. (c) Effect of ethanolic and aqueous extracts of *L. camara* (aculeata) at high (400 mg/kg) and low dose (200 mg/kg) on ESR of CFA-induced arthritic rats. Values are expressed as mean ± SEM for six animals and analysis by One-way ANOVA followed by Dunnett’s multiple comparison test using GraphPad Software; **P* < 0.0001 compared to CFA control

Table 9: Effect of ethanolic and aqueous extract of *Lantana camara* (aculeata) at high (400 mg/kg) and low dose (200 mg/kg) on hematological parameters of CFA-induced arthritic rats

Hematological parameters				
S. No.	Treatment Group	Hb (gm%) (Mean±SEM)	RBC count (million/cm) (Mean±SEM)	ESR (mm/h) (Mean±SEM)
1.	Normal	15.23±0.15	6.15±0.4	0.69±0.14
2.	Control	12.54±0.16	5.1±0.12	4.92±0.24
3.	Prednisolone 10	14.81±0.11	5.95±0.18	1.15±0.07
4.	LC Ethanol High	15.02±0.14	6±0.26	1.25±0.16
5.	LC Ethanol Low	14.85±0.12	5.62±0.21	1.38±0.14
6.	LC Aqueous High	14.60±0.08	5.84±0.02	1.53±0.09
7.	LC Aqueous Low	14.20±0.21	5.23±0.26	1.78±0.15

CFA: Complete Freund’s adjuvant, LC: *Lantana camara*, Hb: Haemoglobin, RBC: Red blood cells, ESR: Erythrocyte sedimentation rate

and antibody production against the injected adjuvant were dramatically increased in CFA control group rats. The standard drug Prednisolone and *L. camara* extract treatment causes significant reduction in the elevated levels of both CRP and RF in the serum.

The *L. camara* exerted its anti-arthritic effect by suppressing the elevated level of serum RF and CRP significantly (*P* = 0.001) as compared to CFA controlled group. Nevertheless, the effect was found to be dose-dependent in both the ethanolic and aqueous extracts and ethanolic extract displayed maximum efficacy in reducing serum RF (28.33 ± 0.26 IU/mL) as well as CRP (4.0 ± 0.27 mg/L) at its dose of 400 mg/kg which is shown in Figure 7a and b.

Estimation of biochemical parameters

Cellular enzymes, such as AST, ALT, membrane bound indicator of type II cell secretory activity or the lysosomal enzyme

glucuronidase, and an indicator of phagocytic activity, can also be used as sensitive markers of cellular integrity and cellular toxicity induced by pathological conditions.^[41] However, the ALP function is associated with annihilation of the bone and is an assessment of lysosomal integrity.^[42] Increased activities of AST, ALT, and ALP were observed in arthritic rats, which may be attributed towards persistent inflammation and also useful in the evaluation of liver damage.^[43]

As a result of inflammation induced by CFA, the levels of all the biochemical parameters were increased in all arthritis rats as compared to control rats. However, the treatment of animals with prednisolone significantly reduced the elevated levels of serum enzymes as shown in Table 11.

Treatment with ethanolic and aqueous extracts of *L. camara* found to be promising in stabilizing the altered biochemical changes effectively. Among all the groups, ethanolic extract of

Table 10: Effect of the ethanolic and aqueous extract of *Lantana camara* (aculeata) at high (400 mg/kg) and low dose (200 mg/kg) on RF and CRP of CFA-induced arthritic rats

Rheumatoid factor			
S. No.	Treatment Group	RF (IU/ml) (Mean±SEM)	CRP (mg/L) (Mean±SEM)
1.	Normal	0±0	3.07±0.2
2.	Control	49.44±0.35	12.8±0.63
3.	Prednisolone 10	25.78±0.22	4.2±0.23
4.	LC Ethanol High	28.33±0.26	4.0±0.27
5.	LC Ethanol Low	34.25±0.23	4.95±0.21
6.	LC Aqueous High	30.12±0.32	5.2±0.32
7.	LC Aqueous Low	35.12±0.21	5.6±0.28

RF: Rheumatoid factors, CRP: C-reactive protein, CFA: Complete Freund's adjuvant, LC: *Lantana camara*

Table 11: Effect of ethanolic and aqueous extract of *Lantana camara* (aculeata) at high (400 mg/kg) and low dose (200 mg/kg) on biochemical parameters (AST, ALT, and ALP) of CFA-induced arthritic rats

