eISSN: 2231-0541 CAS CODEN: PHARN8 An EMBASE, EMCare Covered Journal

PHARMANEST

An International Journal of Advances in Pharmaceutical Sciences

Volume 5 | Issue 1 | January-February 2014 | Pages 1829-1833

Original Research Article

ANTITHROMBOTIC AND THROMBOLYTIC ACTIVITY OF *NIGELLA SATIVA* SEED EXTRACTS

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Received: 21-11-2013 Revised: 11-12-2013
Accepted: 29-12-2013 Available online: 01-01-2014

ABSTRACT

Thrombolytic agents are used to dissolve the preformed clots in the blood vessels. However, the available drugs have certain limitations such as allergic reactions and expensive. The aim of the present study was to investigate whether preparations of *Nigella sativa* seeds possess thrombolytic and antithrombotic activity. An in vitro model was used to check the thrombolytic and antithrombotic effect of *Nigella sativa* seeds against Streptokinase as a positive control. It was found that after addition of Streptokinase, clot formation is delayed upto more than 90 min. Different concentrations of *Nigella setiva* extract delayed the clot formation. The maximum delay in the clot formation of 27 min was recorded at 1.00 mg/dl. For thrombolytic activity, at this concentration the clot dissolution time was 79 min with aqueous extracts. Although in present study, we found that *Nigella sativa* seeds possess thrombolytic and antithrombotic activity but in vivo clot dissolving properties and active component of *Nigella sativa* for clot lysis are to be studied.

Key Words: *Nigella sativa*, Antithrombotic, Thrombolytic, Streptokinase.

INTRODUCTION

Atherothrombotic diseases such as deep vein thrombosis, myocardial or cerebral infarction is one of the most common causes of death world wide [1,2]. Myocardial or cerebral infarctions are serious outcome of the thrombus formed in blood vessels. Various thrombolytic agents are used to dissolve the preformed clots in the blood system. However, the available drugs have certain limitations such as allergic reactions and expensive [1,3]. If a clot (thrombus) developes in the circulatory system then it may cause vascular blockage and leads to serious outcome such as myocardial or cerebral infarction, deep vein thrombosis [4]. Available thrombolytic agents such as streptokinase (SK), tissue plasminogen activator (t-PA), urokinase (UK) etc. are used worldwide for the treatment of such diseases [4]. In India, comparatively lower cost thrombolytic agent such as SK and UK are used but as compared to other thrombolytic drugs (tPA), these are having high risk of hemorrhage, severe allergic/anaphylactic reaction and have no specificity. Furthermore, due to the immunogenic reactions of SK, repeated dose/treatment is restricted. Due to these limitations of the available thrombolytic drugs, efforts are going on to develop better recombinant variants of such drugs. On another hand many available antithrombotic drugs may have a harmful effect on normal haemostasis which may lead to bleeding problems. Hence it is necessary to develop a new drug which shows less unfavorable effect[1]. In recent times, precautionary measures against thrombosis have been tried. Oral administration of the fibrinolytic enzyme nattokinase is one example, which has been reported to enhance fibrinolytic activity and the production of tPA [5]. Significant efforts have been aimed in the direction of the development of natural products from a variety

of plant and animal sources which have antithrombotic, antiplatelet, anticoagulant and thrombolytic activity. Studies have offered evidence that foods with antithrombotic effect could reduce risk of unwanted thrombus formation [6].

The aim of the present work was to investigate whether extracts of *Nigella sativa* (seeds) possess thrombolytic and antithrombotic activity in an in-vitro model using streptokinase as positive control.

MATERIALS AND METHODS

Streptokinase (SK): Commercially available lyophilized SK vial (**ST-Pase®**

Cadila pharma) of 15, 00,000 I.U., mixed in sterile Normal Saline properly. This suspension was used as a stock from which working solution (5,000 I.U) were prepared freshly by normal saline each time. [1]

Specimen: Whole blood was drawn from healthy rabbits. Required volume of blood taken in to each of the micro-centrifuge tubes (MCTs).

Plant material: The seeds of *Nigella sativa* were purchased from local market and were identified and authenticated by NBRI Lucknow.

Preparation of Extract: The seeds of *Nigella sativa* were air dried for 3 days, and then kept in an oven at 45°C for 72 hours. The extraction was carried out by maceration process. 50 gm of dried powder was taken each time for both alcoholic and aqueous extraction. After completion of the extraction process the extract was collected and concentrated by evaporation to obtain a semisolid extract.

Dilution of extracts:

Two dilutions (5 times and 10 times) was been made in normal saline (NS) for both extracts i.e. 0.5g of extract dissolves in 2.5ml and 5ml of NS respectively.

