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ORIGINAL ARTICLE

Analgesic, Anti-Inflammatory and Anti-Arthritic Activity of *Cordia subcordata* Extract

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ABSTRACT
This study evaluated the analgesic, anti-inflammatory and anti-arthritic properties of Cordia subcordata (CS) extract using various in vivo models, including von Frey test, tail immersion test, carrageenan-induced paw edema, acetic acid-induced vascular permeability, tail immersion test and adjuvant-induced arthritis (AIA). The von Frey test revealed that CS at 400 mg/kg significantly reduced pain thresholds, while CS at 200 mg/kg showed no effect at 0 minutes but progressively inhibited hypersensitivity ($p < 0.001$) over time.Tail immersion studies demonstrated significant analgesic activity for CS 400 ($p < 0.001$), though slightly less effective than diclofenac. In the carrageenan-induced paw edema model, CS significantly inhibited paw edema at the 3rd and 5th hours post-administration ($p < 0.001$), comparable to diclofenac sodium in the acetic acid-induced vascular permeability test, CS markedly reduced dye leakage, indicating potent anti-inflammatory effects. In the AIA model, CS effectively reduced paw volume and normalized hematological parameters, including WBC, RBC, platelet count, and ESR. These findings underscore the therapeutic potential of ***Cordia subcordata*** *extract as a natural anti-inflammatory and analgesic agent, warranting further investigation for clinical applications.*
Keywords: *CS extract; Carrageenan; Anti-inflammatory; Mechanical Allodynia*

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INTRODUCTION

Inflammation is a complex biological response of the body's immune system to harmful stimuli such as pathogens, injuries, or irritants. While acute inflammation is a vital protective mechanism, chronic inflammation can lead to a host of debilitating conditions, including arthritis, cardiovascular diseases, and neurodegenerative disorders. Despite the availability of numerous synthetic anti-inflammatory drugs, many are associated with significant side effects, such as gastrointestinal distress and organ toxicity. This has fueled the search for natural alternatives with potent anti-inflammatory properties and minimal adverse effects. In this context, plant-derived compounds have garnered considerable attention as a source of safe and effective therapeutic agents [1,2].

Cordia subcordata, commonly known as the sea trumpet, is a traditional medicinal plant distributed widely in tropical regions. Historically, various parts of the plant, including leaves, bark, and flowers, have been used in traditional medicine to treat inflammation, wounds, and skin disorders. Phytochemical investigations of *Cordia subcordata* have revealed the presence of bioactive compounds such as flavonoids, alkaloids, tannins, and phenolics, which are known for their anti-inflammatory, antioxidant, and analgesic activities. However, despite its traditional uses and rich phytochemical profile, the scientific validation of its anti-inflammatory properties remains limited, particularly through *in vivo* studies [3–5].

The carrageenan-induced paw edema model in rats is a well-established experimental method for evaluating the anti-inflammatory activity of plant extracts. This model mimics acute inflammation by inducing localized swelling, mediated by the release of inflammatory mediators such as histamine, prostaglandins, and cytokines. It provides a robust platform for studying the efficacy of anti-inflammatory agents and understanding their mechanisms of action [6,7].

In this study, we aim to investigate the in vivo anti-inflammatory potential of *Cordia subcordata* extract using the carrageenan-induced rat paw edema model. By quantifying the extent of edema reduction and exploring the extract's impact on inflammation-related biochemical parameters, we seek to validate its traditional use and provide a scientific foundation for its development as a natural anti-inflammatory agent. This work contributes to the growing field of plant-based therapeutics and underscores the potential of *Cordia subcordata* (CS) in managing inflammatory conditions effectively.

MATERIAL AND METHODS

Materials

Cordia subcordata herb was collected in the month of July 2022 from local area of Tirupati, District-Tirupati, State-Andra Pradesh, India. From the collected plant material, the herbarium was prepared and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Botanical Department of Sri Venkateswara University, Tirupati-517 502, Andhra Pradesh, India. A voucher specimen no. 0559 was deposited. After collecting authentication certificate, the extraction procedure was performed. Oxycodone (Sigma Aldrich, St. Louis, MO, USA), and 0.9% sodium chloride (Hospira, Lake Forest, IL, USA), Folin Ciocalteu reagent, 2,2-diphenyl-1-picryl hydrazyl radical (DPPH), phenazine methosulfate, nicotinamide adenine dinucleotide, sodium nitroprusside (SNP), trichloroacetic acid (TCA), thiobarbituric acid (TBA), and L-ascorbic acid were purchased and procured from Lab trading laboratory, Aurangabad. All other chemicals and solvents used were of analytical grade available commercially.

