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การปรับควบคุมการแสดงออกของ eNOS และ p47phox ในไตด้วยกาแลนจินลดการบาดเจ็บของไตในหนูเบาหวาน

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Modulation of Intrarenal eNOS and p47phox Expression by Galangin Attenuates Kidney Injury in Diabetic Rats

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บทคัดย่อ

หลักการและวัตถุประสงค์: โรคไตจากเบาหวาน (diabetic nephropathy) เกิดขึ้นจากภาวะน้ำตาลในเลือดสูงเรื้อรัง ซึ่งกระตุ้นให้เกิดการแสดงออกมากเกินไปของโปรตีน p47phox พร้อมทั้งยับยั้งการแสดงออกของเอนไซม์ endothelial nitric oxide synthase (eNOS) กลไกคู่นี้ส่งเสริมภาวะเครียดออกซิเดชัน การอักเสบ และรบกวนการทำงานของไต นำไปสู่การบาดเจ็บของไตซึ่งเป็นขั้นตอนทางพยาธิวิทยาเริ่มแรกในการพัฒนาของโรคไตจากเบาหวาน วัตถุประสงค์ของการศึกษานี้คือการตรวจสอบศักยภาพในการบรรเทาการบาดเจ็บของไตในหนูเบาหวานด้วยกาแลนจิน (galangin; GA)

วิธีการศึกษา: หนูเพศผู้สายพันธุ์ Wistar ถูกแบ่งออกเป็นสองกลุ่มหลัก ได้แก่ หนูที่ไม่ได้เป็นโรคเบาหวานและหนูที่เป็นโรคเบาหวาน กลุ่มที่ไม่ได้เป็นโรคเบาหวานประกอบด้วยกลุ่มควบคุมที่ได้รับยาหลอกและกลุ่มควบคุมที่ได้รับ GA 50 มิลลิกรัม/กิโลกรัม/วัน กลุ่มที่เป็นโรคเบาหวานประกอบด้วยกลุ่มเบาหวานที่ได้รับยาหลอก กลุ่มเบาหวานที่ได้รับ GA ในขนาด 25 หรือ 50 มิลลิกรัม/กิโลกรัม/วัน (n = 6-8 ต่อกลุ่ม) หนูถูกเหนี่ยวนำให้เป็นโรคเบาหวานชนิดที่ 2 ผ่านการฉีดนิโคตินาไมด์ (110 มิลลิกรัม/กิโลกรัม/วัน ครั้งเดียว ทางช่องท่อง) ตามด้วยสเตรปโตโซโทซิน (55 มิลลิกรัม/กิโลกรัม/วัน ครั้งเดียว ทางช่องท่อง) หลังจากการเหนี่ยวนำโรคเบาหวานเป็นเวลาแปดสัปดาห์ หนูได้รับการรักษาด้วย GA ในขนาด 25 หรือ 50 มิลลิกรัม/กิโลกรัม/วัน เป็นเวลาสี่สัปดาห์ มีการประเมินการทำงานของระบบเมแทบอลิซึมหน้าที่ของไต ระดับการแสดงออกของโปรตีน p47phox โปรตีน eNOS ตัวบ่งชี้ภาวะเครียดออกซิเดชัน และการวิเคราะห์โครงสร้างทางจุลกายวิภาคของโกลเมอรูลัส

ผลการศึกษา: GA สามารถลดระดับน้ำตาลในเลือดขณะอดอาหารและภาวะดื้อต่ออินซูลินอย่างมีนัยสำคัญ พร้อมทั้งปรับปรุงการทำงานของไตเมื่อเปรียบเทียบกับกลุ่มหนูเบาหวาน ($p < 0.05$) การผลิตซูเปอร์ออกไซด์มากเกินไปและภาวะเครียดออกซิเดชันในไตของหนูเบาหวานลดลงอย่างเด่นชัดหลังการรักษาด้วย GA ($p < 0.05$) การให้ GA โดยเฉพาะในขนาด 50 มิลลิกรัม/กิโลกรัม/วัน ยับยั้งการขยายใหญ่ของโกลเมอรูลัสและการเปลี่ยนแปลงโครงสร้างจุลภาคของไตอย่างมีนัยสำคัญ นอกจากนี้ GA ยังยับยั้งการแสดงออกของ p47phox ขณะที่กระตุ้นการแสดงออกของ eNOS ในเนื้อเยื่อไตของหนูเบาหวาน

