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Effect of 5-hydroxymethylfurfural derived from processed *Cornus officinalis* on the prevention of high glucose-induced oxidative stress in human umbilical vein endothelial cells and its mechanism

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ABSTRACT

The aim of this study was to investigate the protective effect of 5-HMF on human umbilical vein endothelial cells (HUVECs) injured by high glucose *in vitro*, and the mechanism underlying this process. Our results demonstrated that high glucose-induced oxidative stress in HUVECs was mainly mediated through activation of reactive oxygen species (ROS), Jun N-kinase 2/3 (JNK2/3) and plasma interleukin-8 (IL-8), and inactivation of phosphorylated protein kinase B (P-Akt). Treatment of HUVECs with media containing high glucose (4.5%) in the presence of 5-HMF (100, 200 and 400 μM) resulted in significant inhibition of high glucose-induced oxidative stress and expression of JNK1 and JNK2/3. Furthermore, 5-HMF rapidly inhibited high glucose-induced activation of IL-8, a downstream activator of P-Akt. Diabetes mellitus can cause a wide variety of vascular complications and high glucose can induce vascular endothelial cell apoptosis. Free radicals are formed disproportionately in diabetes by glucose oxidation. The finding of this study highlights the pharmacological application of 5-HMF for preventing cardiovascular and diabetes mellitus diseases, and provides the theoretical basis for further development of a *Cornus officinalis* agent for diabetes-associated vascular diseases.

1. Introduction

Cornus officinalis is derived from the dry ripe sarcocarp of *C officinalis* Sieb. et Zucc. In traditional Chinese medicine, Crude *C officinalis* and its processed products of jiuzheng pin (JZP) are used clinically for nourishing the liver and kidney (Cao, Zhang, Cong, Cai, & Cai, 2009a,b; Zhou, Wu, Liu, & Liu, 2008). Clinically, it is one of the most popular and widely used herbal medicines in the world and can be used in medicine, food sanitation and cosmetics, due to its anti-inflammation, anti-viral and anti-oxidative effects (Liu, Sun, Wu, & Liu, 2009; Yokozawa, Park, Noh, Tanaka, & Cho, 2009). 5-HMF is an endogenous product found in plants, and in free or bound forms. It is found in large quantities in processed *C officinalis*, from which it is extracted in hot aqueous infusions. Pharmacological studies of its components have shown that 5-HMF has biological activities, such as antioxidant, anti-myocardial ischemia and improving hemorheology effects (Fu, Wang, & Cai, 2008; Kagami, Onda, Oka, & Hirano, 2008; Luo, Zhao, Yang, Shen, & Rao, 2009). Furthermore, 5-HMF is the chief bioactive ingredient in processed *C officinalis*. However, the cytoprotective effects of 5-HMF are not well understood with only a few studies existing in the literature.

Injury to the human umbilical vein endothelial cells (HUVECs) underlying the lumen of blood vessels contributes significantly to the development of atherosclerosis and hypertension. Numerous studies have shown that exposure to local reactive oxygen species (ROS) is one of the main causes leading to injury of human umbilical vein endothelial cells. High glucose is known to be one of the common ROS, which can easily penetrate the plasma membrane and affect neighbouring cells, as well as high glucose-producing cells (Cao et al., 2009a,b; Chao, Hou, Chao, Weng, & Ho, 2009; Taye, Saad, Kumar, & Morawietz, 2010).

Apoptosis is an evolutionarily conserved process that plays an important role in the development, homeostasis and disease pathology of the cardiovascular system (Ho, Chan, Hsieh, & Chen, 2009; Chai et al., 2008). Apoptosis of endothelial cells underlying the lumen of blood vessels has recently been recognized as a cellular mechanism resulting in the development of atherosclerosis (Chen, Chen, Chiu, & Chang, 2005). Several factors, including oxidized lipoprotein, high concentration of glucose, postprandial ser-

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um condition, and ROS, have been proposed to cause apoptosis and/or cytochrome release in endothelial cells (Jiang et al., 2009; Piconi et al., 2008). High glucose and its reactive by-products are potential mediators of cell death induced by diverse stimuli.

The aim of the present study was to examine the protective effect of 5-HMF from processed *C officinalis* and its possible mechanism in HUVECs injured by high glucose. Cell viability and the percentage of cell apoptosis were assessed to evaluate the protective effect of 5-HMF against cytotoxicity induced by high glucose. ROS and TUNEL assay, quantitation, using flow cytometry, propidium iodide (PI) staining, and western blotting, was used to explore these mechanisms.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM), dimethylsulphoxide (DMSO), acridine orange (AO), ethidium bromide (EB), 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT), and fetal bovine serum (FBS), were purchased from Hangzhou Biological Products Co., Ltd. (Zhejiang, China). Glucose was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 0.25% trypsin was provided by Merck Co. Inc., propidium iodide (PI) was provided by Sigma Co., IL-8, JNK1, JNK2/3 and p-Akt antibody were purchased from Bioworld Company. Human anti-β-actin and goat anti-human IgG/HRP were obtained from Nanjing KeyGen Biotech. Inc. (Nanjing, China). All other chemicals used were of commercially grade and purchased from Nanjing Ronghua Reagent Co. (Nanjing, PR China).

