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# The Effect of Diosmin on the blood Proteome in a rat Model of Venous Thrombosis

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## Abstract

This study was designed to find the protein changes of venous thrombosis in Wistar rats with and without diosmin treatment by two-dimensional gel electrophoresis (2-DE) and investigate the effect of a crucial protein known as CEP350 on human vascular endothelial cell growth.

Diosmin was prepared from *G. verum* L. by solvent extraction and identified 98% purification by HPLC after chromatographic purification using macroporous absorption resin (AB-8) and polyamide. Wistar rats were divided into three groups respectively control group, model group and prevention group. The venous thrombosis tissue segments were cut to prepare for histopathologic examination and detection of plasma protein C (PC). Then proteomics provides the tools for next analyses. The low-abundance proteins of each animal in three groups was separated by two-dimensional gel electrophoresis (2-DE). 2-DE analysis revealed 191 protein spots were differentially expressed among three groups. We selected 6 spots to identify by MALDI-TOF-MS and received credible results. These proteins are identified with NCBI respectively. Considering the characteristic of these proteins, we proposed CEP350 as a possible target protein related with spindle assembly. Furthermore, we selected CEP350 siRNA to transfect into HUVECs with Lipofectamine 2000 and evaluated transcriptional gene silencing by RT-PCR. Moreover, the cells were stained for immunofluorescence with Tubulin-tracker Red and structural changes were analyzed by laser scanning confocal microscope.

We concluded that CEP350 depletion decreased microtubule stability. Diosmin could modulate the assemble of spindle from unevenly distributing and protect body from varicose veins by regulating CEP350.

**Key words:** diosmin; venous thrombosis; proteome; CEP350.

## Introduction

Epidemiological studies have advocated that dietary supplements rich in flavonoids. Minor dietary non-nutrients such as flavonoids, derived from flavones with various degrees of hydroxylation and glycosidic substitutions, are showing promising results as potential chemopreventive agents<sup>[1]</sup>. Recently, researchers all over the world have focused their interest on the isolation, elucidation and effectiveness of naturally occurring anti-inflammatory and anticancer agents that are usually active ingredients of the daily diet<sup>[2]</sup>. Diosmin (diosmetin 7-O-rutinoside), a naturally occurring flavone glycoside readily obtained by the dehydrogenation of hesperidin, is found abundantly in the pericarp of various citrus fruits, which has been shown to possess

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