Animals Used and Ethical Considerations

Male Wistar rats and mice weighing approximately 150-200 grams and 25-30 grams, respectively, were used for the research. They were fed a standard pellet diet with water adlibitum and housed under a 12-h light/dark cycle at a controlled temperature (22-250°C). The animals were allowed to acclimate for atleast a week before starting the experiment. Animal care and experimental procedures were performed following the guidelines of the Animal Ethics Committee (IAEC). The study was approved by Institutional animal ethical committee of Institute of Pharmaceutical education, Boradi, Shirpur, India, registered under CPCSEA, India (Registration No.1268/PO/Re/S/09/CPCSEA in resolution no. IPE/IAEC/2023 at Institute of Pharmaceutical education, Boradi, Shirpur, India.

Acute Toxicity Studies

Toxicity guidelines 423 were followed to test the extract toxicity. Animals should be fasted prior to dosing (e.g. with the rat, food but not water should be withheld over-night, with the mouse, food but not water should be withheld for 3-4 hours). Following the period of fasting, the animals should be weighed and the test substance administered. The animals were divided into different groups separately and were treated orally with individual drugs samples at a doses of 5, 50, 300 and 2000 mg/kg b.w. The animals were continuously observed for 1 hr., then frequently for 14 days. The animals were observed continuously for the initial 4 h and intermittently for the next six hours and then again at 24h and 48h following drug administration. No deaths were observed any of the groups and hence 200 and 400 mg/kg dose was selected for the extracts for the study [8,9].

Effect of CS on Paw withdrawal threshold (Von Frey Test)

Mechanical Allodynia: The Rat was placed individually on an acrylic cage elevated maze and adopted for the test environment for a minimum of 15 min. From the base of the mesh floor, Von Frey filament was applied to the planter aspect of the hind paw of the rat. Enough force from the filament was applied to the paw thus causing slight bending and holding for a second. Paw withdrawal was considered a positive response. Diclofenac was used as a standard drug; it was dissolved in 0.9% sodium chloride and administered at a dose of 100 mg/kg [10,11].

Effect of CS on Tail immersion test

Rats were divided into 6 animals in each group. The lower part of the tail (5 cm) was immersed in a beaker containing water maintained at 55 ± 0.5^0C. The time taken for the withdrawal of the tail from the water was recorded as a reaction time, with 10 sec as a cut-off time. The reaction time was noted one hour before the administration of drugs and as well one hour after the administration. The control group was provided with saline, whereas treatment groups were provided with CS (200 and 400 mg/kg) and, Diclofenac (100 mg/kg) was administered as a standard drug orally, 30 min before the test.

Effect of CS on Carrageenan-induced Paw Edema: -

Anti-inflammatory activity of CS as evaluated by carrageenan-induced rat paw edema method in rat models. Rats were divided into five groups of six animals each. Group 1 (Normal control group) was injected with saline and provided with the vehicle, group 2 (carrageenan control) was injected with carrageenan and was orally treated with the vehicle, groups 3 and 4 (Treatment 1) were treated with CS 200 and 400 mg/kg p.o., and group 5 (Standard group) treated with diclofenac sodium 100 mg/kg p.o.

The inflammation-inducing agent carrageenan, standard, and test drugs were administered in solution form using normal saline water as a vehicle. In this study, initially, animals were treated with drugs (vehicle, standard, and three treatments) as per the groups mentioned above. Subsequently, 1 h after the above treatment, 0.1 ml of 1% solution of carrageenan was injected subcutaneously into the subplantar region of the right hind paw to induce edema. The edema was expressed as the increment in paw volume due to carrageenan administration. The paw volume was measured initially and at 0, 3, and 5 h after carrageenan injection using a Plethysmometer. The percentage inhibition of paw thickness was calculated using the following formula,

$$\text{Inhibition of Paw Thickness (\%)} = 1 - \frac{V_t}{V_c} \times 100$$

Where, V_t is the mean relative change in paw volume in test groups and V_c is the mean relative change in paw volume in the control group [12–14].

Acetic acid-induced vascular permeability

Rats were treated with either CS or diclofenac at 100 mg/kg dose p.o. or with a vehicle. 1 h after the treatments, the individual mouse was injected i.v. with 2% Evan's blue solution at 10 mL/kg body weight through the tail vein. 10 mins later each mouse was injected with 06% acetic acid solution (in saline) i.p. at 10 mL/kg body weight. After 30 min of acetic acid injection, the mice were sacrificed, and the peritoneal cavity was washed with saline (10 mL) three times. The saline washes were subjected to centrifuge for 5 min at 3500 rpm. The supernatant was collected, and the absorbance was measured at 590 nm with a plate reader. Evans blue extravasation was enumerated from a standard curve and was expressed in a microgram [15].

