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IN VITRO ANTI-INFLAMMATORY ACTIVITY OF QUERCITRIN ISOLATED FROM *ALLAMANDA CATHARTICA LINN*

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ABSTRACT

In the present work, the flavonoid quercitrin obtained from the fresh flowers of *Allamanda Cathartica Linn*. was evaluated for anti-inflammatory activity by *in vitro* hemolytic membrane stabilizing study. The effect of inflammation condition was studied on erythrocyte exposed to hypotonic solution. The results of the evaluation indicate that the obtained compound was found to show membrane stabilizing activity, which was optimum at 75µg.

KEYWORDS: **Allamanda Cathartica Linn., anti-inflammatory, quercitrin.**

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INTRODUCTION

It is well known from the literatures that approximately 36% of the naturally occurring compounds of plants contain phenolic hydroxyls and of these about one-third are flavonoid types [1]. About 2% of the total carbon photosynthesized by plants is converted to flavonoids [2]. The role of flavonoids in such clinical conditions such as hypertension, rheumatism, arthritis and pregnancy and also their beneficial effects in frost-bite and cold injury have been studied [3]. RBC (Red Blood Cells) has been used as a model system by a number of workers for the study of interaction of drugs with membranes [4-5]. The stabilization of RBC against hypotonic haemolysis has attracted the attention of a number of workers [6-7]. Drugs like anesthetics stabilize RBC against hypotonic haemolysis at low concentrate but cause haemolysis at high concentration [8]. Inflammation is a complex and dynamic biological process generally described as the characteristic response of body tissues to injury of any kind. It is classified as either acute or chronic, depending on whether it involves a short response or a prolonged one, respectively [9]. Anti- Inflammatory drugs may be defined as components that inhibit the whole or any portion of an acute or chronic inflammation reaction irrespective of whether the drug is clinically useful or not. Clinically anti-inflammatory drugs are judged by their effect on the pain, stiffness or swelling of the affected part, their action on swelling [10-12]. Many compounds have anti-inflammatory activity have been isolated from herbs and many crude herbs have been reported to have significant anti-inflammatory activity. Scientists at the Central Drug Research Institute, Lucknow have explored 41 medicinal plants possessing significant anti-inflammatory activity. The leaves of *streblusasper* [13] and *Argemonemexicana* [14] have been observed to the active against carrageenan induced rat paw oedema in rats and their activities are comparable to that of phenyl butazone. There is a positive evidence to show that lysosomal enzymes play an importantrole in the development of acute and chronic inflammation. Increased enzyme activity has been reported in certain types of experimental inflammation including rat paw madeodematous by phlogistic agents [15]. Aspirin and sodium salicylate have been widely used as remedial drug for inflammation. The hormonal and metabolic side effects of the steroidal drugs have led to the development of non-steroidal anti-inflammatory drugs (NSAIDS) [16]. The inhibitory effects (inhibit the synthesis or block the activity of prostaglandins which mediate the inflammatory response) of these drugs on lysosomal enzymes have been proposed as an explanation for one of their many mechanisms of action *in vitro* [17]. Acidic anti-inflammatory agents such as phenyl butazone and indomethacin either inhibit the activities of released lysosomal enzymes or stabilize the lysosomal membrane. It has been reported that the structure of Human RBC membranes are similar to lysosomal membrane, hence the lysosomal membrane stabilization effects have been studied using RBC. When the RBC is subjected to hypotonic stress, the release of haemoglobin from RBC is prevented by anti-inflammatory drugs; this is because of the membrane stabilization of the drugs against hypotonicity induced haemolysis. This serves as a very useful *in vitro* method for assessing the anti-inflammatory activity of various compounds. Flavonoids like quercetin, rutinhyperoside, naringenin and naringin have been reported to exert *in vitro* stabilizing action of the RBC membrane against hypotonicity haemolysis [18]. Anti-inflammatory and anti-histaminic activities of *Daturastramonium* containing kaempferol and quercetin have been reported [19]. *Carica papaya L.* leaf extracts showed a substantial inhibition of hemolysis *in vitro* and could have a possible therapeutic effect on disease processes causing destabilization of biological membranes [20]. *Allamanda Cathartica Linn.* belonging to the floral family of Apocynacea and it is a perennial shrub used in traditional medicine for treating malaria and jaundice. The leaf extract of *Allamanda Cathartica Linn.* was found to promote wound healing in Sprague Dawley rats and also the source of many bioactive compounds with anti-inflammatory activity. The flowers of *Allamanda Cathartica Linn*. is also used as a laxative [21]. In this present investigation, the ethyl acetate concentrates of

Allamanda Cathartica Linn. have tested for its anti-inflammatory activity and the results are exhibited here under.

II. MATERIALS AND METHODS

The mature flowers of *Allamanda Cathartica Linn*. were collected in and around Kumbakonam, India, during the months of May and December. The collected fresh flowers were extracted with 90% methanol under reflux. The alcoholic extract was concentrated in vacuo and the aqueous concentrate was fractionated by using solvents like benzene, peroxide free diethyl ether and ethyl acetate successively. The ethyl acetate fraction was concentrated in vacuo and left in an ice chest for a few days. The yellow solid obtained was quercitrin (quercetin 3-O-rhamnoside) (Figure:1). The isolation and characterization of the extracted compound has been already reported in previous work [22] and in the present work the isolated compound was tested for its anti-inflammatory activity.

