

## In vitro induction of endothelial cell fibrinolytic alterations by *Nigella sativa*

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### Abstract

The effect of *Nigella sativa* (NS) L. oil (blackseed oil) on the fibrinolytic system of the human umbilical vein (HUV) and human uterine arterial (HUA) endothelial cells (ECs) in culture was studied. Both of them showed a concentration-dependent increase in tissue-type plasminogen activator (t-PA). A maximum effect was achieved with 50 µg oil/ml conditioned medium (CM) ( $1.3 \pm 0.15$  ng/10<sup>4</sup> cells/24 h vs. control  $0.7 \pm 0.06$  ng/10<sup>4</sup> cells/24 h, and  $0.38 \pm 0.04$  ng/10<sup>4</sup> cells/24 h vs. control  $0.24 \pm 0.02$  ng/10<sup>4</sup> cells/24 h, for HUVEC and HUA-EC, respectively). At 100 µg/ml, there was a significant change in the amount of t-PA antigen produced by either HUVEC or HUA-EC ( $1.0 \pm 0.1$  ng/10<sup>4</sup> cells/24 h or  $0.28 \pm 0.02$  ng/10<sup>4</sup> cells/24 h) as compared to control CM from cells grown under control conditions, but still less than that recorded at 50 µg oil/ml. Plasminogen activator inhibitor-type 1 increased the CM significantly and concentration-dependently in both cells. For HUVEC, the maximum effect was achieved at a concentration of 100 µg/ml ( $257.7 \pm 8.0$  ng/10<sup>4</sup> cells/24 h vs. control  $72.7 \pm 3.8$  ng/10<sup>4</sup> cells/24 h). HUA-EC showed the maximum effect at a concentration of 100 µg/ml ( $171.6 \pm 4.4$  ng/10<sup>4</sup> cells/24 h vs. control  $53.8 \pm 3.7$  ng/10<sup>4</sup> cells/24 h). This study suggests a role for NS oil in modulating the balance of fibrinolysis/thrombus formation by modulating the fibrinolytic potential of endothelial cells.

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**Keywords:** *Nigella sativa*; Endothelial cells; Fibrinolytic system; t-PA; PAI-1

### Introduction

A free vascular blood flow is maintained by a dynamic equilibrium between blood coagulation and fibrinolysis. The main function of the fibrinolytic system is to dissolve fibrin clots in the circulation, composed of the inactive precursor plasminogen (Plg) which can be converted into the proteolytic enzyme plasmin by the

plasminogen activators (PAs), tissue-type PA (t-PA), and urokinase-type PA (u-PA) (Wun and Capuano, 1985). The fibrinolytic capacity of plasma is considered to be strongly dependent on the concentration of circulating t-PA, which is presumed to be derived mainly from the vascular wall, where it is localized in endothelial cells (ECs) (Emeis, 1988). The fibrinolytic capacity of human ECs is maintained by the balance between PAs and plasminogen activator inhibitor type 1 (PAI-1). This balance is regulated by a wide variety of stimuli (Schleef and Loskutoff, 1988; Van Hingsbergh, 1988). The fibrinolytic system is important not only for dissolving blood clots but also for extracellular

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processes like vascularization, and plays a role in the body's defense system.

Insight into the regulation of t-PA synthesis may be helpful in developing drugs that counteract insufficient endogenous t-PA by increasing its production. Because t-PA and PAI-1 are produced by the endothelium, regulation of their synthesis and secretion at the EC level represents a rapid and a direct way of modulating the fibrinolytic potential of blood. Previous studies have shown that production of PAs and inhibitors in various cell types are modulated in some instances by some herbal drugs, such as 20 (S)-protopanaxatriol notoginsenoside R1 (NR1) (Zhang et al., 1994) or quercetin (Zhao et al., 1998).

The seeds of *Nigella sativa* (NS) L. (Ranunculaceae) have been employed for thousand of years as a spice, food preservative and curative or medicinal remedy for numerous disorders (Nadakarni, 1976; Chopra et al., 1956). The blackseeds were referred to by the prophet Mohammed as having healing powers, identified as the curative black cumin in the Holy Bible, described as the Melanthion of Hippocrates and Dioscorides and as the Gith of Pliny (Atta-ur Rahman et al., 1985). Several beneficial pharmacological effects have been attributed to various crude and purified components of blackseeds, including antihistaminergic, antihypertensive, hypoglycemic, antimicrobial, mast cell stabilizing and anti-inflammatory activities (El-Dakhkhny, 1965; Marozzi et al., 1970; Salomi et al., 1991, 1992; Chakravarty, 1993; El-Tahir et al., 1993; Hailat et al., 1995; Houghton et al., 1995; Zaoui et al., 2000). The crude gum and its constituents also represent protective and immunomodulating agents against carbon tetrachloride hepatotoxicity in mice, proteinuria and hyperlipidemia associated with nephrotic syndrome (Nagi et al., 1999; Haq et al., 1999; Badary et al., 2000). In vitro and in vivo, blackseed preparations have also demonstrated significant antineoplastic activity (Salomi et al., 1991, 1992; Hailat et al., 1995; Worthen et al., 1998; Badary, 1999; Badary et al., 1999; Swamy and Tan, 2000).

The effect of NS on hemostasis is known in traditional medicine. Ibn Sina (1037) mentioned that NS causes menorrhagia when used along with honey. Contrary to this, it was claimed that in certain cases of epistaxis, a natural fat extract of NS was useful in the management of such cases with no recurrence of bleeding after several applications of the drug to the nostrils (Ghoneim et al., 1982). The pharmacological investigations of the effect of NS on hemostasis are few. Ghoneim et al. (1982) reported an enhancement of coagulation after parenteral administration of NS extract, while El-Naggar and El-Deib (1992) reported an anticoagulant effect after oral administration of NS powdered seeds. Recently it has been shown that either fixed oil or NS seed affected blood homeostasis in rats and seems to induce transient changes in the coagulation

activity (Zaoui et al., 2002; Al-Jishi and Abu Hozaifa, 2003).

