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Research Article

# *IN VITRO* ANTI-INFLAMMATORY ACITIVTY OF FLOWER EXTRACT OF *COUROUPITA GUIANENSIS* AUBL.

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**ABSTRACT**

**Objective:** This study states that evaluation of "anti-inflammatory effect" on *Couroupita guianensis* flower for the improvement of medicinal uses.

**Methods:** *In vitro* anti-inflammatory activity was evaluated using human red blood cell membrane stabilization. Diclofenac sodium was used as a standard drug. The percentage of membrane stabilization for *C. guianensis* ethanolic flower (CGEF) extract, *C. guianensis* methanolic flower (CGMF) extract, and diclofenac sodium was done at different (100, 200, 300, 400 and 500 µg/ml) concentrations.

**Results:** The maximum membrane stabilization of CGMF extract was found to be 70.58 ± 7.1 at a dose of 500 µg/ml compared with CGEF extract (67.90 ± 5.8) and a standard drug (66.88 ± 4.3).

**Conclusion:** It was concluded that the medicinal study has given the highest anti-inflammatory effect against the standard drug.

**Keywords:** *Couroupita guianensis* ethanolic flower extract, *Couroupita guianensis m*ethanolic flower extract, *In vitro* anti-inflammatory.

## INTRODUCTION

The natural product is a source for bioactive compounds and has the potential for developing some novel therapeutic agent over the last decade there has been a growing interest in drugs of plant origin and such drugs formed an important class for diseases control. Herbs are staging a comeback and herbal "renaissance" is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment [1].

An inflammation is a complex biological response of vascular tissues to harmful stimuli. It is also a protective attempt by the organism to remove the injurious stimuli and initiate the healing process [2]. At the onset of an inflammation, the cells undergo activation and release inflammation mediators. These mediators include histamine, serotonin, slow reacting substances of anaphylaxis, prostaglandins and some plasma enzyme systems such as the complement system, the clotting system, the fibrinolytic system, and the kinin system [3]. These mediators' molecules work collectively to cause increased vasodilatation and permeability of blood vessels. Thus, leading to increased blood flow, exudation of plasma proteins and fluids, and migration of leukocytes, mainly neutrophils, outside the blood vessels into the injured tissues [4].

Inflammation can be classified as either acute or chronic inflammation. Acute inflammation is the initial response of the body to injurious stimuli and is achieved by increased movement of plasma and a leukocyte from the blood into the injured tissues. The process of acute inflammation is initiated by cells already present in the tissues. This is characterized by marked vascular changes including vasodilatation and increased capillary permeability which is induced by the actions of the various inflammatory mediators [5]. Chronic inflammation is a prolonged inflammatory response that leads to a progressive shift in the type of cells present at the site of inflammation and is characterized healing of the tissues from the inflammatory process [6].

*Couroupita guianensis*, also called as cannonball tree, is a native of India, Sri Lanka or South America. The tree is deciduous and large, have been reported for various pharmacological activities to treat diseases such as gastritis, scabies, bleeding piles, dysentery, and scorpion poison [7].

## METHODS

### Identification and collection of flower

The flowers of *C. guianensis* were collected from the Mannargudi, Thiruvarur District, Tamil Nadu, India. They were identified and authenticated by Dr. John Britto, The Rapient Herbarium and Centre for Molecular Systematics, St. Joseph's College, Trichirapalli, Tamil Nadu, India.

### Extraction and preparation of flower

The flowers were garbled and dried under shade and powdered. 25 g of dried powdered flower materials were extracted separately with ethanol and methanol using soxhlet apparatus for 48 hrs. The solvent was distilled at a lower temperature under reduced pressure and concentrated on water bath to get the crude extract which is stored in desiccator for future use.

### *In vitro* anti-inflammatory activity

*The human red blood cells membrane stabilization method [8-10]*

The blood was collected from healthy human volunteer who had not taken any nonsteroidal anti-inflammatory drug (NSAIDS) for 2 weeks before the experiment and mixed with an equal volume of Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride in water) and centrifuged at 3000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various concentrations of extracts were prepared (100, 200, 300, 400 and 500 µ/ml) using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hyposaline, and 0.5 ml of human red blood cells (HRBC) suspension were added. It was incubated at 37°C for 30 minutes and centrifuged at 3000 rpm for 20 minutes, and the hemoglobin content of the supernatant solution was estimated on an ultraviolet spectrophotometer at 560 nm. Diclofenac was used as standard and control was prepared by omitting the extracts.

$$\text{Percentage of inhibition} = 100 - \left(\frac{\text{Optical density of drug treated sample}}{\text{Optical density of control}}\right) \times 100$$

**Statistical analysis**

Three replicates of each sample were used for each test to facilitate statistical analysis and the data were represented as a mean ± standard deviation.

## RESULTS AND DISCUSSION

The flower extract exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The lysosomal enzymes released during inflammation produce a variety of disorders. Since HRBC membrane is similar to lysosomal membrane components the prevention of hypotonicity-induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity of drugs. The results were reported in Table 1. It was observed from the Table 1 and Fig. 1 that the methanolic extract shows significant anti-inflammatory activity at the concentration 0f 500 mg/ml which is comparable to the standard drug (66.88 ± 4.3) and *C. guianensis* ethanolic flower (CGEF) (67.90 ± 5.8) extract. The anti-inflammatory activity of the extracts was concentration dependent with the increasing concentration the activity is also increased.

