J Appl Biomed
DOI: 10.32725/jab.2023.006
journal homepage: http://jab.zsf.jcu.cz

Journal of Applied Biomedicine

Original research article

In vitro study of *Nigella sativa* and thymoquinone activity on endothelial activation and monocyte adhesion

Al'Aina Yuhainis Firus Khan [1], Fahmi Mohtar [2], Thuhairah Abdul Rahman [1, 3, 4], Suhaila Abdul Muid [1, 5], Gabriele Ruth Anisah Froemming [1, 6], Hapizah Nawawi [1, 3] *

[1] *Universiti Teknologi MARA, Institute for Pathology, Laboratory and Forensic Medicine (I-PPerForM), Sungai Buloh, Malaysia*
[2] *Universiti Teknologi MARA, Centre for Pathology Diagnostic and Research Laboratories (CPDRL), Sungai Buloh, Malaysia*
[3] *Universiti Teknologi MARA, Faculty of Medicine, Department of Pathology, Sungai Buloh, Malaysia*
[4] *Universiti Teknologi MARA, Integrative Pharmacogenomics Institute (iPROMISE), Puncak Alam, Malaysia*
[5] *Universiti Teknologi MARA, Faculty of Medicine, Department of Biochemistry and Molecular Medicine, Sungai Buloh, Malaysia*
[6] *Universiti Malaysia Sarawak, Faculty of Medicine and Health Sciences, Department of Basic Medical Sciences, Kota Samarahan, Malaysia*

Abstract
Introduction: Thymoquinone (TQ) is one of the bioactive compounds in *Nigella sativa* (NS). Also known as black seeds/cumin, it has been postulated to possess anti-atherogenic properties. However, research on the effects of NS oil (NSO) and TQ on atherogenesis remain scarce. The aim of this study is to determine gene and protein expression of Intercellular Adhesion Molecule-1 (ICAM-1), Vascular Cell Adhesion Molecule-1 (VCAM-1), and Endothelial-eukocyte adhesion molecule (E-selectin) in Human Coronary Artery Endothelial Cells (HCAECs).
Methods: HCAECs were stimulated for 24 hours (h) with 200 μg/ml of Lipopolysaccharides (LPS) and different concentrations of NSO (55, 110, 220, 440 μg/ml) or TQ (4.5, 9.0, 18.0, 36.0 μm). The effects of NSO and TQ on gene and protein expressions were measured using multiplex gene assay and ELISA assay, respectively. Rose Bengal assay was used to analyse monocyte binding activity.
Results: NSO and TQ significantly reduced ICAM-1 and VCAM-1 gene and protein expressions. TQ showed significant reduction activity of the biomarkers in dose dependent manner. HCAECs pre-treated with NSO and TQ for 24 h significantly lowered monocytes adherence compared to non-treated HCAECs.
Conclusions: NSO and TQ supplementation have anti-atherogenic properties and inhibit monocytes' adherence to HCAECs via down-regulation of ICAM-1 expression. NSO could potentially be incorporated in standard treatment regimens to prevent atherosclerosis and its related complications.

Keywords: Anti-atherogenic; Endothelial activation biomarkers; Monocytes binding thymoquinone; *Nigella sativa*

Highlights:
- *Nigella sativa* is used in traditional medicine for its various beneficial effects.
- Limited studies have been performed on its anti-atherogenesis effect in HCAECs.
- Results showed NS oil or TQ exhibited anti-atherogenesis biomarkers.

Introduction

Atherosclerosis is a chronic inflammatory process involving key events such as endothelial dysfunction, inflammation, oxidative stress, and prothrombogenesis. Over a period of time it can lead to complications such as coronary artery disease (CAD), cerebrovascular accidents (CVA), and peripheral vascular disease (PVD) (Steven et al., 2019). These non-communicable diseases remain the leading cause of mortality and morbidity worldwide (Lozano et al., 2012). Several risk factors such as oxidised low-density lipoprotein and hypertension have been shown to reduce nitric oxide concentrations, leading to endothelial dysfunction and unregulated expression of endothelial activation markers during the initiation stage of atherogenesis. These endothelial activation markers (ICAM-1, VCAM-1, E-selectin) promote monocyte adherence; a key pathogenesis to the formation of atherosclerotic plaques (Marchio et al., 2019).

The management of CAD has long aimed to control established risk factors such as dyslipidaemia, hypertension, and hyperglycaemia/diabetes mellitus through drug treatment and lifestyle modifications (Malakar et al., 2019). However, in recent years, there has been a move towards identifying the therapeutic values of natural compounds to incorporate them into standard treatment regimens for reducing the risk of de-

* **Corresponding author:** Hapizah Nawawi, Universiti Teknologi MARA, Institute for Pathology, Laboratory and Forensic Medicine (I-PPerForM), Sungai Buloh, Malaysia; e-mail: hapizah.nawawi@gmail.com
http://doi.org/10.32725/jab.2023.006
Submitted: 2022-04-22 • Accepted: 2023-04-04 • Prepublished online: 2023-05-04
J Appl Biomed 21/2: 73–79 • EISSN 1214-0287 • ISSN 1214-021X
© 2023 The Authors. Published by University of South Bohemia in České Budějovice, Faculty of Health and Social Sciences.
This is an open access article under the CC BY-NC-ND license.

veloping CAD (Yuan et al., 2016). One such natural product with a compound that has been of interest is *Nigella sativa* (NS), or as it is more commonly known, the black seed or black cumin.

