Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(1):182-185

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

In vitro study for antiplatelet activity of '*Kalonji*' (*Nigella sativa*) extracts using aspirin as standard

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ABSTRACT

A thrombus formation in the circulatory system due to failure of haemostasis causes vascular blockage and leads to serious consequences such as myocardial or cerebral infarction. Antiplatelet drugs are used to prevent formation of unwanted blood clot but their uses are associated with serious side effects. In association of efforts to develop natural products from plant origin as antiplatelet agent, Nigella sativa seeds were investigated for same. The platelet aggregation was monitored using spectrophotometer. The well known antiplatelet drug aspirin was taken as standard drug and activity was compared with it. Collagen was used as platelet aggregation inducer. 0.4 ml of 0.9mg/dl aspirin solution was used as standard dose. The final working concentration of aspirin and collagen was 18µg /ml and 2 µg /ml respectively. The aqueous extract was found to have antiplatelet activity. The 5.0µg/ml of seed extract was found 67% active against 18µg/ml of aspirin.

Keywords: Antiplatelet, *Nigella sativa*, antithrombotic, myocardial infarction, deep vein thrombosis.

INTRODUCTION

Atherothrombotic coronary artery diseases are one of the most common causes of death worldwide [1, 2, 8]. Atherothrombotic diseases such as myocardial or cerebral infarction are serious outcome of the unwanted thrombus formed in blood vessels. Antiplatelet drugs are used to prevent unwanted thrombus formation. However, these drugs have certain limitations which cause serious and sometimes fatal consequences [1, 3, 8].

A blood clot (thrombus) developed in the circulatory system due to failure of haemostasis causes vascular blockage and leads to serious consequences in atherothrombotic diseases such as myocardial or cerebral infarction [4,8]. Antiplatelet drugs are used to prevent formation of unwanted blood clot and thrombolytic agents that include tissue plasminogen activator (t-PA), Urokinase (UK), streptokinase (SK) etc are used for the treatment of these diseases [4,8] but their uses are associated with hyper risk of GI irritation , gastric erosions with bleeding, neutropenia, hemorrhage, severe anaphylactic reaction and lacks specificity. Many antithrombotic drugs may have a deleterious effect on normal haemostasis leading to bleeding complications. Therefore it is necessary to find out a new drug which shows less adverse effect. [1, 8]

Considerable efforts have been directed towards the discovery and development of natural products from various plant and animal sources which have antiplatelet, anticoagulant, antithrombotic, and thrombolytic activity. Epidemiologic studies have provided evidence that foods with experimentally proved antithrombotic effect could

reduce risk of thrombosis. Herbs showing antithrombotic and thrombolytic activity have been studied and some significant observations have been reported [6, 8].

Nigella sativa (belong family *Ranunculaceae*) also known as Black Cumin, Small Fennel, Kaalaajaaji, Kalikaa, Prthvikaa, Sthulajiraka, Sushavi, Upkunchikaa, Kalonji, Kamaazaruus, is generally found in Punjab, Bengal, Assam and Bihar regions in India. [7, 8]

The aim of our work was to investigate whether our selected herbal preparations of *Nigella sativa* (seeds) possess antiplatelet activity or not by using an in-vitro procedure taking aspirin as positive control.

EXPERIMENTAL SECTION

Plant material:
The seeds of *Nigella sativa* were procured from local market of Lucknow, UP, India and authenticated by Dr. Muhamma Arif, at faculty of Pharmacy, Integral University, Lucknow, Uttar Pradesh (India). A the accession no. of the specimen is IU/PHAR/HRB/15/06.

Extraction:
The seeds were dried in shade under hot air blower and powdered and defatted with petroleum ether. The extraction was done by maceration. The aqueous and methanolic extracts were obtained and concentrated over water bath at 40°C and stored in airtight containers.

Specimen:
Whole blood was drawn from healthy rabbits. Required volume of blood taken in to each of the test tubes. The Experimental protocol was approved by the Institutional Ethical Committee (Approval no. IU/Pharm/Ph.D./CPCSEA/10/03) following the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) which complies with International norms of INSA (Indian National Science Academy).

