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IN VITRO ANTI PLATELET AGGREGATION ACTIVITY AND THROMBOLYTIC ACTIVITY OF *CHEENALINGA CHENDHURAM*

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ABSTRACT

Cellular metabolism plays a vital role in the homeostasis of the body. This metabolism is disturbed in conditions like thrombus which inhibits the blood supply to the body tissues leading to ischemia and finally necrosis of the cells. Hence there is an urgent need for research in the field of antiplatelet and thrombolytic agents in order to prevent and cure thrombus which leads to the pathological conditions like Myocardial Infarction (MI), Hemiplegia, etc. Siddha system of medicine boasts of a variety of medicines to prevent, cure and to revive the body from various diseases which are coded based on the known 96 principles. Though, herbal medicines have the above mentioned properties, it is essential to search for mineral medicines of similar nature which are considered to be cost effective, with a speedy recovery and having a longer shelf life. *Cheenalinga chendhuram* [CLC] is a mineral drug and as in literature is mentioned for its use in cardiac ailments predominantly angina and MI in which thrombus is the pathological background. In this study, CLC was evaluated for its in vitro antiplatelet aggregation and thrombolytic activity which was showed effective at the dose of 300µg/ml and 75µg/ml respectively. We conclude therefore, that CLC is an effective drug in the treatment of cardiovascular diseases and cerebrovascular accidents.

Keywords: Cheenalinga Chendhuram, Anti-platelet activity, thrombolytic, angina and MI.

1. INTRODUCTION

In this modernised world, we are facing major threats against many stress related disease. Thrombus is a pathological condition that plays a vital role in causing many diseases like Stroke, Deep vein thrombosis and Myocardial infarction. Major clinical manifestation of thrombus is stroke. Stroke or Cerebro Vascular Accident (CVA) Causes 2,00,000 deaths each year in the world and are a major cause of disability. The most common forms of Cerebro Vascular Accident (CVA) are Cerebral Thrombosis (40% of cases), Cerebral embolism (30% of cases) and cerebral hemorrhage (20% of cases).Long before inventions of various modern techniques and drugs, siddhars spiritually and holistically emphasized various wonderful medicines to prevent stroke, *cheenalinga chendhurum* is one among them that posses thrombolytic activity and anti platelet aggregation activity. There is many Antiplatelet aggregation and Thrombolytic medicine in siddha, yet there is no proper scientifical evaluation or an attempt to establish *cheenalinga chendhuram* as good anti-platelet aggregation thrombolytic drug in siddha system.These beneficial effects result in the improvement of blood fluidity. However, as siddha drug significantly enhances fibrinolytic activity, it is theoretically possible that its over-activity could cause platelets to aggregate through the release of fibrinogen degradation products

because it has been reported that excessive fibrinolysis is associated with the release of FDP. These findings clearly showed that siddha drug has anticoagulant properties. Its medicinal uses include treatment for including Stroke, Deep vein thrombosis and Myocardial infarction. The interaction between platelets and blood vessel walls are important in the development of thrombosis and cardiovascular diseases such as myocardial infarction, stroke and atherosclerosis. Among the family of platelet activating factors (PAF), Arachidonic acid, and ADP are three important platelet stimulants, which induce platelet aggregation via different mechanism. Platelet plays a key role in the physiological hemostatic process and pathologic thrombosis. *CheenaLinga Chendhuram* possess anti-platelet activity in a dose-dependent manner. A physically active life style has an important role in preventing thrombotic events and decreasing the risk of cardiovascular disease. Platelets are involved in the pathogenesis and progression of cardiovascular diseases. These beneficial effects result in the return of normal blood flow.

2. MATERIALS AND METHODS

2.1 *PREPARATION OF CHENDHURAM*

The preparation was collected from *ANUBOGA VAITHIYA NAVANEETHAM – PART 4*, P.no-54 has written by *HAKKIM P.MOHAMMED ABDULLAH SAIBU.*

2.2 COLLECTION AND AUTHENTICATION OF THE TEST DRUG

The raw drugs 500mg of lingam, 2kg of vediuppu and 2kg of padikaram which are ingredients of *CHEENALINGA CHENDHURAM* collected from raw drug shop Tampcol at Chennai.They were identified and confirmed by gunapadam experts, PG Department of Gunapadam G.S.M. college at Chennai-106. These specimens were kept in PG Department of Gunapadam, G.S.M. College, Chennai-106, for further reference.

