



# EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *CATHARANTHUS ROSEUS* FLOWERS IN CHICKS

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## ABSTRACT

**AIM:** The present study investigates the anti-inflammatory activity of hydroalcoholic extract from *Catharanthus roseus* flowers in chicks. Recognized for its traditional medicinal applications, *Catharanthus roseus* contains bioactive compounds with potential therapeutic effects. The study begins with the collection and authentication of plant material from Virudhunagar District, Tamil Nadu, followed by extraction using a hydroalcoholic solution. Preliminary phytochemical analysis confirms the presence of flavonoids, alkaloids, glycosides, steroids, terpenoids, reducing sugars, quinones, tannins, and phenolic compounds.

**METHOD:** In the pharmacological evaluation, acute toxicity studies guided dose selection. Chicks were divided into five groups, including a normal control, a carrageenan-induced inflammation control, a standard treatment with indomethacin, and two test groups receiving *Catharanthus roseus* flower extract (CRFE) at 100 mg/kg and 150 mg/kg. Inflammation was induced using a carrageenan-induced foot edema model, with subsequent measurement of foot volume to assess inflammation.

**RESULTS:** It demonstrated a dose-dependent anti-inflammatory effect of CRFE, with the higher dose (150 mg/kg) showing significant activity compared to the lower dose (100 mg/kg) and the control group. Statistical analysis using ANOVA confirmed the significance of these findings ( $p < 0.05$ ). These findings suggest that CRFE possesses substantial anti-inflammatory properties, warranting further investigation into its therapeutic potential and underlying mechanisms.

**CONCLUSION:** It based on the results, it appears that the hydroalcoholic extract of *Catharanthus Roseus* flowers may have anti-inflammatory effects in chicks, with the higher dose showing s more prominent effect. Further studies, including additional parameters and mechanisms, would be valuable to better understand the potential therapeutic applications of CRFE in inflammation.

**KEYWORDS:** *Catharanthus roseus*, Anti-inflammatory, Chicks, Carrageenan-induced inflammation, Herbal medicine.

## INTRODUCTION

Natural products have been a main source to identify the new molecule of drugs<sup>1</sup>. Herbal medicines, also known as phytomedicine, are a blend of chemical components that produce specific effects. Around 75% of the world's population relies on herbs for medicine, with 25-30% of modern drugs being chemical intermediates derived from plant constituents. Despite the rise of synthetic medicine, herbal medicines remain in high demand due to their nontoxicity, accessibility, and affordability<sup>2</sup>. A herbal Renaissance is emerging worldwide, with folk medicine incorporating plant, animal, and mineral-based medicines, spiritual therapies, manual techniques, and exercise to treat, diagnose, and prevent illness or maintain well-being<sup>3</sup>. Inflammation, a process where white blood cells and their products protect against infection, can cause diseases like arthritis. Chronic inflammation is an ongoing, long-term response to inflammatory stimuli, characterized by the accumulation of mononuclear leukocytes and tissue injury<sup>4,5</sup>. *Catharanthus Roseus* flower is traditionally used for anti-inflammatory effects, treating various conditions like cancer, diabetes, and fever. It stabilizes and prevents denaturation in non-steroidal anti-inflammatory medicines<sup>6</sup>. The study aims to evaluate the anti-inflammatory activity of a hydroalcoholic extract of *Catharanthus roseus* flowers in chicks. The process includes selecting, collecting, and authenticating plant material, extracting it with hydro alcohol, conducting preliminary phytochemical analysis, evaluating the anti-inflammatory activity, and analyzing the results statistically.

## MATERIALS AND METHOD

### Plant collection and authentication:

The plant *Catharanthus roseus* was collected from Virudhunagar District, Tamil Nadu, and authenticated from Ayya Nadar Janaki Ammal College. The flowers were dried, crushed to a coarse powder, and stored in an airtight plastic container for further use.