Determination of standard dose of Aspirin:
The standard dose of Aspirin of was determined in laboratory for in vitro evaluation as according to method published [9, 10, 11]. Platelet rich plasma (PRP) was prepared by centrifugating rabbit whole blood [containing 0.9% sodium citrate as anticoagulant] at 1500 rpm for 15 mins. The concentration of PRP was adjusted to optical density (OD) 1.0 at 400nm λ by adding normal saline. 1ml of PRP was taken in each tube. The increasing amounts of aspirin (0.9mg/dl) were added in consequent test tubes and final volume of each tube was made upto 2 ml by adding normal saline (Table 2). All tubes were incubated for at 37°C for 3 mins and then 0.2ml of collagen (0.2mg/dl) was added in each tube[9, 10,11]. Aggregation was induced under continuous stirring at 1000 rpm for 3 mins and the aggregation was monitored under spectrophotometer at 400 nm λ.

Antiplatelet activity testing:
1ml of PRP (prepared as explained earlier) was taken in each tube. First two tubes were taken as positive and negative control and test samples were added in increasing amount in consequent tubes and final volume of each tube was made upto 2 ml by adding normal saline. All tubes were incubated for at 37°C for 3 mins and then 0.2ml of collagen (0.2mg/dl) was added in each tube [9, 10,11]. Aggregation was induced under continuous stirring at 1000 rpm for 3 mins and the aggregation was monitored under spectrophotometer at 400 nm λ. In tube no. 2 normal saline and tube no. 1, 0.4ml aspirin (0.9mg/dl) were added in place of test sample.

RESULTS

Determination of standard dose of Aspirin:
The maximum antiplatelet activity was obtained at 0.4ml, 0.5ml and 0.6ml of aspirin (at given conc.). Hence 0.4 ml was taken as standard dose (conc. 0.9mg/dl). (TABLE 1) (FIGURE 1)

Table 1: Determination of standard dose of aspirin

Tube no.	Vol. of PRP (diluted)	Vol. of Aspirin soln.(0.9mg/dl)	N.S.(ml)	Vol. of collagen soln.(1mg/ml)	O.D. *
1.	1.0ml	--	0.98ml	0.02ml	0.1±0.1
2.	1.0ml	0.1 ml	0.88ml	0.02ml	0.2±0.1
3.	1.0ml	0.2 ml	0.78ml	0.02ml	0.3±0.2
4.	1.0ml	0.3 ml	0.68ml	0.02ml	0.5±0.1
5.	1.0ml	0.4 ml	0.58ml	0.02ml	0.6±0.1
6.	1.0ml	0.5 ml	0.48ml	0.02ml	0.6±0.2
7.	1.0ml	0.6 ml	0.38ml	0.02ml	0.6±0.1
8.	1.0ml	0.7 ml	0.28ml	0.02ml	0.5±0.1
9.	1.0ml	0.8 ml	0.18ml	0.02ml	0.5±0.2
10.	1.0ml	0.9 ml	0.08ml	0.02ml	0.4±0.1
11.	1.0ml	1.0 ml	0.00ml	0.02ml	0.4±0.2