2.3 PREPARATION OF THE DRUG

Lingam : 35gm
Vediuppu : 70gm
Padikaram : 70gm

Purified *Vediuppu* and *Padikaram* were powdered, taken together in new mud vessel and it was burnt on low flame. When it melts, purified *lingam* bar was added and fried. Then *Lingam* was taken from it and scrubbed. Then it was washed in distilled water and grinded in stone mortar into fine powder. This powdered medicine was kept in a closed air tight glass vessel for the further use.

2.4 TOXICITY STUDY

Animals:

Healthy adult Wistar albino rats (200-250g) and Swiss albino mice (28-35g); individually in polypropylene cages, maintained under standard conditions (12 h light and 12 h dark cycle; 25± 30^{o}C; 35 - 60% humidity). The animals were fed with standard rat pellet diet and water *ad libitum*. The Institutional Animal Ethical Committee (IAEC/VELS/08/10-2011) approved the study

ACUTE TOXICITY STUDY:

Acute toxicity studies were conducted to determine the safe dose as per OECD-423 guidelines. Drugs were administered orally to overnight fasted animals. After drug administration the animals were observed continuously for 1 hour, frequently for the next four hours, and then after 24 hours. After administration, Irwin's test was conducted, where the animals were observed for gross behavioral changes. The toxic dose was determined by observing the mortality rate in the drug treated groups. From this the therapeutic dose was selected for the further study.

2.5 PHARMACOLOGICAL STUDY

Drugs and Chemicals

The drugs and reagents used in this study were as follows: Heparin sodium and adenosine diphosphate (ADP) (Sigma Chemical Co., St. Louis, MO, U.S.A). Other reagents used were of analytical grade. On the day of the assay the CLC was dissolved in 2% CMC in saline to yield a final concentration of 0.5% (v/v) for *InVitro Anti-platelet Aggregation* studies.

ANTI-PLATELET AGGREGATION ACTIVITY

Platelet rich plasma (PRP) was prepared by centrifugation (1000 rpm for 5min) of blood collected from normal aspirin free blood bank donors. 1.5 ml of acid citrate dextrose was used as anticoagulant for every 8.5 ml of blood. PRP was

taken into siliconized glass cuvettes. Platelet poor plasma (PPP) collected by centrifugation (3000 rpm for 5 min) was kept as reference. The cuvettes were incubated at 37°C for 5 min. The aggregation was initiated by `adding 20 µl of ADP (10 µM) to 1ml of PRP. The aggregation was recorded for 5 min at 600 nm. The effect of different concentrations (50–250µg) of *Cheenalinga Chendhuram* was studied by incubation with PRP at 37 °C for 5 min before the addition of ADP. Commercial heparin (20µg/ml) was used as reference standard. The maximal aggregation was recorded.

The aggregation is expressed as % inhibition (*X*) calculated by using the following equation: *X(%)=(A-B)/A×100.* where A=maximal aggregation of the control, and B=maximal aggregation of drug-treated PRP.

ADP induced platelet aggregation

The reaction mixture contains different concentration of drug CLC 0.5 ml (100, 200, 400 µg/ml) solution and platelet rich plasma 0.5 ml. These reaction mixtures was maintained at 37^0C and kept for 2 min with constant stirring, 0.5 ml of ADP solution was added and incubated for 4 mins and absorbance was measured at 414 nm. ADP induced platelet aggregation was calculated by following formula:

$$\% \text{ ADP induced platelet aggregation} = \frac{(T1-T2) - (T1-T3)}{(T1-T2)} \times 100$$

Where, T1= ADP + Platelet, T2= ADP + CLC +Platelet and T3= ADP + CLC +Platelet.

Statistical analysis

The data of I*n vitro* anti-platelet aggregation activity of *Cheenalinga Chendhuram* was analyzed statistically using One-Way ANOVA followed by Dunnett's t test by INSTAT-V3 computer software programme.

2.7 THROMBOLYTIC ACTIVITY

Method

In the present study the thrombolytic activity was analysed by the modified invitro method. The 0.2ml of fresh blood of a donor was added in seven sterile cuvettes were added serially and the test drug SLC at different concentration from 25-125microgram was added after fifteen minutes of blood collection. Then the contents were mixed slowly by tilting the cuvettes and then a small amount of thrombus was transferred carefully with the help of capillary tube to the plain glass plate and observed for the cell distribution under microscope.