สรุป: GA มีบทบาทในการปกป้องไตในหนูเบาหวานผ่านกลไกการลดน้ำตาลและต้านอนุมูลอิสระผ่านการยับยั้งการแสดงออกของ p47phox ฟื้นฟูการแสดงออกของ eNOS ทำให้การทำงานของไตเป็นปกติ และรักษาโครงสร้างของโกลเมอรูลัสผลการศึกษานี้สนับสนุนศักยภาพของ GA ในการป้องกันหรือชะลอโรคไตจากเบาหวานในระยะเริ่มแรก

คำสำคัญ: eNOS, p47phox, กาแลนจิน, การบาดเจ็บของไต, หนูเบาหวาน

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Abstract

Background and Objective: Diabetic nephropathy (DN) develops as a consequence of chronic hyperglycemia, which triggers the overexpression of p47phox while simultaneously suppressing endothelial nitric oxide synthase (eNOS) expression. This dual mechanism promotes oxidative stress, inflammation, and disrupts renal function, leading to kidney injury that represents the initial pathological step in DN progression. The objective of this study was to investigate the therapeutic potential of galangin (GA) in addressing kidney injury in diabetic rats.

Methods: Male Wistar rats were divided into two main groups: non-diabetic and diabetic. The non-diabetic group comprised a vehicle-treated control and a GA-treated (50 mg/kg) subgroup. The diabetic group comprised a vehicle-treated control, a GA 25 mg/kg subgroup, and a GA 50 mg/kg subgroup (n = 6-8 per group). Type 2 diabetes was induced in male Wistar rats by a single intraperitoneal injection of nicotinamide (110 mg/kg/day), followed by a single intraperitoneal injection of streptozotocin (55 mg/kg/day). After eight weeks of diabetes induction, rats received GA supplementation at doses 25 or 50 mg/kg/day for four weeks. Metabolic parameters and renal functions, kidney tissue of p47phox and eNOS proteins, oxidative stress markers, and glomerular morphology by light microscopy and ultrastructure by transmission electron microscopy were evaluated.

Results: GA supplementation significantly reduced elevated fasting blood glucose and insulin resistance while improving kidney function compared to diabetic controls ($p < 0.05$). Excessive superoxide production and oxidative stress in diabetic kidneys were markedly attenuated following GA treatment ($p < 0.05$). GA administration, particularly at 50 mg/kg/day, significantly inhibited glomerular hypertrophy and ultrastructural kidney changes. Additionally, GA suppressed renal p47phox expression while upregulating eNOS expression in diabetic kidney tissues.

Conclusions: GA provides renoprotection in diabetic rats through glucose-lowering and antioxidant mechanisms that suppress p47phox-mediated reactive oxygen species generation, restore eNOS expression, normalize renal function, and preserve glomerular architecture. These findings support GA's therapeutic potential for preventing or delaying early diabetic nephropathy.

Keywords: eNOS, p47phox, galangin, kidney injury, diabetic rats

Introduction

Diabetes mellitus (DM) is a significant global public health concern and serves as the primary instigator of diabetic kidney disease (DKD), also known as diabetic nephropathy (DN)[1]. DN significantly contributes to mortality in individuals with diabetes[2]. The early stages of DN are characterized by glomerular hyperfiltration, kidney injury, and subsequent structural changes within the glomeruli[3]. These alterations are accompanied by a gradual decline in renal function and the occurrence of albuminuria.

The pathogenesis of kidney injury is intricately associated with the extent of chronic hyperglycemia. Elevated plasma glucose levels are postulated to initiate kidney injury through multifaceted mechanisms[4]. A growing body of evidence underscores the critical role of hyperglycemia in eliciting the excessive generation of reactive oxygen species (ROS), which plays a central role in renal injury. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidases are identified as the primary sources of ROS production within the kidney[5]. Furthermore, heightened ROS levels can directly impede the activity of nitric oxide (•NO) and induce dysfunction of endothelial nitric oxide synthase (eNOS), leading to •NO depletion (eNOS uncoupling). Intrarenal •NO governs a multitude of physiological processes that exert influence over renal function[6,7].