## Materials and methods

*Nigella sativa* L. seed oil (El-Baraka seed oil, 100 mg/capsules) was purchased as soft gelatin capsules from Pharco-pharmaceuticals (Alexandria, Egypt). This natural oil in Baraka capsules contains various constituents, including nigellone, fatty acids, glycosides, phenolic components, carotene, minerals (as phosphorus and iron), and some digestive enzymes. The oil was dissolved in dimethylsulfoxide (DMSO) and then diluted in incubation medium to yield 100 µg/ml of oil. Dulbecco's Modified Eagle's Medium (DME) and Ham's F-12 (F-12) culture media, Hank's Balanced Salt Solution (HBSS), and normal bovine, calf, and horse sera, were purchased from GIBCO (Grand Island, NY). Collagenase (Worthington type IV) was obtained from Cooper Biochemicals (Malvern, PA) and antibody to human factor VIII antigen (Cat. no. 0210-3207) was obtained from Cooper Biomedical (Malvern, PA). Culture flasks, plates, and sterile plasticware were purchased from Costar (Cambridge, MA). Other materials used in the methods described below have been specified in detail in the pertinent references.

## Cell culture

### Human umbilical vein endothelial cells (HUVECs)

Human umbilical vein endothelial cells (HUVECs) were isolated from fresh human umbilical cord veins with a collagenase technique (Sigma) similar to that described by Jaffe et al. (1973). Cells from five to seven cords were pooled and plated in 75-cm<sup>2</sup> tissue-culture flasks (Costar) coated with 1% calf skin gelatin (Sigma). Cells were grown to confluence at 37 °C in a humidified 95% air-5% CO<sub>2</sub> atmosphere in medium 199 (Sigma) supplemented with 20% heat-inactivated supplemented calf serum (SCS, HyClone), 100 µg/ml streptomycin, 100 IU/ml penicillin, 250 ng/ml amphotericin B, 1 mmol/l glutamine (JHR Biosciences), 2 IU/ml heparin (Liquemin Roche, Hoffmann-La Roche), and 50 µg/ml ECGS Technoclone, Vienna, Austria). Cells were confirmed as endothelial by their "cobblestone" morphology, positive immunofluorescence with anti-von Willebrand factor VIII antibodies (Booyse et al., 1975; Jaffe et al., 1980) and uptake of acetylated low-density lipoprotein (Stein and Stein, 1980). Primary cultures were harvested at confluence with 0.05% trypsin-0.02% EDTA (JHR Biosciences) and plated at a split ratio of 1:3 in 75-cm<sup>2</sup> flasks. Subconfluent cells were allowed to grow to

confluence under the same conditions, harvested during the exponential cell-growth phase with trypsin-EDTA, and frozen in 1-ml aliquots of medium 199 containing 10% dimethyl sulfoxide in liquid nitrogen. Vials were thawed at 37 °C and cells were grown in Petri-dishes (Costar, Cambridge, MA) in medium 199 containing SCS, ECGS, and heparin at the concentration described above until confluence. First- through fourth-passage cells were subcultured into gelatin-coated 24-well cluster plates (Costar) by exposing them to 0.5 g/l trypsin and 0.2 g/l EDTA. The cells were always fed with fresh medium the day before the experiment.

## Human uterine arterial endothelial cells (HUA-ECs)

### Sources of tissue

Tissue was obtained from the uteri of 20 normally cycling premenopausal women between the ages 21 and 37 years who were undergoing endometrial biopsy as part of their infertility evaluation. Biopsy samples were taken using a Pipelle Endometrial Curette (Unimar, Inc., Wilton, CT) as described previously (Hill et al., 1989). Biopsy specimens were placed immediately in an ice-cold mixture of Dulbecco's Modified Eagle's Medium and Ham's F-12 (DME/F-12) for transport to the laboratory of Institute of Vascular Biology and Thrombosis Research, Vienna University, where the cell cultures were prepared.

### Tissue dissociation and cell purification

The tissue was gently dissected to isolate the uterine artery, which was then dissected into small pieces (1–2 mm<sup>3</sup>) and washed by centrifugation (400g) in fresh medium to remove any debris or excess blood cells. Human uterine artery EC (HUA-EC) were isolated by mild collagenase treatment following the methods of Gimbrone et al. (1974) and Booyse et al. (1975). HUA-ECs were grown in gelatin-coated Petri dishes, using M199 containing 20% SCS, 50 µg/ml ECGS, and 5 U/ml heparin, and subcultured using a split ratio of 1:3.

The cells were confirmed as endothelial by their "cobblestone" morphology (Booyse et al., 1975; Jaffe et al., 1980), positive staining with anti-von Willebrand Factor (vWF) VIII antibodies, and uptake of acetylated low-density lipoprotein (Stein and Stein, 1980). All ECs used in this study were between passage 2 and 4.

### Preparation of conditioned medium (CM)

Subconfluent cells were washed twice gently with 0.5 ml of serum-free growth medium pre-warmed to

37 °C, followed by incubation for 24 h at 37 °C, with various concentrations of oil in serum-free growth medium (0.6 ml final volume in each well). The aliquots were removed after 24 h incubation and were frozen immediately at –80 °C for later measurement of t-PA and PAI-1 (see below). At the end of each experiment, the cells were treated with trypsin/EDTA for 2–3 min at room temperature, and were counted in an electronic counter (Coulter). Each concentration was tested in triplicate. Triplicate control wells containing only serum-free growth medium were included in each experiment. Viability of cells was confirmed at 24 h in each experiment by visual inspection.

