

IN VITRO ANTIPLATELET ACTIVITY- ETHANOLIC EXTRACT OF RHIZOME
OF *CURCUMA LONGA* LINN

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ABSTRACT

Curcuma longa Linn belongs to the family Zingiberaceae, which is commonly called as turmeric. The traditional report indicates that *Curcuma longa* Linn is used in the treatment of blood clot. Hence an attempt has been made to screen the effect of *Curcuma longa* Linn in prevention of platelet aggregation induced by ADP. The ethanolic extract was prepared by continuous hot percolation method. Ethanolic extract was tested in concentration of 62 µg/ml, 125 µg/ml, 250 µg/ml & 500 µg/ml. Aspirin was used as standard. The extract showed comparable antiplatelet activity at 250 µg/ml. The inhibition of platelet aggregation may be due to presence of curcumin in *curcuma longa*.

KEY WORDS: *Curcuma longa* Linn., Anti-platelet activity, ADP

INTRODUCTION

Curcuma longa Linn is an erect perennial herb. The rhizome is pungent and bitter. It helps to improve the complexion; useful in diseases of blood, scabies, urinary discharges, inflammation, bad taste in mouth, elephantiasis, snake bite, small pox etc. An attempt has been made to study the efficacy of rhizome of *Curcuma longa* Linn in platelet aggregation. Curcumin and sesquiterpenes are the major active constituent present^{1,2}. Blood platelets are involved in haemostasis. The normal haemostatic system limits blood loss by precisely regulated interactions between components of blood vessel, circulating blood platelets and plasma protein. Platelets can adhere to the walls of the blood vessel release bioactive compounds and aggregate to each other. These properties increase to a well established level in

conditions of arterial thrombosis and atherogenesis. Several agonists such as ADP, thrombin, collagen and serotonin induce the release of arachidonic acid, after the phospholipase activation through calcium mobilisation. Several drugs have been developed to block the different steps in platelet activation pathway. Inhibition of platelet function by aspirin has been very well established. One of the most popular mechanism by which flavonoid appear to inhibit platelet aggregation is by mediating increase in platelet cyclic AMP levels by either stimulation of adenylate cyclase or inhibition of cAMP phosphodiesterase activity. Therefore the following method is used to evaluate the antiplatelet activity of ethanolic fraction of *Curcuma longa* Linn and try to elucidate the inhibitory mechanism of flavonoid on platelet aggregation³.

METHODS AND MATERIALS

Extraction

Rhizomes were collected in November 2009 from botanical garden in our campus which was authenticated by Dr.P.Jayaraman, Director, PARC, West Tambaram, Chennai. Voucher specimens were deposited in our college herbarium for future reference. The voucher specimen number is SRU/COPS/063. The *Curcuma longa* Linn rhizomes were air dried and powdered (100 gm). It was extracted with ethanol using Soxhlet apparatus for 48 hrs. The solvent was concentrated under reduced pressure to get the crude extract which is stored in desiccator for future use. (Yield: 6 %).

DETERMINATION OF ANTIPLATELET ACTIVITY

Reagents Required

Tyrode buffer-AR, Platelet rich plasma suspension, Dimethyl sulphoxide (DMSO)-AR, various concentration of *Curcuma longa* Linn ethanolic extracts (62.5, 125, 250, 500 mcg/ml), Adenosine diphosphate (ADP)-AR, Aspirin [standard]⁵ were used.

Preparation Of Tyrode Buffer^{6,8}

The Tyrode buffer was prepared using Sodium chloride-149 mM, Potassium chloride 2.6mM, Sodium carbonate 9.5 mM, Sodium dihydrogen phosphate 0.5 mM, Magnesium chloride 0.6 mM, Glucose 5.5 mM, gelatin 0.25%. First the gelatin is made to dissolve in water by gradual addition and heating, later the other ingredients are added. Tyrode buffer is used to neutralize the PRP suspension for the activity. Tyrode buffer is used to neutralise the PRP suspensions for the activity.

Preparation of PRP (Platelet Rich Plasma)^{6,9}

Platelets were isolated from the blood samples of experimental subjects. The blood was centrifuged at 275 g for 10 minutes at room temperature to obtain platelet rich plasma. The PRP was once again centrifuged at 400 g for 10 minutes at room temperature to remove any contaminating red blood cells. Then PRP was subjected to 1000 g to pellet out the platelets and it was subjected to washing in platelet washing buffer I (0.12 M NaCl, 0.0129 M Trisodium citrate and 0.03 M glucose) followed by washing in washing buffer II (0.154 M NaCl and 0.001 M EDTA) at a pH of 7.4. The washing procedure was continued till the platelets were erythrocyte free and its purity was confirmed. The platelet pellets were suspended in platelet storage buffer containing 0.109 M NaCl, 4.3 mM K₂HPO₄, 8.3 mM NaH₂PO₄ and 5.5mM glucose at a pH of 7.5 and stored at 4° C. All the estimations were performed concurrently within 5 hours of sample collection under sterile conditions^{6,9}.