NS is a herbaceous plant belonging to the Ranunculaceae family. It is widely distributed in Eastern Europe, the Middle East, and Western Asia and is harvested annually (Ahmad et al., 2013). Commonly, NS is known as "black cumin", in the middle east as "habbat us sauda", and in southern Asia as "kalonji" (Ahmad et al., 2021; Yimer et al., 2019). NS has been mentioned in various historical and religious textbooks as a potential plant with medicinal properties (Ahmad et al., 2021). The discovery of several inscriptions on NS found within the tomb of Pharaoh Tutankhamen prove it has been used as a remedy for ailments since ancient civilisation.

The historical accounts of the medicinal properties of NS have sparked a growing research interest in identifying the mechanism of its efficacy in various diseases (Padhye et al., 2008). NS has been shown to be an antioxidant (Bordoni et al., 2019), anti-diabetes (Sheikh, 2016; Tiji et al., 2021), anti-tumour (Rahmani and Aly, 2015), and anti-convulsant agent (Hosseinzadeh and Parvardeh, 2004). Previous studies from animal models and clinical trials have also shown that NS can be administered either orally (Abdel-Daim and Ghazy, 2015; Basheer and Qureshi, 2018; Heshmati and Namazi, 2015) or through intraperitoneal injection (Hosseini et al., 2015) in two forms; crushed black seeds in powder form or as extract including fixed and volatile oils (Samir Bashandy, 2007; Sultan et al., 2014, 2015; Zaoui, et al., 2002a, b).

Oil is the main constituent of NS, making up 32–40% of it, followed by carbohydrates (29.2%), protein (18.1%), fibre (6.4%), moisture (5.5%), ash (4.7%), and volatile oil (0.4–0.45%) (Mamun and Absar, 2018). Fixed oil dominates the constituents of the NS oil, whereas volatile oil only accounts for 0.5–1.4% of the seeds' weight (Kiralan, 2014). The volatile oil of NS has valuable phytomedicinal features and contains a variety of compounds. There have been various reports on the active compounds contained in NS, with thymoquinone (TQ) as the main constituent and other oils such as carvacrol, junipene, *p*-cymen, 4-Terpineol, longipene, and bornylacetate present in minor quantities (Edris, 2010; Kiralan, 2014).

TQ is the main active compound of NS that has abundant therapeutic values (Majdalawieh et al., 2021). The wide variety of medicinal uses of TQ are mainly due to its antioxidant activity. Thymoquinone acts as a reducing agent and plays an essential role in preventing oxidative stress (Cobourne-Duval et al., 2016). Oxidative stress is caused by reactive oxygen species (ROS), which oxidises and damages proteins, lipids, and nucleic acids including DNA. ROS are typically produced as a by-product of mitochondrial ATP production, but under certain circumstances, the production of ROS will escalate resulting in cellular apoptosis and death (Zorov et al., 2014). Certain diseases such as diabetes mellitus and cancer have been shown to excessively produce ROS (Leong et al., 2013). TQ has been reported to reduce oxidative stress as it acts as a scavenger of ROS (Cobourne-Duval et al., 2016).

Several studies have been conducted to determine the effects of NS and its bioactive compound, TQ, in the mechanisms of various diseases. Many studies have shown that TQ is the major component of NSO, and responsible for its therapeutic benefits (Mazaheri et al., 2019; Srinivasan, 2018). Therefore, the present study aims to evaluate NSO and TQ for anti-atherogenic activity. However, to the best of our knowledge, the role of NS and TQ in the atherogenesis pathway – particularly their influence on endothelial activation and monocyte binding – have not been well addressed. Therefore, this study aims to investigate the effects of NS and TQ on the gene and protein expressions of endothelial activation markers in stimulated human coronary artery endothelial cells (HCAECs).

Materials and methods

Reagents and chemicals

Nigella sativa oil (NSO) was purchased from Kausar, Iran, as a pure oil with no additives. TQ was purchased from Sigma-Aldrich (USA) with a purity of 99% and kept at 4 °C. All chemicals used in this study were of analytical grade, including 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), and lipopolysaccharides (LPS) obtained from Sigma-Aldrich. HCAECs and its growth medium of endothelial basal medium-2 (EBM-2) kit were acquired from Lonza, Switzerland. The RPMI 1640 (Corning, New York), Foetal Bovine Serum (FBS) and penicillin from Gibco, USA, were used for treatment media. Rose Bengal stain was acquired from Sigma-Aldrich, USA. Commercially available ProcartaPlexTM Assay was used for protein expression quantification, while gene was measured by QuantiGene® 2.0 Plex Assay from Affymetrix, USA.

Cell culture maintenance

The human coronary artery endothelial cells (HCAECs) were maintained in complete growth medium (CGM), supplemented with EBM-2 growth medium, 10% fetal bovine serum, and 1% of penicillin antibiotic at 37 °C and in a 5% CO_2 humidified incubator. The number of HCAEC passages was maintained between 5–7 passage during the study.

Cytotoxicity assay

The HCAECs (2×10^4 per well) were seeded into 96-well culture plates, and the media was replaced the next day with a fresh medium containing NSO (3.4–800.0 μg/ml) or TQ (2.4–42.2 μm) diluted from the stock solution. Following 24 h of incubation, the MTT solution was added and incubated further for another 2 h. MTT formazan formed by metabolically viable cells was dissolved in isopropanol, and absorbance was measured at 595 nm on a plate reader (TECAN, Switzerland).

HCAECs treatment for gene and protein expression

HCAECs were maintained in continuous exponential growth prior to the assay. HCAECs untreated cells were used as negative control and cells only treated with LPS as positive control. All groups of NSO (55.0–440.0 μg/ml) and TQ (4.5–36.0 μm) treated cells were stimulated with 200 μg/ml of LPS. After 24 h of treatment, the cells were harvested and resuspended in RPMI medium and stored at –80 °C for gene expression study. The supernatants were collected and stored at –20 °C for protein expression study.