### Assay for tissue type PA (t-PA) antigen

t-PA antigen in endothelial cell-CM was determined by two-site, 'sandwich'-specific, commercially available enzyme-linked immunosorbent assays (ELISA) (Technoclone, Austria) according to manufacturer's instructions. The test range for this assay is 0.2–2.5 ng/ml. The t-PA ELISA detects free t-PA and t-PA complexed with PAI-1. The t-PA secretion observed with each oil concentration was expressed as nanogram (ng) per 10 000 cells to correct for any differences in cellular proliferation induced by the various agents used.

### Assay for plasminogen activator inhibitor type-1 (PAI-1) antigen

PAI-1 antigen in endothelial cell-CM was measured by a two-site 'sandwich' ELISA. Briefly, a monoclonal anti-PAI-1 antibody (5PAI12) that recognizes active PAI-1, latent PAI-1 and PAI-1 in complex with t-PA immobilized to a micro-ELISA plate, was used to bind the PAI-1 contained in the samples. A second peroxidase-labeled monoclonal anti-PAI-1 antibody (3PAI15) that also recognizes active and latent PAI-1 as well as PAI-1 in complex with t-PA was used to quantify the amount of bound PAI-1. Purified melanoma PAI-1 (Wagner and Binder, 1986) was used as a calibration standard (Resh et al., 1989). The PAI-1 levels in endothelial cell-CM were expressed as nanogram (ng) per 10 000 cells to correct for variations in cellular proliferation induced by the various agents used.

### Statistical analysis

The significance of differences in t-PA and PAI-1 secretion in response to various oil concentrations was assessed using student's two-tailed *t*-test, (StatGraph Program) with *p* < 0.05 considered significant. The figures were plotted using "Sigma Plot Scientific Graphing Software, Version 2.01".

## Results

### Effects of blackseed oil on release of t-PA antigen

As shown in Table 1 and Fig. 1, treatment of HUVEC and HUA-EC with increasing concentrations of blackseed oil for 24 h resulted in a concentration-dependent increase of t-PA antigen in the CM of such treated cells. Maximal effects were achieved with 25 and 50  $\mu\text{g/ml}$  [(1.2 $\pm$ 0.06 and 1.3 $\pm$ 0.15 ng/10<sup>4</sup> cells/24 h vs. control 0.7 $\pm$ 0.06 ng/10<sup>4</sup> cells/24 h,  $n = 9$ ,  $p < 0.001$ , and 0.34 $\pm$ 0.03 and 0.38 $\pm$ 0.04 ng/10<sup>4</sup> cells/24 h vs. control 0.24 $\pm$ 0.02 ng/10<sup>4</sup> cells/24 h,  $n = 9$ ,  $p < 0.01$ , for HUVEC [a] and HUA-EC [b], respectively)].

### Effects of blackseed oil on release of PAI-1 antigen

As can be seen from the Table 1 and Fig. 2, PAI-1 antigen increased significantly in the CM in a concentration-dependent manner, when either HUVEC [a] or HUA-EC [b] were incubated for 24 h with increasing amounts of blackseed oil. For HUVEC, a dramatic

concentration-dependent increase in PAI-1 was measured up to 100  $\mu\text{g/ml}$  (257.7 $\pm$ 8.0 ng/10<sup>4</sup> cells/24 h vs. control 72.7 $\pm$ 3.8 ng/10<sup>4</sup> cells/24 h, [ $n = 9$ ,  $p < 0.001$ ]). Similarly, PAI-1 concentration increased in a concentration-dependent manner in HUA-EC up to 100  $\mu\text{g/ml}$  (171.6 $\pm$ 4.4 ng/10<sup>4</sup> cells/24 h vs. control 53.8 $\pm$ 3.7 ng/10<sup>4</sup> cells/24 h [ $n = 9$ ,  $p < 0.001$ ]).

## Discussion

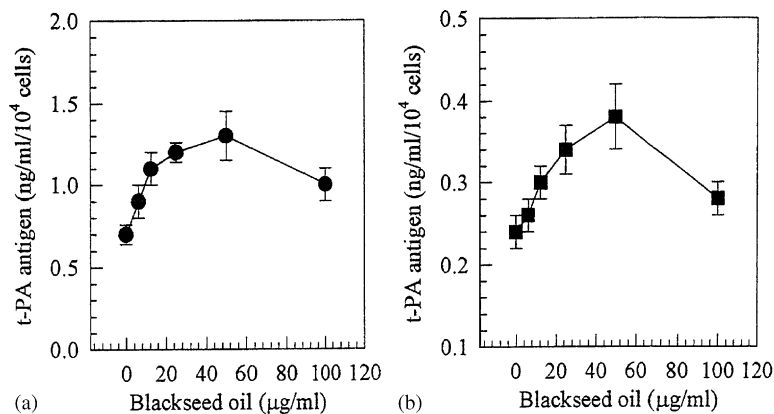
ECs play a major role in maintaining normal hemostasis and vascular patency by regulating the balance between synthesis of the coagulant proteins that promote clot formation and the fibrinolytic proteins that facilitate clot lysis. These cells are major site of synthesis of fibrinolytic proteins, t-PA, u-PA, PAI-1, and the various binding proteins involved in the regulation and surface-localization of EC-mediated fibrinolysis. In vivo, vascular ECs are thought to be the main source of circulating plasma t-PA, which is considered to be mainly responsible for intravascular