Previous investigations have consistently indicated an overall reduction in •NO production in the context of chronic kidney disease (CKD)[8]. Additionally, in an experimental model of acute renal failure, diminished glomerular •NO production significantly contributes to impaired renal function[9].

DN also gives rise to structural modifications in the kidney, including thickening of the glomerular basement membrane, fusion of foot processes, and loss of podocytes, ultimately leading to albuminuria and a decreased glomerular filtration rate (GFR)[10]. Effectively managing intrarenal metabolic and hemodynamic disturbances is crucial for impeding the progression of DN. Consequently, mitigating hyperglycemia and addressing oxidative stress in diabetic individuals represent pivotal strategies for averting DN. Galangin (GA) is natural flavonoid compounds of the flavanol subclass (3,5,7-trihydroxy flavone; C15H10O5) isolated from the rhizomes of *Alpinia officinarum*, has shown potential in reducing hyperglycemia and modulating glucose metabolism by inhibiting α-amylase activity, enhancing glucose uptake, and activating transcription factors[11-13]. Nevertheless, research on GA's effects on glycemic profiles, renal function, and oxidative stress under diabetic conditions remains relatively limited.

In this study, our objective was to comprehensively investigate the impact of GA on the regulation of blood glucose levels, antioxidant activity, and protection of renal function in diabetic rats.

Methods

Chemicals

GA was acquired from Aktin Chemicals, Inc. (Mianyang City, Sichuan, China). All other chemicals and solvents used in the study were of analytical grade and were procured from reputable suppliers.

Animals and diabetic induction

Male Wistar rats (6–8 weeks old, 180–200 g) were obtained from the Northeast Laboratory Animal Center, Khon Kaen University, Thailand, and housed in individually ventilated cages within an Heating, Ventilation, and Air Conditioning (HVAC)-controlled environment (22 ± 2 °C, 50 ± 10% relative humidity, 12 h light/12 h dark) with ad libitum access to standard chow and water. All procedures were approved by the Institutional Animal Ethics Committee of Khon Kaen University (AEKKU 35/65)

Following one week of acclimatization, forty rats were randomized into five treatment groups (n=8 each). Control groups received standard water with either vehicle or GA 50 mg/kg daily during the final four weeks. Diabetic groups received 25% fructose water for twelve weeks to induce metabolic dysfunction, combined with either vehicle, GA (25 or 50 mg/kg), or

metformin (Met; 200 mg/kg) during the last four weeks. All treatments were administered via oral gavage.

Diabetes was chemically induced using sequential intraperitoneal injections of nicotinamide (NA; 110 mg/kg/day) followed by streptozotocin (STZ; 55 mg/kg/day) according to established protocols[12]. Hyperglycemia was confirmed to be 48 hours post-injection with fasting glucose levels of more than 200 mg/dL. Treatment doses were selected based on previously validated renoprotective and antidiabetic effects in rodent studies.

Measurement of metabolic parameters

Rats were fasted overnight (approximately 8 hours) before metabolic parameter assessment. Blood was collected from the tail vein after gentle cleaning of the tail. Fasting blood glucose was measured immediately using a glucometer and glucose strip (Accu-Chek, Roche Diabetes Care, Switzerland).

Following fasting, an intraperitoneal glucose tolerance test (IPGTT) was performed. Rats received an intraperitoneal injection of glucose at 2 g/kg body weight. Blood glucose levels were monitored from the tail vein at baseline (0 minutes) and at 15-, 30-, 60-, and 120-minutes post-injection over a 2-hour period, and the area under the curve (AUC) was calculated.

Fasting plasma insulin concentrations were determined using a commercial ELISA kit (INVITROGEN, Carlsbad, CA, USA) as per the manufacturer's instructions. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was calculated as: HOMA-IR = [fasting glucose (mg/dL) × fasting insulin (μIU/mL)] / 405[14]. Lipid profiles, including total cholesterol, triglycerides, and HDL cholesterol, were analyzed using enzymatic colorimetric methods at Srinagarind Hospital, Khon Kaen University, Thailand.