Gene expression

Frozen cells were thawed and lysed for gene expression quantification using QuantiGene® 2.0 Plex Assay (Thermo Fisher Scientific, USA) for *ICAM-1, VCAM-1*, and *E-selectin* expression. The target-specific RNA molecules of LPS-stimulated HCAECs were *ICAM-1*: NM_000201, *VCAM-1*: NM_001078 and *E-selectin*: NM_000450. The genes were normalised with geometric means of three references genes, namely Glyceraldehyde-3-phosphate dehydrogenase (GAPDH): NM_002046; Glucuronidase beta (GUSB): NM_000181; and Hypoxanthine

phosphoribosyltransferase 1 (HPRT1): NM_000194. The Quanti-Gene Plex 2.0 assay combines branched DNA (bDNA) signal amplification and multi-analyte profiling beads (xMAP) technologies to enable the detection and quantitation of multiple RNA targets simultaneously. The gene expression of treated cells was presented as fold changes relatively to LPS group.

Protein expression

Supernatants were thawed at room temperature and protein expression of ICAM-1, VCAM-1, and E-selectin were measured by ProcartaPlexTM Assay. The assay was performed following manufacturer instructions. The absorbance was determined at 540 nm on a plate reader (TECAN, Switzerland).

Monocyte binding assay

HCAECs were stimulated with 1 μg/ml of LPS for 24 h at 37 °C, and 5% CO_2 humidified incubation. The LPS stimulated HCAECs were then treated with different concentrations of NSO (55.0, 110.0, 220.0, 440.0 μg/ml) and TQ (4.5, 9.0, 18.0, 36.0 μm). Monocyte cell line (U937) obtained from ATCC, USA, was then added and incubated for 1 h. Rose Bengal stain which was prepared in phosphate-buffered saline (PBS) was added. The absorbance data was measured using spectrophotometer at 570 nm.

Statistical analysis

Each concentration was performed in biological triplicate and all the experiments were conducted in triplicate. Data were recorded as mean ± standard error mean (SEM) and analysed by SPSS version 22.0. One-way ANOVA and *Post Hoc* analysis (Bonferroni) were used, where $p < 0.05$, $p < 0.01$ or $p < 0.001$ was deemed to be statistically significant.

Results

Effects of NSO and TQ on HCAECs cell survival

The characterisation of the cytotoxicity of NSO (3.4–880 μg/ml) and TQ (2.4–42.2 μm) revealed that high concentrations of NSO and TQ caused a reduction in cell viability by approximately 60% and 14%, respectively (Fig. 1A, B). Thus, NSO (3.4–440 μg/ml) and TQ (4.5–36 μm) concentrations were considered not to be cytotoxic to HCAECs and used for further studies.

NSO and TQ attenuate LPS-mediated endothelial activation in HCAECs

LPS-induced injury in HCAECs is related to increased endothelial activation. The mRNA expression of *ICAM-1, VCAM-1* and *E-selectin* were measured by QuantiGene Plex assay, and the protein levels by ELISA. Fig. 2A depicts the HCAECs NSO treated gene and protein expression of VCAM-1, where the gene evidenced down-regulation in dose dependent relatively to HCAECs LPS induced significantly ($p < 0.001$). Similarly, VCAM-1 protein expression were down-regulated significantly ($p < 0.01$) across all concentrations (55–440 μg/ml) in comparison to LPS group. The gene expression of ICAM-1 in NSO treated cells showed down-regulation in all concentrations, with $p < 0.01$, for 55–110 μg/ml and $p < 0.001$, for 220–440 μg/ml. As depicted in Fig. 2B, the ICAM-1 protein expression demonstrated NSO down-regulated ICAM-1 across all concentrations significantly ($p < 0.001$). As for E-selectin, NSO down-regulate the E-selectin gene expression significantly in dose dependent with for 55–110 μg/ml ($p < 0.01$) and 220–440 μg/ml ($p < 0.001$). While for the protein expression, NSO was down-regulated significantly ($p < 0.001$) in cells treated with 110–440 μg/ml groups. The concentration of NSO at 55 μg/ml increased the protein expression of E-selectin, although this did not reach a statistically significant level.

The endothelial activation biomarkers of HCAECs cells treated with various concentrations of TQ are depicted in Fig. 3A VCAM-1; Fig. 3B ICAM-1; and Fig. 3C E-selectin. LPS stimulated HCAECs treated with TQ showed significantly ($p < 0.001$) down-regulated VCAM-1 genes in dose-dependent manner relatively compared to LPS group. Similarly, the protein expression of TQ treated cells demonstrated significant down regulation of VCAM-1 in dose dependent pattern ($p < 0.001$). As for ICAM-1, the gene expression of TQ treated cells demonstrated significant down regulation at all concentrations. At concentration of 4.5–9 μm and 18–36 μm ICAM-1 gene down regulated in dose dependent pattern significantly ($p < 0.001$). The protein expression of ICAM-1 upon treated with TQ demonstrated down regulates at all concentrations in dose dependent manner ($p < 0.001$). As for E-selectin, the gene exhibited down regulation significantly in dose-proportional as well ($p < 0.001$). While for the E-selectin protein expression, though all concentrations evidenced significant down regulation ($p < 0.001$), but only 9–36 μm were in dose-dependently.

