International Journal of Herbal Medicine
Available online at www.florajournal.com

IJHM International Journal of Herbal Medicine

E-ISSN: 2321-2187
P-ISSN: 2394-0514
IJHM 2016; 4(5): 05-08
Received: 02-07-2016
Accepted: 03-08-2016

S Sumathi
Research Scholar, PG and Research Department of Biochemistry, Sengamala Thayaar Educational Trust Women's College, Mannargudi, Tamil Nadu, India – 614 001

R Anuradha
PG and Research Department of Biochemistry, Sengamala Thayaar Educational Trust Women's College, Mannargudi, Tamil Nadu, India –614001.

Correspondence
S Sumathi
Research Scholar, PG and Research Department of Biochemistry, Sengamala Thayaar Educational Trust Women's College, Mannargudi, Tamil Nadu, India – 614 001

In vitro anti-inflammatory activity of flower extract of *Couroupita guianensis* Aubl

S Sumathi and R Anuradha

Abstract
The nature has provided abundant plant wealth for all the living creatures, which possess medicinal virtues. Hence, the present study aims to open new avenues for the improvement of medicinal uses of *Couroupita guianensis* flower for the selected area for *in vitro* anti-inflammatory activity was evaluated using human red blood cell membrane stabilization. Diclofenac sodium was used as a standard standard drug. The percentage of membrane stabilization for CGEF (*Couroupita guianensis* thanolic flower) extract, CGMF (*Couroupita guianensis* methanolic flower) extract and diclofenac sodium were done at different concentrations. The maximum membrane stabilization of CGMF extract was found to be 70.58±7.1 at a dose of 500µg/ml compared with CGEF extract and standard drug.

Keywords: CGEF extract, CGMF extract, *in vitro* anti-inflammatory

1. Introduction
Natural product is a source for bioactive compounds and has 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].

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 are 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 like gastritis, scabies, bleeding piles, dysentery, and scorpion poison [7].

2. Materials and methods
2.1 Identification and collection of flower
The flowers of *Couroupita guianensis* were collected from the Mannargudi, Thiruvarur District, Tamil Nadu, and India. They were identified and authenticated by Dr. John Britto, The Rapient Herbarium and Centre for Molecular Systematics, St. Joseph's college, Tiruchirappalli, Tamil Nadu, India.

2.2 Extraction and preparation of flower

The flowers were garbled and dried under shade and powdered. 25g of dried powdered flower materials were extracted separately with ethanol and methanol using soxhlet apparatus for 48hrs.The solvent was distilled at 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

2.3 The human red blood cells (HRBC) membrane stabilization method [8-10]

The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05 % citric acid and 0.42% sodium chloride in water) and centrifuged at 3,000 rpm. The packed cells were washed with iso saline and a 10% suspension was made. Various concentration of extracts were prepared (100,200,300,400 and 500µ/ml) using distilled water and to each concentration 1ml of phosphate buffer, 2ml hyposaline and 0.5ml of HRBC suspension were added.it was incubated at 37 ºC for 30min and centrifuged at 3,000 rpm for 20min, and the haemoglobin content of the supernatant solution was estimated on UV spectrophotometer at 560nm. Diclofenac was used as standard and a control was prepared by omitting the extracts.

$$\text{Percentage of inhibition} = 100 - \frac{\text{optical density of drug treated sample}}{\text{Optical density of control}} \times 100$$

2.4 Statistical analysis

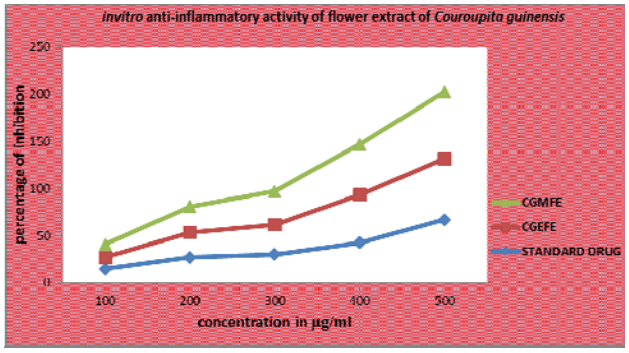
Three replicates of each sample were used for each test to facilitate statistical analysis and the data were represented as mean ±standard deviation.

3. Results and discussion

The flower extract exhibited membrane stabilization effect by inhibiting hypo tonicity induced lysis of erythrocyte membrane. The lysosomal enzymes released during inflammation produce a variety of disorders. Since HRBC membrane are 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 figure 1 that the methanolic extract shows significant anti-inflammatory activity at the concentration 0f 500mg/ml which is comparable to the standard drug (66.88±4.3) and CGEF (67.90±5.8) extract. The anti-inflammatory activity of the extracts were concentration dependent, with the increasing concentration the activity is also increased.

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



The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize Lysosomal membranes [11]. 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 upon extra cellular release [12].