Biochemical Parameters				
S. No.	Treatment Group	AST (U/L) (Mean±SEM)	ALT (U/L) (Mean±SEM)	ALP (U/L) (Mean±SEM)
1.	Normal	42±2.3	42±1.7	73±3.3
2.	Control	127±4.4	173±2.8	475±1.6
3.	Prednisolone 10	59±3.3	57±2.0	127±2.7
4.	LC Ethanol High	68±2.09	72±1.46	138±1.67
5.	LC Ethanol Low	76±1.86	98±2.38	156±2.85
6.	LC Aqueous High	78±1.68	86±1.38	152±1.85
7.	LC Aqueous Low	86±1.95	97±1.67	163±1.73

AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: Alkaline phosphatase, LC: *Lantana camara*

L. camara (400 mg/kg) treated group prevented alterations in serum biomarkers AST (68 ± 2.09 U/L), ALT (72 ± 1.46 U/L), and ALP (138 ± 1.67 U/L) to a greater extent as shown in Figure 8.

Radiological Study

The severity of RA is indicated by the radiographic changes and hence it is considered as useful diagnostic measures. Early phase RA is characterized by soft tissue swelling, whereas the prominent signs such as bony erosions and narrowing of joint spaces can be observed only in the developed stages of RA.^[39]

The arthritic control rats show the narrowing of joint space, severe soft-tissue swelling, pronounced decrease in bone density, marked destruction of bones and abnormal ossification in the joints of interphalangeal regions. All these symptoms symbolize the presence of subchondral erosion in arthritic conditions. However, it is found that the animals treated with the different extracts and standard drug prednisolone have contributed to noticeable reduction in joint erosion. The treatment also resulted in normalization of other changes. The standard prednisolone showed better effect compared to all other group. The radiographs of the rat joints in CFA-induced arthritic rat model are shown in Figure 9.

The animals administered with ethanolic and aqueous extract of aerial parts of *L. camara* caused diminution of all symptoms including soft-tissue swelling, bony destruction, and narrowing of joint spaces. The effect of 400 mg/kg of the ethanolic extract of *L. camara* resulted in better antiarthritic effect by causing minimal bone damage, less soft-tissue swelling, and reducing the narrowing of the joint spaces. This effect was superior as compared to other groups of animals treated with *L. camara*.