NSO and TQ effects on HCAECs monocyte binding

To investigate the effect of NSO and TQ on monocyte adherence inhibition, rose Bengal assay was performed. As shown in Fig. 4A NSO and Fig. 4B TQ, HCAECs pre-treated with 55, 110, 220, 440 μg/ml NSO for 24 hours showed significant ($p < 0.001$) lower adherence of monocytes compared to HCAECs incubated with LPS mean ± SD/(% inhibition); 1.33 ± 3.3×10^{-3}/(9.75%), 1.38 ± 0.01/(6.12%), 1.35 ± 1.9×10^{-2}/(8.39%), 1.33 ± 8.9×10^{-3}/[(9.3%) versus 1.47 ± 5.77×10^{-3}.

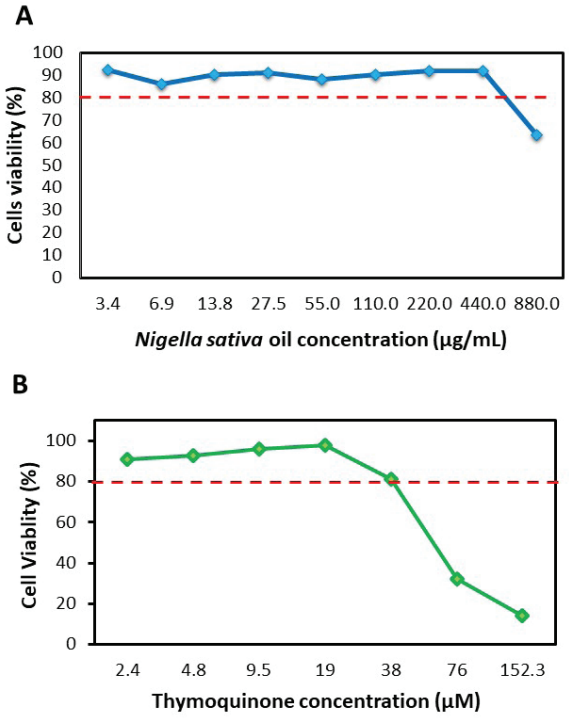

Fig. 1. Cell viability of HCAECs. HCAECs were treated with different concentrations of (**A**) NSO (3.4–880 μg/ml) and (**B**) thymoquinone (TQ) (2.4–42.2 μm) for 24 h. Cell viability was measured using MTT assay.

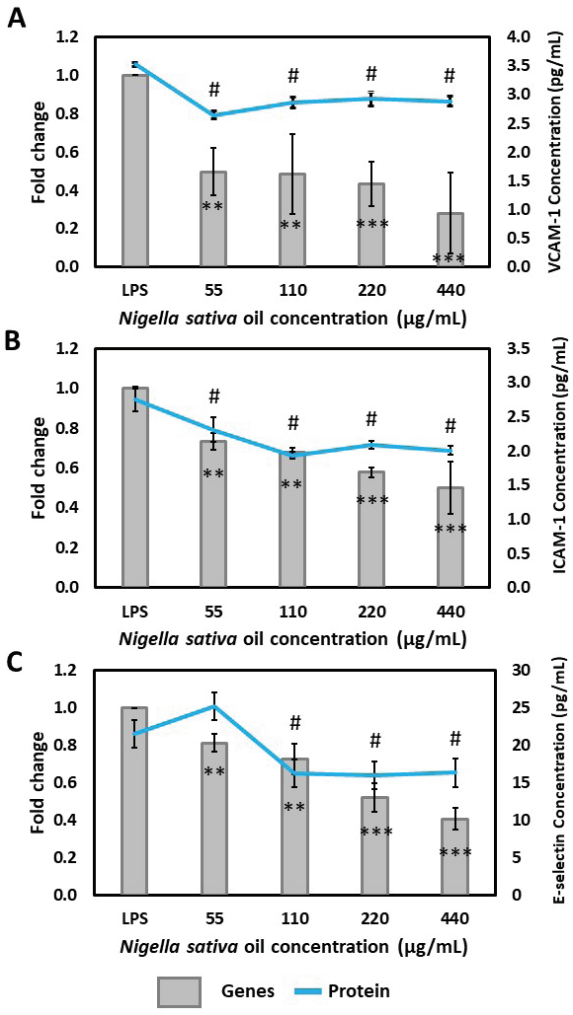

Fig. 2. Attenuated effect of NSO on endothelial activation biomarkers (**A**) VCAM-1, (**B**) ICAM-1 and (**C**) E-selectin in HCAECs. The gene expressions of targeted genes are expressed as fold change based on a calculation using GAPDH, GUSB and HPRT1 as the reference gene. LPS treated cells were assigned as 1. Data expressed are mean ± SD (n = 3). Statistical analysis: ANOVA, *post-hoc* with Bonferroni correction; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ for gene expression and # $p < 0.001$ for protein expression compared to HCAECs incubated with LPS alone.