**Table 1.** Effect of blackseed oil on some fibrinolytic parameters in the CM of subconfluent HUVECs and HUA-ECs

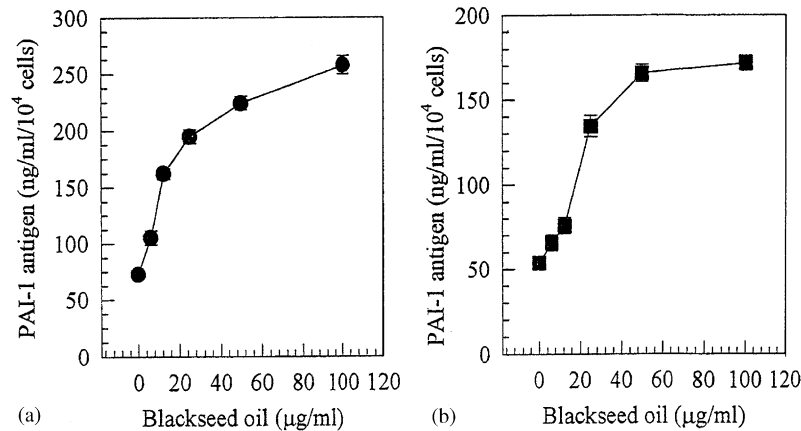
Cells	HUVECs		HAU-ECs	
	t-PA antigen (ng/ml/10 <sup>4</sup> cells)	PAI-1 antigen (ng/ml/10 <sup>4</sup> cells)	t-PA antigen (ng/ml/10 <sup>4</sup> cells)	PAI-1 antigen (ng/ml/10 <sup>4</sup> cells)
Control	0.7 $\pm$ 0.06	72.7 $\pm$ 3.8	0.24 $\pm$ 0.02	53.8 $\pm$ 3.7
6.25	0.9 $\pm$ 0.1*	105.3 $\pm$ 6.5**	0.26 $\pm$ 0.02	65.8 $\pm$ 4.6**
12.5	1.1 $\pm$ 0.1**	162.3 $\pm$ 4.9***	0.30 $\pm$ 0.02**	75.8 $\pm$ 4.8***
25	1.2 $\pm$ 0.06***	194.7 $\pm$ 6.1***	0.34 $\pm$ 0.03***	134.4 $\pm$ 6.3***
50	1.3 $\pm$ 0.15**	224.3 $\pm$ 6.0***	0.38 $\pm$ 0.04***	165.6 $\pm$ 5.1***
100	1.0 $\pm$ 0.1*	257.7 $\pm$ 8.0***	0.28 $\pm$ 0.02*	171.6 $\pm$ 4.4***

Results are expressed as mean $\pm$ SD values of three experiments, each performed in triplicate.

\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .



**Fig. 1.** Dose-response of blackseed oil on t-PA release from HUVECs (a) and HUA-ECs (b). Cells were incubated with serum-free media containing 0–100  $\mu\text{g/ml}$  of oil for 24 h. Values are expressed as mean $\pm$ SD of triplicate cultures. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$  vs. the cultures without oil.



**Fig. 2.** Dose-response of blackseed oil on PAI-1 release from HUVECs (a) and HAU-ECs (b). Cells were incubated with serum-free media containing 0–100 μg/ml of oil for 24 h. Values are expressed as mean ± SD of triplicate cultures. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$  vs. the cultures without oil.

lysis of fibrin clots (Emeis, 1988). Human ECs also express t-PA and PAI-1 in vitro and, therefore, provide a useful tool to investigate mechanisms involved in the regulation of t-PA and PAI-1 expression (Kooistra, 1990). Secretion of t-PA in its active (uninhibited) form is the essential first step in the generation of the fibrinolytic enzyme in plasma. The balance between endothelial secretion of t-PA and PAI-1 has been proposed as a key determinant of the level of activity of the plasmin-dependent fibrinolytic pathway (Hekman and Loskutoff, 1985).

The pharmacological investigations of the effect of NS on hemostasis are few. Ghoneim et al. (1982) reported an enhancement of coagulation after parenteral administration of NS extract while El-Naggar and El-Deib (1992) reported an anticoagulant effect after oral administration of NS powdered seeds. It has been shown recently that either fixed oil or NS seed affects blood homeostasis in rats and seems to induce transient changes in the coagulation activity (Zaoui et al., 2002; Al-Jishi and Abu Hozaifa, 2003) and that NS-induced prolongation of prothrombin time (PT) can be due to deficiency in any of the factors involved in the extrinsic and common pathway of blood coagulation or the presence of inhibitors (DeGruchy, 1983). NS induced a reduction in activated partial thromboplastin time (APTT) when given to the rats at a high dose, 2 or 3 times, of 180 mg/kg/body wt/day (Ghoneim et al., 1982; Al-Jishi and Abu Hozaifa, 2003). It is likely that the mechanism responsible for APTT reduction, whether operating via enzyme induction or any other metabolic process involved in the synthesis of any of intrinsic pathway factors, requires a high level of NS (Ghoneim et al., 1982; Al-Jishi and Abu Hozaifa, 2003). This study may provide evidence to confirm these mechanisms since both t-PA and PAI-1 showed a concentration-dependent increase treated with NS oil as compared

to controls. However, decline of t-PA at high concentrations of oil might explain the intrinsic pathway for the reduction of APTT.

Oral administration of NS powder produced transient reduction in thrombin time (TT) and a concomitant increase in fibrinogen level, which was also transient (Ghoneim et al., 1982; Al-Jishi and Abu Hozaifa, 2003). It is likely that the mechanism responsible for fibrinogen elevation, whether occurring through enzyme induction or any other metabolic process involved in fibrinogen synthesis, requires a certain level of NS. The transient effect of oil on t-PA level might explain the transient reduction of TT and increase of fibrinogen. This effect can be explained by the development of pharmacokinetic tolerance to NS (Nies, 1992) and/or the implementation of different active ingredient of NS.

The current data on PAI-1 and t-PA antigen level may explain partially the development of coagulant/anticoagulant balance through the intrinsic pathway. A common mechanism seems to affect both of PAI-1 and t-PA, leading to their parallel response, which may be an enzyme induction by the NS. This may be attributed to the effect of different oil constituents on enzyme levels for each pathway. Therefore, NS oil modulates the balance of fibrinolysis/thrombus formation.

