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Zingerone attenuates diabetic nephropathy through inhibition of nicotinamide adenine dinucleotide phosphate oxidase 4

Yan Cui, Yan Shi, Yan Bao, Shulong Wang, Qiuju Hua, Yun Liu*

The Department of Nephrology, The First Affiliated Hospital of Xinxiang Medical University, Weihui, 453100, Henan, China

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ABSTRACT

Diabetes affects a large proportion of population wide across the world and kidney is a main target organ of diabetic complications. Zingerone is a stable active component derived from dry ginger rhizome. We investigated the effect of zingerone on diabetic nephropathy and explored the possible mechanisms. We showed that zingerone decreased the levels of serum insulin, C-peptide and glycosylated hemoglobin A1c. The levels of blood urea nitrogen (BUN), serum creatinine, urinary albumin content and albumin/creatinine ratio (ACR) were reduced by zingerone. Moreover, zingerone attenuated the pathological injuries of kidneys, reduced the surface area of Bowman's capsule, Bowman's space, glomerular tuft, and decreased the expression of collagen IV and fibronectin in kidneys in db/db mice. The high levels of triglyceride and cholesterol, and high expression of TNFα and IL-6 were decreased by zingerone. Furthermore, zingerone decreased the level of MDA and increased the content of glutathione (GSH). NADPH oxidase 4 (NOX4) expression was significantly increased in kidneys of db/db mice and in HK-2 cells after exposure to high glucose. Zingerone significantly decreased the expression of NOX4 in vivo and in vitro. Upregualtion of NOX4 significantly inhibited zingerone-induced protective effects against the cytotoxicity of high glucose. Downregulation of NOX4 was responsible for zingerone-exhibited pharmacological activities and reduction of diabetic nephropathy. Overall, zingerone is a promising therapeutic treatment to attenuate diabetic nephropathy.

1. Introduction

Diabetes affects a large proportion of population wide across the world [1,2]. The kidney has been believed to be a main target organ of complications of diabetes [3]. Approximately, 40 percent of patients suffer from diabetes may develop diabetic nephropathy, which is a major cause of end-stage renal disease [4] and can result in disability and mortality of diabetic patients [5]. To date, the molecular mechanisms underlying diabetic nephropathy are still not completely understood and the therapy is limited [6]. Diabetic nephropathy is characterized by increase of lipid profile, inflammation, oxidative stress, accumulation of extracellular matrix (ECM) proteins and an irreversible decline in renal function [7]. Therapeutic strategies are designed to target hyperglycemia, hyperlipidemia, oxidative stress, inflammatory cytokines as well as genetic disposition [8]. The most common way is to decrease blood glucose levels and lower hypertension through blockage of the renin-angiotensin system [9,10]. However, the efficiency of these approaches is not satisfied [11]. Therefore, new alternative treatments are urgently needed to control the progression of diabetic nephropathy.

The herbal plant Zingiber officinale, commonly known as ginger, is consumed worldwide as a natural dietary spice and flavoring agent [12]. Zingerone [4-(4-hydroxy-3-methoxyphenyl) butan-2-one] is a stable active component derived from dry ginger rhizome (Fig. 1A) [13]. It has been reported that zingerone exhibit various pharmacological activities such as anti-inflammatory, anti-apoptotic, antioxidant, anti-cancer, lipolytic, and radioprotective effects [14–18]. Zingerone ameliorates lipopolysaccharide-induced acute kidney injury by inhibiting Toll-like receptor 4 signaling pathway [19]. Zingerone also exhibits nephro-protective effect against CCl_4-induced renal toxicity in Swiss albino mice [20]. The findings suggest that zingerone possesses a potent nephro-protective effect. However, whether zingerone possesses biological effects on kidney function under diabetic condition is not known.

The present study aimed to assess the possible effect of zingerone on diabetic nephropathy in db/db mice and to explore the possible mechanisms. We found that zingerone could attenuate the progression of diabetic nephropathy as reflected by amelioration of renal structural and functional injuries. Zingerone exhibited anti-inflammatory, hypolipidemic, anti-oxidative activities in db/db mice. Downregualtion of NADPH oxidase 4 (NOX4) was involved in the protective effect of

* Corresponding author at: The Department of Nephrology, The First Affiliated Hospital of Xinxiang Medical University, No. 88 Jiankang Road, Weihui 453100, Henan, China.
E-mail address: yunliu3324@163.com (Y. Liu).