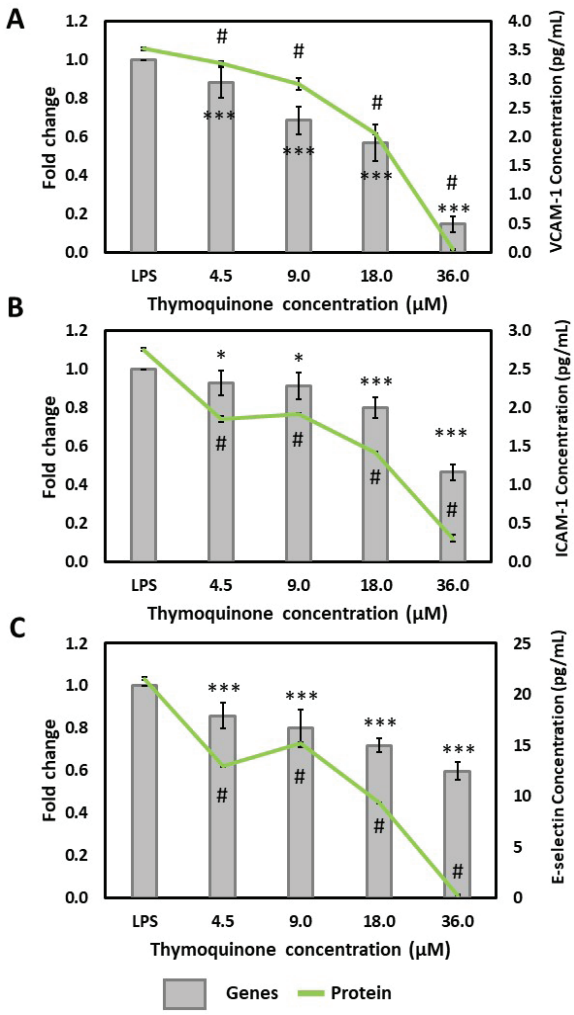

Fig. 3. Attenuated effect of TQ on endothelial activation biomarkers (**A**) VCAM-1, (**B**) ICAM-1 and (**C**) E-selectin in HCAECs. The gene expressions of targeted genes are expressed as fold change based on a calculation using GAPDH, GUSB, and HPRT1 as the reference gene. LPS treated cells were assigned as 1. Data expressed are mean ± SD (n = 3). Statistical analysis: ANOVA, *post-hoc* with Bonferroni correction; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ for gene expression, and # $p < 0.001$ for protein expression compared to HCAECs incubated with LPS alone.

Similarly, the concentrations of 4.5, 9.0, 18.0, 36.0 µg/ml TQ for 24 hours demonstrated significant ($p < 0.001$) lower adherence for monocytes compared to HCAECs incubated with LPS mean ± SD/(% inhibition); $1.18 \pm 3.3 \times 10^{-3}$/(8.33%), $1.17 \pm 17.6 \times 10^{-3}$/(8.33%), $1.22 \pm 3.3 \times 10^{-3}$/(4.43%), $0.89 \pm 3.3 \times 10^{-3}$/(30.73%) versus $1.28 \pm 11.5 \times 10^{-3}$.

Discussion

Cardiovascular disease is the global leading cause of death and is mainly caused by atherosclerosis. Over the years, researchers have been studying natural product compounds to attempt to reduce the atherosclerosis activity. The present study evaluated NSO and its bioactive compound, TQ, as potential anti-atherosclerotic activity on HCAECs as limited data was found regarding its mechanism in *in vitro* study. In this study, NSO and TQ supplementation inhibited LPS-induced endothelial activation and monocyte adherence to HCAECs, possibly via downregulation of ICAM-1, VCAM-1, and E-selectin expressions were evaluated.

It is crucial to identify a potential anti-atherosclerosis agent with minimal toxicity on the surrounding normal cells as the aim is to decrease plaque production in normal HCAECs cells. NSO and TQ are in line as the cytotoxicity assay on HCAECs revealed NSO and TQ only exhibited toxicity on HCAECs cells at high concentration upon treated for 24 h. Gene and protein analysis of endothelial activation biomarkers were performed to evaluate the level of expression upon those treated with NSO and TQ. Further analysis of NSO on endothelial activation biomarkers revealed that NSO significantly decreased the gene expression of VCAM-1, ICAM-1, and E-selectin in a dose dependent pattern. For protein expression, NSO significantly reduced the expression of the endothelial biomarkers as well.

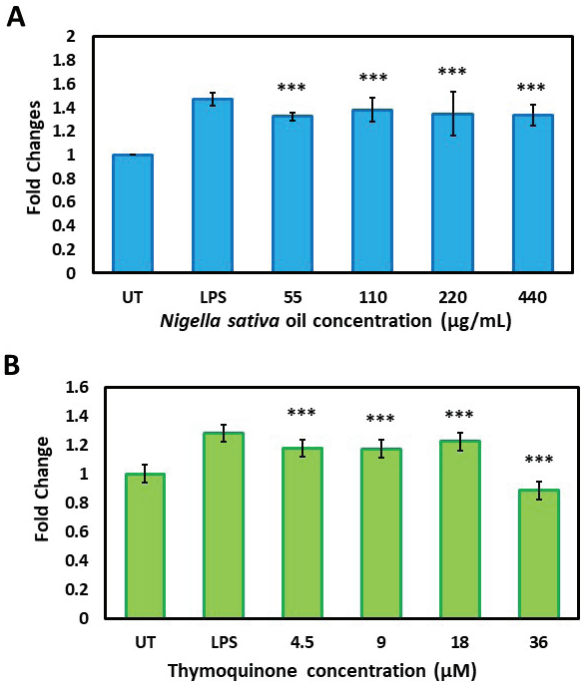

Fig. 4. NSO inhibited monocyte binding to HCAECs. HCAECs were stimulated with LPS or treated with (**A**) NSO and (**B**) TQ for 24 h at 37 °C with 5% CO2 humidity. The absorbance was measured using spectrophotometer at 570 nm. Percentage of inhibition are expressed as mean ± SEM (n = 3). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. LPS group (200 µg/ml).