### Preparation of crude extracts

The drug was extracted by the maceration method. The coarsely powdered flowers (250 mg) were taken in a round bottom flask with a hydroalcoholic solution (1:1) ratio for 48 hours at room temperature. After extraction, the extracts were evaporated or concentrated by using a rotary evaporator and dried at room temperature. The obtained crude extracts were weighed and stored at 4 °C for further analysis and the percentage yield of Aqueous flower extract of *Catharanthus Roseus* (CRFE) was 10% w/w<sup>7</sup>.

## QUALITATIVE SCREENING OF PHYTOCHEMICALS PRESENT IN CATHARANTHUS ROSEUS FLOWER EXTRACT

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**Preparation of test sample:** A small quantity of the extract was dissolved in 5 ml of distilled water and filtered. The filtrate was tested to detect the presence of various phytochemical constituents in the sample<sup>8</sup>.

**Phytochemical screening:** Tests include frothing for saponins, tannins, triterpenes, alkaloids, flavonoids, sugar reduction, quinines, and proteins. The extract is boiled with water for two minutes, then mixed vigorously and left for three minutes. The presence of frothing indicates the presence of saponins. The extract is then mixed with sodium chloride to make a 2% strength solution, filtered, and mixed with a gelatin solution. The presence of triterpenes is indicated by the red color of the chloroform solution. The extract is then digested with 2 M HCl and mixed with amyl alcohol to detect the pink color of alkaloids. Flavonoids are determined using a 1% aluminum chloride solution in methanol, concentrated HCl, magnesium turnin, and potassium hydroxide solution. The presence of sugar is indicated by the red color of Fehling's A and B solutions. Quinines are indicated by the blue-green or red appearance of 1% NaOH. Finally, the presence of proteins is indicated by the yellow color of mercuric chloride<sup>9, 10</sup>.

## INDUCTION OF INFLAMMATION

The study evaluated inflammation in seven-day-old chicks using a carrageenan-induced foot edema model. Indomethacin was used as a positive control, and various extracts were suspended in sterile distilled water. Carrageenan was injected sub-plantar into the chicks' footpads to induce edema. Extracts were administered at different concentrations, and foot volume was measured before and after injection<sup>12</sup>. The control animals received tween-20 in sterile water. All drugs and extracts were orally administered in volumes not exceeding 100 mL/kg<sup>13</sup>.

## MEASUREMENT OF INFLAMMATION INDUCTION

measured inflammation induction by normalizing foot volume increase scores for each chick, averaging them for each treatment group, and computing the increase using the following equation<sup>14</sup>.

$$\% \text{Increase of foot volume} = \frac{\text{Foot volume at time t} - \text{Foot volume at time zero}}{\text{Foot volume at the time}} \times 100$$

Area under the curve (AUC), The percentage inhibition of edema for each treatment group was then determined as follows:

$$\% \text{ Inhibition of edema} = \frac{\text{AUC control} - \text{AUC treatment}}{\text{AUC control}} \times 100$$

**Pharmacological evaluation of Catharanthus roseus leaves extract**

Acute toxicity study Based on a previously conducted study, the dose was selected. the experimental model was Chicks were divided into 5 groups, each containing 3 animals, as follows: The chicks were given the following as in the table: normal, control, standard, and CRS (*Catharanthus roseus* extract) injection (tests 1 & 2), and the results are noted<sup>11</sup>.

**Table 1 : Experimetnal design**

GROUP	GROUP SPECIFICATION	INTERVENTION
Group I	Normal	Saline
Group II	Control	Carrageenan
Group III	Standard	Indomethacin(100mg)
Group IV	Test 1	CRS Extract (100mg)
Group V	Test 2	CRS Extract (150mg)

**STATISTICAL ANALYSIS**

Data' s of all the parameters were analyzed using the Graph pad 5.0 software. Analysis of Variance (ANOVA); one-way ANOVA followed by Dunnett's comparison test was performed. The values were expressed as Mean  $\pm$  SEM. P value <0.05 was considered as significant<sup>15</sup>.

**RESULTS****PRELIMINARY PHYTOCHEMICAL ANALYSIS OF CRFE**

The present study was carried out on extracts of *Catharanthus Roseus* leaves. The results have confirmed that *Catharanthus Roseus* leaves contain flavonoids, alkaloids, glycosides, and less extent steroids, terpenoids, Reducing Sugar, quinine, tannins, and phenolic compounds. Complete details of the presence of phytochemicals in each crude extract are presented in Table 2.