Adjuvant-Induced Arthritis (AIA) in Rats

Inoculation of Freund's complete adjuvant (CFA) in rats caused the induction of arthritis. On day 0, the rats were anesthetized with ketamine and xylazine mixture (80:10 mg/kg, i.p.) and then the rats were injected with 0.1 ml CFA 1 mg/ml of heat-inactivated Mycobacterium tuberculosis in 85% paraffin oil and 15% mannide monooleate (Sigma Aldrich, St. Louis, MO, USA) intra-dermally at the base of the tail. Control group rats were injected with an equal volume of saline. Grouping was done as follows: Control (no adjuvant, saline), AIA (adjuvant, no treatment), CS (adjuvant, 200 and 400 mg/kg), and 100 mg/kg diclofenac was used as a standard drug administered p.o. Treatments were given daily from the first injection for 27 days. For the determination of hematological parameters blood samples were collected from the retro-orbital plexus for laboratory tests. Hematological parameters determined include Red Blood Cell (RBC), White Blood Cell (WBC), Platelet count, and Erythrocyte Sedimentation Rate (ESR) [16,17].

RESULTS AND DISCUSSION

Effect of CS on Paw Withdrawal Threshold

In Figure 1, table 1, the von Frey test illustrating pain threshold measurement is presented. A diminished withdrawal response was noted following non-noxious mechanical stimulation of the paw (akin to allodynia) in carrageenan-treated animals, which sustained a plateau post-treatment. Treatment with CS 400 and the standard medication ($p < 0.05$ and $p < 0.001$, respectively) exhibited a significant reduction in pain at 0 minutes, whereas CS 200 did not yield any notable effect at 0 minutes. Conversely, both CS and the standard medication demonstrated a complete blockade of carrageenan-induced hypersensitivity ($p < 0.001$) from 15 to 60 minutes.

Table 1: Effect of CS on paw withdrawal threshold.

Treatment	Paw withdrawal threshold (g)				
	0 min	15 min	30 min	45 min	60 min
Normal control	0.63±0.0097	0.66±0.009	0.67±0.01	0.68±0.012	0.65±0.01
Carrageenan control	0.28±0.014	0.22±0.006$^{\alpha}$	0.26±0.006$^{\alpha}$	0.28±0.009$^{\alpha}$	0.26±0.0076$^{\alpha}$
CS 200	0.30±0.016	0.56±0.017^{a}	0.59±0.012^{a}	0.51±0.012^{a}	0.49±0.016^{a}
CS 400	0.34±0.015^{c}	0.72±0.015^{a}	0.78±0.011^{a}	0.71± 0.014^{a}	0.69±0.014^{a}
Standard	0.38±0.014^{a}	0.79±0.025^{a}	0.83±0.020^{a}	0.78±0.018^{a}	0.77±0.019^{a}

Values are expressed as mean ± SEM. $^{\alpha}p < 0.001$, compared to the control group, $^{a}p < 0.001$, compared to the carrageenan control group. Difference between the groups was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test.

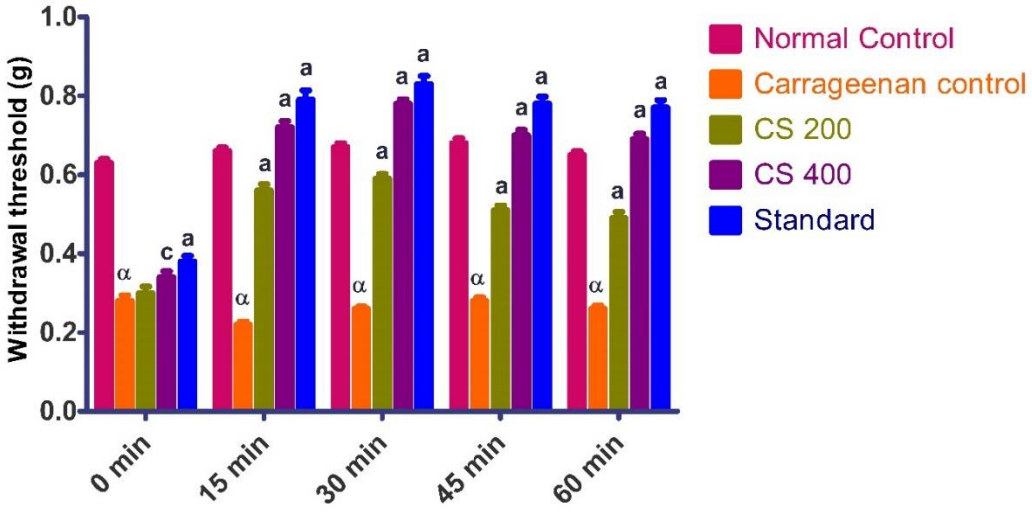


Figure 1: Effect of CS on paw withdrawal threshold

Effect of CS on Tail Withdrawal Reflexes-induced by Tail Immersion Method in Rats