Some of the NSAIDs are known to possess membrane stabilization properties which may contribute to the potency of their anti-inflammatory effect. Though the exact mechanism of the membrane stabilization by the extract is not known yet; hypotonicity –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].

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's a reaction of living tissues towards 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 extra cellular 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 haemoglobin. 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 has been correlated with the peroxidative decomposition of lysosomal lipids [21].

Achyranthes Aspera was reported to possess very low haemolytic activity towards human erythrocytes [22]. Aqueous extract of *Lantana camara* and its various solvent fractions were reported to possess moderate haemolytic activity towards human erythrocytes [23]. The haemolytic activity of seventy one extracts prepared from twelve plants. Only three extracts prepared from E. nuda showed significant haemolytic activity [24]. Chloroform and aqueous extract of leaves of *Acanthus ilicifolius* were reported to possess significant haemolytic activity towards the chick red blood cells [25].

During inflammation, there are lysis of lysosomal membrane which release 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 interaction of drugs with membranes. Haemolysis is due to red blood cells destruction which resulted from lysis of membrane lipid bilayer. This haemolysis relates to concentration and potency of extract. Furthermore the haemolytic activity of each extract is related to their chemical composition [27].

Ethanolic extract kadam leaves exhibited membrane stabilization or heat induced haemolytic effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is an alogusto the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membrane [28].

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 inflammation. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The main action of anti-inflammatory agents is the inhibition of cyclooxygenase enzyme which is responsible for conversion of arachidonic acid to prostaglandins (PG) [29].

The non-steroidal drugs (NSAIDs) act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membranes by means of inhibiting the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes (cyclooxygenase) and proteases, which cause further tissue inflammation and damage upon extra cellular release or by stabilizing the lysosomal membrane [30].

The erythrocyte membrane may be considered a model of the lysosomal membrane which plays an important role in inflammation [31]. The compounds which prevent the lysis of membrane caused by the release of hydrolytic enzymes contained within the lysosomes may relieve some symptoms of inflammation [32]. When the human RBC is subjected to hypotonic stress, the haemoglobin release from RBC will be prevented by anti-inflammatory drugs because of the membrane stabilization. It has been demonstrated that certain herbal preparations were capable of stabilizing the red blood cell membrane and this may be indicative of their ability to exert anti-inflammatory activity [33].

Lysosomes are single membrane structures that contain digestive enzymes. When certain white blood cells engulf bacteria, the bacteria are digested and destroyed by these lysosomal enzymes. Worn out cell parts and dead cells are also digested by these enzymes. This is a beneficial process and is necessary before tissue repair can begin. But it does not have a disadvantage in that lysosomal digestion contributes to inflammation in damaged tissues. An excessive inflammation can start a vicious cycle, actually a positive feedback mechanism that results in extensive tissue damage [34].

During inflammation, histamine from damaged tissues makes capillaries more permeable and the lysosomes of damaged cells release their enzymes which help breakdown damaged tissue but may also cause destruction of nearby healthy tissue. Some of the NSAIDS and glucocorticoids stabilize lysosomes in tissue cells and there by prevent the release of lysosomal enzymes into the cytoplasm of the cells, thus preventing deterioration from this source [35].

4. Conclusion

This is the first comparative *in vitro* study on anti-inflammatory activity of *Couroupita guianensis* flower. The current study provides evidence for the traditional use of *Couroupita guianensis* against inflammatory disorders. The methanolic extract of the *Couroupita 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.*

5. References

1. Bhatt RH, Khurana ML. Indian Journal of pharmacy. 195:208-211.
2. Ferrero-Miloani L, Nielson OH, Anderson PS. Chronic inflammation: importance of NoD2 and NALP3 in interleukin-1β generation Clin Exp Immunol. 2007; 147(2):227-235.
3. Perianayagam JB, Sharma SK, Pillai SK. Anti-inflammatory activity of *Trichodes maindicum* root extracts in experimental animals J ethnopharmacol. 2006; 104:410-414.
4. Chaitanya R, Sandhya S, David B. HRBC Membrane stabilizing property of root stems and leaf of Glochidion velutinum, Int J Res Pharmaceut Biomed Sci. 2011; 2(1):256-259.
5. Emig SA, Krieg J, Davidson JM. Inflammation is wound repair molecular and cellular mechanisms Jinvest Dermatol.
6. Mounnissamy VM, kavimani S, Balu V. Evaluation of anti-inflammatory and membrane stabilizing properties of ethanol extract of Canjerarehedi Iranian J Pharmacol Therapeut. 2008; 6:235-237.
7. Farrukh Aqil, Iqbal Ahmad, Zafar Mehmood. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants Turk J Biol. 2006; 30:177-183.
8. Chaudhary RD. Herbal drug industry 1st Ed, Eastern Publication New Delhi, 1996, 1.
9. Nakayoma JM Yamada. Isolate three chemical constituents from *Anthocephalus cadamba* Biochemical pharmacology. 1995; 45:265-267.
10. Samnelsson G, kyeremter G, Farah MH. Journal of ethnopharmacology. 1985; 14:193.
11. Chou CT. The anti-inflammatory effect of *Tripterygium wilfordii* Hook F. on adjuvant induced paw edema in rats and anti-inflammatory mediator' srelease Phytother Res. 1997; 11:152.
12. Murugasan N, Vember S, Damodharan C. Studies on erythrocyte membrane *in-vitro* haemolytic activity of Oleander extract Toxicol Lett. 1981; 8:33-38.