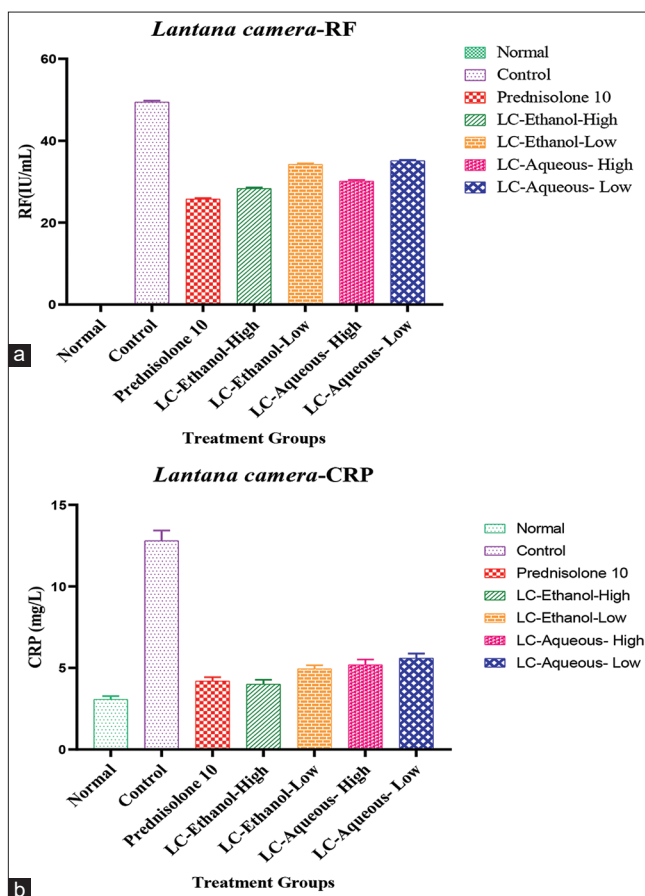


Figure 7: (a) Effect of the ethanolic and aqueous extracts of *Lantana camara* (aculeata) at high (400 mg/kg) and low dose (200 mg/kg) on RF of CFA-induced arthritic rats. Values are expressed as mean \pm SEM for six animals and analysis by one-way ANOVA followed by Dunnett's multiple comparison test using GraphPad Software; * $P < 0.0001$ compared to CFA control. (b) Effect of the ethanolic and aqueous extracts of *L. camara* (aculeata) at high (400 mg/kg) and low dose (200 mg/kg) on CRP of CFA-induced arthritic rats. Values are expressed as mean \pm SEM for six animals and analysis by One-way ANOVA followed by Dunnett's multiple comparison test using GraphPad Software; * $P < 0.0001$ compared to CFA control

Histopathological Analysis

The finding of joint histopathological study 28 days post-CFA induction revealed noticeable changes in hind paw joints. This significant changes includes synovial hyperplasia, infiltration of leukocytes, erosion of bone and cartilage, joint space narrowing, pannus formation, infiltration of inflammatory cells, as seen in picture of disease control animals (B) compared to vehicle control animals (A).

After treatment with the standard drug prednisolone the intensity of the changes were drastically reduced as seen in picture (C). The effect of *L. camara* ethanolic and aqueous extract at their dose of 400 mg/kg resulted in normalization of changes to great extent and in many cases the outcome is comparable to standard prednisolone as seen in Figure 10.

Histopathological studies of animal's revealed effect of ethanol extracts of *L. camara* at 400 mg/kg as superior effect as it shows no evidence of leukocyte infiltration, synovial hyperplasia, bone and cartilage erosion, and pannus formation.

DISCUSSION

As the recent healthcare scenario is mainly focused on use of synthetic drugs though having numerous side effects associated with them, the use of herbal drugs always remains in unique

position because of less side effects, easy availability, and affordability. To increase the widespread use of herbal drugs, efforts are taken to validate the ethno medical claim of various medicinal plants for treatment of various disorders.

The plant *L. camara* (*aculeata*) has been traditionally claimed for use in Rheumatism. To explore the potential of *L. camara* for treatment of arthritis, it has been subjected for continuous Soxhlet extraction using various solvents of increasing order of polarity. It has been found from the percentage yield of Pet. ether, ethyl acetate, ethanol, and aqueous extracts of *L. camara*, maximum phytoconstituents are present in ethanolic extract followed by aqueous extract. The amount of phytoconstituents that are soluble in solvents like pet ether and ethyl acetate is comparatively less. Initially, all the four extracts were screened for anti-arthritis activity using *in vitro* models of inhibition of protein, that is, egg albumin and BSA denaturation.

Denaturation of the protein involves the disruption of secondary, tertiary and quaternary structure of the molecules and finally leads to cell death, it occurs due to stress like a high level of salt, high temperature and high level of acidity. Denaturation of protein is one of the causes of RA due to the production of auto-antigens in certain rheumatic diseases.^[44] It has been reported that inhibition of denaturation of BSA at pathological pH (6.2–6.5) was accountable for anti-inflammatory action of various NSAIDs.^[45]

Agents that can prevent protein denaturation, therefore, would be worthwhile for antiarthritic drug development. The anti-arthritis activity was also shown in a concentration dependent manner and the activity was increased on increasing the concentration of extracts. The increments in absorbance of

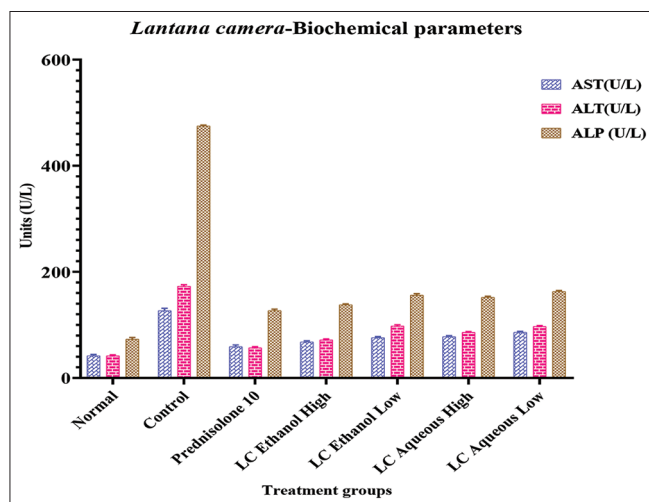


Figure 8: Effect of the ethanolic and aqueous extracts of *Lantana camara* (*aculeata*) at high (400 mg/kg) and low dose (200 mg/kg) on Biochemical parameters of CFA-induced arthritic rats. Values are expressed as mean \pm SEM for six animals and analysis by Two-way ANOVA followed by Dunnett's multiple comparison test using GraphPad Software; * $P < 0.0001$ compared to CFA control