The CS 400 and standard treatment groups demonstrated significant inhibition compared to the control group ($p < 0.001$), whereas the CS 200 group did not exhibit any significant effect compared to the normal control group. Analysis of Table 2 and Figure 2 reveals that CS exhibited noteworthy analgesic activity, albeit slightly lower than that of the standard drug.

Table 2: Effect of CS on tail withdrawal reflexes induced by tail immersion method in rats.

Drug (dose)	Before treatment (sec)	After treatment (sec)
Normal control	5.8 ± 0.087	5.9 ± 0.13
CS 200	5.6 ± 0.18	6.9 ± 0.35
CS 400	5.9 ± 0.19	8.7 ± 0.36[a]
Standard	5.6 ± 0.15	9.9 ± 0.25[a]

Values are expressed as mean ± SEM. [α]$p < 0.001$, compared to the control group, and [a]$p < 0.001$, compared to the carrageenan control group. Difference between the groups was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test.

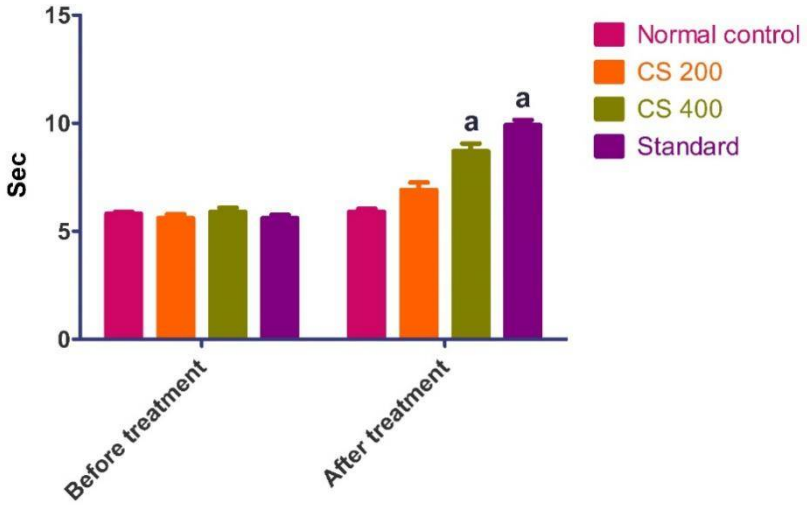


Figure 2: Effect of CS on tail withdrawal reflexes induced by tail immersion method in rats

Effect of CS on Carrageenan-induced Paw Edema

In the carrageenan-induced paw edema test in rats, subplantar injection of carrageenan resulted in a progressive increase in paw edema over time. Notably, at the 5th hour following carrageenan administration in the carrageenan control group, the maximum increase in edema was observed [18].

However, CS demonstrated significant inhibition of paw edema at both the 3rd and 5th hours after carrageenan administration ($p < 0.001$). Similarly, the standard anti-inflammatory drug diclofenac sodium also significantly reduced paw edema ($p < 0.001$) at the 3rd and 5th hours following carrageenan administration (Table 3, Figure 3).

Table 3: Effect of CS on carrageenan-induced paw edema.

Treatment		**Increase in paw edema (mL)**	
	O hours	**After 3 h**	**After 5 h**
Normal control	0.20 ± 0.009	0.22 ± 0.013	0.23 ± 0.012
Carrageenan control	0.63 ± 0.030α	0.92 ± 0.052$^{\alpha}$	0.90 ± 0.022 $^{\alpha}$
CS 200	0.53 ± 0.025	0.47 ± 0.020^{a}	0.45 ± 0.020^{a}
CS 400	0.47 ± 0.044b	0.32 ± 0.016^{a}	0.30 ± 0.018^{a}
Standard	0.34 ± 0.036a	0.21 ± 0.012^{a}	0.20 ± 0.011^{a}

Values are expressed as mean ± SEM. $^{\alpha}p < 0.001$, compared to the control group, and $^{a}p < 0.001$, compared to the carrageenan control group. Difference between the groups was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test.