Procedure for the Activity^{6,8}

The platelet rich plasma 0.13×10^{-7} for each assay was resuspended in tyrode buffer (pH adjusted to 7.4 with 0.25 M HCl). The platelet aggregation was recorded as transmittance values of spectrophotometer measurement. To determine the invitro inhibition of platelet aggregation different concentrations of extract from the rhizome like 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml in DMSO (Dimethyl sulphoxide) were used. The platelet aggregation was induced with ADP at a concentration of 5 µM which is used as control. The Aspirin 100 µg/ml is used as a standard⁵. The difference concentration of extract was added to platelet suspension and kept at 37° C for one minute then it

must be treated with the platelet aggregating agent. The transmittance is recorded at interval of 1 min for every 5 minutes. The platelet aggregation was recorded by increasing the transmittance value of spectrophotometric measurement. The lambda max was found to be 610 nm. The percentage transmittance of ethanolic extract was given in the table and figure no 1^{6,8}.

RESULTS AND DISCUSSION

The extract and aspirin showed significant antiplatelet activity. Platelet plays an important role in the process of a thrombosis by adhering to the damaged regions (caused by reactive oxygen species) of the endothelial surface. The activated platelet to platelet bond binds to leucocyte bringing them into a complex process of plaque formation and growth. The antiplatelet therapy constitutes the best available tool for ameliorating the mechanism related to atherogenesis and have interestingly inhibited platelet aggregation. Platelet sticks to damaged vessel wall, stick to each other (aggregate) and release ADP. Thromboxane A₂ (TxA₂) which promote further aggregation. Thus a platelet plug is formed. In the vein, due to sluggish blood flow the fibrous tail is formed, which traps RBC'S the red tail. In arteries platelet mass is the main constituent of the thrombus. Antiplatelet drugs are more useful in arterial thrombosis, while anticoagulants are more effective in venous thrombosis.

The flavonoids present in the extract might have prevented the adhesion and aggregation of platelets, besides the release of cytoplasmic calcium that stimulates the release of ADP and 5 HT. The above mentioned results prove that *Curcuma*

longa Linn prevents aggregation of platelets^{4,7}.

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Table-1 Antiplatelet activity of ethanolic extract of *Curcuma longa* Linn

S.No	Concentrations in mcg/ml	% Transmittance					
		0minutes	1minutes	2 minutes	3 minutes	4 minutes	5 minutes
1	Standard	17.36	17.48	17.455	17.51	17.55	17.6
2	Control	12.6	12.2	12.3	12.4	12.5	12.7
3	62 $\mu\text{g/ml}$	13.4	13.61	13.63	13.72	13.77	13.92
4	125 $\mu\text{g/ml}$	14.58	14.75	14.7	14.74	14.76	14.84
5	250 $\mu\text{g/ml}$	15.72	15.63	15.65	15.71	15.71	15.8
6	500 $\mu\text{g/ml}$	16.21	16.53	16.58	16.7	16.79	16.92

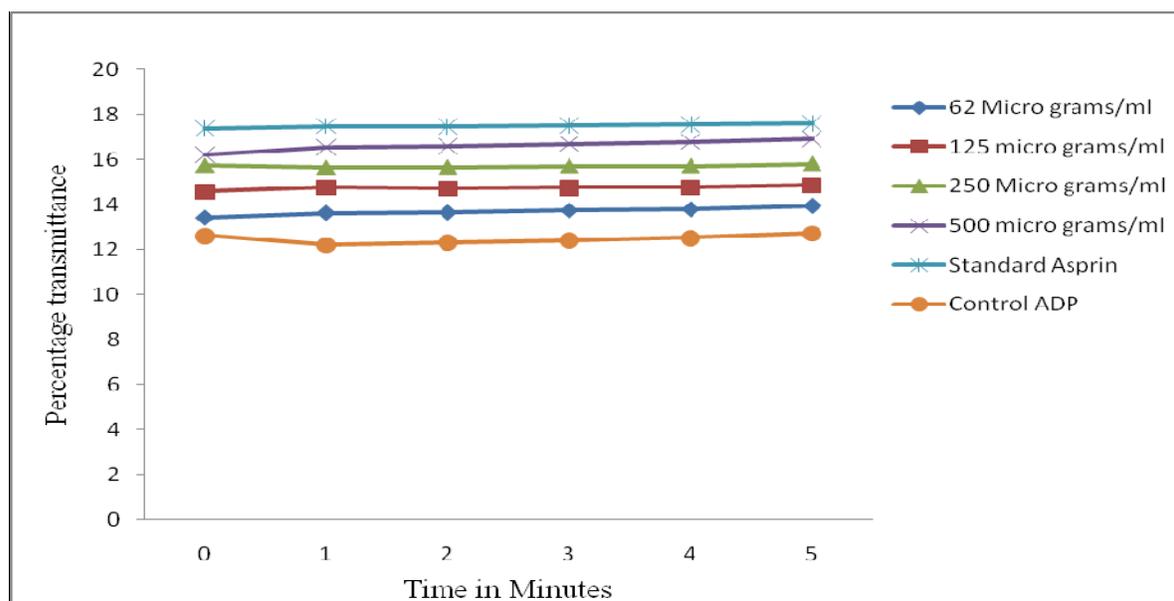


Figure-1 Antiplatelet activity of ethanolic extract of *Curcuma longa* Linn