Clot lysis and antithrombotic activity:

Experiments for clot lysis and antithrombotic activity were carried as reported earlier [1,7]. Different concentrations of both extracts (alcoholic and aqueous) were added in previously washed micro centrifuge tubes (MCTs) containing 0.5 ml of blood in each tube. Streptokinase was used as positive control. In first test tube only normal saline (N.S.) was added and taken as blank. SK was added in tube no. 2. Extract of *Nigella sativa* was added in increasing order in tube no. 3 to 6 (Table 1). Time was noted before each addition. The volume of each tube was made upto 1ml. For antithrombotic activity reactants (extract solution, SK, NS) was added before clot formation (immediately after taking blood in tubes) and for thrombolytic activity, the reactants were added just after clot formation. All reactions have been maintained at 37°C in water bath. The experiment was repeated 4 times with all 4 dilutions of both the extracts.

Table.1.Method- various volumes of solutions used

Tube no.	Vol. of blood (ml)	Vol. of solutions in ml.					
		For anti- thrombotic activity			For thrombolytic activity		
		S.K.	Test solution	N.S.	S.K.	Test solution	N.S.
1.	0.5	---	---	0.50	---	---	0.5
2.	0.5	0.2	---	0.30	0.5	---	---
3.	0.5	---	0.1	0.48	---	0.1	0.4
4.	0.5	---	0.2	0.46	---	0.2	0.3
5.	0.5	---	0.3	0.44	---	0.3	0.2
6.	0.5	---	0.5	0.40	---	0.5	---

The volume of each tube was made up to 1ml by adding sufficient amount of NS. Same method was applied for both (5X and 10X) dilutions of aqueous and alcoholic extracts.

RESULTS

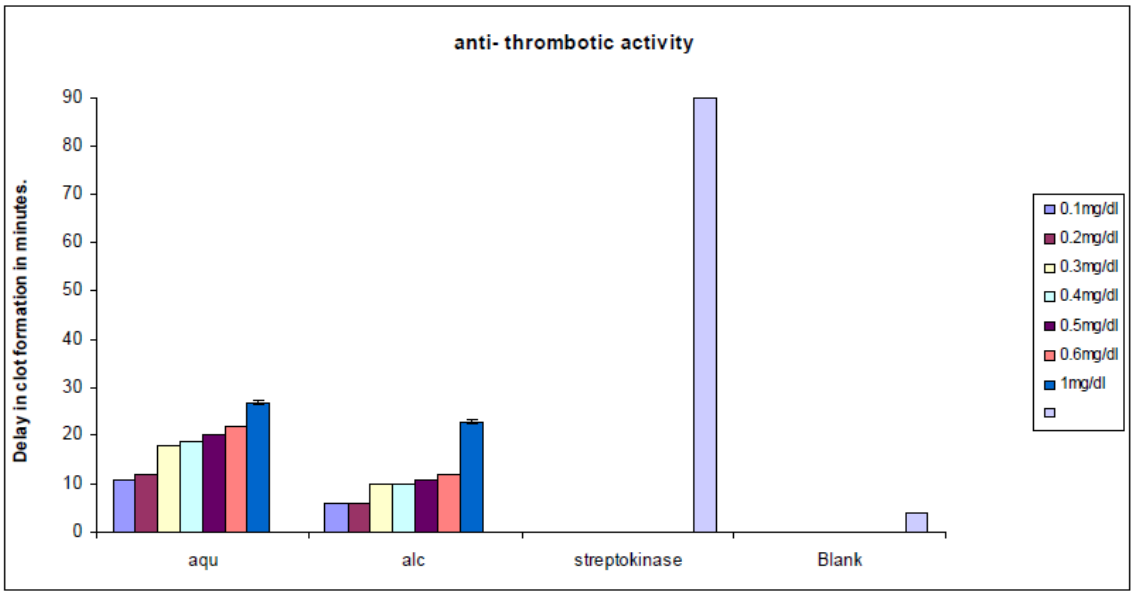
When NS was added to the control (tube# 1) the clot lysis was negligible. Where as tube to which SK was added, the clot lysis can be seen in 40-50 minutes and in case of extract solutions, significant clot lysis could be seen as according to concentration. Maximum clot lysis was observed in tube #6 in which maximum concentration of extract was added. In case of antithrombotic experiment, the clot was formed in normal time or slight delay when NS was added to the control (tube# 1). Where as the tube in which SK was added, the clot was not formed and in case of extract solutions, significant delay in clot formation time was noted according to concentration. Maximum delay in clot formation time was observed in tube #6 in which maximum concentration of extract was added (Table 2).