Assessment of kidney function

The rate of urine excretion, urine creatinine, and albumin were regularly assessed throughout the experiment. After a 12-hour fast, rats were individually housed in metabolic cages for urine collection.

Creatinine clearance (Ccr) was measured to estimate glomerular filtration rate (GFR). Urine volume and urinary and plasma creatinine concentrations were determined. Ccr was calculated using the formula: Ccr=Ux×VP[15]; where Ux is urinary creatinine concentration, V is urine flow rate (mL/min), and P is plasma creatinine concentration. Blood and urinary creatinine, and urine albumin were analyzed by the laboratory of Associated Medical Sciences, Khon Kaen University, Thailand.

Assay of systemic oxidative stress markers

Superoxide anion ($O_2^{\bullet-}$) production in kidney tissue was measured using lucigenin-enhanced chemiluminescence, following a modified established method[14]. Lipid peroxidation was assessed by measuring malondialdehyde (MDA) levels using thiobarbituric acid (TBA), as previously described[14]. Protein oxidation, indicated by carbonyl group generation, was evaluated by quantifying carbonyl levels using established procedures[14]. Redox status in whole blood was assessed by measuring glutathione (GSH), a major antioxidant compound, using previously described methods[14].

Measurement of renal oxidative stress by western blot analysis for protein expression

The protein expressions of eNOS and the NADPH oxidase subunit p47phox were determined in renal homogenates using a previously described method with minor modifications[15]. The intensities of the specific eNOS, p47phox, and β-actin bands were visualized and captured using ImageQuant (GE Healthcare, USA). Band densities were analyzed using the ImageJ software (National Institutes of Health, USA). The intensity of each target protein band was normalized to the corresponding β-actin band from the same sample.

Histological examination of the glomerulus

Paraffin-embedded kidney tissues were stained with hematoxylin and eosin (H&E) to examine glomerular histopathology. The H&E-stained sections were visualized at 400× magnification using a microscope equipped with a charge-coupled device camera (Pulnix, Sunnyvale, CA, USA) to determine glomerular volume and area. Renal fibrosis was further investigated through immunohistochemistry (IHC) to assess the expression of fibronectin and type IV collagen (COL IV). The IHC staining was performed as previously described by Sangartit et al[16]. Briefly, deparaffinized sections were incubated with primary antibodies against fibronectin and COL IV, followed by detection with appropriate secondary antibodies and a chromogenic substrate. Images of the stained sections were captured, and the staining intensity was quantitatively analyzed using ImageJ software.

Ultrastructural examination of glomerular changes

The renal cortex was partially excised and immediately immersed in a buffer solution for transmission electron microscopy (TEM) analysis. Sample preparation was performed by the Electron Microscopy Unit, Department of Anatomy, Faculty of Medicine, Khon Kaen University, Thailand.

Glomerular basement membrane (GBM) thickness, podocyte foot process width, and slit pore density were captured and measured using a transmission electron microscope at 15,000× magnification. This imaging was conducted at the Research Instruments Center, Faculty of Science, Khon Kaen University, Thailand.

Statistical analysis

Results were expressed as mean ± Standard Error of the Mean (SEM). Differences among groups were compared using one-way analysis of variance (ANOVA) followed by a post-hoc Tukey test. All analyses were performed using SigmaStat software version 3.1. The value of $p < 0.05$ was considered statistically significant.

Results

GA attenuated metabolic changes of DM rats.

The successful induction of type 2 diabetes in rats through the administration of NA and STZ, coupled with a 25% fructose solution, was confirmed by the presence of hyperglycemia, insulin resistance, and impaired insulin secretion in comparison to normal control rats (Table 1, $p < 0.05$). These findings indicate that the STZ-NA treatment led to partial destruction of pancreatic β-cells, resulting in impaired insulin secretion. Additionally, diabetic rats displayed typical type 2 diabetes characteristics, including reduced body weight, increased urine excretion, and dyslipidemia ($p < 0.05$ compared to normal controls; Table 1). After 8 weeks of diabetic induction, there was a significant increase in urinary albumin excretion and a notable decrease in creatinine clearance, suggesting alterations in renal function due to diabetes.

