



Research Article

PHARMACOLOGICAL EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF VALLARAI CHOORANAM USING CARRAGEENAN-INDUCED PAW EDEMA IN WISTAR ALBINO RATS

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ABSTRACT

The aim of the study was to explore the anti-inflammatory activity of Siddha polyherbal formulation *Vallarai Chooranam* in albino rats, and to compared with the standard drug indomethacin. The Siddha formulation *Vallarai Chooranam* is indicated for *Azhal Neerchurukku* which are comparable to urinary tract infection in the Siddha system of medicine. The current study used a carrageenan-induced paw edema model in Wistar albino rats to assess the anti-inflammatory properties of *Vallarai Chooranam*. The animals were divided into four groups (n=6): two test groups given VC at doses of 200mg/kg and 400mg/kg of body weight, a standard group treated with indomethacin at 10mg/kg and a disease control group. Acute inflammation was induced by injecting carrageenan, and paw edema was measured hourly for up to five hours. Additionally, histopathological analysis and hematological parameters were carried out. Dunnett's test and one-way ANOVA were used to analyze the data, which were expressed as mean±SEM. p-value<0.05 was deemed statistically significant. VC administration significantly reduced paw edema, with effects that increased with higher doses; the most effective dose showed results similar to the standard treatment. Histopathological results showed decreased infiltration of inflammatory cells, and hematological parameters were considerably improved. The study results support the traditional use of *Vallarai Chooranam*, confirming its strong anti-inflammatory properties.

INTRODUCTION

Urinary tract infection (UTI) is defined as the bacterial invasion of the urinary tract. It can occur anywhere between the urethra and the kidney. UTI is the commonest of all bacterial infections. It is more common in women because of a short urethra (4cm), and up to 50% of women have a UTI at some time. [1] The clinical features of UTI are abrupt onset of frequency of micturition and urgency, oliguria, haematuria, dysuria, incontinence, and suprapubic pain during and after voiding. The Urine may appear cloudy and have an unpleasant odor. The Siddha system of medicine, one of the oldest traditional medical systems practiced in South India, employs

numerous herbal formulations for *Azhal Neerchurukku* compared to urinary tract infection. *Vallarai Chooranam* (VC) is a polyherbal formulation composed of medicinal plants such as *Centella asiatica*, *Myristica fragrans*, *Elettaria cardamomum*, *Syzygium aromaticum*, *Embllica officinalis*, *Terminalia chebula*, and *Terminalia bellerica*. It is traditionally indicated for *Megam* (syphilis), *Piramiyam* (gonorrhoea), *Neererivu* (burning micturition), *Neerozhukal* (incontinence of urine), *Kan erivu* (burning sensation of the eyes), urinary tract infections, leucorrhoea, etc.[2] *Centella asiatica* is the main ingredient in VC, and its leaf extract was very effective at reducing inflammation by inhibiting nitric oxide (NO) production, which, in turn, suppresses inflammatory processes.[3] The study also showed that the extract could eliminate biofilm-forming bacteria, which are often pathogenic. These actions are linked to the bioactive compounds present in the ethanolic extract, including asiatic acid, madasiatic acid, and madecassic acid.[4] Inflammation is a complex process involving immune pathways, such

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as cyclooxygenase (COX) enzymes, and the release of pro-inflammatory cytokines like tumor necrosis factor- α , interleukin-1 β , and interleukin-6, as well as increased prostaglandin production.^[5,6] The carrageenan-induced paw edema model is widely used to study acute inflammation in animals, as it shows both the early and later stages of inflammation, making it useful for testing anti-inflammatory agents.^[7] Despite its widespread traditional use, scientific validation of its pharmacological activity remains limited. The standardization of VC was done in the Regional Research Institute of Unani Medicine, Chennai. The quantitative test for preliminary phytochemical screening of *Vallarai chooranam* shows the presence of carbohydrates, reducing sugar, flavonoids and phytosterols. Therefore, the present study was undertaken to evaluate the anti-inflammatory activity of *Vallarai Chooranam* using the carrageenan-induced paw edema model in Wistar albino rats.

