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Thymoquinone Reverses Homocysteine-Induced Endothelial Dysfunction via Inhibition of Endoplasmic Reticulum-Stress Induced Oxidative Stress Pathway

(Timoquinon Membalikkan Disfungsi Endotelium Aruhan Homosistein melalui Perencatan Laluan Tekanan Oksidatif Aruhan Retikulum Endoplasma)

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ABSTRACT

Hyperhomocysteinemia causes endoplasmic reticulum (ER) stress, which elevates reactive oxygen species (ROS) and induces endothelial dysfunction, the hallmark of cardiovascular diseases. *Nigella sativa* seeds contain thymoquinone (TQ), a cardioprotective bioactive component. Nevertheless, research on investigating the effectiveness of TQ in preventing endothelial dysfunction caused by homocysteine (Hcy) is scarce. Therefore, the purpose of this work was to examine the role of TQ in restoring Hcy-induced endothelial dysfunction as well as the mechanisms behind this role. Male Sprague-Dawley (SD) rat aortas were isolated and then co-treated in an organ bath with Hcy and TQ, tauroursodeoxycholic acid (TUDCA), apocynin, or Tempol to examine vascular function. Furthermore, human umbilical vein endothelial cells (HUVECs) were treated with Hcy and TQ, Tempol, apocynin, TUDCA or H_2O_2 to determine the cell viability via a phase contrast microscope and dye exclusion test. ER stress pathway involvement, ROS and NO bioavailability were investigated using immunoassays and fluorescence staining, respectively. The binding affinity of TQ to GRP78 has been identified using molecular docking. According to our findings, Hcy hindered endothelium-dependent relaxation in an isolated aorta and caused apoptosis in HUVECs. TQ, TUDCA, Tempol, and apocynin were able to counteract these negative effects. In HUVECs, treatment with TQ decreased ROS levels, increased NO bioavailability, and decreased GRP78 and NOX4 protein. According to the molecular docking study outcomes, TQ could attach to GRP78 effectively via a hydrogen bond and a hydrophobic connection to the amino acid at GRP78 ATP binding pocket. Taken together, the findings show that TQ protected endothelial function caused by Hcy via inhibiting ER stress-mediated ROS and eNOS uncoupling.

Keywords: Endoplasmic reticulum stress; endothelial dysfunction; homocysteine; oxidative stress; thymoquinone

ABSTRAK

Hiperhomosisteinemia meninggikan tekanan retikulum endoplasma (RE) yang boleh meningkatkan spesies oksigen reaktif (SOR), yang membawa kepada disfungsi endotelium sel dan penyakit kardiovaskular. Biji *Nigella sativa* mengandungi timoquinon (TQ), komponen bioaktif berkardio-protektif. Walau bagaimanapun, tiada kajian yang menilai kesan TQ terhadap disfungsi endotelial yang disebabkan oleh homosistein (Hcy). Oleh itu, penyelidikan ini bertujuan untuk mengkaji kesan dan mekanisme TQ dalam menormalkan disfungsi endotelial yang disebabkan oleh Hcy. Aorta diasingkan daripada tikus Sprague-Dawley (SD) jantan yang diinkubasi dengan Hcy dan dirawat bersama dengan atau tanpa TQ, TUDCA, apocynin atau Tempol dalam mandian organ untuk mengkaji fungsi vaskular. Di samping itu, sel endotelial vena umbilik manusia (HUVECs) diinkubasi dengan Hcy dan TQ, Tempol, apocynin, TUDCA atau H_2O_2 untuk menilai keviabelan sel dengan menggunakan mikroskop kontras fasa dan ujian pengecualian pewarna. Penglibatan laluan tekanan ER, ROS dan NO bioketersediaan diakses masing-masing melalui immunoasai dan pewarnaan pendarfluor. Dok molekul telah dilakukan untuk menilai pertalian mengikat TQ kepada GRP78. Keputusan kami mendedahkan bahawa Hcy merosakkan disfungsi endotelial dalam aorta dan apoptosis dalam HUVECs. Kesan ini telah dinormalkan oleh TQ, TUDCA, Tempol dan apocynin. Rawatan dengan TQ mengurangkan tahap ROS, meningkatkan bioketersediaan NO serta mengurangkan protein GRP78 dan NOX4 dalam HUVECs. Hasil kajian dok molekul menunjukkan bahawa TQ boleh mengikat dengan baik kepada GRP78 melalui ikatan hidrogen dan interaksi hidrofobik dengan asid amino pada poket pengikat ATP GRP78. Kesimpulannya, TQ membaikipulih fungsi endotelial yang dirosakkan oleh Hcy melalui perencatan ROS pengantara tekanan ER dan meningkatkan bioketersediaan NO.