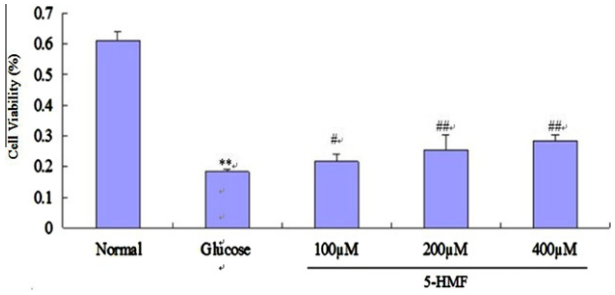

Fig. 1. Protective effects of 5-HMF on HUVECs from oxidative stress at different concentrations. Cells were pretreated with high glucose (4.5%) and followed by treatment with various concentrations of 5-HMF for 72 h. Cell viability was measured by MTT assay. The data represent group means and standard deviation from the means of three independent experiments. **Mean value was significantly different from that of the control group ($p < 0.01$). ##Mean value was significantly different from that of the high glucose treatment group ($p < 0.01$).

2.2. 5-HMF extraction

Processed *C officinalis* was collected from Henan suppliers and authenticated by an expert in the field. Ultrasonic extraction of processed *C officinalis* was carried out twice with 10 volumes of methanol for 45 min. The extract was filtered and the filtrate was concentrated under reduced pressure at 40 °C to free the solvent. The dried extract was then water-precipitated. 5-HMF was separated and concentrated by D101 macroporous resin, and eluate was extracted with dichloromethane. The quality of 5-HMF was analyzed by high performance liquid chromatography (HPLC). HPLC analysis consisted of an Agilent Zorbax Extend C_{18} column (250 × 4.6 mm, particle size 5 μm) at a column temperature of 30 °C. 5-HMF was then eluted with 2% acetonitrile and 98% aqueous phosphoric acid (0.1%, v/v) at a flow rate of 1.0 ml/min and a detection wavelength of 284 nm.

2.3. Cell culture and drug treatment

HUVECs were purchased from Nanjing KeyGen Biotech. Inc., and were cultured in DMEM, supplemented with 10% heart-inactivated fetal bovine serum (FBS), benzylpenicillin (100 ku/l), and streptomycin (100 mg/l). The cultures were maintained in a humidified atmosphere containing 5% CO_2 at 37 °C. HUVECs were passed every 3–4 days and elevated glucose concentrations (high glucose 4.5%)

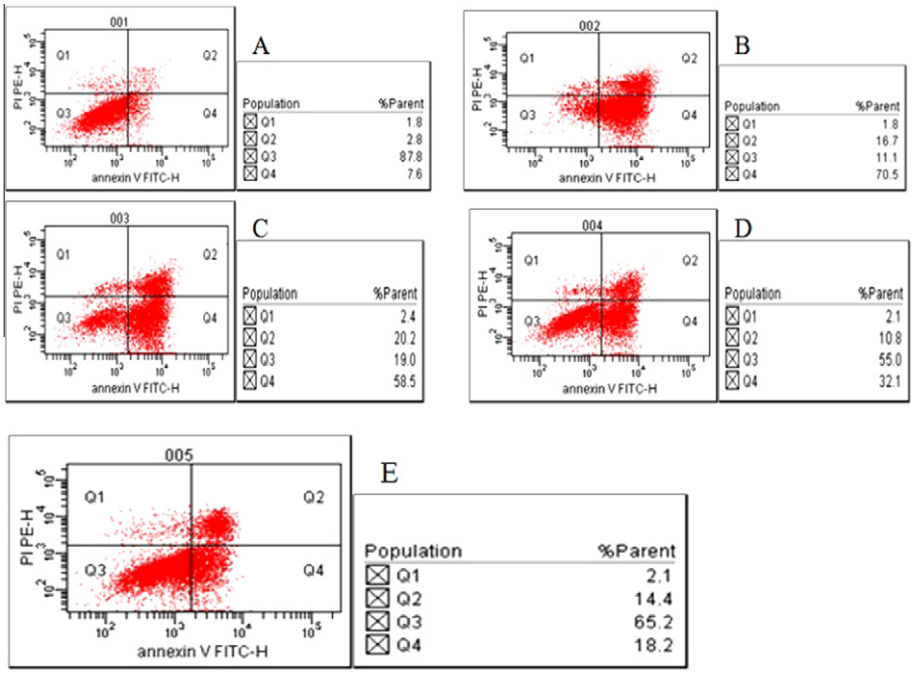

Fig. 2. Effect of 5-HMF on apoptosis in high glucose-injured HUVECs. (A) control, (B) high glucose treatment (4.5%), (C) high glucose + 5-HMF (100 μM), (D) high glucose + 5-HMF (200 μM), (E) high glucose + 5-HMF (400 μM).