** n= 4, mean±SD*

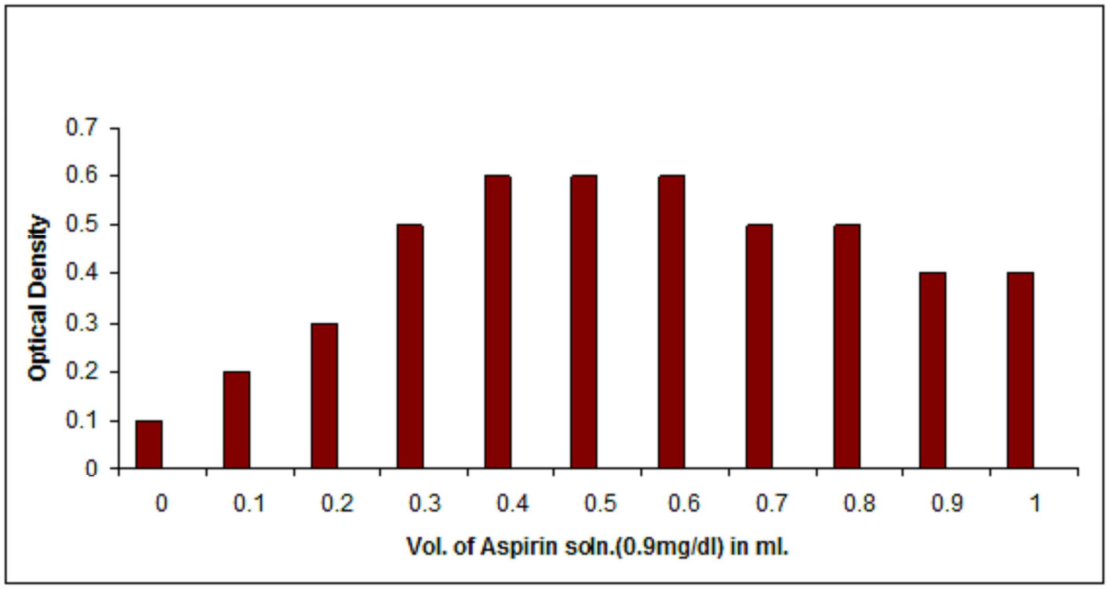

Fig 1: Determination of standard dose of Aspirin

Antiplatelet activity:

After several investigations, the *Nigella sativa* was found nominally active as antiplatelet agent, only maximum used concentration (5.0µg/ml) was showing bit remarkable activity in case of aqueous extract. The alcoholic extract doesn't show any remarkable activity in all concentrations used. (TABLE 2) (FIGURE 2)

Table 2: Observations for antiplatelet activity of aqueous and alcoholic extracts of *Nigella sativa* at different concentrations

S.No.	Conc. Of drug (µg/ml)	O.D. (Optical Density)	
		Aqueous	Alcoholic
1.	0.00 (blank)	0.1	0.1
2.	0.5	0.1	0.1
3.	1.0	0.1	0.1
4.	1.5	0.1	0.1
5.	2.0	0.2	0.1
6.	2.5	0.2	0.1
7.	3.0	0.3	0.1
8.	5.0	0.4	0.2
9.	Aspirin	0.6	0.6

Note:

1. Working concentration of collagen was 2 µg /ml
Working concentration of Aspirin was 18µg/ml

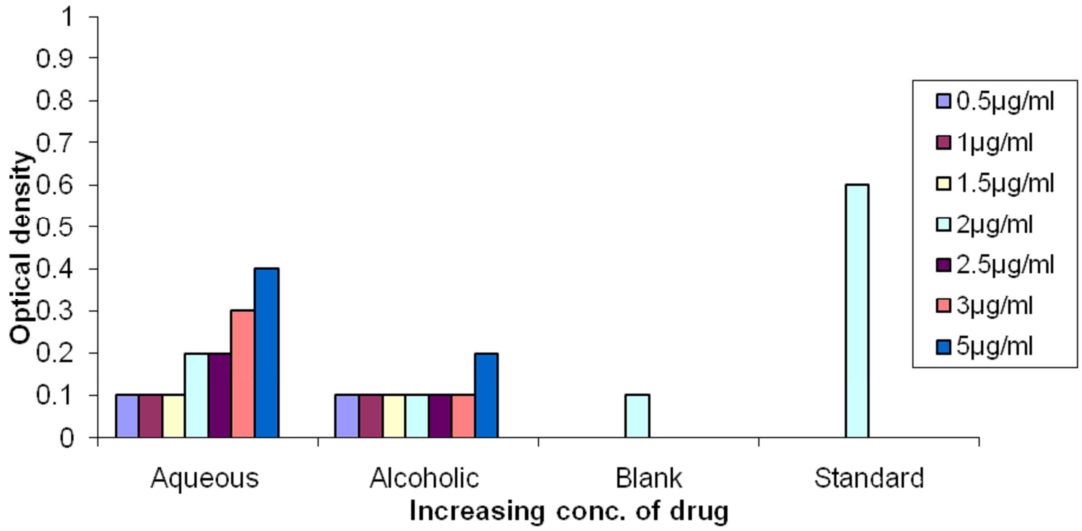

Fig 2: Antiplatelet activity at different concentrations for aqueous and alcoholic extracts of *Nigella sativa*

DISCUSSION

Nigella sativa showed remarkable antiplatelet activity in case of maximum used concentration (5.0µg/ml) of aqueous extract. The alcoholic extract doesn't show any remarkable activity in all concentrations used. The minimum concentration showing antiplatelet activity was 2.0µg/ml. The maximum used concentration (5.0µg/ml) was found 67% active against 18µg/ml of aspirin. Increased concentration of extracts may show the desired activity as it's indicated by results. The *Nigella sativa* seed extract may be further investigated for in vivo antiplatelet activity since plant origin agents are believed to have less unwanted side effects.

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