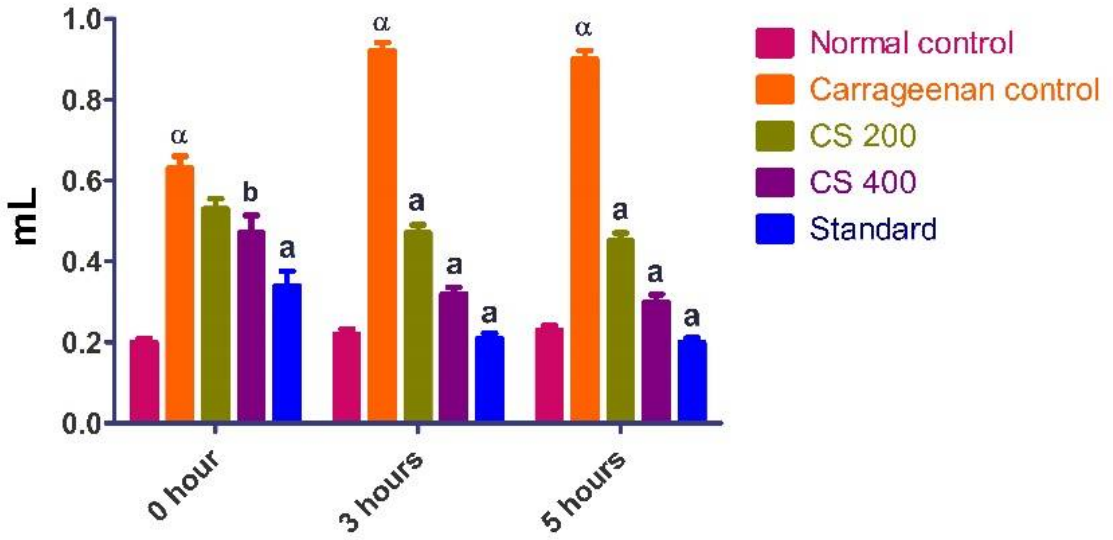


Figure 3: Effect of CS on carrageenan-induced paw edema

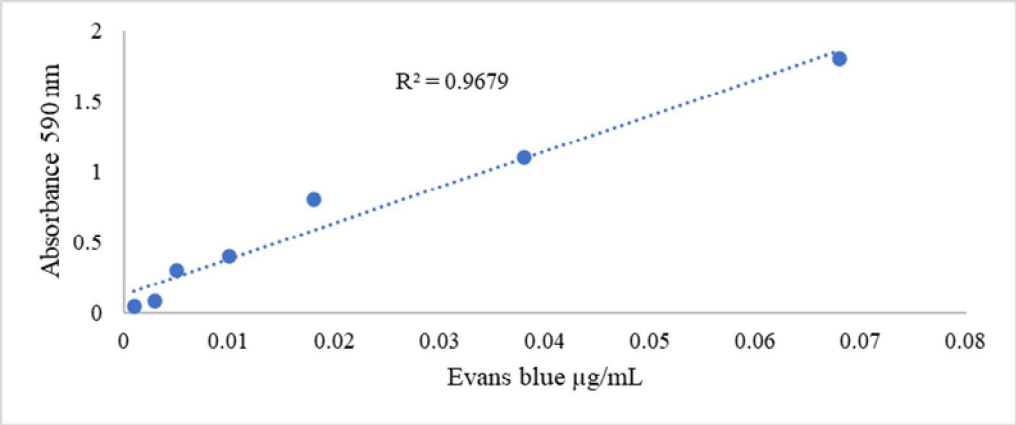
Effect of CS on Acetic acid-induced vascular permeability

CS demonstrated a greater reduction in dye leakage into the peritoneum, suggesting its enhanced anti-inflammatory action attributable to decreased vessel permeability (Table 4). In this experimental setup, both CS ($p < 0.001$) and Diclofenac sodium ($p < 0.001$) exhibited significant inhibition of dye leakage (Figure 4b).

