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Effect of Andrographolide on *in vitro* Thrombolytic Activity

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ABSTRACT

Background: Thrombolytic therapy holds great promise for becoming an important therapeutic adjunct in the treatment of acute vascular occlusions, but such therapy has not reached the stage for general clinical use. Hence, the aim of present study was to evaluate the thrombolytic activity of andrographolide, an active constituent present in the plant *Andrographis paniculata*. **Method:** Venous bloods drawn from animals (rats) was transferred in 6 pre weighed sterile micro centrifuge tubes and were incubated at 37 degree centigrade (°C) for 45 min to form clots. After formation of clot, the tubes were reweight to calculate clot weight (weight of the tube with clot-pre weight of the tube = clot weigh) and then in 3 micro centrifuge tubes containing clot, 500 microlitre of isolated andrographolide was added and in remaining 3 tubes, 1% w/v tween 80 in distilled water was added which serves as negative control. All the test tubes were then incubated at 37 degree centigrade (°C) for 90 minutes and observed for clot lysis. **Results:** Addition of 500μl of andrographolide to the clots along with 90 min of incubation at 37 degree centigrade (°C) showed 21.22±0.62% clot lysis. Distilled water, showed only negligible clot lysis of 4.20±0.06% compared with andrographolide. Which indicate that andrographolide have mild thrombolytic activity. **Conclusion:** From the present study, it may be concluded andrographolide may be used as mild thrombolytic agent and as a natural source for the treatment of blood clot.

Key words: *Andrographis paniculata*, andrographolide, thrombolytic, blood clot, clot lysis

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INTRODUCTION

Cerebral venous sinus thrombosis is a common disorder that is often accompanied by significant morbidity and mortality. A blood clot developed in the circulatory system due to failure of hemostasis causes vascular blockage and while recovering leads to serious consequences in atherothrombotic diseases such as myocardial or cerebral infraction at times leading to death (Lee, 1995). The interaction between platelets and blood vessels is important in the development of thrombosis and cardio vascular diseases (Gorden, 1981). Uncontrolled platelets aggregation is critical in arterial thrombosis and may cause life threatening disorders (Davies and Thomas, 1985). Antiplatelet agents are therefore considered as a key tool in the treatment and prevention of cardiovascular thrombotic diseases (De Meyer *et al*., 2008). Although, well established that aspirin will provides an effective secondary prevention of ischemic cardiovascular disorders, this drug can produced hemorrhagic events and upper gastrointestinal bleeding as major drawbacks (Johnson, 2008).

Thrombolytic agents that include tissue plasminogen activator, urokinase and streptokinase are used all over the world for the treatment of these diseases. In India though streptokinase and urokinase are widely used due to lower cost as compared to other thrombolytic agents their use is associated with hyper risk of hemorrhage, severe anaphylactic reaction and lacks specificity. Moreover, as a result of immunogenicity multiple treatments with streptokinase in a given patient are restricted. Because of the shortcomings of the available thrombolytic drugs attempts are underway to develop improved recombinant variants of these drugs (Prasad *et al*., 2007). The Indian pharmacopoeia mentioned that *Andrographis paniculata* is a predominant constituent of at least 26 ayurvedic formulations. Extensive research has revealed that *Andrographis paniculata* has a broad range of pharmacological effect (Radhika and Lakshmi, 2010).

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Andrographis paniculata is an herbaceous plant belongs to family Acanthaceae, native to India and Srilanka mostly leaves and roots were used for medicinal purpose (Kumar *et al*., 2010). Andrographolide, Neo andrographolide and kalmeghnin present in the plant have been reported to be a active principles (Rajani *et al*., 2000). Andrographolide is a bicyclic terpenoid lactone having bitter taste with various activities (Maiti *et al*., 2006). Herbal drug therapy is the most trusted system of medicine in countries like India, where people strongly believe in Ayurveda as herbs are the part of rural Indian life style. Most of the diseases which have no medicine in allopathic system can be cured successfully using traditional medicines (Roopashree *et al*., 2009). The present study was undertaken to evaluate the thrombolytic activity of andrographolide isolated from *Andrographis paniculata*.

MATERIALS AND METHODS

Collection and identification of plant materials: The aerial parts of *Andrographis paniculata*, were collected from Thirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India, Palayamkottai. The aerial part of *Andrographis paniculata*, were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Animals: Experiment animals (rat) were obtained from the central animal house, Raja Muthiah Medical College Annamalai University. They were acclimatized to the standard laboratory conditions for two weeks prior to initiation of the study. All experiments were carried out according to the guideline for care and use of experimental animals and approved by Institution Animal Ethical Committee Affiliated to CPCSEA India.

Extraction isolation and identification: Aerial parts of *Andrographis paniculata* powder was extracted exhaustively with a 1:1 mixture of dichloromethane and methanol by cold maceration method. Solvents were removed by vacuum evaporation. The obtained extract was washed with toluene several times for removal of colored matters then the toluene was removed from the residue. The crystalline material left behind was dissolved in hot methanol and cooled in a refrigerator for crystallization. The isolated Andrographolide identified by compared with standard Andrographolide by IR and NMR (Rajani *et al*., 2000).

Acute toxicity: The acute toxicity study of isolated andrographolide was carried out in female swiss albino mice as per Organization Economic Cooperation Development (OECD 423) guidelines. For the evaluation of acute toxicity, isolated andrographolide 2000 mg kg^{-1} b.wt. was used (Prakash and Manavalan, 2011).