Treatment with GA, particularly at a dosage of 50 mg/kg/day for four weeks, effectively prevented the decrease in body weight in diabetic rats ($p < 0.05$ compared to diabetic rats). Furthermore, GA ameliorated impaired glucose regulation, insulin resistance, and dyslipidemia in diabetic rats ($p < 0.05$ compared to diabetic rats; refer to Table 1). This study also revealed the beneficial impact of GA on kidney injury in diabetic rats. GA supplementation significantly reduced urinary albumin excretion and improved glomerular filtration, as indicated by increased creatinine clearance ($p < 0.05$ compared to diabetic rats; Table 1).

Although the metabolic parameters suggest a positive effect of GA mitigating diabetes and kidney injury, the precise mechanism underlying GA's action remains to be elucidated.

Table 1 Effect of GA on metabolic parameters in all experimental groups at the end of experiment.

Variables	Control		DM		
	Veh	GA50	Veh	GA25	GA50
Body weight (g)	481.46 ± 1.65	489.96 ± 1.62	462.70 ± 1.35*	452.41 ±1.27*	480.23 ±1.54*,†
FBG (mg/dL)	93.74 ± 1.96	94.18 ± 2.31	300.93 ± 1.30*	234.32 ± 1.28*,†	211.82 ± 1.15*,†,#
AUC (mg/dL/120 min)	18,756.30 ± 54.76	18,459.80 ± 32.56	26,844.20 ± 34.68*	23,450.10 ± 39*,†	20,454.50 ± 66.32*,†,#
HOMA-IR	4.51 ± 0.01	4.38 ± 0.05	10.68 ± 0.22*	9.48 ± 0.04*,†	7.32 ± 0.41*,†,#
Insulin (µU/mL)	15.37± 0.59	15.70 ± 0.13	13.21 ± 0.38*	13.92 ± 0.41*	14.25 ± 0.52†,#
Cholesterol (mg/dL)	25.67 ± 2.40	25.37 ± 1.25	52.28 ± 0.57*	45.56 ± 0.46*,†	34.00 ± 0.56*,†,#
Triglyceride (mg/dL)	139.67 ± 2.02	138.01 ± 0.32	525 ± 14.46*	319 ± 5.31*,†	212.83 ± 6.29*,†,#
HDL cholesterol (mg/dL)	19.33 ± 0.88	21.10 ± 1.13	8.67 ± 0.33*	12.00 ± 0.53*,†	16.78 ± 0.33*,†,#
Kidney's weight (g)	2.47 ± 0.24	2.51 ± 0.18	3.51 ± 0.41*	3.56 ± 0.62*,†	3.22 ± 0.17*,†
Urine (mL/day)	25.02 ± 2.47	26.11 ± 1.32	110.78 ± 3.81*	112.78 ± 4.01*	87.33 ± 2.77*,†
Creatinine clearance (mL/min)	1.02 ± 0.08	1.04 ± 0.12	0.66 ± 0.03*	0.65 ± 0.03*	0.99 ± 0.07*,†
Urinary albumin (g/L)	1.24 ± 0.46	1.37 ± 0.23	5.41 ± 0.33*	5.28 ± 0.54*	4.79 ± 0.11*,†

Rats received STZ-NA-induced DM with 25% W/V fructose solution and GA 25 or 50 mg/kg/day. Data are expressed as mean ± SEM, n=6-8/group. *p<0.05 vs. control; †p<0.05 vs. DM+Veh; #p<0.05 vs. DM+GA25. Abbreviations: Vehicle (Veh), Gallic acid 25 mg/kg/day (GA25), Gallic acid 50 mg/kg/day (GA50), Fasting Blood Glucose (FBG), Area Under the Curve (AUC), Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) and High-Density Lipoprotein (HDL)

GA mitigated systemic and intrarenal oxidative stress of DM rats.

Increased oxidative stress was clearly observed in STZ-NA-induced diabetic rats. This was marked by an elevation in plasma MDA and plasma protein carbonyl levels, along with a reduction in GSH levels and the GSH/GSSG ratio ($p<0.05$ compared with control; Figure 1 A-D). Furthermore, diabetic rats exhibited heightened renal oxidative stress, as indicated by elevated kidney malondialdehyde levels and an increased rate of renal superoxide production ($p<0.05$ compared with control; Figure 1 E-F). These findings suggest that under diabetic conditions, oxidative stress is exacerbated and may contribute to the development of diabetic kidney injury.