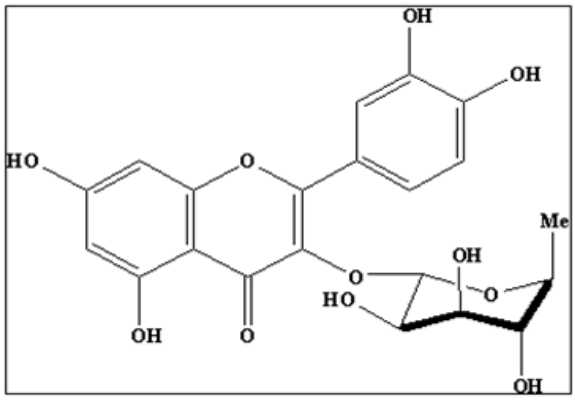


Figure 1
Structure of Quercetin 3-O-rhamnoside

II. 1. Anti-Inflammatory test (Hypotonicity induced RBC membrane stabilization)

II. 1.1.Collection of Blood

Blood was collected from healthy human volunteers using sterile 22 gauze hypodermic needle and it was mixed with equal volume of sterilized alsever solution [23] and stored at 4°C.

II. 1.2.Preparation of saline

Saline at different concentrations was prepared (Isosaline 0.85% and hyposaline 0.25%).

II. 1.3.Preparation of RBC suspension

The blood was centrifuged at 3000 RPM and the packed cells obtained were washed with isosaline (0.85% pH7.2) thrice and a 2% (v/v) suspension was made with isosaline.

II. 1.4.Determination of RBC membrane stabilization

Assay mixture which contains the ethyl acetate fraction in different concentrations as mentioned in (Table-1), 1ml of phosphate buffer (0.15M, pH 7.4), 2ml of hyposaline (0.25%) and 0.5ml of 2% RBC suspension were taken in different tubes. In another tube instead of drug, 2 ml of distilled water was taken and this served as the control. Both the tubes were incubated at 37°C for 30 min. After incubation they were centrifuged and haemoglobin content in the supernatant was estimated using a photoelectric colorimeter at 560nm. The percentage inhibition of heamolysis (membrane stabilization) was calculated [24] by using the formula given below and has been mentioned in Table-1. The different concentrations mention in Table-1 was plotted against percentage inhibition as shown in the Graph-1.

Percentage inhibition of heamolysis = 100 X {OD1+OD2/OD1}

where;

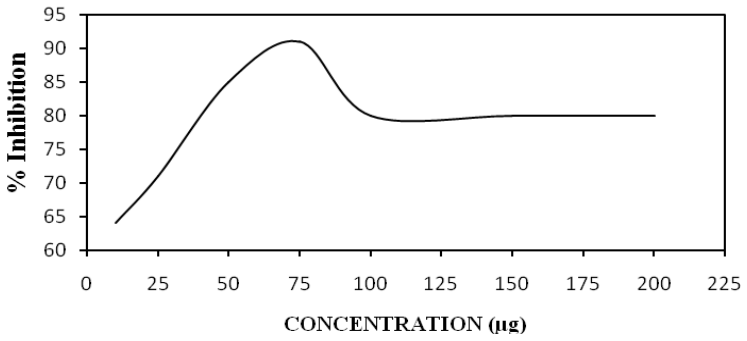
OD1= Optical density of the hypotonic+buffered saline solution alone

OD2 = Optical density of test sample in hypotonic solution

Table 1
Effect of ethyl acetate fraction of Allamanda Cathartica Linn. on hypotonicity induced RBC membrane stabilization

S. No.	Ethyl acetate fraction (µg)	% inhibition of heamolysis
1.	10	64
2.	25	71
3.	50	85
4.	75	91
5.	100	80
6.	150	80
7.	200	80

Graph.1 **HRBC Membrane Stabilization Studies On Allamanda Cathartica Linn.**



III. RESULTS AND DISCUSSION

From the above discussion, it is clear that the hypotonicity induced membrane stabilization studies on the ethyl acetate extract (quercitrin) from *Allamanda Cathartica Linn*. showed stabilization property. When the concentration of the isolated compound increased from 10µg to 75µg, the percentage inhibition of heamolysis is also increased (up to 91%). The percentage inhibition of heamolysis decreases at 100µg. After 100µg percentage inhibition of heamolysis becomes constant (80%), which indicates that the stabilization effect is unaltered by change of concentration. This is attributed biphasic property of the ethyl acetate fraction of *Allamanda Cathartica Linn*. which has been encountered frequently in literatures. Such kind of property is a rare phenomenon in flavonoids.

IV. CONCLUSION

The flavonoid isolated from the floral species *Allamanda Cathartica Linn*. was tested for its hypotonicity induced RBC membrane stabilization activity. The quercitrin compound has been proved to have significant anti-inflammatory activity, even at a very low concentration of 75µg. Thus, the isolated compound quercitrin was found to be very effective against the whole or any portion of an acute or chronic inflammation.

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