Original Article

In vitro Anti-atherothrombosis Activity of *Nigella sativa* Oil, Phytol and their Combinations

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ABSTRACT

Background: Black seed oil and Phytol (PHY) are evident for their promising biological effects in various test systems. **Aim:** This study evaluates anti-atherothrombosis activity of *Nigella sativa* oil (NSO) and Phytol (PHY). **Materials and Methods:** The clotlysis activity of the NSO and/or PHY has been investigated by taking Streptokinase (SK) as a standard clotlysis drug. **Results:** The results suggest that the NSO and PHY exhibited a concentration-dependent clotlysis activity in human clotted blood. The highest clotlysis of the NSO and PHY was seen by 57.83 ± 0.23% and 53.05 ± 2.93% with 80 µL and 150 µg per tube, respectively. NSO co-treated with PHY showed a better clotlysis capacity than the NSO and PHY. The vehicle showed negligible clotlysis (2.86 ± 4.33%) capacity, while the standard SK (100 I.U.) showed 78.96 ± 1.08% clotlysis activity. **Conclusion:** NSO and/or PHY exhibited clotlysis activity in human clotted blood in *in vitro*.

Key words: *Nigella sativa* oil, Phytol, Antioxidant, Atherothrombosis, Cardiovascular diseases.

INTRODUCTION

Atherothrombosis is characterized by atherosclerotic lesion disruption with superimposed thrombus formation, which is the major cause of Acute Coronary Syndromes (ACS) and cardiovascular death worldwide.[1] Viles-Gonzalez *et al.* in an excellent review, have been summarized the causes, current treatments and challenges.[1] Among the clotlysis agents, Streptokinase (SK) is a vastly used thrombolytic medication.[2] However, SK is evident to cause some side effects, including nausea, bleeding, low blood pressure and allergic reactions, therefore, should be restricted its use during pregnancy. Urokinase, isolated from human urine, the other clot lysis drug, in an elevated expression level is found to correlate with tumor malignancy.[3]

Nowadays, plant-derived compounds come into the spotlight due to their diverse biological effects. *Nigella sativa* L. is also known as black cumin or black seeds. On the other hand, Phytol (PHY) is a chlorophyll-derived diterpenoid abundantly present in nature. *N. sativa* and PHY are evident to possess many important biological activities, including antioxidant, anti-inflammatory and organ protective activities.[4,5] However, their anti-atherothrombosis activity is yet to be checked. Therefore, this study aimed at the evaluation of anti-atherothrombosis (*in vitro*) of *N. sativa* oil and/or PHY in human clotted blood.

MATERIALS AND METHODS

Reagents and chemicals

N. sativa oil was purchased from a local market of Narayanganj, Bangladesh, while PHY and other reagents and chemicals used

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in this study were purchased from the Sigma Aldrich USA.

Clot lysis test (in vitro)

The *N. sativa* oil (NSO) at 5 to 80 µL, PHY at 25 to 150 µg and NSO and PHY combinations were used in this study. Experiments for clot lysis were carried as reported earlier Prasad *et al*.[6] Briefly, 3 mL venous blood drawn from healthy volunteers (n= 5, male and female who are not currently using oral contraceptive or anticoagulant therapy) and was distributed in: Vehicle (VEH: 0.05% Tween 80 dissolved in 0.9% NaCl solution); Standard (SK: Streptokinase) and different concentrations of the NSO and/or PHY pre-weighed sterile microcentrifuge (alpin/ependorf) tubes (0.5 mL/tube) and incubated at 37°C for 45 min. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of the tube alone). To each micro-centrifuge tube containing pre-weighed clot, 100 µL of test sample/control were added separately. 100 µL of SK (100 I.U.) and 100 µL of VEH were used as positive and negative control, respectively. All the tubes were then incubated at 37°C for 90 min and observed for clot lysis. After incubation, fluid released was removed and tubes were again weighed to observe the difference in weight after discarding the lysed weight. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clotlysis. The experiment was carried out in triplicate.