MATERIALS AND METHODS

Experimental Animals

Healthy Wistar albino rats weighing 150–200 g were selected and housed in polypropylene cages with rice husk bedding under standard laboratory conditions (temperature 24 \pm 1 $^{\circ}$ C, relative humidity 55 \pm 15%, 12-hour light/dark cycle). The rats had free access to standard rat food (Sai Durga Feeds and Foods, Bangalore, India) and water. Ethical approval for animal care and use was obtained from the Institute Animal Ethics Committee (IAEC) -proposal

Group	Treatment	Dose
Group I	Disease control [carrageenan]	Normal saline + carrageenan
Group II	VC low dose [200 mg/kg]	[200mg/kg]
Group III	VC high dose [400 mg/kg]	[400mg/kg]
Group IV	Standard	[carrageenan + indomethacin 10mg/kg]

Hematological Evaluation

Blood samples were obtained by retro-orbital puncture under light anesthesia at the conclusion of the trial period. An automated hematology analyzer was used to examine hematological parameters such as the platelet count, total leukocyte count (WBC), hemoglobin (Hb), red blood cell count (RBC), and differential leukocyte count.

Histopathological Analysis

After completion of the experiment rats were euthanized by cervical dislocation, paw tissues were collected, fixed in formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The sections were examined microscopically for inflammatory changes.

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Chemicals and Drugs

Carrageenan (1% solution) was used for induction of inflammation. Indomethacin (10mg/kg) was used as the standard drug. All chemicals used were of analytical grade.

Drug Administration

The test drug *Vallarai Chooranam* and indomethacin were administered orally using an oral gavage tube. The required doses were freshly prepared before administration.

Anti-Inflammatory Activity

Acute inflammation was induced by injecting 1% carrageenan into the right hind paw. After acclimatization, Animals were divided into four groups (n=6). The rats were given different doses of *V.Chooranam*, indomethacin, and regular saline an hour before the carrageenan injection. All treatments were given one hour before the carrageenan injection.^[8] Paw volume was measured before injection and at one, two, three, four, and five hours after induction using a plethysmometer. The percentage inhibition of edema, indicating the anti-inflammatory effect was calculated using the following formula: [9]

$$\text{Inhibition of paw edema (\%)} = (O_c - O_t) / O_c \times 100$$

Where O_c represents the mean paw volume of the disease control group and O_t represents the mean paw volume of the treated group.

Statistical Analysis

Data were expressed as mean \pm SEM. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test. A p-value <0.05 was considered statistically significant.

RESULTS

Effect of *Vallarai Chooranam* on Paw Edema

One hour after giving the rats low or high doses of VC or indomethacin (10mg/kg body weight) by mouth, 1% carrageenan was injected into the right hind paw to induce acute inflammation. Paw swelling was measured every hour for five hours. The disease control group showed a steady increase in paw volume, reaching its highest point at the fifth hour. Both VC and indomethacin treatments significantly

reduced paw swelling at all time points compared to the disease control group (Table 1, Figure 1).

Table 1: Effect of Vallarai Chooranam on carrageenan-induced paw edema

Group	1 st Hr	2 nd Hr	3 rd Hr	4 th Hr	5 th Hr
Group 1 (Carrageenan)	1.72±0.01	1.78±0.01	1.83±0.01	1.88±0.03	1.92±0.04
Group 2 - Low	1.64±0.02	1.56±0.02	1.62±0.02	1.49±0.02	1.46±0.03
Group 3 - High	1.50±0.02	1.42±0.02	1.47±0.03	1.37±0.02	1.22±0.02
Group 4 - (Indomethacin (10mg/Kg/Bw))	1.55±0.01	1.44±0.02	1.54±0.02	1.48±0.02	1.30±0.03

All values are expressed as mean ± SEM (n=6). *p<0.05, **p<0.01, ***p<0.001. VC showed significant inhibition of paw edema compared to the disease control group. The high dose exhibited maximum inhibition at the 5th hour.