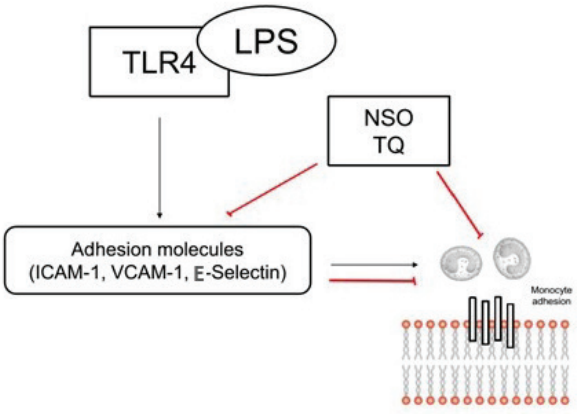

Fig. 5. Scheme summarising the anti-atherogenic effects of NSO and TQ in human coronary artery endothelial cells (HCAECs). The mammalian Toll-like receptors 4 (TLR4) are the germline-encoded receptors expressed by the innate immune system cells. LPS stimulations leads to interactions which resulted in TLR4 complex conformational change on cell surface. This leads to intracellular adaptor protein recruitment which is essential to activating the endothelial signalling pathway and causes the excess production of adhesion molecules; namely ICAM-1, VCAM-1, and E-selectin. This study showed that NSO and TQ inhibit the molecules that are responsible for the monocyte binding to the endothelium, which occurs at the beginning of the atherosclerotic plaque formation.

Similarly, for NSO bioactive compound (TQ), it significantly reduced both the protein and gene expression of the targeted biomarkers in a dose dependent pattern.

The present study suggests NSO and TQ reduce the activation of leukocyte trafficking in inflammation. This discovery points to the essential role of NSO and TQ in the regulation of vascular endothelial dysfunction. Atherosclerosis is a chronic inflammation which is characterised by plaque deposition on the arterial wall, leading to endothelial cell dysfunction, and leukocyte cells crossing the endothelial, resulting in effector of immune cells accumulate in arterial wall (Herrero-Fernandez et al., 2019). Activated endothelial cells are reported to play a critical role in the process of atherosclerosis. HCAECs become activated when stimulated by LPS through the secretion of several chemokines and cytokines (Zeuke et al., 2002).

This study has shown that the activation of endothelial cells increases protein expressions of ICAM-1, VCAM-1, and E-selectin, which mediated at a transcriptional level. E-selectin induces leukocytes rolling. The VCAM-1 and ICAM-1 instigate leukocyte to bind Leukocyte function Antigen (LFA1) and Very Late Antigen-4 (VLA-4) respectively, which resulted in leukocyte migrates through the endothelial cells (Jones et al., 2017). In this study, NSO and TQ reduced the gene and protein expressions of E-selectin, VCAM-1 and ICAM-1 (Fig. 5). The potential anti-atherogenesis mechanism of NSO and TQ is possibly mediated through various mechanisms such as nuclear factor-kappa beta (NF-κB), substance P-1, activating protein-1, and interferon regulatory factor-1 (Cook-Mills et al., 2011). The administration of TQ markedly increased the high-density lipoprotein-cholesterol (HDL-C) levels, while decreasing triglycerides (TG), total cholesterol (TC), and low-density lipoprotein-cholesterol (LDL-C) (Al-Naqeep et al., 2011; Nader et al., 2010). Yimer et al. (2019) also reported that possible mechanisms of NS might be mediated via the modulation of oxidative status in the upregulation of endogenous antioxidants, or the reduction of reactive oxygen species and attenuation of inflammation in anti-diabetic assessment.

These studies showed the influence of NS on atherogenesis, but only with respect to the lipid lowering and antioxidant properties of the natural product. Our study highlights the influence of NS and its bioactive compound TQ on the biomarkers of inflammation and endothelial activation, which, to our knowledge, has not been addressed. Furthermore, this study adopted HCAECs which best represent the mechanisms involved in atherogenesis within endothelial cells of coronary arteries.

One of essential components of inflammatory response observed in early stage of atherosclerosis pathogenesis is monocyte adhesion to the vascular endothelium. The foam cell formation is a result of monocytes recruitment into subendothelial arteries. The VCAM-1, ICAM-1, and E-selectin are among the adhesion molecules involved in facilitating the interaction between monocyte and endothelial cells (Xu et al., 2019). In this study, both NSO and TQ reduced the adhesion of monocytes to HCAECs. However, this was not observed to be in a dose-dependent manner. From the results, it appears that NSO shows the highest monocyte adhesion inhibition at its lowest dose 55 µg/ml, while the highest dose of TQ (36.0 µg/ml) had the best inhibition of monocyte binding. The effect of TQ can be explained by the reduction of the adhesion molecule markers, but for NSO, probably through other mechanisms, such as inflammation, oxidative stress, prothrombogenesis, or immunomodulation (Ahmad et al., 2013).

Conclusions

The present study has revealed the protective effects of NSO and TQ on endothelial cell injury by elucidating the possible mechanism underlying the anti-atherogenic effect of NSO and TQ. NSO and TQ inhibit monocytes' adherence to HCAECs via downregulation of ICAM-1, VCAM-1, and E-selectin at the transcription and translation phases of the protein. In addition, the differences in the monocyte adhesion capabilities of NSO and TQ suggest the possibility that some form of synergism may be at play, making NSO more effective at inhibiting monocyte adhesion in lower doses compared to TQ. The limitation of this study is that it was focused on only one reported signalling pathway. The study is also limited by the absence of *in vivo* research and stability or standardised extraction of the NSO. Though the present study evidenced the potential anti-atherogenesis of NSO, future studies are warranted to address the mentioned limitations before determining the potential of NSO as a supplement to improve vascular endothelial function.

Author contributions

AYFK edited and reviewed the original manuscript and finalised the final draft. FM performed the benchwork. TAR and SAM assisted in the conceptualisation, co-supervised the project, and reviewed the manuscript. GRAF supervised the benchwork. HN performed conceptualisation of the project, funding acquisition, project administration, supervision of the project, and reviewed the manuscript.

Data availability

The data from this study are available from the corresponding author upon reasonable request.