Table.2.Result- observations of different concentrations of both aqueous and alcoholic extracts

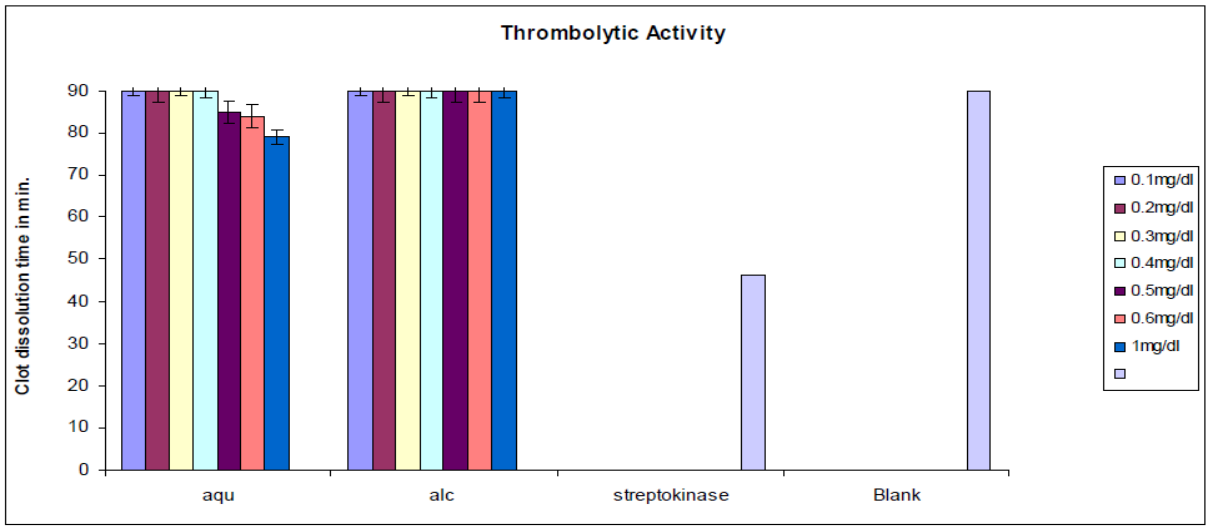
S.No.	For Anti- thrombotic activity			For Thrombolytic activity		
	Conc. of drug (mg/dl)	Delay in clot formation in minutes		Conc. of drug (mg/dl)	Clot dissolution time in minutes	
		For Aqu.	For Alc.		For Aqu.	For Alc.
1.	0.00 (blank)	04	04	0.00 (blank)	No*	No*
2.	0.10	11	06	0.10	No*	No*
3.	0.20	12	06	0.20	No*	No*
4.	0.30	18	10	0.30	No*	No*
5.	0.40	19	10	0.40	No*	No*
6.	0.50	20	11	0.50	85	No*
7.	0.60	22	12	0.60	84	No*
8.	1.00	27	23	1.00	79	No*
9.	SK	No*	No*	SK	43	41

*More than 90 minutes.

Observation for both anti- thrombotic and thrombolytic activity is presented here. In case of both activities, monitoring was done for 90 min. but clot formation of positive control (in case of anti-thrombotic activity) and clot dissolution of negative control (in case of thrombotic activity) not occurred hence 90 min. is taken as reference value for graphical expression.



Normally clot formation was observed within four minutes in blank whereas addition of SK delays clot formation more than 90 min. Hence monitoring was done for 90 mins, this value have been taken as maximum (and it is believed that clot formation will not occur after such a long time in normal conditions). Activity of aqueous and alcoholic extracts can be seen increasing as increase of drug concentration.



Clot lysis activity expressed here. Clot was not dissolved till 90 mins. In case of blank to which NS was added and in case of positive control to which SK was added clot was dissolved significantly earlier. It can be seen that aqueous and alcoholic extracts of drug are decreasing clot lysis time as concentration increases.

DISCUSSION

Several studies have been conducted to find out antithrombotic effect of the herbs and natural food sources and their supplements. There is evidence that consuming such food leads to prevention of atherothrombotic diseases [3]. The present study of antithrombotic and thrombolytic activity of *Nigella sativa* has been studied. SK was used as a positive control and normal saline (NS) was taken as a negative control. The positive and negative controls clearly revealed the appropriateness of the methods under investigation. Nigella Sativa showed anti-thrombotic activity and delaying clot formation time. Alcoholic extract was less potent than aqueous extract. Increased concentration of extracts showed antithrombotic and thrombolytic activity as it's indicated by results. Increase in dilutions decreased the activity. Both extracts were well dissolved in normal saline. *Nigella sativa* showed anti thrombotic and thrombolytic activity while in vivo thrombolytic and anti thrombotic properties and responsible component(s) of *Nigella sativa* for these properties are to be studied.

CONFLICT OF INTEREST

The Authors declare no conflict of interest

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HOW TO CITE THIS ARTICLE

Vaseem A Ansari*, H H Siddiqui, R K Dixit. (2014 January 1). Antithrombotic and Thrombolytic Activity of *Nigella Sativa* Seed Extracts *PHARMANEST, 5(1),* 1829-1833. http://www.pharmanest.net