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Evaluation of Anti-Inflammatory Activity of Gloriosa Superba Using Various In-Vitro Methods

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Abstract **Original Research Article**

Background: Inflammation is a biological response to harmful stimuli, and traditional medicinal plants have been widely explored for their anti-inflammatory potential. Gloriosa Superba, a medicinal plant, is known for its therapeutic properties. This study evaluates the in-vitro anti-inflammatory activity of Gloriosa Superba tubers. ***Methods:*** The anti-inflammatory activity of the ethanolic extract of Gloriosa Superba tubers was assessed using the human red blood cell (HRBC) membrane stabilization method, including heat-induced hemolysis and hypotonicity-induced hemolysis, along with an egg albumin denaturation assay. Acetylsalicylic acid (ASA) was used as a standard reference drug. ***Results:*** The crude ethanolic extract exhibited significant anti-inflammatory activity in a concentration-dependent manner. The heat-induced membrane stabilization method revealed that the extract (500 µg/ml) and ASA (500 µg/ml) exhibited 52.667% and 78% inhibition, respectively. The hypotonicity-induced hemolysis method showed 35.67% and 59% inhibition of RBC hemolysis, respectively. The egg albumin denaturation assay demonstrated that the crude ethanolic extract (1000 µg/ml) and ASA (1000 µg/ml) inhibited egg albumin denaturation by 60% and 97.12%, respectively. ***Conclusion:*** The tubers of Gloriosa Superba exhibit promising anti-inflammatory activity, supporting their potential medicinal application. Further in-vivo studies are recommended to establish its efficacy and safety.

Keywords: Gloriosa Superba, Anti-inflammatory, Denaturation, Hypotonicity-induced haemolysis, Heat-induced Homolysis.

1. INTRODUCTION

Inflammation is a protective response triggered by various stimuli, including pathogens, irritants, and cellular damage. It is characterized by redness, swelling, heat, pain, and loss of function. While inflammation is essential for immune response and tissue repair, chronic inflammation contributes to various diseases, including arthritis and cardiovascular conditions. Synthetic anti-inflammatory drugs are widely used; however, they are associated with side effects, necessitating the search for natural alternatives.

Gloriosa Superba, a medicinal plant traditionally used in Ayurveda and folk medicine, contains bioactive compounds with potential therapeutic applications. This study aims to evaluate the in-vitro anti-inflammatory activity of the ethanolic extract of Gloriosa Superba tubers using membrane stabilization and protein denaturation assays.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents: All chemicals and reagents used in this study were of analytical grade. Acetylsalicylic acid (ASA) was used as the standard anti-inflammatory agent.

2.2 Plant Collection and Extraction
Gloriosa Superba Tubers Extraction Procedures:

2.3 Plant material: The Gloriosa Superba Tubers were collected from Botanical Garden of Lidya college of Pharmacy, East Godavari, Andhra Pradesh and identified by a taxonomist at Botany department, Andhra University.

Fig. No:01, Gloriosa superba Plant and Tubers

2.4 Sampling and sample treatment:

The Gloriosa Superba Tubers were washed with distilled water and cool dried for two weeks. Mortar and pestle were used to pound the dried Gloriosa Superba Tubers into powder, then sieved and stored in a covered plastic container for Invitro Anti-inflammatory Testing. All reagents were of analytical reagent grade unless otherwise stated. Distilled water was used in the preparation of solutions and dilution unless otherwise stated. The physiochemical analyses were carried out in triplicates unless otherwise stated.

2.5 Sample Preparation and Extraction:

The Gloriosa Superba Tubers was extracted by using Ethanol as a solvent in Soxhlet apparatus for several hours, then filtered and extract was dried by heating mantle under reduced pressure.

2.6 Phytochemical Screening:

Standard phytochemical methods were used to test for the presence of saponins, alkaloids, tannins, anthraquinones, cardiac glycosides, cyanogenetic glycosides, amino acid & protein and flavonoids.