The excess of PAI-1 antigen produced in response to oil may be explained as follows: (1) PAI-1 released under stimulus is released mainly into CM (Ehrlich et al., 1991; Cockell et al., 1995); (2) cultured ECs produce active PAI-1, but the material they secrete is rapidly inactivated (Levin, 1983; Van Mourik et al., 1984; Hekman and Loskutoff, 1985; Levin, 1986); (3) upon exposure to the appropriate stimulus, the form of PAI-1 obtained from ECs, exhibited a comparable specific activity (Handt et al., 1994) but most of the PAI-1 secreted by ECs is rapidly inactivated (Levin, 1986; Mimuro et al., 1987; Handt et al., 1994). For example,

the ratio of inactive to active PAI-1 found in the CM of cultured HUVEC ranged from 12:1 after 4 h in culture to 56:1 after 24 h (Levin, 1986); (4) it has been reported that PAI-1 concentration in cell culture systems were frequently considerably higher than in plasma (Hekman and Loskutoff, 1987; Schleef and Loskutoff, 1988; Loskutoff, 1991); (5) the observation of van den Berg et al. (1988) that freshly isolated ECs from umbilical arteries do not contain detectable amounts of PAI-1 mRNA supports the hypothesis that the high PAI-1 synthesis by EC reflects changes in the biosynthetic properties of EC as they have adapted to the culture conditions (Christ et al., 1993); and (6) previous studies demonstrated that the majority of cell-associated PAI-1 in cultured ECs exists in the extracellular matrix (Mimuro et al., 1987; De Fouw et al., 1987; Ehrlich et al., 1991). It might be that one constituent of oil makes a complex with PAI-1 in EC cell matrix and the complex released into the supernatant of the culture represents another PAI-1 compartment secreted in CM. This is in agreement with Ehrlich et al. (1991) and Cockell et al. (1995) who found that thrombin released PAI-1 from cell-free extracellular matrix secreted into CM by ECs and smooth muscle cells, respectively. The increase in PAI-1 secretion caused by oil is, however, moderate as compared to the increase in response to some cytokines. This contrasts with the anti-fibrinolytic effect of cytokines, which inhibit t-PA secretion while strongly stimulating PAI-1 secretion (Schleef et al., 1988).

On the addition of thrombin, acute release of t-PA relating to the constitutive secretion (i.e., t-PA synthesis) occurred, and most if not all endothelial t-PA was released into the medium not complexed to an inhibitor (van den Eijnden-Schrauwen et al., 1995). Because t-PA in the subcellular matrix and t-PA on the plasma membrane are complexed to PAI-1 (Kooistra et al., 1986), the t-PA released must be derived from an intracellular compartment but not from plasma membrane-bound t-PA (Emeis et al., 1993; Schrauwen et al., 1994; van den Eijnden-Schrauwen et al., 1995). t-PA that is not acutely released from this pool into the medium still disappears from the pool, because the time t-PA stays in the pool is only about 1 h. This missing t-PA is called “lost t-PA” because it is not secreted in the constitutive or in the acute release pathway (van den Eijnden-Schrauwen et al., 1995). Two possible explanations account for this missing t-PA. Either the t-PA is deposited in the subcellular matrix (Korner et al., 1993), or the t-PA is degraded intracellularly, possibly in a lysosomal compartment, but is not recovered in the constitutive pathway (van den Eijnden-Schrauwen et al., 1995).

In light of previous studies, one could speculate that the stimulation of ECs by the NS oil at 100 µg/ml may cause an acute release of t-PA, which is then stored for a short period before either being deposited in the

subcellular matrix or degraded intracellularly. This acute t-PA release and subsequent binding to PAI-1 may provide a mechanism for (play an important role in) speeding up thrombolysis. Not only is the t-PA thus released itself enzymatically active, but acute release will also result in a local t-PA concentration much larger than the basal level of t-PA and also than that of its inhibitor PAI-1 (van den Eijnden-Schrauwen et al., 1995).

The changes in t-PA and PAI-1 might be due to decreased intracellular cAMP (Francis and Neely, 1989) and/or antagonizing the stimulatory effect of the protein kinase C activator as in response to phorbol ester (Santell and Levin, 1988), and via stimulation of endothelial phosphoinositide metabolism and protein kinase C activity (Lambert et al., 1986; Jaffe et al., 1987). It has been shown that the tumor suppressor p53 represses the transcription from the promoter of the human u-PA and t-PA gene and activates the promoter of the PAI-1 gene (Kunz et al., 1995). The t-PA/PAI-1 induced with high concentration of NS oil might result from a similar mechanism involving interaction of some oil constituents or another factor downstream in the signaling cascade with the respective promoter of t-PA and PAI-1. Such coordinated regulation leading to an increase in the production of fibrinolytic proteins would result in the balance of fibrinolysis/thrombus formation of endothelial cells and would allow for a greater potential for hemostasis in the endothelial cell environment (Zhang et al., 1997).

It can be suggested that the oil-induced t-PA/PAI-1 antigens at any given concentration reflect the dynamic balance between t-PA and PAI-1 levels rather than the effects of some chemical stimuli like alcohol, which represent an impact upon the plasminogen activator/plasmin system that participates in extracellular proteolysis, and thus indirectly influence the development of vascular lesions in the vessel (Vassali et al., 1991; Venkov et al., 1997). The observation that endothelial cells of different sites (HUVECs and HUA-ECs) respond similarly to blackseed oil confirms the universality of this pattern across different vascular tissues. This is the first report of a possible therapeutic role of NS oil-modulation of the fibrinolytic potential in ECs. Clinical studies should clarify if the modulation of the fibrinolytic system by this oil is also operative in vivo and if this modulation, might therefore contribute to the effect of NS in the treatment of cardiovascular diseases.