Funding

This study was funded by the Ministry of Higher Education, Malaysia, under the Fundamental Research Grant Scheme (FRGS) [Grant code: 600-RMI/ST/FRGS 5/3/Fst (49/2011)] awarded to the corresponding author, HN. The funders had no involvement in the study design; collection, analysis, and interpretation of data; the writing of the report; or the decision to submit the article for publication.

Ethical aspects and conflict of interests

The authors have no conflict of interests to declare.

Acknowledgments

This study acknowledges the Institute for Pathology, Laboratory and Forensic Medicine (I-PPerForM), the Institute for Medical Molecular Biotechnology (IMMB), and the Centre for Pathology Diagnostic and Research Laboratories (CPDRL) staff for their assistance throughout the study.

References

Abdel-Daim MM, Ghazy EW (2015). Effects of *Nigella sativa* oil and ascorbic acid against oxytetracycline-induced hepato-renal toxicity in rabbits. Iran J Basic Med Sci 18(3): 221–227.

Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, et al. (2013). A review on therapeutic potential of *Nigella sativa*: A miracle herb. Asian Pac J Trop Biomed 3(5): 337–352. DOI: 10.1016/S2221-1691(13)60075-1.

Ahmad MF, Ahmad FA, Ashraf SA, Saad HH, Wahab S, Khan MI, et al. (2021). An updated knowledge of Black seed (*Nigella sativa* Linn.): Review of phytochemical constituents and pharmacological properties. J Herb Med 25: 100404. DOI: 10.1016/j.hermed.2020.100404.

Al-Naqeep G, Al-Zubairi AS, Ismail M, Amom ZH, Esa NM (2011). Antiatherogenic potential of *Nigella sativa* seeds and oil in diet-induced hypercholesterolemia in rabbits. Evid Based Complement Alternat Med 2011: 213628. DOI: 10.1093/ecam/neq071.

Basheer I, Qureshi IZ (2018). Short and long term modulation of tissue minerals concentrations following oral administration of black cumin (*Nigella sativa* L.) seed oil to laboratory rats. Phytomedicine 39: 56–65. DOI: 10.1016/J.PHYMED.2017.12.022.

Bordoni L, Fedeli D, Nasuti C, Maggi F, Papa F, Wabitsch M, et al. (2019). Antioxidant and Anti-Inflammatory Properties of *Nigella sativa* Oil in Human Pre-Adipocytes. Antioxidants 8(2): 51. DOI: 10.3390/antiox8020051.

Cobourne-Duval MK, Taka E, Mendonca P, Bauer D, Soliman KFA (2016). The Antioxidant Effects of Thymoquinone in Activated BV-2 Murine Microglial Cells. Neurochem Res 41(12): 3227–3238. DOI: 10.1007/s11064-016-2047-1.

Cook-Mills JM, Marchese ME, Abdala-Valencia H (2011). Vascular cell adhesion molecule-1 expression and signaling during disease: Regulation by reactive oxygen species and antioxidants. Antioxid Redox Signal 15(6): 1607–1638. DOI: 10.1089/ars.2010.3522.

Edris AE (2010). Evaluation of the volatile oils from different local cultivars of *Nigella sativa* L. Grown in Egypt with emphasis on the effect of extraction method on thymoquinone. J Essent Oil-Bear Plants 13(2): 154–164. DOI: 10.1080/0972060X.2010.10643805.

Herrero-Fernandez B, Gomez-Bris R, Somovilla-Crespo B, Gonzalez-Granado JM (2019). Immunobiology of atherosclerosis: A complex net of interactions. Int J Mol Sci 20 (21): 5293. DOI: 10.3390/ijms20215293.

Heshmati J, Namazi N (2015). Effects of black seed (*Nigella sativa*) on metabolic parameters in diabetes mellitus: A systematic review. Complement Ther Med 23(2): 275–282. DOI: 10.1016/J.CTIM.2015.01.013.

Hosseini M, Mohammadpour T, Karami R, Rajaei Z, Reza-Sadeghnia H, Soukhtanloo M (2015). Effects of the hydro-alcoholic extract of *Nigella sativa* on scopolamine-induced spatial memory impairment in rats and its possible mechanism. Chin J Integr Med 21(6): 438–444. DOI: 10.1007/s11655-014-1742-5.

Hosseinzadeh H, Parvardeh S (2004). Anticonvulsant effects of thymoquinone, the major constituent of *Nigella sativa* seeds, in mice. Phytomedicine 11(1): 56–64. DOI: 10.1078/0944-7113-00376.

Jones DP, True HD, Patel J (2017). Leukocyte Trafficking in Cardiovascular Disease: Insights from Experimental Models. Mediators Inflamm 2017: 9746169. DOI: 10.1155/2017/9746169.

Kiralan M (2014). Changes in volatile compounds of black cumin (*Nigella sativa* L.) seed oil during thermal oxidation. Int J Food Prop 17(7): 1482–1489. DOI: 10.1080/10942912.2012.723231.

Leong XF, Rais Mustafa M, Jaarin K (2013). *Nigella sativa* and its protective role in oxidative stress and hypertension. Evid Based Complement Alternat Med 2013: 120732. DOI: 10.1155/2013/120732.

Lozano R, Naghavi M, Foreman F, Lim S, Shibuya K, Aboyans V, et al. (2012). Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380: 2095–2128. DOI: 10.1016/S0140-6736(12)61728-0.

Majdalawieh AF, Yousef SM, Abu-Yousef IA (2021). Thymoquinone, a major constituent in *Nigella sativa* seeds, is a potential preventative and treatment option for atherosclerosis. Eur J Pharmacol 909: 174420. DOI: 10.1016/j.ejphar.2021.174420.