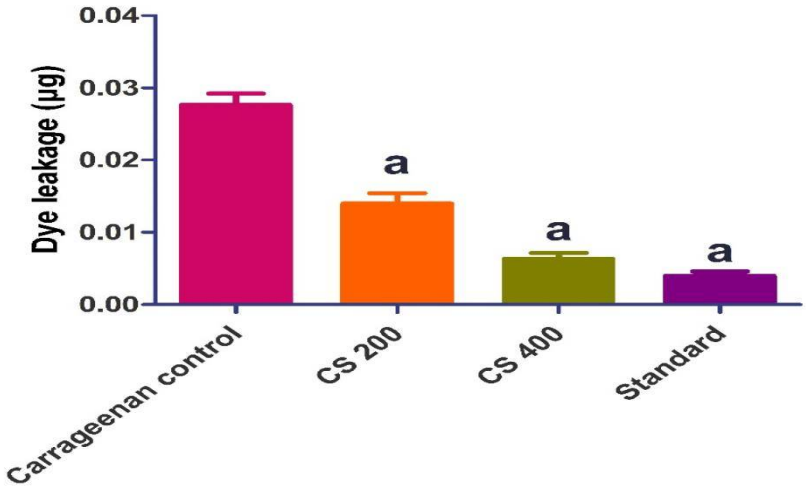
Table 4. Evans blue dye extravasation into the peritoneal cavity of Rats

Groups	**Dye Leakage (µg)**
Carrageenan control	0.028±0.0016
CS 200	0.014±0.0014^{a}
CS 400	0.0063±0.0008^{a}
Standard	0.004±0.0006^{a}

Values are expressed as mean ± SEM. $^{\alpha}p < 0.001$, compared to the control group, and $^{a}p < 0.001$, compared to the carrageenan control group. Difference between the groups was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test.



(A)



(B)

Figure 4. A) Evan blue dye standard curve; B) Effect of CS on Evans blue dye extravasation into the peritoneal cavity of mice

Adjuvant-induced Arthritis (AIA) in Rats

The paw volume exhibited a significant increase in the AIA group compared to the normal control group ($p < 0.001$) from day 8 to day 28 (Table 5 and 6). However, both the CS and diclofenac-treated groups displayed a notable reduction in paw edema compared to the normal control group ($p < 0.001$) (Figure 5).

Table 5: Effect of CS on increase in paw volume (Day 2-14)

Group	2nd day	4th day	6th day	8th day	10th day	12th day	14th day
Normal control	0.12±0.043	0.12±0.006	0.12±0.007	0.12±0.007	0.12±0.007	0.12±0.0058	0.14±0.0072
AIA control	0.14±0.004	0.14±0.004$^{\gamma}$	0.30±0.022$^{\alpha}$	0.35±0.014$^{\alpha}$	0.38±0.016$^{\alpha}$	0.39±0.010$^{\alpha}$	0.41±0.013$^{\alpha}$
CS 200	0.13±0.005	0.13±0.004^{c}	0.25±0.010^{c}	0.27±0.012^{a}	0.27±0.007^{a}	0.29±0.016^{a}	0.29±0.017^{a}
CS 400	0.12±0.006	0.12±0.007^{b}	0.17±0.007^{a}	0.17±0.007^{a}	0.17±0.01^{a}	0.19±0.006^{a}	0.21±0.01^{a}
Standard	0.12±0.005	0.12±0.0056^{b}	0.12±0.0073^{a}	0.12±0.007^{a}	0.12±0.008^{a}	0.14±0.005^{a}	0.17±0.005^{a}

Table 6: Effect of CS on increase in paw volume (Day 16-28)

Group	16th day	18th day	20th day	22nd day	24th day	26th day	28th day
Normal control	0.13±0.005	0.14±0.006	0.16±0.007	0.17±0.076	0.16±0.011	0.16±0.008	0.17±0.008
AIA control	0.45±0.0085$^{\alpha}$	0.47±0.007$^{\alpha}$	0.55±0.017$^{\alpha}$	0.73±0.029$^{\alpha}$	1.0±0.066$^{\alpha}$	1.1±0.068$^{\alpha}$	1.2±0.056$^{\alpha}$
CS 200	0.31±0.018^{a}	0.32±0.018^{a}	0.37±0.014^{a}	0.46±0.020^{a}	0.49±0.014^{a}	0.52±0.014^{a}	0.53±0.02^{a}
CS 400	0.27±0.014^{a}	0.26±0.018^{a}	0.28±0.010^{a}	0.40±0.022^{a}	0.42±0.023^{a}	0.44±0.030^{a}	0.45±0.023^{a}
Standard	0.20±0.011^{a}	0.21±0.011^{a}	0.22±0.009^{a}	0.34±0.026^{a}	0.36±0.021^{a}	0.37±0.021^{a}	0.39±0.018^{a}