## Conclusion

The fibrinolytic capacity of plasma is considered to be strongly dependent on the concentration of circulating t-PA. Plasma t-PA is presumed to be derived mainly

from the vascular wall, where it localizes in endothelial cells (Emeis, 1988). Insight into the regulation of t-PA and PAI-1 synthesis may be helpful in developing drugs that may counteract insufficient endogenous t-PA or PAI-1 by increasing its production. These data provide an evidence that blackseed oil can modulate the fibrinolytic potential of HUVEC and HUA-EC in vitro by increasing the expression of t-PA and PAI-1 in these cells. This effect on endothelial t-PA and PAI-1 was evident on the level of the respective antigens. Further in vivo investigations are needed to evaluate this effect of *Nigella sativa* oil on the fibrinolytic system and to identify the constituents of blackseed oil responsible for modulating EC fibrinolytic potential.

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## References

- Al-Jishi, S.A., Abuo Hozafa, B., 2003. Effect of *Nigella sativa* on blood hemostatic function in rats. *J. Ethnopharmacol.* 85 (1), 7–14.
- Atta-ur Rahman, Malik, S., Cunheng, H., Clardy, J., 1985. Isolation and structure determination of nigellicine a novel alkaloid from the seeds of *Nigella sativa*. *Tetrahedron Lett.* 26, 2759–2762.
- Badary, O.A., 1999. Thymoquinone attenuates ifosfamide-induced Fanconi syndrome in rats and enhances its antitumor activity in mice. *J. Ethnopharmacol.* 67 (2), 135–142.
- Badary, O.A., Al-Shabanah, O.A., Nagi, M.N., Al-Rikabi, A.C., Elmazar, M.M., 1999. Inhibition of benzo(a)pyrene-induced forestomach carcinogenesis in mice by thymoquinone. *Eur. J. Cancer Prev.* 8 (5), 435–440.
- Badary, O.A., Abdel-Naim, A.B., Abdel-Wahab, M.H., Hamada, F.M., 2000. The influence of thymoquinone on doxorubicin-induced hyperlipidemic nephropathy in rats. *Toxicology* 143 (3), 219–226.
- Booyse, F.M., Sedlak, B.J., Rafelson, R., 1975. Culture of arterial endothelial cells: characterization and growth of bovine aortic endothelial cells. *Thromb. Diath. Haemorrh.* 34, 825–839.
- Chakravarty, N., 1993. Inhibition of histamine release from mast cells by nigellicone. *Ann. Allergy* 70, 237–242.
- Chopra, R.N., Nayar, S.L., Chopra, I.C., 1956. *Glossary of Indian Medicinal Plants*. CSIR, New Delhi, India 175p.
- Christ, G., Seiffert, D., Hufnagl, P., Gessl, A., Wojta, J., Binder, B.R., 1993. Type 1 plasminogen activator inhibitor synthesis of endothelial cells is downregulated by smooth muscle cells. *Blood* 18 (5), 1277–1283.
- Cockell, K.A., Ren, S., Sun, J., Angel, A., Shen, G.X., 1995. Effect of thrombin on release of plasminogen activator inhibitor-1 from cultured primate arterial smooth muscle cells. *Thromb. Res.* 77 (2), 119–131.
- de Fouw, N.J., Van Hinsbergh, V.W., de Jong, Y.F., Haverkate, F., Bertina, R.M., 1987. The interaction of activated protein C and thrombin with plasminogen activator inhibitor released from human endothelial cells. *Thromb. Haemost.* 57, 176–182.
- DeGruchy, G.C., 1983. *Clinical Haematology in Medical Practice*. Blackwell Scientific Publications, UK, p. 698.
- Ehrlich, H.J., Gebbink, R.K., Preissner, K.T., Keijer, J., Esmon, N.L., Mertens, K., Pannekoek, H., 1991. Thrombin neutralizes plasminogen activator inhibitor 1 (PAI-1) that is complexed with vitronectin in the endothelial cell matrix. *J. Cell Biol.* 115, 1773–1781.
- El-Dakhakhny, M., 1965. Studies on the Egyptian *Nigella sativa* L. part IV: some pharmacological properties of the seed's active principle in comparison to its dihydro compound and its polymer. *Arzneim. Forsch.* 15, 1227–1229.
- El-Naggar, A.R.M., El-Deib, A.R.M., 1992. A Study of some biological activities of *Nigella sativa* (black seeds). *Habat El-Baraka J. Egypt. Soc. Pharmacol. Exp. Therap.* 11, 781–800.
- El-Tahir, K., Ashour, M., Alharbi, M., 1993. The cardiovascular actions of the volatile oil of the blackseed (*Nigella sativa*) in rats elucidation of the mechanism of actions. *Gen. Pharmacol.* 24, 1123–1131.
- Emeis, J.J., 1988. Mechanisms involved in short-term changes in blood levels of t-PA. In: Kluft, C. (Ed.), *Tissue-type plasminogen activator (t-PA): Physiological and Clinical Aspects*, Vol. 2. Boca Raton Fla, CRC Press, pp. 21–35.
- Emeis, J.J., van den Hoogen, C.M., Schrauwen, Y., 1993. An endothelial storage granule for tissue-type plasminogen activator. *Thromb. Haemost.* 69, 609 (abstr).
- Francis Jr., R.B., Neely, S., 1989. Inhibition of endothelial secretion of tissue-type plasminogen activator and its rapid inhibitor by agents which increase intracellular cyclic AMP. *Biochim. Biophys. Acta* 1012, 207–213.
- Ghoneim, M.T., El-Gindy, A.R., El-almi, R., Shoukry, E., Yassen, S., 1982. Possible effect of some extracts of *Nigella sativa* L. seeds on blood coagulation system and fibrinolytic activity. In: *Proceeding of the Second International Conference on Islamic Medicine in Kuwait*, pp. 528–535.
- Gimbrone, M.A., Cotran, R., Folkman, J.J., 1974. Human vascular endothelial cells in culture growth and DNA synthesis. *J. Cell Biol.* 60, 673.
- Hailat, N., Bataneh, Z., Lafi, S., Raweily, E., Aqil, M., Al-Katib, M., Hanash, S., 1995. Effect of *Nigella sativa* volatile oil on Jurkat T cell leukemia polypeptides. *Int. J. Pharmacog.* 33, 16–20.
- Handt, S., Jerome, W.G., Braaten, J.V., Lewis, J.C., Kirkpatrick, C.J., Hantgen, R.R., 1994. PAI-1 released from cultured human endothelial cells delays fibrinolysis and is incorporated into the developing fibrin clot. *Fibrinolysis* 8, 104–112.
- Haq, A., Lobo, P.I., Al-Tufail, M., Rama, N.R., Al-Sedairy, S.T., 1999. Immunomodulatory effect of *Nigella sativa*