Figure 9: X-ray radiographic assessment of hind paws of CFA-induced arthritis rats after treatment with standard and different plant extracts. Where, (a) normal control, (b) disease control, (c) standard prednisolone, (d) 400 mg/kg of *Lantana camara* (*aculeata*) aqueous extract, (e) 400 mg/kg of *L. camara* (*aculeata*) ethanolic extract

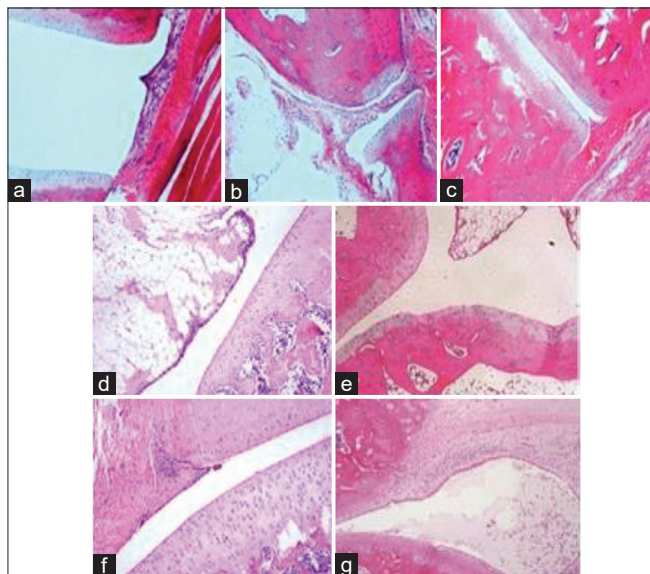


Figure 10: Histopathological assessment of hind paws of CFA-induced arthritis rats after treatment with standard and different extracts of *Lantana camara (aculeata)* where, (a) normal control, (b) disease control, (c) standard prednisolone, (d) 200 mg/kg of *L. camara (aculeata)* ethanolic extract, (e) 400 mg/kg of *L. camara (aculeata)* ethanol extract, (f) 200 mg/kg of *L. camara (aculeata)* aqueous extract, (g) 400 mg/kg of *L. Camara (aculeata)* aqueous extract, respectively

test samples with respect to control indicated stabilization of protein, that is, inhibition of heat induced protein denaturation by plant extract. Hence, maximum activity was reported at highest concentration (1000 µg/ml) of the ethanolic extract of *L. camara*.

The *in vitro* anti-arthritis protein denaturation assay showed that among all extracts, only ethanolic and aqueous extract are significant and promising and hence evaluated further for *in vivo* anti-arthritis activity.

The purpose of preliminary acute toxicity studies is to determine the LD50 values to help us in evaluating the safe dose range at which the drug can be used without any harmful or lethal effect on the experimental animals. Accordingly 5000 mg/kg is fixed as cut off LD50.

In vivo anti-arthritis activity of *L. camara* was validated using CFA induced arthritis model and different parameters were observed such as changes in Paw volume, Body weight, arthritic index, hematological system (RBC, Hb, and ESR), biochemical marker (AST, ALT, and ALP), CRP level, RF level, radiological, and histopathological study.

In Freund's Adjuvant Arthritic rat model, treatment with ethanolic and aqueous extract of *L. camara* at 400 mg/kg showed significant inhibitory effect on injected hind paw edema, body weight changes, arthritic index, hematological changes, biochemical changes and maximum inhibition was observed on the group receiving ethanolic extract. The study also illustrates the superior effect of ethanolic extract in other parameters such as level of CRP and RF in serum, radiological changes, and histopathological changes.

CONCLUSION

The present study can serve as great useful information in validating the traditional claim for Rheumatism of *L. camara*

(*aculeata*). The study has demonstrated that the ethanolic extract of *L. camara* shows superior antiarthritic activity as compared to any other extract as seen from *in vitro* and *in vivo* activity.

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