Malakar AK, Choudhury D, Halder B, Paul P, Uddin A, Chakraborty S (2019). A review on coronary artery disease, its risk factors, and therapeutics. J Cell Physiol 234(10): 16812–16823. DOI: 10.1002/jcp.28350.

Mamun MA, Absar N (2018). Major nutritional compositions of black cumin seeds – cultivated in Bangladesh and the physicochemical characteristics of its oil. Int Food Res J 25(6): 2634–2639.

Marchio P, Guerra-Ojeda S, Vila JM, Aldasoro M, Victor VM, Mauricio MD (2019). Targeting early atherosclerosis: A focus on oxidative stress and inflammation. Oxidative Med Cell Longev 2019: 8563845. DOI: 10.1155/2019/8563845.

Mazaheri Y, Torbati M, Azadmard-Damirchi S, Savage GP (2019). A comprehensive review of the physicochemical, quality and nutritional properties of *Nigella sativa* oil. Food Rev Int 35:4: 342–362. DOI: 10.1080/87559129.2018.1563793.

Nader MA, El-Agamy DS, Suddek GM (2010). Protective effects of propolis and thymoquinone on development of atherosclerosis in cholesterol-fed rabbits. Arch Pharm Res 33(4): 637–643. DOI: 10.1007/s12272-010-0420-1.

Padhye S, Banerjee S, Ahmad A, Mohammad R, Sarkar FH (2008). From here to eternity-the secret of Pharaohs: Therapeutic potential of black cumin seeds and beyond. Cancer Ther 6(b): 495–510. PMID: 19018291.

Rahmani AH, Aly SM (2015). *Nigella sativa* and its active constituents thymoquinone shows pivotal role in the diseases prevention and treatment. Asian J Pharm Clin Res 8(1): 48–53.

Samir Bashandy AE (2007). Effect of fixed oil of *Nigella sativa* on male fertility in normal and hyperlipidemic rats. Int J Pharmacol 3(1): 27–33. DOI: 10.3923/ijp.2007.27.33.

Sheikh BY (2016). The role of prophetic medicine in the management of diabetes mellitus: A review of literature. J Taibah Univ Medical Sci 11(4): 339–352. DOI: 10.1016/J.JTUMED.2015.12.002.

Srinivasan K (2018). Cumin (*Cuminum cyminum*) and black cumin (*Nigella sativa*) seeds: Traditional uses, chemical constituents, and nutraceutical effects. Food Qual Saf 2(1): 1–16. DOI: 10.1093/fqsafe/fyx031.

Steven S, Frenis K, Oelze M, Kalinovic S, Kuntic M, Jimenez MTB, et al. (2019). Vascular inflammation and oxidative stress: Major triggers for cardiovascular disease. Oxidative Med Cell Longev 2019: 1–26. DOI: 10.1155/2019/7092151.

Sultan MT, Butt MS, Karim R, Ahmed W, Kaka U, Ahmad S, et al. (2015). *Nigella sativa* fixed and essential oil modulates glutathione redox enzymes in potassium bromate induced oxidative stress. BMC Complement Altern Med 15: 330. DOI: 10.1186/s12906-015-0853-7.

Sultan MT, Butt MS, Karim R, Iqbal SZ, Ahmad S, Zia-Ul-Haq M, et al. (2014). Effect of *Nigella sativa* fixed and essential oils on antioxidant status, hepatic enzymes, and immunity in streptozotocin induced diabetes mellitus. BMC Complement Altern Med 14: 193. DOI: 10.1186/1472-6882-14-193.

Tiji S, Bouhrim M, Addi M, Drouet S, Lorenzo JM, Hano C, et al. (2021). Linking the phytochemicals and the α-glucosidase and α-amylase enzyme inhibitory effects of *Nigella sativa* seed extracts. Foods 10(8): 1818. DOI: 10.3390/foods10081818.

Xu H, Jiang J, Chen W, Li W, Chen Z, Medbury H (2019). Vascular Macrophages in Atherosclerosis. J Immunol Res 2019: 4354786. DOI: 10.1155/2019/4354786.

Yimer EM, Tuem KB, Karim A, Ur-Rehman N, Anwar F (2019). *Nigella sativa* L. (Black Cumin): A Promising Natural Remedy for Wide Range of Illnesses. Evid Based Complement Alternat Med 2019: 1528635. DOI: 10.1155/2019/1528635.

Yuan H, Ma Q, Ye L, Piao G (2016). The traditional medicine and modern medicine from natural products. Molecules 21(5): 559. DOI: 10.3390/molecules21050559.

Zaoui A, Cherrah Y, Alaoui K, Mahassine N, Amarouch H, Hassar M (2002a). Effects of *Nigella sativa* fixed oil on blood homeostasis in rat. J Ethnopharmacol 79: 23–26. DOI: 10.1016/s0378-8741(01)00342-7.

Zaoui A, Cherrah Y, Mahassini N, Alaoui K, Amarouch H, Hassar M (2002b). Acute and chronic toxicity of *Nigella sativa* fixed oil. Phytomedicine 9(1): 69–74. DOI: 10.1078/0944-7113-00084.

Zeuke S, Ulmer AJ, Kusumoto S, Katus HA, Heine H (2002). TLR4-mediated inflammatory activation of human coronary artery endothelial cells by LPS. Cardiovasc Res 56(1): 126–134. DOI: 10.1016/s0008-6363(02)00512-6.

Zorov DB, Juhaszova M, Sollott SJ (2014). Mitochondrial Reactive Oxygen Species (ROS) and ROS-Induced ROS Release. Physiol Rev 94: 909–950. DOI: 10.1152/physrev.00026.2013.