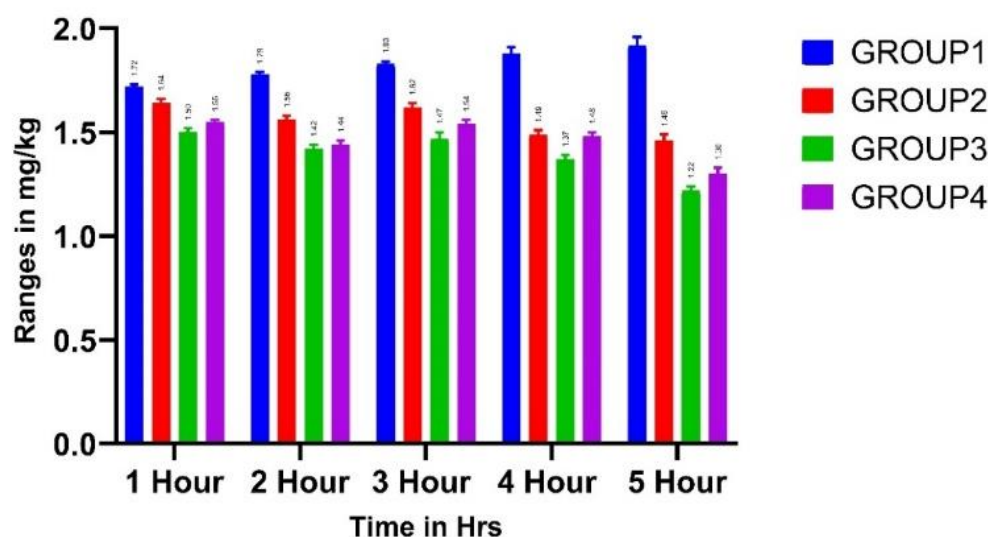


Figure 1: Effect of Vallarai Chooranam on carrageenan-induced paw edema in rats

Percentage Inhibition of Carrageenan-Induced Edema

Table 2 and Figure 2 show the percentage inhibition of paw edema in comparison to disease control. VC clearly showed that inflammation was inhibited in a dose-dependent manner. In contrast to the 38.92% shown with indomethacin, the percentage inhibition at the fifth hour was 27.64% in the VC 200mg/kg group and 42.58% in the VC 400mg/kg group. At the fifth hour, the high dose of VC showed the greatest reduction in paw swelling, followed by indomethacin and the low dose of VC (Table 2). Significant differences between the treatment groups and the disease control were found by statistical analysis (P<0.05–0.001). Sustained anti-inflammatory activity is indicated by the percentage inhibition gradually increasing over time.

Table 2: % Inhibition of paw edema in Vallarai Chooranam and Carrageenan treated rats

Groups	1 st Hour	2 nd Hour	3 rd Hour	4 th Hour	5 th Hour
Disease control (carrageenan)	-	-	-	-	-
Low Dose	5.42	12.86	16.94	22.38	27.64
High Dose	16.28	24.92	28.46	35.12	42.58
Indomethacin	12.64	22.48	25.96	31.78	38.92

*All values were expressed as mean ± SEM, n = 6, * P < 0.05, **P < 0.01, ***P < 0.001 as compared to the disease control group. Results were done by one-way ANOVA followed by Dunnett's test

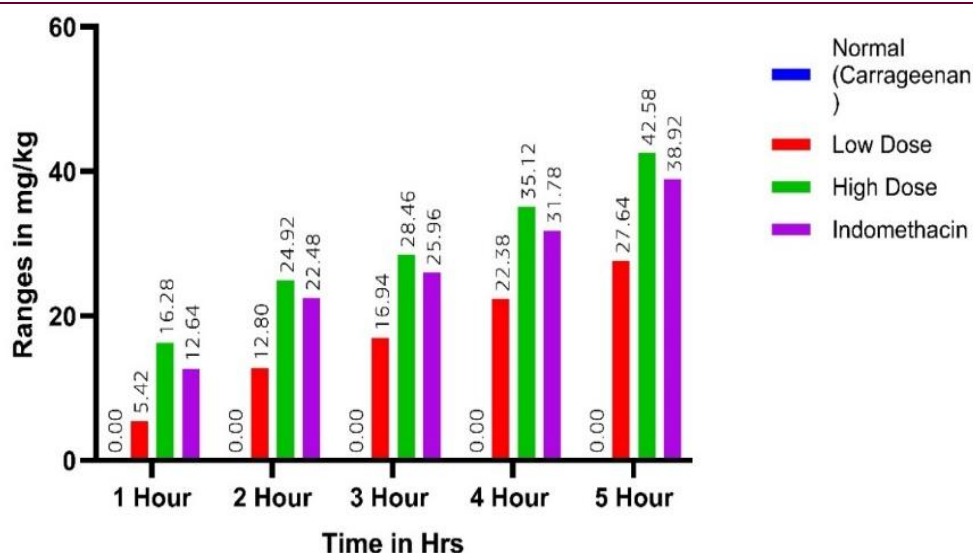


Figure 2: % Inhibition of paw edema in *Vallarai Chooranam* and Carrageenan treated rats

Effect on Hematological Parameters

Systemic inflammatory activation was demonstrated by the significant increase in total leukocyte count ($12.86 \pm 0.56 \times 10^9/L$) and granulocyte and eosinophil fractions that followed carrageenan-induced inflammation (Table 3).