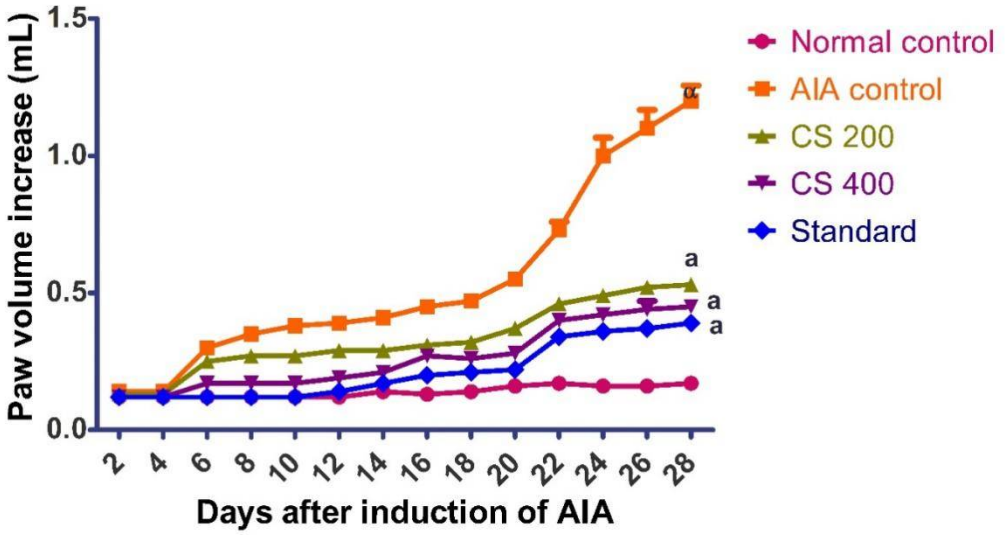


Figure 5. Anti-inflammatory effect of CS on AIA in rats.

Values are expressed as mean ± SEM. $^{\alpha}p < 0.001$, compared to the control group, and $^{a}p < 0.001$, compared to the carrageenan control group. Difference between the groups was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test.

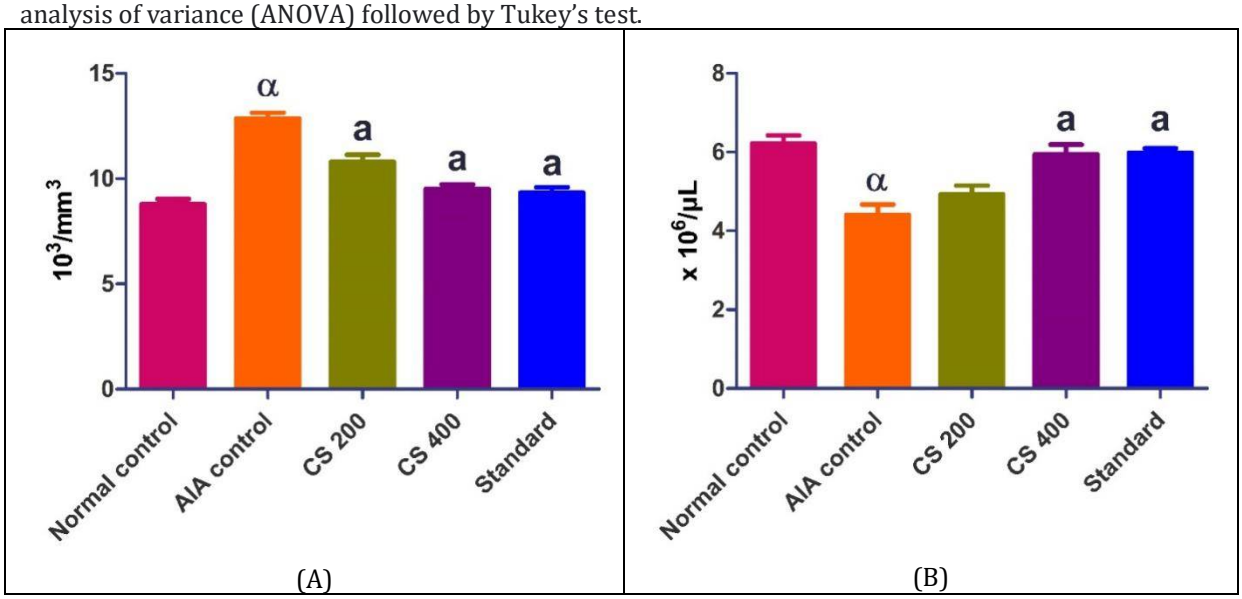
Effect of CS on hematological parameters of rats treated with Freund's complete adjuvant

Table 7 and Figure 6 illustrate the hematological alterations linked to the arthritic condition. In arthritic rats, the levels of white blood cells (WBC), platelet count, and erythrocyte sedimentation rate (ESR) were elevated, while the level of red blood cells (RBC) was decreased. However, upon treatment with CS and the standard drug, these levels were observed to approach normal levels compared to the AIA group.