Kata kunci: Disfungsi endotelium; homosistein; tekanan oksidatif; tekanan retikulum endoplasma; timoquinon

INTRODUCTION

One of the main risk factors for cardiovascular disease is hyperhomocysteinemia (HHcy). Based on data from the World Health Organisation, 17.9 million deaths from cardiovascular disease were reported in 2019. These deaths accounted for 32% of all worldwide fatalities, and 85% of them were due to a heart attack or stroke (Mahadir Naidu et al. 2019). Endothelial dysfunction (ED) is the initial symptom of atherosclerosis and vascular disease. Hcy adversely affects endothelial function, according to various studies (Barroso et al. 2016; Da Silva et al. 2018; Dubey et al. 2022; Kern et al. 2022; Wu et al. 2019; Zhang et al. 2017). Hhcy could hinder endothelial-dependent dilatation via oxidative stress, which disrupts nitric oxide (NO) synthase activity uncoupling, endoplasmic reticulum stress (ER stress) and inflammation.

Increased Hcy levels trigger ER stress by interfering with the ER's normal protein folding and processing, which causes a buildup of misfolded proteins via activating the GRP78 protein (Lindholm et al. 2017). Unfolded protein response (UPR) reduces the impact of misfolded proteins by upregulating ER protein folding capacity through enhanced chaperone synthesis and reducing ER protein load through suppression of protein transcription and translation (Amen et al. 2019). This reaction is mediated by three concurrent signal transduction pathways which are inositol requiring enzyme 1 (IRE1), RNA-activated protein kinase-like ER kinase (PERK), and activating transcription factor 6 (ATF6) (Oslowski & Urano 2011). Oxidative stress and the ER stress response are subsequently elevated as a result of UPR activation. The role of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 4 (NOX4) in the production of reactive oxygen species (ROS) in ER stress-induced disease states has been reported in several investigations (Amanso, Debbas & Laurindo 2011; Loughlin & Artlett 2010; Santos et al. 2009). The availability of nicotinamide adenine dinucleotide phosphate (NADPH), a cofactor for NOX4, regulates NOX4 activity (Vermot et al. 2021). Superoxide radicals (O_2^-) are created when NADPH binds to NOX4, which causes the enzyme to change in conformation and allows the transfer of electrons from NADPH to molecule oxygen (Sies & Jones 2020). NOX4-mediated ROS production from ER stress can enable the uncoupling of endothelial nitric oxide synthase (eNOS), leading to decreased nitric oxide (NO) formation when O_2^- interacts rapidly with NO to produce peroxynitrite, another toxic radical, which subsequently results in eNOS to uncouple and discharge additional O_2^-. Furthermore, ROS can directly deactivate NO via a process called 'oxidation', which reduces NO bioavailability. When NO bioavailability is diminished, the endothelium's ability to initiate and maintain vasodilation is impaired, leading to a reduction in blood flow and an increase in blood pressure. Additionally, decreased NO bioavailability can increase oxidative stress, inflammation, and thrombosis, all of the contributing factors in the development of cardiovascular disease (Medina-Leyte et al. 2021; Panda et al. 2022; Rotariu et al. 2022) These alterations aid in the emergence of endothelial dysfunction, a fundamental component of many cardiovascular illnesses.

There is an unmet need for better clinical outcomes in the treatment of cardiovascular disease. This need can be filled by discovering a novel fundamental mechanism from natural materials that may improve the safety and