Statistical analysis

For this purpose Graph Pad Prism 6.0 (Graph pad Inc., San Diego, CA) was used. One-way analysis of variance (ANOVA) followed by Tukey post hoc test was applied for multiple comparisons between the study groups. The calculated values are expressed as mean ± standard deviation (SD) and considered statistically significant with $p< 0.05$, $p< 0.01$ and $p< 0.001$.

RESULTS

Table 1 displays that NSO reconstituted with the vehicle, concentration-dependently exerted clot lysis activity, where maximum clot lysis was seen with the 80 µL/tube. In comparison to the vehicle group, NSO significantly ($p< 0.05$, $p< 0.01$, $p< 0.001$) lysed the clot at 10 to 80 µL (EC_{50}: 48.83 ± 0.20; CI: 35.16 to 67.86; R^2: 0.98). PHY also concentration-dependently showed clot lysis activity. PHY, at all the concentration exerted significant ($p< 0.05$, $p< 0.01$, $p< 0.001$) clotlysis (EC_{50}: 120.40 ± 0.45; CI: 93.39 to 155.30.86; R^2: 0.95). NSO at 40 and 80 µL showed a better clot lysis capacity than PHY 100 and 150 µg.

Table 1: Clotlysis capacity of *Nigella sativa* oil and/or phytol and controls.

NSO		PHY	
Concentration (µL/tube)	***%Clot lysis***	***Concentration (µg/tube)***	***%Clot lysis***
5	5.57 ± 1.78	25	8.49 ± 6.09*
10	12.24 ± 0.21*	50	14.84 ± 2.68*
20	24.37 ± 2.26**	75	29.53 ± 3.12**
40	46.99 ± 3.89***	100	38.98 ± 3.05***
80	57.83 ± 0.23***	150	53.05 ± 2.93***
NSO (µL/tube) + PHY (µg/tube)		***VEH (0.05% Tween 80 + 0.9% NaCl solution)***	
5 + 25	13.09 ± 1.09*	100 µL	2.86 ± 4.33
10 + 50	23.75 ± 2.11**		
20 + 75	32.70 ± 2.79**	***SK***	
40 ± 100	56.44 ± 3.69***	100 I.U.	78.96 ± 1.08***
80 ± 150	69.15 ± 1.84***		

Values are mean ± SD (n= 5); *$p< 0.05$, **$p< 0.01$ and ***$p< 0.001$ compared to the VEH (0.05% Tween 80 dissolved in 0.9% NaCl solution); two-way ANOVA (with non-parametric test) followed by Tukey's test; NSO: *Nigella sativa* oil; PHY: Phytol.

NSO co-treated with the PHY at all concentrations augmented clot lysis effects than the NSO and PHY individual groups. NSO combined with the PHY at highest concentration showed clotlysis activity by 69.15 ± 1.84%. Regarding to the vehicle group, the co-treated group at all concentrations exerted significant clot lysis activity. SK at 100 I.U. produced clotlysis by 78.96 ± 1.08%.

DISCUSSION

Atherosclerosis may start early in life and progress asymptomatically through adult life. Clinically it is manifested as coronary artery disease, stroke, transient ischaemic attack and peripheral arterial disease.[1] Besides drug therapy, dietary supplements are the major driving force to treat many diseases, including atherosclerosis.[7] NSO is a dietary supplement in many countries.[8] On the other hand, PHY, an essential oil, is a chlorophyll side chain and it is a daily consumed compound, especially by the ruminant animals.[9] *N. sativa* and its vastly studied component thymoquinone are evident for cardioprotective effects in various test systems.[10,11] On the other hand, PHY has antioxidant, anti-inflammatory, lipid lowering and organ protective effects.[9]

The atherosclerotic plaque is prone to disruption, thus leading to local platelet activation and aggregation, which is a major consequence of thrombus formation.[12]

In this study, we have seen that the NSO and PHY caused lysis of clot at all concentrations.

