Prayogik Rasayan

In vitro Thrombolytic Activity of Flowers of *Nerium oleander*

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Abstract

The study was carried out to investigate the *in vitro* thrombolytic potential of crude methanolic extract of flowers of *Nerium oleander*, a plant widely employed in traditional medicine to treat cardiovascular diseases. Using the blood of selected sixteen individuals, the thrombolytic activity was assessed in four different concentrations of the crude plant extract where streptokinase and normal saline were used as the positive and negative control respectively. The clot lysis percentage was calculated using weight reduction of the blood clot in comparison to the initial clot weight. The highest thrombolytic potential was observed at 10 mg/mL concentration of the extract and there was no significant difference between mean clot lysis percentages of streptokinase and this extract. Therefore, 10.0 mg/mL concentration of methanolic extract of flowers of *N. oleander* has a good thrombolytic ability, however, further investigations on its chemical profile and toxic properties are required before developing it as a therapeutic agent.

Keywords: *Nerium oleander*, streptokinase, thrombolytic activity.

1. Introduction

The term “thrombosis” is used to describe the formation of blood clots inside blood vessels and this is a condition which could lead to disturbed blood flow through the circulatory system due to blockage of blood vessels. If this occurs inside an artery, it is known as arterial thrombosis and it is a main cause of myocardial infarction, ischemic stroke, and limb gangrene. When this occurs inside a vein, it is known as venous thrombosis and it leads to deep vein thrombosis which can be complicated by the post-thrombotic syndrome, pulmonary embolism, chronic thrombo-embolism and pulmonary hypertension.[1] Thrombolytic therapy, which is known as thrombolysis, is a treatment to dissolve blood clots formed in blood vessels and improve blood flow through blood vessels. This prevents damage to tissues and organs due to the loss of blood supply. Thrombolysis is mainly done by the injection of clot-busting drugs through an intravenous line or through a long catheter that delivers drugs directly to the site of blockage. Streptokinase, urokinase and tissue plasminogen activator are widely employed in clinical practice as thrombolytic agents. However all these agents have significant deficiencies that include the necessity of large doses to be maximally effective, limited fibrin specificity and hyper-risk of bleeding tendency. Due to such kinds of limitations, attempts are taken to find out new thrombolytic agents with minimum side effects[2] and plants that have been used in the indigenous medicinal systems would be a potent source.

Nerium oleander (Family: Apocynaceae) is widely employed in traditional medicine in Sri Lanka to treat cardiovascular disorders while the flowers and leaves are used as a cardio tonic in other Asian countries as well.[3] In addition, it is used as treatment options for various conditions such as asthma, epilepsy, bronchitis, leprosy, malaria, kidney diseases and skin disorders.[4] Due to the traditional utility of *N. oleander* as a remedy for cardiovascular diseases, the present study is undertaken to evaluate its thrombolytic potential and thereby to scientifically validate the folklore claims.

2. Methodology

2.1. Preparation of plant extract

Flowers of *N. oleander* were collected from Gampaha district in Western Province of Sri Lanka in 2018 and authenticated from the National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka. A voucher specimen (WP2018-NO_13) was deposited at the Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Sri Lanka. The flowers were air dried, cut into small pieces and extracted in methanol for two days. Thereafter, the liquid aliquot obtained by filtration was evaporated into complete dryness using the rotary

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evaporator. Then four test concentrations (0.5, 1, 5 and 10 mg/mL) were prepared using this crude extract.

2.2. Determination of thrombolytic activity

Blood samples were drawn from healthy volunteers (n=16) without a history of anticoagulant therapy and those who have not taken any form of medicaments for the past two weeks. Five milliliter of blood was drawn from each individual at a time and was aliquoted into six equal parts (0.8 mL each) which were transferred into the previously weighed microcentrifuged tubes (W_e) immediately. These samples were incubated at 37 °C for 45 minutes for clot formation. Thereafter, the samples were centrifuged and serum was completely removed without disturbing the clot. The serum was used for total cholesterol measurement. The weight of the tube with the clot (W_c) was measured.

100 µL of reconstituted streptokinase (positive control), 100 µL of normal saline (negative control), and 100 µL of crude extracts were separately added to the microcentrifuge tubes. All the tubes were then incubated at 37 °C for 90 minutes and observed for clot lysis. After incubation, the tubes were centrifuged and releasing fluid was completely removed. Final weights of tubes were measured (W_f) and the clot lysis percentage was calculated following the method of Tabassum et al.[5]

W_e = Weight of the empty microcentrifuge tube
W_c = Weight of the microcentrifuge tube with initial blood clot
W_f = Weight of the microcentrifuge tube after clot lysis in 90 minutes
Reduction of clot weight = $W_c - W_f$
Initial clot weight = $W_c - W_e$

Clot lysis % = (Reduction of clot weight / Initial clot weight) × 100

Clot lysis % = $(W_c - W_f / W_c - W_e) \times 100$

The experiment was performed in duplicate. The statistical analysis of the data was performed by Analysis of Variance (ANOVA) using Post hoc test. P value <0.05 was considered to denote a statistically significance.