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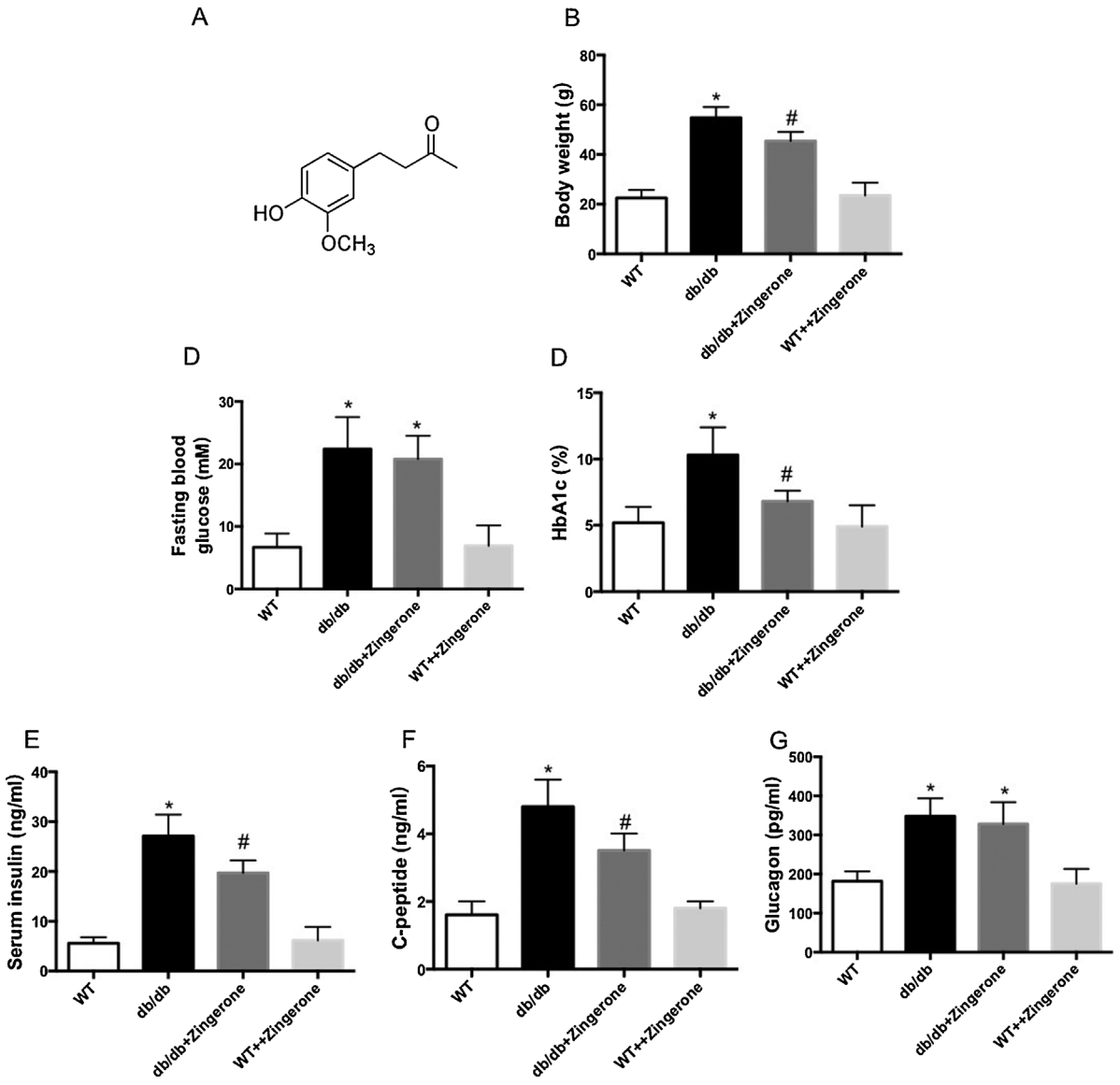

Fig. 1. Zingerone inhibited the changes of metabolic parameters in db/db mice.
(A) Structure of zingerone. The db/db mice were injected with zingerone daily for 10 weeks. Body weights (B), fasting blood glucose (C), HbA1c level (D), insulin concentration (E), C-peptide level (F), and glucagon concentration (G) were measured. $^{*}p < 0.05$, compared with that of control; $^{\#}p < 0.05$, compared with that of db/db mice.

zingerone against diabetic nephropathy.

2. Materials and methods

2.1. Reagents

Zingerone was obtained from Sigma-Aldrich Inc. (Saint Louis, MO, USA). 5-(and-6)-carboxy-2', 7'-dichlorodihydroflourescein diacetate (DCFDA) was obtained from Invitrogen. NOX4 antibody was purchased from Cell Signaling Biotechnology (CST, USA). β-actin antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Secondary antibodies were obtained from Thermofisher Scientific Biotechnology.

2.2. Animals and treatment

The animal procedures and experimental protocols have been approved by the Ethics Committee and the Institutional Animal Care and Use Committee of the First Affiliated Hospital of Xinxiang Medical University. C57BL/KsJ db/db mice (10 weeks old), which carried a point mutation in the leptin receptor and developed obesity and type 2 diabetes, and age-matched wild type (WT) nondiabetic (db/m) mice were used in the study. Previous literature has proved that mice on the C57BL/BLKS background recapitulates similar characteristic of the glomerular and tubulointerstitial injury associated with progressive diabetic nephropathy in humans [21]. 20 male WT mice and 30 male db/db obese mice were purchased from Model Animal Research Center of Nanjing University. Animals were housed under specific pathogen-free (SPF) conditions in an air-conditioned animal facility with a controlled temperature (23 ± 3 °C) and relative humidity (50 ± 20%) on a 12 h light–dark cycle. The mice were provided standard chow with free access to tap water.

The blood glucoses of db/db mice are > 11.1 mM. The db/db mice were randomly allocated into two groups, with 15 mice in each group: db/db (DMSO in saline) and db/db + Zingerone (50 mg/kg/day). Zingerone was dissolved in DMSO and diluted in saline solution. db/db + Zingerone mice were injected intraperitoneally with Zingerone solution. The WT mice were randomly allocated into two groups, with 10 mice in each group: WT (DMSO in saline) and WT + Zingerone (50 mg/kg/day). WT + Zingerone mice were injected intraperitoneally