- proteins fractionated by ion exchange chromatography. *Int. J. Immunopharmacol.* 21 (4), 283–295.
- Hekman, C.M., Loskutoff, D.J., 1985. Endothelial cells produce a latent inhibitor of plasminogen activators that can be activated by denaturants. *J. Biol. Chem.* 260, 11581–11587.
- Hekman, C.M., Loskutoff, D.J., 1987. Fibrinolytic pathways and endothelium. *Sem. Thromb. Haostas* 13, 514.
- Hill, G.A., Herbert, C.M., Parker, R.A., Wentz, A.C., 1989. Comparison of late luteal phase endometrial biopsies utilizing the Novak curette or Pipelle Endometrial Suction Curette. *Obstet. Gynecol.* 73, 443.
- Houghton, P., Zarka, R., Heras, B., Hoult, J., 1995. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosenoid generation in leukocytes and membrane lipid peroxidation. *Planta Med.* 16, 33–36.
- Ibn Sina (avescenna), 1037, *Al-Qanoon Fit-Tib* (Principles in medicine). Reprinted in 1970 from Bulaq, edition Sader Publishing House Beirut, Lebanon.
- Jaffe, E.A., Nachman, R.L., Becker, C.G., Minick, C.R., 1973. Culture of human endothelial cells derived from umbilical cord veins: identification by morphologic and immunologic criteria. *J. Clin. Invest.* 52, 2745–2756.
- Jaffe, E.A., Hoyer, L.W., Nachman, R., 1980. Synthesis of antihepophilic factor antigen by cultured human endothelial cells. *J. Clin. Invest.* 52, 2757–2764.
- Jaffe, E.A., Grulich, J., Weksler, B.B., Hampel, G., Watanabe, K., 1987. Correlation between thrombin-induced prostacyclin production and inositol trisphosphate and cytosolic free calcium levels in cultured human endothelial cells. *J. Biol. Chem.* 262, 8557–8565.
- Kooistra, T., 1990. The use of cultured human endothelial cells and hepatocytes as an in vitro model system to study modulation of endogenous fibrinolysis. *Fibrinolysis* 4 (suppl 2), 33–39.
- Kooistra, T., Sprengers, E.D., Van Hinsbergh, V.W.M., 1986. Rapid inactivation of the plasminogen-activator inhibitor upon secretion from cultured human endothelial cells. *Biochem. J.* 239, 497.
- Korner, G., Bjornsson, T.D., Vlodavsky, I., 1993. Extracellular matrix produced by cultured corneal and aortic endothelial cells contains active tissue-type and urokinase-type plasminogen activators. *J. Cell Physiol.* 154, 456.
- Kunz, C., Pebler, S., Otte, J., von der Ahe, D., 1995. Differential regulation of plasminogen activator and inhibitor gene transcription by the tumor suppressor p53. *Nucl. Acid Res.* 23, 3710–3717.
- Lambert, T.L., Kent, R.S., Whorton, A.R., 1986. Bradykinin stimulation of inositol polyphosphate production in porcine aortic endothelial cells. *J. Biol. Chem.* 261, 15288–15293.
- Levin, E.G., 1983. Latent tissue plasminogen activator produced by human endothelial cells in culture: evidence for an enzyme-inhibitor complex. *Proc. Natl. Acad. Sci. USA* 80, 6804.
- Levin, E.G., 1986. Quantitation and properties of the active and latent plasminogen activator inhibitors in cultures of human endothelial cells. *Blood* 67, 1309–1313.
- Loskutoff, D.J., 1991. Regulation of PAI-1 expression. *Fibrinolysis* 5, 197.
- Marozzi, E.J., Kocialiski, A.B., Malone, M.H., 1970. Studies on the antihistaminic effects of thymoquinone thymohydroquinone and quercetin. *Arzneim. Forsch.* 20, 1574–1578.
- Mimuro, J., Schleeff, R.R., Loskutoff, D.J., 1987. Extracellular matrix of cultured bovine endothelial cells contains functionally active type I plasminogen activator inhibitor. *Blood* 70, 721–728.
- Nadakarni, K.M., 1976. *Corcus sativus Nigella sativa*. In: Nadkarni, K.M. (Ed.), *Indian Materia Medica*. Popular Prakashan, Bombay, India, pp. 386–411.
- Nagi, M.N., Alam, K., Badary, O.A., al-Shabanah, O.A., al-Sawaf, H.A., al-Bekairi, A.M., 1999. Thymoquinone protects against carbon tetrachloride hepatotoxicity in mice via an antioxidant mechanism. *Biochem. Mol. Biol. Int.* 47 (1), 153–159.
- Nies, A.S., 1992. *The pharmacological Basis of Therapeutics*. McGraw-Hill, New York, PP, pp. 62–83.
- Resh, I., Krutisch, G., Geiger, M., Binder, B.R., 1989. ELISA systems for active and total plasminogen activator inhibitor 1 (PAI-1) antigen. *Thromb. Haemost.* 62, 299.
- Salomi, N.J., Nair, S.C., Panikkar, K.R., 1991. Inhibitory effects of *Nigella sativa* and Saffron (*Crocus sativus*) on chemical carcinogenesis in mice. *Nutr. Cancer* 16, 67–72.
- Salomi, N.J., Nair, S.C., Jayawardhanan, K.K., Vargghese, C.D., Pantikkar, K.R., 1992. Anti-tumor principles from *Nigella sativa* seeds. *Cancer Lett.* 63, 41–46.
- Santell, E.G., Levin, E.G., 1988. Cyclic AMP potentiates phorbol ester stimulation of tissue plasminogen activator release and inhibits secretion of plasminogen activator inhibitor-1 from human endothelial cells. *J. Biol. Chem.* 263, 16802–16808.
- Schleeff, R.R., Loskutoff, D.J., 1988. Fibrinolytic system of vascular endothelial cells. *Haemosatasis* 18, 328.
- Schleeff, R.R., Bevilacqua, M.P., Sawdey, M., Gimbrone Jr., M.A., Schleeff, R.R., Loskutoff, D.J., 1988. Fibrinolytic system of vascular endothelial cells. *Haemostasis* 18, 328–341.
- Schrauwen, Y., Emeis, J.J., Kooistra, T., 1994. A sensitive ELISA for human tissue-type plasminogen activator applicable to the study of acute release from cultured human endothelial cells. *Thromb. Haemost.* 71, 225.
- Stein, O., Stein, Y., 1980. Bovine aortic endothelial cells display macrophage-like properties towards acetylated <sup>125</sup>I-labeled low density lipoprotein. *Biochem. Biophys. Acta* 620, 631–635.
- Swamy, S.M., Tan, B.K., 2000. Cytotoxic and immunopotentiating effects of ethanolic extract of *Nigella sativa* L seeds. *J. Ethnopharmacol.* 70 (1), 1–7.
- van den Berg, E.A., Sprengers, E.D., Jaye, M., Burgess, W., Maciag, T., van Hinsbergh, V.W.M., 1988. Regulation of plasminogen activator inhibitor-1 mRNA in human endothelial cells. *Thromb. Haemostas.* 60, 63.
- van den Eijnden-Schrauwen, Y., Kooistra, T., de Vries, R.E.M., Emeis, J.J., 1995. Studies on the acute release of tissue-type plasminogen activator from human endothelial cells in vitro and in rats in vivo: evidence for a dynamic storage pool. *Blood* 85 (12), 3510–3517.