The erythrocyte membrane is analogous to the lysosomal membrane [11] and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage on extracellular release [12].

A some of the NSAIDs are known to possess membrane stabilization properties which may contribute to the potency of their anti-inflammatory effect. Although the exact mechanism of the membrane stabilization by the extract is not known yet; hypotonicty – induced hemolysis may arise from shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components. The extract may inhibit the processes, which may stimulate or enhance the efflux of these intracellular components [13].

**Table 1: *In vitro* anti-inflammatory activity of flower extract of *Couroupita guianensis***

| S. No | Concentration (µg/ml) | Percentage of inhibition | | |
|---|---|---|---|---|
| | | Standard drug (diclofenac sodium) | CGEF extract | CGMF extract |
| 1 | 100 | 14.72±6.2 | 12.27±4.9 | 13.72±6.8 |
| 2 | 200 | 27.22±7.0 | 26.31±7.85 | 27.44±7.8 |
| 3 | 300 | 30.09±4.3 | 31.59±5.5 | 35.29±5.3 |
| 4 | 400 | 42.55±4.3 | 50.87±3.9 | 52.93±6.3 |
| 5 | 500 | 66.88±4.3 | 67.90±5.8 | 70.58±7.1 |

CGEF: *Couroupita guianensis* ethanolic flower, CGMF: *Couroupita guianensis* methanolic flower

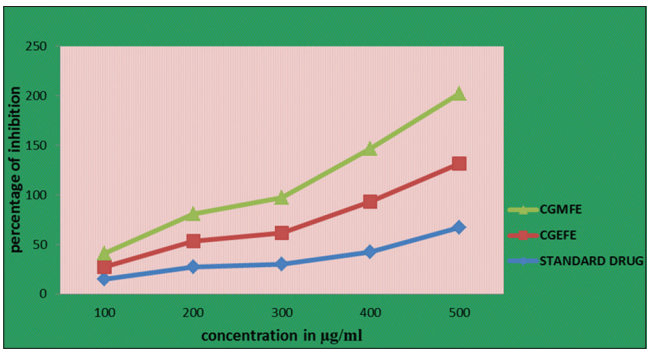


**Fig. 1: *In vitro* anti-inflammatory activity of flower extract of *Couroupita guianensis***

Inflammation is probably the fastest growing metabolic disease in the world and as knowledge of the multifactorial or heterogeneous nature of the diseases increases so does the need for more challenging and appropriate therapies. Traditional plant remedies have been used for centuries [14]. Inflammation is a common phenomenon and it is a reaction of living tissues toward injury. NSAIDs possibly induce the redistribution of lymphocytes which cause rapid and transient decrease in peripheral blood lymphocyte counts to effect longer term response [15]. The lysosomal enzymes released during inflammation produce a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic by inhibiting the lysosomal membrane [16].

The erythrocyte membrane resembles to lysosomal membrane and as such the erythrocyte could be extrapolated to the stabilization of lysosomal membrane [17]. The vitality of cells depends on the integrity of their membranes, exposure of RBC's to injurious substances such as hypotonic medium results in lysis of its membrane accompanied by haemolysis and oxidation of hemoglobin. An injury to RBC membrane will further render cell more susceptible to secondary damage through free radical induced lipid peroxidation [18].

Similar to studies suggested that the high membrane stabilizing activity of the extract of *Celosia argentea* which has potential to protect the erythrocyte membrane from free radical damage [19]. Protection against free radical lipid peroxidation by plant extract is of great significance for their traditional use against inflammatory disorders, many of which are associated with membrane damage and tissue recovery [20]. Lipid peroxidation results in mitochondrial swellings and disintegration degradation of lysosomes have been correlated with the peroxidative decomposition of lysosomal lipids [21].

*Achyranthes aspera* was reported to possess very low hemolytic activity toward human erythrocytes [22]. Aqueous extract of *Lantana camara* and its various solvent fractions were reported to possess moderate hemolytic activity toward human erythrocytes [23]. The hemolytic activity of 71 extracts prepared from 12 plants. Only three extracts prepared from *Elaeoluma nuda* showed significant hemolytic activity [24]. Chloroform and aqueous extract of leaves of *Acanthus ilicifolius* were reported to possess significant hemolytic activity toward the chick red blood cells [25].

During inflammation, there is lysis of lysosomal membrane which releases their components enzymes that produce a variety of disorders. Non-steroidal anti-inflammatory drugs extract their beneficial effects by either inhibiting the release of lysosomal enzymes or by stabilizing the lysosomal membranes [26]. Erythrocytes have been used as a model system by a number of workers for the study of the interaction of drugs with membranes. Hemolysis is due to red blood cells destruction which resulted from lysis of membrane lipid bilayer. This hemolysis relates to concentration and potency of the extract. Furthermore, the hemolytic activity of each extract is related to their chemical composition [27].

Ethanolic extract kadam leaves exhibited membrane stabilization or heat induced hemolytic effect by inhibiting hypotoncity inducedlysis of erythrocyte membrane. The erythrocyte membrane is analogusto thelysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membrane [28].

## CONCLUSION

This is the first comparative *in vitro* study on anti-inflammatory activity of *C. guianensis* flower. The current study provides evidence for the traditional use of *C. guianensis* against inflammatory disorders. The methanolic extract of the *C. guianensis* flower showed maximum anti-inflammatory activity as compared to standard drug and CGEF extract. Thus, further investigation would be carried out in isolation of the active compounds and elucidate their inhibitory mechanism in *in vivo.*

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