**Table 2. Phytochemical Analysis of *Catharanthus Roseus* Flowers Extract**

S.NO	PHYTOCHEMICAL CONSTITUENTS	CRFE RESULT
1	Saponins	Positive
2	Tannins	Positive
3	Triterpenes	Positive
4	Alkaloids	Positive
5	Flavonoids	Positive
6	Reducing Sugar	Positive
7	Quinines	Positive
8	Proteins	Positive
9	Steroids	Positive

**Evaluation of the anti-inflammatory effect of CRFE in the carrageenan-induced inflammation model**

**Figures 1 and 2: The induction of inflammation using carrageenan in chick paw.**



**Table 3: Experimental Design for Induced Inflammation**

S. NO	GROUP	INITIAL FOOT VOLUME(MM)	INFLAMMATION INDUCED(MM)	FINAL FOOT VOLUME(MM)
1	Normal (Saline)	3.52±0.36	-	3.52±0.36
2	Control (Carrageenan)	3.53±0.07	4.12±0.08	4.38±0.06
3	Standard (Indomethacin 100 mg per kg)	3.48±0.03	4.03±0.06	3.50±0.04
4	Test 1 (CRFE 100 mg per Kg Low Dose)	3.68±0.07	4.10±0.03	3.79±0.02
5	Test 2 (CRFE 150 mg per kg High Dose)	3.54±0.03	4.13±0.06	3.58±0.08

In Test 1, where a low dose of CRFE (*Catharanthus Roseus* flower extract) at 100 mg per kg was administered, there was a noticeable but moderate increase in the anti-inflammatory effect. The measured parameters showed a consistent trend, with values of 3.68±0.07, 4.10±0.03, and 3.79±0.02. This suggests that even at a lower dose, the extract has the potential to exert anti-inflammatory effects, albeit to a lesser extent.

In Test 2, a higher dose of CRFE at 150 mg per kg was administered, resulting in a more significant anti-inflammatory response. The measured parameters increased to 3.54±0.03, 4.13±0.06, and 3.58±0.08. This indicates a dose-dependent enhancement of the anti-inflammatory activity, reinforcing the potential of the hydroalcoholic extract of *Catharanthus Roseus* flower as an anti-inflammatory agent. Complete details of results of the anti-inflammatory effect of CRFE in the carrageenan-induced inflammation model

## DISCUSSION

The results suggest that both the low and high doses of CRFE may possess anti-inflammatory properties, as indicated by the reduction in foot volume compared to the carrageenan-induced inflammation in the control group.

The effect of CRFE seems to be dose-dependent, with the higher dose (Test 2) showing a slightly more pronounced effect than the lower dose (Test 1). Indomethacin, the standard anti-inflammatory drug, serves as a positive control, validating the efficacy of the experimental setup.

## CONCLUSION

Based on the results obtained from the evaluation of the anti-inflammatory activity of the hydro-alcoholic extract of *Catharanthus Roseus* flower in chicks, it is evident that the extract exhibited a dose-dependent response.

In conclusion, based on the results, it appears that the hydroalcoholic extract of *Catharanthus Roseus* flowers may have anti-inflammatory effects in chicks, with the higher dose (Test 1) showing a more prominent effect. Further studies, including additional parameters and mechanisms, would be valuable to better understand the potential therapeutic applications of CRFE in inflammation.

Overall, these findings support the hypothesis that the hydroalcoholic extract of *Catharanthus Roseus* flower possesses anti-inflammatory properties in chicks, and the effect is more pronounced with higher doses. Further studies and investigations are warranted to elucidate the underlying mechanisms and assess the safety and efficacy of this extract for potential therapeutic applications in inflammatory conditions.

## ABBREVIATIONS:

CRFE – *Catharanthus roseus*.

## FUNDING:

Nil.

## AUTHOR CONTRIBUTIONS:

All the authors have contributed equally.

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