Herbal medications, now-a-days are commonly used in all medical settings. It is due to their effectiveness and affordability and decreases the risk of allergies, adverse reactions, or cross-reactivity with other pharmaceuticals and supplements.[13] The therapeutic efficiency of herbal combination product has been clinically proven according to the highest standards.[14] In our study, the combined concentrations of the NSO and PHY were found to show clot lysis capacity better than the each concentration of the NSO and PHY used in individual group. It may be due to their synergistic effect on clot.

In a recent study, a number of spices, such as pepper, ginger, garlic, onion have been found to act against atherosclerosis.[15] Wu *et al*. suggest that andrographolide (a bitter diterpene lactone) ameliorates atherosclerosis by suppressing pro-inflammation and Reactive Oxygen Species (ROS) generation pathways.[16] Both, NSO and PHY are evident to possess ROS scavenging and anti-inflammatory effects. In another study, pseudolaric acid B (diterpene acid) was found to attenuate atherosclerosis progression and inflammation by suppressing peroxisome proliferator-activated receptor-gamma (PPARγ)-mediated nuclear factor kappa B (NF-κB) activation in mice.[17] NSO and PHY are also evident to act through these pathways.[4,18-20]

Moreover, atherosclerosis is also characterized by vascular inflammation, accumulation of lipids in the arterial wall and formation and growth of atherosclerotic plaques followed by ischemia. Traditional anti-atherosclerotic therapy is mainly focused on improving blood lipid profile and does not target various stages of plaque progression. The retention of cholesterol precedes plaque formation. Therefore, targeting the latter pathway may be beneficial for preventing further atherogenic progression. In this regard, herbal preparations can be used, due to their good tolerability and suitability for long-lasting treatment.[21]

CONCLUSION

NSO and PHY concentration-dependently exerted clotlysis effect on human clotted blood. NSO and PHY combindly exhibited better clotlysis effect at all concentrations. NSO and PHY may act synergistically. Both NSO and PHY may be good candidates for the treatment of atherombosis and related other cardiovascular diseases.

Ethical Statement

This study was approved by the Ethical Committee under the Department of Pharmacy, Bangabandhu Sheikh Mujibur Rahman Science and Technology University (BSMRSTU) (Approval No. BSMRSTU-2018/12).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

NF-κB: Nuclear factor kappa B; **NSO:** *Nigella sativa* oil; **PHY:** Phytol; **PPARγ:** Peroxisome proliferator-activated receptor-gamma; **ROS:** Reactive oxygen species; **SK:** Streptokinase.

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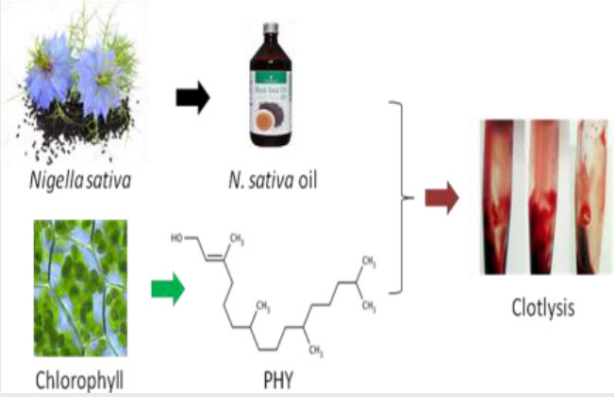
PICTORIAL ABSTRACT



SUMMARY

Thrombosis, the formation of a blood clot inside a blood vessel that obstructs the blood flow through the circulatory system is one of the major consequences of cardiovascular diseases. In this study, NSO and/or PHY were evaluated for anti-atherothrombosis activity in clotted human blood depicts that both NSO and PHY exhibited a concentration-dependent clot lysis activity. The combination of the NSO and PHY was found to exhibit a better clot lysis capacity than the individual treatment of the NSO and PHY. Although, the clot lysis capacity of the NSO and/or PHY was lower than the standard drug SK, but in comparison to the NC group, both of them and their combination group showed significant effects. So far we know, this is the first time where we have claimed that the NSO and/or PHY may have anti-atherothrombosis activity.

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