- van Hingsbergh, V.W.M., 1988. Regulation of the synthesis and secretion of plasminogen activators by endothelial cells. *Haemostasis* 18, 307–327.
- van Mourik, J.A., Lawrence, D.A., Loskutoff, D.J., 1984. Purification of an inhibitor of plasminogen activator (antiactivator) synthesized by endothelial cells. *J. Biol. Chem.* 259, 14914–14921.
- Vassali, J.D., Sappino, A.P., Belin, D., 1991. The plasminogen activator/plasmin system. *J. Clin. Invest.* 88, 1067–1072.
- Venkov, C.D., Su, M., Shyr, Y., Vaughan, D.E., 1997. Ethanol-induced alterations in the expression of endothelial-derived fibrinolytic components. *Fibrinol. Proteol.* 11 (2), 115–118.
- Wagner, O.F., Binder, B.R., 1986. Purification of an active plasminogen activator inhibitor immunologically related to the endothelial type plasminogen activator inhibitor from the conditioned media of a human melanoma cell line. *J. Biol. Chem.* 261, 14474–14481.
- Worthen, D.R., Ghosheh, O.A., Crooks, P.A., 1998. The in vitro anti-tumor activity of some crude and purified components of blackseed *Nigella sativa* L. *Anticancer Res.* 18 (3A), 1527–1532.
- Wun, T.-C., Capuano, A., 1985. Spontaneous fibrinolysis in whole human plasma: identification of tissue activator-related protein as the major plasminogen activator causing spontaneous activity in vitro. *J. Biol. Chem.* 260, 5061–5066.
- Zaoui, A., Cherrah, Y., Lacaille-Dubois, M.A., Settaf, A., Amarouch, H., Hassar, M., 2000. Diuretic and hypotensive effects of *Nigella sativa* in the spontaneously hypertensive rat. *Therapie* 55 (3), 379–382.
- Zaoui, A., Cherrah, Y., Alaoui, K., Mahassine, N., Amarouch, H., Hassar, M., 2002. Effects of *Nigella sativa* fixed oil on blood homeostasis in rat. *J. Ethnopharmacol.* 79 (1), 23–26.
- Zhang, W., Wojta, J., Binder, B.R., 1994. Effect of Notoginsenoside R1 on the synthesis of tissue-type plasminogen activator and plasminogen activator inhibitor-1 in cultured human umbilical vein endothelial cells. *Arterioscler. Thromb.* 14, 1040–1046.
- Zhang, W., Wojta, J., Binder, B.R., 1997. Regulation of the fibrinolytic potential of cultured human umbilical vein endothelial cells: astragaloside IV downregulates plasminogen. *J. Vasc. Res.* 34, 273–280.
- Zhao, X., Gu, Z., Attele, A.S., Yuan, C.-S., 1998. Effects of quercetin on the release of endothelin, prostacyclin and tissue plasminogen activator from human endothelial cells in culture. *J. Ethnopharmacol.* 67, 279–285.