2.7 Determination of percentage yield:

The oil which was recovered by complete distilling of the solvent on a heating mantle was then transferred into a measuring cylinder. The measuring cylinder is then placed over water bath for complete evaporation of solvent for about 2-3 hours in accordance with the method reported and volume of the oil was recorded and expressed as oil content (%) as follow.

Oil content (%) = Volume of the oil / Weight of sample x 100%

3 Anti-inflammatory Assays:

In Vitro Anti-Inflammatory Activity:

The following standard methods were used for Invitro anti-inflammatory evaluation of any crude ethanolic extract of Gloriosa Superba Tubers.

1. Human red blood cell (HRBC) membrane stabilization assay
   - Heat-induced haemolysis of RBC membrane.
   - Hypotonicity-induced hemolysis of RBC membrane.
2. Egg albumin denaturation assay.

3.1 Human Red Blood Cell (HRBC) Membrane Stabilization Assay:

To examine the in-vitro anti-inflammatory action of the extract, the HRBC membrane stabilization technique was employed following Tarannum Naz et al. NSAIDs were used as the standard, and anti-inflammatory activity was expressed as the percentage of RBC lysis. HRBC membranes mimic lysosomal membranes; thus, stabilization by the extract also suggests lysosomal membrane stabilization.

Hemoglobin content in the suspension was measured using a spectrophotometer at 560 nm. Blood was collected from a healthy human volunteer, with prior NSAID use within 2 weeks being the exclusion criterion. Sodium oxalate was used to prevent clotting, and samples were stored at 4 °C for 24 hours. Centrifugation was performed at 2500 rpm for 5 minutes to remove the supernatant.

Washing was done with sterile saline (0.9% NaCl) by centrifuging at 2500 rpm for 5 minutes, repeated thrice. The packed cell volume was used to prepare a 40% v/v suspension in phosphate-buffered saline (10 mM, pH 7.4), prepared with NaH2PO4.2H2O (0.26 g), Na2HPO4 (1.15 g), and NaCl (9 g) per liter of distilled water.

3.2 Heat-induced Hemolysis of RBC Membrane:

5 ml of isotonic buffer containing different concentrations (50, 100, 200, 400, and 800 µg/ml) of the ethanol extract was placed into duplicate centrifuge tubes. A control tube contained only the vehicle. 50 µl of RBC suspension was added and gently mixed. One set was incubated at 54 °C for 20 minutes in a water bath,

while the other was kept at 0–5 °C. After centrifugation at 5000 rpm for 5 minutes, absorbance was measured at 560 nm. ASA (200 µg/ml) served as the reference.

The percent inhibition of hemolysis was calculated using: % inhibition of hemolysis = 100 × (OD1 − OD2) / (OD3 − OD2) Where, OD1 = Test Sample Unheated; OD2 = Test Sample Heated; OD3 = Control Sample Heated.

3.3 Hypotonicity-induced Hemolysis of RBC Membrane

The hypotonic solution was prepared using 154 mM NaCl in 10 mM sodium phosphate buffer (pH 7.4). 50 µl of stock RBC suspension was added to 5 ml of hypotonic solution containing the extract at concentrations of 50, 100, 200, 400, and 800 µg/ml. A control was prepared without the extract. After 10 minutes of incubation at room temperature, samples were centrifuged at 5000 rpm for 5 minutes and absorbance measured at 540 nm. ASA (200 µg/ml) was the reference.

Percent inhibition of hemolysis was calculated as: % inhibition of hemolysis = 100 × (OD1 − OD2) / (OD3 − OD2) OD1 = Test isotonic solution; OD2 = Test Sample hypotonic solution; OD3 = Control sample in hypotonic solution.

3.4 Egg Albumin Denaturation Assay

Protein denaturation contributes to inflammation and arthritic diseases. Agents preventing this can be considered for anti-inflammatory drug development. The ethanolic extract was evaluated for its ability to prevent denaturation of egg albumin.