Table 7: Effect of CS on the hematological parameter of rats treated with Freund's complete adjuvant.

Group	WBC count (10^3/mm^3)	RBC (x 10^6/µL)	Platelet count (10^5/mm^3)	ESR (mm/hr)
Normal control	8.8±0.25	6.2 ± 0.20	2.3 ± 0.12	4.0 ± 0.21
AIA control	13±0.27$^{\alpha}$	4.4 ± 0.27$^{\alpha}$	3.7 ± 0.08$^{\alpha}$	5.9 ± 0.15$^{\alpha}$
CS 200	11±0.34^{a}	4.9 ± 0.23	3.1 ± 0.11^{b}	5.3 ± 0.14^{c}
CS 400	9.5±0.23^{a}	5.9 ± 0.25^{a}	2.8 ± 0.11^{a}	4.9 ± 0.07^{a}
Standard	9.3±0.27^{a}	6.0 ± 0.12^{a}	2.4 ± 0.049^{a}	4.3 ± 0.07^{a}

Values are expressed as mean ± SEM. $^{\alpha}p < 0.001$, compared to the control group, $^{a}p < 0.001$, $^{b}p < 0.01$, compared to the carrageenan control group. Difference between the groups was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test.



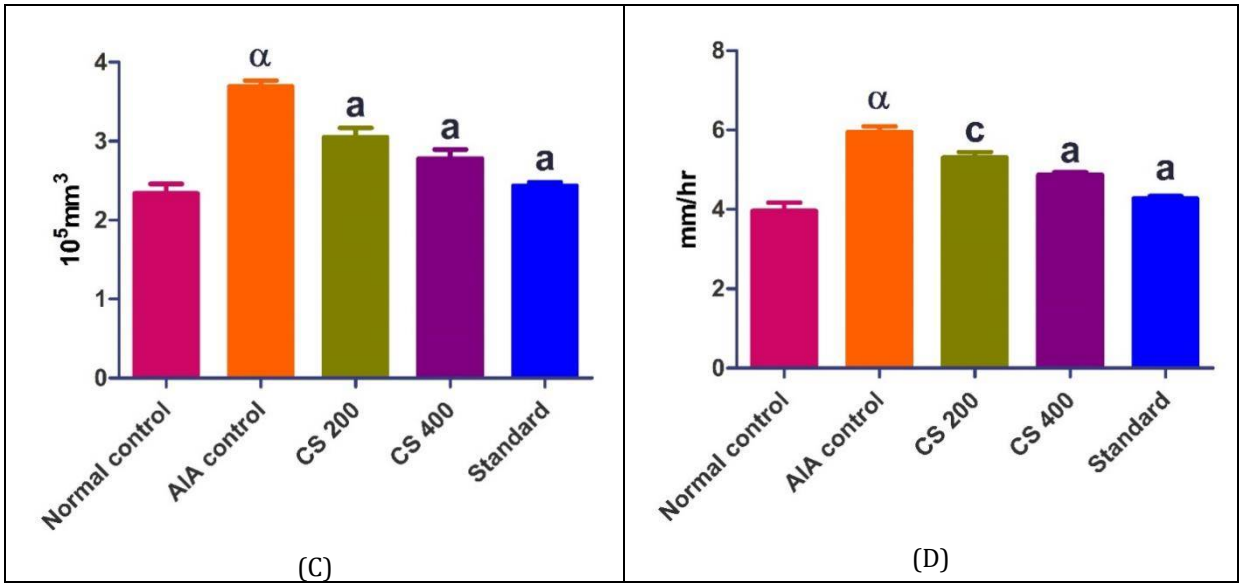


Figure 6. A) Effect of CS on WBC levels; B) Effect of CS on RBC levels; C) Effect of CS on platelet count; D) Effect of CS on ESR levels

CONCLUSION

The findings from this study demonstrate the significant analgesic, anti-inflammatory and anti-arthritic potential of *Cordia subcordata* extract across multiple experimental models. CS effectively inhibited carrageenan-induced paw edema and hypersensitivity, reduced vascular permeability, and alleviated inflammation and pain in adjuvant-induced arthritis. Additionally, CS restored hematological parameters altered by arthritis, highlighting its systemic anti-inflammatory effects. While the 400 mg/kg dose exhibited consistent efficacy, its activity, though substantial, was slightly less potent than the standard drug diclofenac sodium. These results validate the traditional use of *Cordia subcordata* and suggest its potential as a natural alternative to synthetic anti-inflammatory drugs. Further studies to isolate and characterize the bioactive compounds are essential for understanding its mechanism of action and enhancing its therapeutic applications.

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