These hematological changes were considerably altered by VC treatment. The total leukocyte count in the high-dose group decreased to $8.74 \pm 0.52 \times 10^9/L$, which was comparable to the indomethacin group's values of $10.08 \pm 0.44 \times 10^9/L$. When comparing VC-treated animals to disease control, differential leukocyte analysis revealed lower granulocyte and eosinophil/basophil/monocyte counts (Table 3). Furthermore, when compared to untreated inflammatory animals, the VC-treated groups showed improved hemoglobin concentration, and red blood cell count. Compared to the disease control group, VC-treated animals showed higher platelet counts which may indicate that the inflammatory and vascular responses had stabilized.

Table 3: Effects of *Vallarai Chooranam* on hematological parameters of rats induced with inflammation using Carragenan

Parameters	Untreated control	VC Low	VC High	Indomethacin
RBC ($\times 10^{12}/L$)	5.74 ± 0.26	6.82 ± 0.41	7.42 ± 0.24	5.98 ± 0.33
Hematocrit (%)	36.92 ± 1.64	41.36 ± 1.58	44.68 ± 1.22	38.86 ± 1.47
Hemoglobin (g/dL)	11.94 ± 0.55	13.62 ± 0.69	14.54 ± 0.48	12.38 ± 0.62
Platelet ($\times 10^9/L$)	312.40 ± 58.76	536.20 ± 46.92	612.80 ± 34.16	334.80 ± 52.64
WBC ($\times 10^9/L$)	12.86 ± 0.56	9.46 ± 0.38	8.74 ± 0.52	10.08 ± 0.44
Lymphocyte ($\times 10^9/L$)	9.42 ± 0.71	7.62 ± 0.41	7.06 ± 0.47	8.04 ± 0.59
Granulocyte ($\times 10^9/L$)	0.72 ± 0.11	0.66 ± 0.09	0.48 ± 0.05	0.92 ± 0.14
Eosinophil, Basophil, Monocyte ($\times 10^9/L$)	2.72 ± 0.18	0.98 ± 0.16	0.84 ± 0.12	1.12 ± 0.22

*All values were expressed as mean \pm SEM, n = 6, * P < 0.05, **P < 0.01, ***P < 0.001 as compared to the disease control group. Results were done by one-way ANOVA followed by Dunnett's test.

Histopathological Findings

Histopathological analysis of H&E- stained paw tissue revealed clear signs of inflammation in the disease control group, including moderate cell infiltration and widespread chronic granulomatous inflammation (Figure 3A). Low-dose VC treatment led to minimal cell infiltration (Figure 3B), and high-dose VC treatment also showed minimal inflammatory changes (Figure 3C). Rats treated with indomethacin also showed minimal inflammatory cell infiltration (Figure 3D). Notably, the high dose of VC reduced cellular infiltration as much as, or slightly more than, indomethacin.