The reaction mixture (5 ml) contained 0.2 ml egg albumin, 2.8 ml phosphate buffer saline (PBS, pH 6.4), and 2 ml of extract at concentrations of 200, 400, 600, 800, and 1000 µg/ml. A control used distilled water. Samples were incubated at 37.2 °C for 15 minutes and then heated at 70 °C for 5 minutes. Absorbance was recorded at 660 nm. ASA at corresponding concentrations was used as reference.

Percent inhibition of denaturation was calculated using: % inhibition of denaturation = 100 × (OD1 − OD2) / (OD3 − OD2) Where, OD1 = Test Sample Unheated; OD2 = Test Sample Heated; OD3 = Control Sample Heated.

3.5 Statistical Analysis

Results were expressed as mean ± SD using MS Excel 2021. One-way ANOVA, Scheffe's post-hoc test, or Student's t-tests were applied as appropriate. Statistical significance was set at $p < 0.05$.

4. RESULTS AND DISCUSSION

4.1 Heat-induced Hemolysis Inhibition:

The ethanolic extract of Gloriosa Superba (500 µg/ml) inhibited heat-induced hemolysis by 52.667%, whereas ASA (500 µg/ml) exhibited 78% inhibition, indicating moderate membrane stabilization properties.

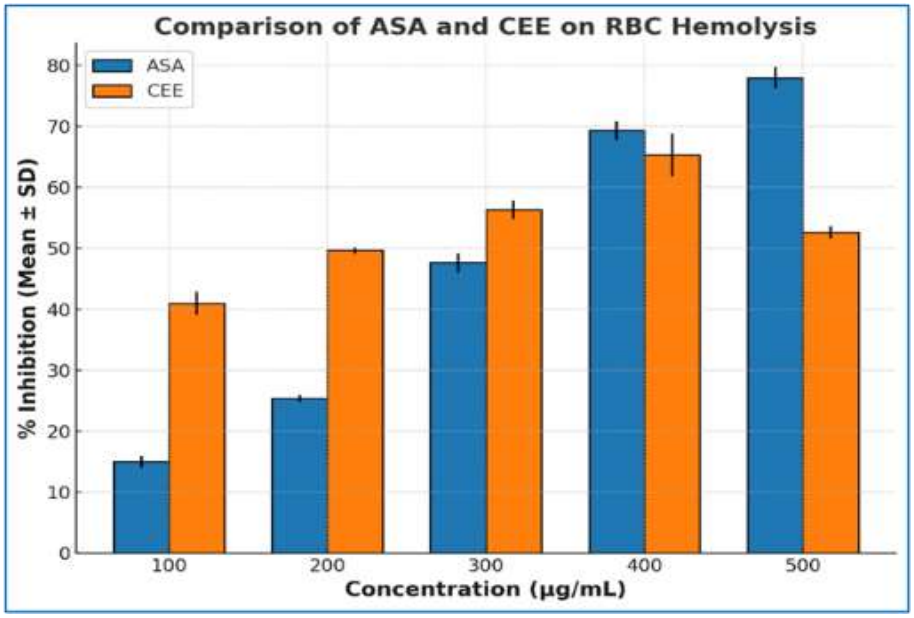

Fig. no: 02: Comparing the % Inhibition of ASA and CEE at different concentrations in Heat-induced haemolysis of RBC membrane Method

4.2 Hypotonicity-induced Hemolysis Inhibition:

The extract (500 µg/ml) and ASA (500 µg/ml) showed 35.67% and 59% inhibition, respectively, confirming its protective effect on RBC membranes against osmotic stress.