13. Vadivu R, Lakshmi KS. *In-vitro* and *in-vivo* anti-inflammatory activity of leaves of *Symplocos cochinchinensis* (Lour) Moore ssplauina J Pharmcol. 2008; 3:121-124.
14. Newman DJ, Crag GM. Natural products as sources of new drugs over the last 25 years J Nat Prod. 70:461-477.
15. Eisenberg DM, Davis RB, Ettner SL, Appel S, Wilkey Van, Rompay M *et al.* Trends in alternative medicine use in the United States, American medical association.1997; 280:1569-1575.
16. Umamaheswara Rao P. Anti-inflammatory and antinociceptive activities of leaves of *Rumex dentatus* in rats, Asian pacific Journal of Tropical biomedicine. 2008; 3:14-17.
17. Omale J, Okafor PN. Comparative antioxidant capacity and cytotoxicity of the leaf and stem of *Cissus multistriata*, African Journal of Biotechnology. 2008; 7(17):3129-3133.
18. Augusto WJ, kunze KL, Montellano PRN. Phenylprotoporphyrin formation in the haemoglobin phenylhydrazine reaction.
19. Olubenga M, Fafunso MA, Makinde JM. Membrane stabilizing activity: A possible mechanism of action of anti-inflammatory property of *Gongronema latifolium* leaves, International Journal of biomedical and health science. 2005; 1(1):1-14.
20. Halliwell B, Guteeridge JM. Free radicals in biology and medicine, Asian Journal of pharmaceutical and clinical research. 1998; 5(1):32-35.
21. Desai ID, Sawant PL, Tappel ALT. Peroxidative and radiation damage to isolated lysosomes Biomed Biophys Acta 1964; 86:277-385.
22. Priya CL, Kumar G, Karthik L, Rao KVB. Antioxidant activity of *Achyranthes aspera* Linn Stem extracts Pharmacology online. 2010; 2(2):228-237.
23. Kalita S, Kumar G, Karthik L, Rao KVB. Phytochemical composition and *in vitro* haemolytic activity of *Lantana camara* L Leaves Pharmacology online Newsletter. 2011; 1:59-67.
24. Oliveraira VMA, Carneiro ALB, Cauper GB, Pohlit AM. *In-vitro* screening of Amazonian plants for hemolytic activity and inhibition of platelet aggregation in human blood Acta Amazonica. 2009; 39(4):973-980.
25. Thirunarukkurasu P, Ramanathan T, Ram Kumar L. Hemolytic and antimicrobial effect in the leaves of *Acanthus Ilicifolius,* Journal of Pharmacology and Toxicology. 2011; 6(2):196-200.
26. Feirrali M, Signormi C, CiccoliliL. Iron release and membrane damage in erythrocyte exposed to oxidizing agent's phenylhy drazine deviance and iso-uranil Biochem J. 1992; 285:295-301.
27. Mohammedi Zohra, Atik Fawazia. Hemolytic activity of different herbal extracts used in Alegeria. Internal Journal of pharama sciences and Research. 2014; 5:08.
28. Chatterjee S, Das SN. Anti-arthritic and anti-inflammatory effects of a polyherbal drug (Ease): its mechanism of action, Indian Journal of pharmacology. 28:116-119.
29. Arun Shirwaikar, Sarala Devi, Siju EN.Anti-Inflammatory activity of *Thespesia populnea* fruits by Membrane Stabilization. International Journal of PharmTech Research. 2011; 3(4): 2060-2063.
30. Seema Chaitanya Chippada, Sharan Suresh Volluri, Srinivasa Rao Bammidi and Meena Vangalapati. *in vitro* anti inflammatory activity of methanolic extract of *centella asiatica* by HRBC membrane stabilisation. Rasayan J Chem. 2011; 4(2):457-460.
31. Weissmann G, Spilberg I, Krakauer K. Arthristis and Rheumatism. 1969; 12(2):103-116.
32. Hess SM, Milonig RM. Assay for anti-inflammatory drugs. In: Lepow IH, and Ward PA, (Eds.), Inflammation: Mechanisms and Control, Academic Press, New York, 1972, 1-12.
33. Olajide OA, Makinde JM, Okpako DT. Awe SOJ. Ethnopharmacol. 2000; 71(1-2):153-160.
34. Valerie C, Scanlon and Tina Sanders. Essentials of Anatomy and Physiology, 6th edition, FA Davis Company, Philadelphia, 2010; 287.
35. Arthur C, Guyton, John E and Hall. Text Book of Medical Physiology, 12th edition, Elsevier Inc, Philadelphia. 2010, 312-345.