3. RESULTS

Table 1. ***Modulatory effect of different concentrations of Cheenalinga Chendhuram on platelet deaggregation activity.***

S.No.	Groups	Inhibition of platelet aggregation (%)
1.	Control	----------
2.	*Cheenalinga Chendhuram* 100 (µg/ml)	23.6 ± 2.62**
3.	*Cheenalinga Chendhuram* 200 (µg/ml)	38.19 ± 2.44**
4.	*Cheenalinga Chendhuram* 300 (µg/ml)	49.60 ± 2.53**
5.	Heparin (20 µg/ml)	78.99 ± 2.84

Values are expressed as mean ± SEM. P<0.01 compared to standard (n=6)

The doses fixed for the Anti platelet aggregation activity were 100, 200, and 300µg/ml to ascertain the dose dependent activity. *In vitro* inhibitory activity of *Cheenalinga Chendhuram* against ADP-induced platelet aggregation were measured. *Cheenalinga Chendhuram* inhibited platelet aggregation *in vitro* potently compared to heparin reference drug widely used as anti-platelet agent in clinical practice.

Intravascular thrombosis is one of the generators of a variety of cardiovascular disease and platelet aggregation is believed to play a crucial role in atherothrombotic processes. *In vitro*, *Cheenalinga Chendhuram* inhibits platelet aggregation in a dose-dependent manner stimulated by a ADP, in human platelet-rich plasma. Tissue factor, also called platelet tissue factor, is necessary for the initiation of thrombin formation from the zymogen prothrombin. Taken together, all these results suggest that *Cheenalinga Chendhuram* has an effective anti-platelet effect *in vitro,* and be a potential therapeutic agent for arterial thrombosis.

THROMBOLYTIC ACTIVITY

The microscopical examination reveals that the *Cheenalinga chenduram* at the concentration of 75microgram added content showed RBC redistribution at dose dependent manner. As a result of drug treatment at various concentrations it was identified that the CLC possess effective deaggregation compared to aspirin and indicates the drug can be an alternative for aspirin in the treatment of cardiac diseases since the activity was almost equivalent to that of the positive control aspirin.

4. DISCUSSION

Platelets play a pivotal role in health and diseases, given their central involvement in homeostasis and thrombosis. Recently several natural antiplatelet agents from natural products including polyphenols and flavonoids have been reported. Plant preparations containing polyphenols/flavonoids have been used for centuries as herbal remedies for a variety of diseases and found to have an impact on diabetes and obesity related disorders. Thrombosis plays an important role in the pathogenesis of acute coronary syndromes, and vessel wall injury leads to the adherence of platelets and subsequent platelet activation. Platelet aggregation is absolutely essential to the formation of a hemostatic plug when normal blood vessels are injured. However, the interactions between platelets and collagen can also cause circulatory disorders, such as thrombosis, atherosclerosis, and myocardial infarction. Inhibition of the platelet-collagen interaction might be a promising approach to the prevention of thrombosis. *Cheenalinga Chendhuram* may have an anti-platelet function by elevating the cyclic adenosine monophosphate (cAMP) level, and then by decreasing the $[Ca^{2+}]$, an essential factor for platelet aggregation. Intravascular thrombosis is one of the generators of a variety of cardiovascular disease and platelet aggregation is believed to play a crucial role in atherothrombotic processes. It is reported that CLC have anti-platelet aggregation activity. *In vitro*, CLC (40–200 μM) inhibits platelet aggregation in a dose-dependent manner stimulated by an agonist ADP, in human platelet-rich plasma. Further investigation reveals that those effects are due to the inhibition of phospholipase C activity, leading to reduced phosphoinositide breakdown, followed by the inhibition of thromboxane A_2 formation, and then inhibition of $[Ca^{2+}]$ mobilization of platelet aggregation stimulated by agonists.

Tissue factor (TF), also called platelet tissue factor, is necessary for the initiation of thrombin formation from the zymogen prothrombin. Besides the above mechanism, the antiplatelet activity of CLC is related to the inhibition of the release of platelet-derived TF by stimulating the synthesis and releases of cGRP *(cyclic g\Guanosine Releasing Peptide).* Taken together, all these results suggest that CLC has an effective anti-platelet effect both *in vivo* and *in vitro,* and be a potential therapeutic agent for arterial thrombosis.

5. CONCLUSION

In conclusion, many cardiovascular diseases can be attributed to excessive platelet aggregation, which has a critical role in thrombus formation. It appears that *Cheenalinga Chendhuram* can inhibit platelet aggregation *in vitro*; therefore, they may be used to treat or prevent some cardiovascular diseases. The present study ensures the anti-platelet aggregation of *Cheenalinga Chendhuram* that is beneficial for cardiovascular disorder patients. However, this drug should be used with caution by patients with bleeding or other haematological disorders as it may increase the risk of bleeding and complications.

6. REFERENCES

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