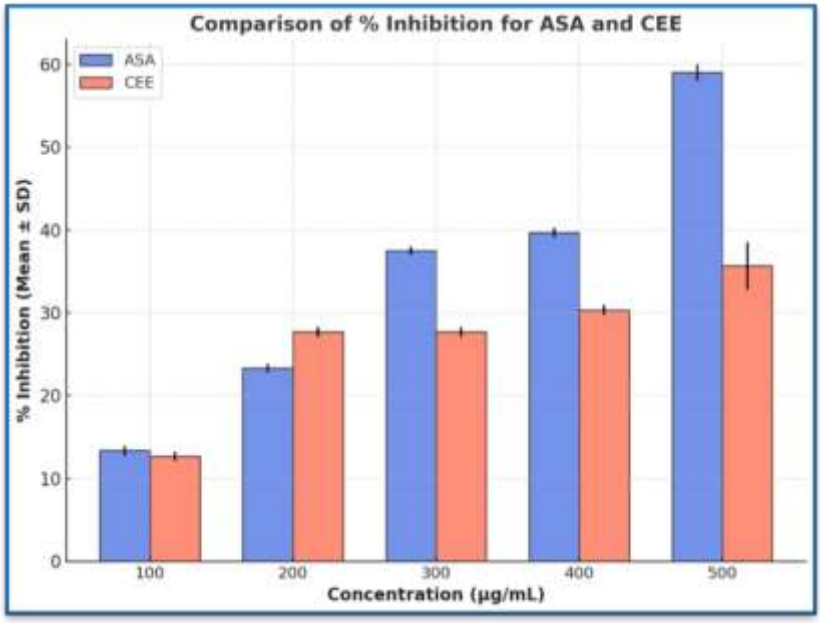

Fig. no:03 Comparing the % Inhibition of ASA and CEE at different concentrations in Hypotonicity-induced hemolysis of RBC membrane Method

4.3 Egg Albumin Denaturation Inhibition:

The ethanolic extract (1000 µg/ml) demonstrated 60% inhibition of egg albumin denaturation, while ASA (1000 µg/ml) exhibited 97.12% inhibition, suggesting its potential to prevent protein denaturation, a key process in inflammation.

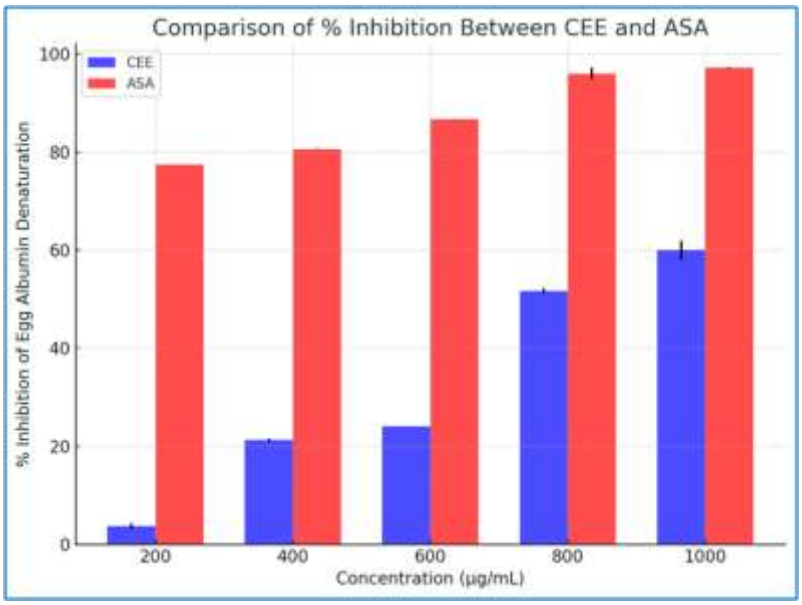

Fig. no:04: Comparing the % Inhibition of ASA and CEE at different concentrations in egg albumin denaturation method

4.4 Comparative Analysis:

The results indicate that Gloriosa Superba possesses moderate anti-inflammatory activity. While its inhibition rates were lower than those of ASA, its membrane-stabilizing and protein-denaturation inhibition properties highlight its potential as a natural anti-inflammatory agent.

5. CONCLUSION

The present study demonstrates that the ethanolic extract of Gloriosa Superba tubers exhibits significant in-vitro anti-inflammatory activity. Although less potent than ASA, the extract shows promise as a natural anti-inflammatory agent. Further in-vivo studies and phytochemical analyses are recommended to explore its full therapeutic potential.

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