2.3 Measurement of total cholesterol level

In order to minimize the variation of blood cholesterol level of the individuals on the clot formation, a correlational study was carried out to assess whether there is a correlation between total cholesterol level and thrombolytic activity. For that, total blood cholesterol level was measured using the separated serum. The colorimetric method was employed to estimate the total cholesterol level using DIALAB cholesterol assay kit (Austria) and the absorbance at 500 nm was measured. Cholesterol level of the samples was calculated according to the following equation.

Serum cholesterol concentration= $Ab_T/Ab_S \times 100$
where Ab_T = Absorbance of the test sample
Ab_S = Absorbance of the standard

All the measurements were conducted in duplicate.

Ethical approval was obtained by the Ethical Review Committee of Faculty of Allied Health Sciences, University of Ruhuna, Sri Lanka.

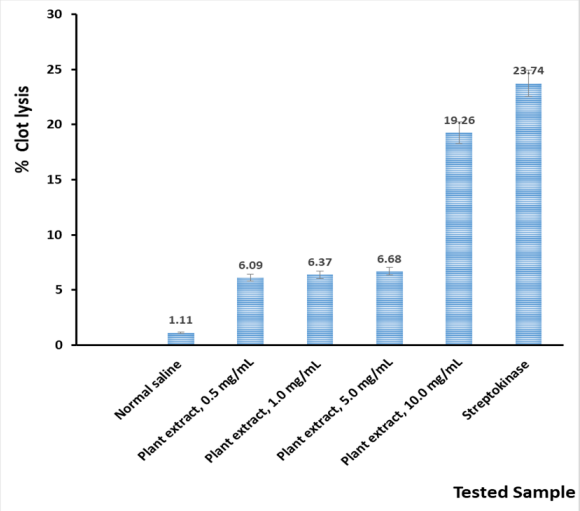

Fig. 1: Mean clot lysis percentage by normal saline, four concentrations of extract of flowers of *N. oleander* and streptokinase.

3. Results and Discussion

Addition of 100 µL of the positive control streptokinase (1,500,000 IU) to the clots and subsequent incubation for 90 minutes at 37°C, resulted in 23.74% lysis of clot where as normal saline which was used as the negative control exhibited a negligible percentage of lysis of clot (1.11%). On the other hand, different concentrations of the crude extract displayed different degrees of clot lysis percentage with the highest activity at a concentration of 10 mg/mL (Fig.1). Interestingly, the post hoc analysis revealed that there is no significant difference of mean clot lysis percentage of streptokinase and the crude extract at 10 mg/mL (p=0.205).

A number of studies have been conducted in other countries over the last few years to assess thrombolytic activity of several medicinal plants. For example, the aqueous extract of *Murraya koenigii* showed a proportional increase in clot lysis with the increase in sample concentration and it exhibited 26.17% lysis at 100 mg/mL, the highest concentration used for the assay.[6] Similarly, we have observed an increase in the clot lysis activity with the increase in sample concentration and the highest activity was observed at 10 mg/mL. This suggests that the above concentration could be used in thrombolytic therapy, however further experiments should be performed to evaluate its suitability to develop as a thrombolytic agent.

Increased levels of total serum cholesterol, low density lipoprotein (LDL) and decreased levels of high density

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lipoprotein (HDL) are well established risk factors for atherothrombotic disorders. Besides their strong effect on atherogenesis, lipoproteins and lipids could influence hemostasis by modulating the expression and function of procoagulant, fibrinolytic, and rheological factors. Triglycerides increase factor VII levels, plasminogen activator inhibitor, and blood viscosity. HDL has anti-atherothrombotic properties that may result from inhibition of platelet aggregation, suppression of tissue factor activity, and reduction of blood viscosity. Due to such kind of effects, lipid levels also contribute to the development of venous thrombosis.[7] Thus the correlation between total serum cholesterol values and percentage clot lysis was also evaluated in this study. The Pearson correlation between cholesterol and percentage clot lysis was determined as 0.061 which is nearly zero. This indicated that there is no correlation between these two parameters, hence, percentage clot lysis is independent of the total serum cholesterol of an individual.

4. Conclusion

The findings of the present study demonstrated that folklore medicinal plant *N. oleander* has significant implications in cardiovascular health indicating the possibility of developing novel thrombolytic agents from this plant. Thus, further studies are in progress to isolate and characterize the compounds responsible for thrombolytic activity and to evaluate possible cytotoxic effects, in order to assess the suitability of this extract to develop as a thrombolytic agent.

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