Thrombolytic activity: Venous blood (1-2 mL) drawn from rat was transferred in different pre weighed (W_1) sterile microcentrifuge tube (500 microlitre (μL)/tube) and incubated at 37°C for 45 min. After clot formation, serum was completely removed (aspirated out without disturbing the clot formed) and each tube having clot was again weighed (W_2) to determine the clot weight (clot weight = weight of clot containing tube-weight of tube alone). Each microcentrifuge tube containing clot was properly labeled and 500 μL of isolated andrographolide (2 mg mL^{-1} suspended in 1% w/v tween 80 in distilled water) was added to the tube.

The concentration of tween 80 (1% w/v) in distilled water was also added to one of the tube containing clot and this serves as a negative control. All the tubes were then incubated at 37°C for 90 min and observed for clot lysis. After incubation, fluid obtained was removed and tubes were again weighed (W_3) to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis (Prasad *et al*., 2007). The experimental results were expressed as the Mean±SEM.

RESULTS AND DISCUSSION

The percentage of weight loss of clot after application of andrographolide was taken as the functional indication of thrombolytic activity. The results from the above study clearly demonstrate that the addition of 500 μL of andrographolide isolated from *Andrographis paniculata* to the clots along with 90 min of incubation at 37°C showed 21.22±0.62% clot lysis. Distilled water, which was taken as a negative control, when compared with andrographolide clearly demonstrated clot dissolution does not occur when water was added to the clot and showed negligible clot lysis 4.20±0.065% (Table 1). Which indicate that Andrographolide may be having mild thrombolytic activity.

Blood clotting process is very complex, involving many factors found in the plasma and tissues. It involves both the intrinsic and extrinsic pathways. One of the major causes of blood circulation problem is the formation of blood clots. Thrombi or emboli can lodge in a blood vessel and block the flow of blood in that location depriving tissue of normal blood flow and oxygen, hence, result in damage destruction or even death of the tissues in the area. Platelets are blood cells that participate in the human primary haemostatic mechanism. Platelet-platelet interaction has the final

Table 1: Data for Thrombolytic activity of andrographolide

Tube No.	Sample name	W1 (g)	W2 (g)	W3 (g)	Clot lysis (%)
1	Distilled water	1.057	1.591	1.568	4.3
2	Distilled water	1.046	1.584	1.562	4.08
3	Distilled water	1.021	1.597	1.574	4.24
Mean±SEM		1.04±0.010	1.59±0.003	1.56±0.003	4.20±0.065
4	Andrographolide	1.0676	1.4075	1.3621	20.51
5	Andrographolide	1.073	1.4232	1.349	22.47
6	Andrographolide	1.0559	1.4554	1.3727	20.7
Mean±SEM		1.06±0.005	1.42±0.014	1.36±0.006	21.22±0.62

purpose to produce a platelet thrombus that constitutes the primary haemostatic plug (Jin *et al*., 2004).

In addition, platelet adhesion and aggregation on blood vessel walls contribute to the occurrence of thrombosis and emboli formation and have relation with other cardiovascular diseases (Ruggeri, 2002; Tang *et al*., 2003; Michelson *et al*., 2006).

Fibrinolytic drugs have been used to dissolve thrombi in accurately occluded coronary arteries there by to restore blood supply to ischemic myocardium to limit necrosis and to improve prognosis. The treatment with any oral anticoagulant must be monitored to ensure that the dose is providing the required effects.

There are several thrombolytic drugs obtained from various sources. Some are modified further with the use of recombinant technology (Verstraete, 2002) in order to make this thrombolytic drug more site specific and effective. Side effects related to these drugs have been reported that lead to further complications (Baruah *et al*., 2006). Sometimes the patients die due to bleeding and embolism (Verstraete, 2002; Gallus, 1998; Wardlaw *et al*., 2004; Capstick and Henry, 2005).

Therefore, now time alarming us to think of some alternative in the field. Hence, efforts are made to think back on our natural products. Herbal preparations, if taken in appropriate dose, can lead to a better option for curing various ailments. Toxicity of plant extract is a major concern of scientists and medical practitioners. Among several methods lethality test has been successfully used to biomonitor the isolation of cytotoxic, antimalarial, insecticide and antifeedansts compound from plant extracts (Krishnaraju *et al*., 2006).

The study of thrombolysis activity was carried out with andrographolide from the plant *A. paniculata*. The study was supported by the earlier studies on herbal plants as *Glycyrrhiza glabra* Linn was reported to have antiplatelet and anti inflammatory activities (Kharb and Singh, 2004). Interestingly *Bacopa monnieri* Linn exhibited nearly 50% of clot lysis and used as a cardiac nerve and brain tonic (Joshi and Parle, 2006). Few product and plant are identified to have fibrolytic activity, Such as *Lumricus rubella* (Jeon *et al*., 1995), *Pleurotus ostreatus* (Choi and Shin, 1998).

CONCLUSION

In conclusion, on basis of the experimental results obtained in the study andrographolide lyses blood clot *in vitro*, however *in vivo* dissolving property for clot lysis is yet to be found out. Andrographolide is used as mild thrombolytic agent for the treatment of blood clot. Clot lysis property could also beneficial in the treatment of clot lysis in patients.

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