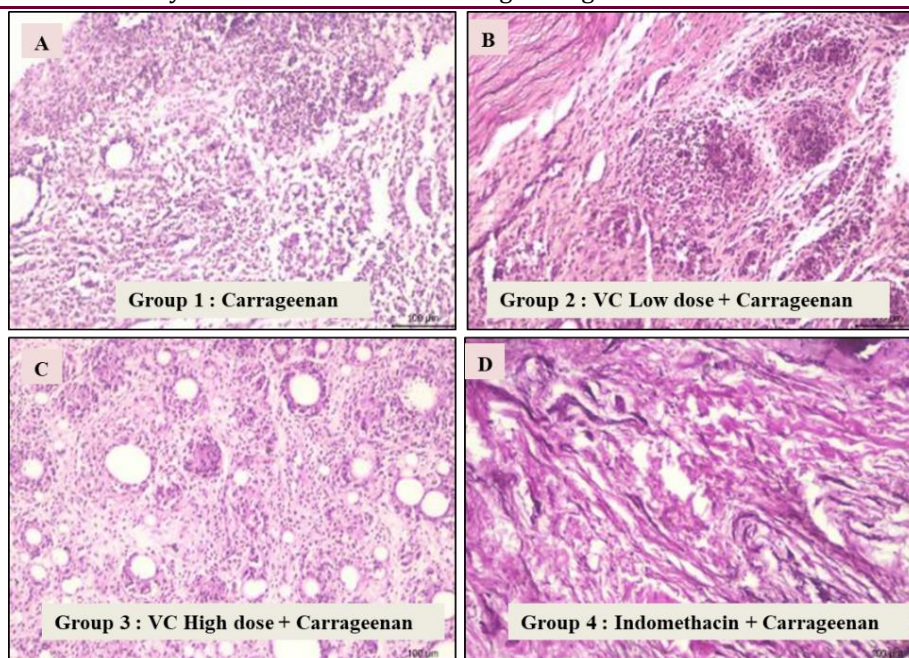


Figure 3: Histopathological Analysis of Paw Tissue (H&E Staining)

DISCUSSION

This study shows that *Vallarai Chooranam* (VC) has strong anti-inflammatory effects in the carrageenan-induced paw edema model in Wistar rats. The carrageenan model is a standard way to study acute inflammation, with an early phase (0–2 hours) driven by histamine and serotonin, and a late phase (3–5 hours) linked to prostaglandin production and COX-2 activation.^[8] The significant reduction in inflammation, especially in the later phase, suggests that VC may affect prostaglandin-related pathways.

The high dose of VC greatly reduced paw swelling, performing as well as or slightly better than indomethacin at the fifth hour. Since NSAIDs like indomethacin work by blocking COX enzymes and lowering prostaglandin production^[10], VC's similar effect suggests it may act on the same inflammatory mediators. The herbal blend contains flavonoids, phenolic compounds, and triterpenoids, which are known to block COX activity, reduce nitric oxide (NO) production, and lower levels of pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6.^[11,12]

Centella asiatica, the main ingredient in VC, has been shown to reduce inflammation by blocking nitric oxide production and affecting NF- κ B signaling pathways.^[13] Triterpenoids like asiatic acid and madecassic acid can lower the release of inflammatory mediators and decrease oxidative stress.^[14] Other ingredients, such as *Embllica officinalis* and *Terminalia* species, also have strong antioxidant and anti-inflammatory effects, which may work together to enhance the overall benefit.^[15]

The blood test results also support VC's anti-inflammatory effects. Carrageenan-induced

inflammation usually causes an increase in white blood cells and changes in immune cell types.^[16] VC treatment, especially at the higher dose, significantly lowered total and differential WBC counts, showing a reduction in the body's inflammatory response. The increases in RBC, hemoglobin, hematocrit, and platelet counts suggest that VC may help stabilize inflammation and support immune function. These changes are linked to lower levels of inflammatory cytokines.^[17]

Histopathological examination corroborated the biochemical findings, revealing reduced cellular infiltration and minimal granulomatous inflammation in VC-treated groups. The reduction in inflammatory cell migration suggests inhibition of chemotactic factors and of vascular permeability changes characteristic of acute inflammation.^[18]

Overall, the present study provides experimental evidence supporting the traditional use of *Vallarai Chooranam* in the treatment of inflammatory conditions. The anti-inflammatory effect may be attributed to inhibition of prostaglandin synthesis, suppression of pro-inflammatory cytokines, antioxidant activity, and modulation of immune responses. Further studies focusing on molecular targets such as COX-2, inducible nitric oxide synthase (iNOS), and NF- κ B signaling pathways are warranted to elucidate the precise mechanisms underlying its therapeutic action.

CONCLUSION

The findings of the present study provide scientific evidence supporting the therapeutic potential of VC as an anti-inflammatory formulation. The observed dose-dependent effects in the

experimental model indicate its promising efficacy in controlling acute inflammatory responses. The improvement in systemic parameters and histological architecture further suggests a beneficial modulatory effect on inflammation-associated alterations.

Overall, this study substantiates the traditional claims of *Vallarai Chooranam* and highlights its potential as a safer plant-based alternative for the management of inflammatory conditions. However, further molecular and clinical investigations are required to confirm its mechanism of action and translational applicability in human inflammatory disorders.

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