**Glossogyne tenuifolia Enhances Posttranslational S-Nitrosylation of Proteins in Vascular Endothelial Cells**

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**ABSTRACT:** *Glossogyne tenuifolia* (GT) is a traditional Chinese herb that possesses strong antioxidant activity and protects against endothelial cell (EC) injury by inhibition of free reactive oxygen species (ROS). The aim of this study was to elucidate the mechanisms by which GT prevents endothelial injury using a proteomics approach. We used a sensitive method to analyze the S-nitrosoproteins utilizing a modified biotin-switch method in order to detect the possible effects of GT on protein posttranslational modification. After treatment of vascular ECs with GT, two proteins HspA9 (IS1), beta-actin (IS2) were observed to have increased posttranslational S-nitrosylation, whereas seven proteins, vimentin (DS2, DS3 and DS5), tropomyosin 3, 4 (DS6 and DS7) and oxidative phosphorylation protein such as ATP synthase, F1 complex (DS1) and 80K-H protein (DS4), were found to have decreased posttranslational S-nitrosylation. Due to S-nitrosylation of HspA9 causing the reduction of intracellular ROS and S-nitrosylation of ATP synthase interfering with ATP production and ROS formation, our study may indicate a novel mechanism in which GT protects EC injury by the inhibition of oxidative reaction.

**KEY WORDS:** Antioxidation, free reactive oxygen species, *Glossogyne tenuifolia*, posttranslational S-nitrosylation of proteins, vascular endothelial cells.

**INTRODUCTION**

*Glossogyne tenuifolia* (GT) is a traditional Chinese herb that is mainly available in Southern Asia, Australia, New Caledonia and southern Taiwan. The effective components of GT identified include luteolin, luteolin-7-glucoside and oleanolic acid (Hsu et al., 2005; Wu et al., 2005). Recent studies have shown that the major effects of GT are as follows: (1) it is cytotoxic to human hepatocellular carcinoma cells and inhibits hepatitis B virus replication; (2) it promotes the anti-oxidation of tumour necrosis factor-alpha (TNF-α), interleukin (IL)-6 and interferon-gamma (IFN-γ) in human whole blood and may prevent atherosclerosis by inhibiting the oxidation of low-density lipoproteins (LDLs) and the production of reactive oxygen species (ROS) in human leukocytes; and (3) it acts as an immunomodulator by inhibiting the synthesis of proinflammatory mediators in activated murine peritoneal macrophages and splenocytes via the NF-κB dependent pathway (Hsu et al., 2005; Wu et al., 2005; Yang et al., 2006; Ha et al., 2006).

The released NO mediates its effects via guanylate cyclase to increase the level of cGMP in the cardiovascular system and thereby results in vasodilatation, anti-thrombosis and anti-proliferation (Murad, 1999; Yang et al., 2006). Previous studies have shown that NO may directly react with cysteine residues so that the latter undergo S-nitrosylation – a generic protein modification that is required for the regulation of diverse protein functions and signalling mechanisms (Ravi et al., 2004). The aim of this study is to elucidate the mechanism of action of GT by using a recently modified method (Huang et al., 2009) to improve the detection sensitivity of S-nitrosoproteins and to examine the possible reactions involved in the prevention of endothelial injury.

**MATERIALS AND METHODS**

Plant material and preparation of the ethanol extract of GT

The raw materials for GT were bought from an herbal store in Penghu Island, and then later confirmed by using DNA sequence identification (Hsu et al., 2005). Dried whole plant materials of GT (5.3 kg) were crushed and soaked in 20 L ethanol for 1 day and then extracted 3 times with the same volume of ethanol.