Treatment with GA, especially at a dosage of 50 mg/kg/day, effectively ameliorated these changes ($p<0.05$ compared with DM+Veh; Figure 1 A-E), underscoring GA's antioxidant properties and its potential to protect against kidney oxidative stress and damage.

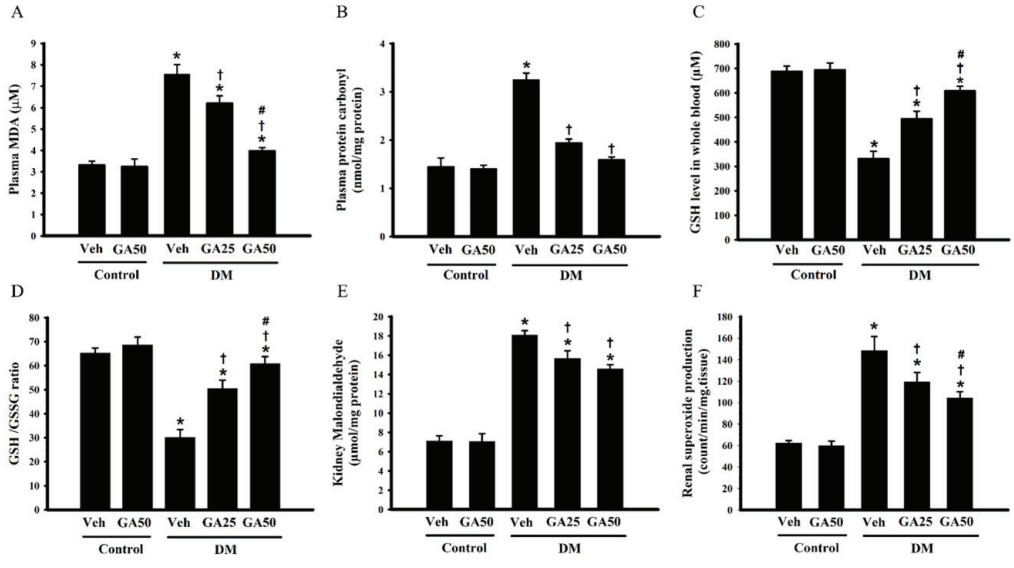

Figure 1 Effect of GA supplementation on intrarenal oxidative stress markers and redox status of DM rats. Plasma MDA (A), plasma protein carbonyl (B), reduced GHS levels (C), redox status of GSH/GSSG (D), kidney MDA levels (E), and renal $O_2^{\bullet-}$ production rate (F). The data is presented as the mean ± SEM. $^{*}p < 0.05$ vs. control; $^{\dagger}p < 0.05$ vs. DM+Veh; $^{\#}p < 0.05$ vs. DM+GA25. Abbreviations: Vehicle (Veh), Gallic acid 25 mg/kg/day (GA25), Gallic acid 50 mg/kg/day (GA50), Malondialdehyde (MDA), Reduced Glutathione (GSH) and Reduced Glutathione (GSSG).

GA modulated the expression of eNOS and p47phox of DM rats.

The expression of key proteins, including eNOS and p47phox, was evaluated in kidney tissue homogenates. p47phox plays a pivotal role in kidney homeostasis, and its overexpression can lead to renal oxidative stress, inflammation, and fibrosis. Conversely, eNOS regulates renal vascular tone and autoregulation, and its reduced expression may contribute to decreased renal perfusion. In this study, diabetic kidneys exhibited significantly increased levels of p47phox, accompanied by decreased eNOS expression compared to the control group ($p < 0.05$; Figure 2A-B).

Following intragastric administration, GA (50 mg/kg/day) effectively restored eNOS and p47phox expressions to levels close to normal when compared to the untreated diabetic group ($p < 0.05$), as illustrated in Figure 2 A-B. These findings suggest that GA exerts a renoprotective effect through the modulation of eNOS and p47phox expression.

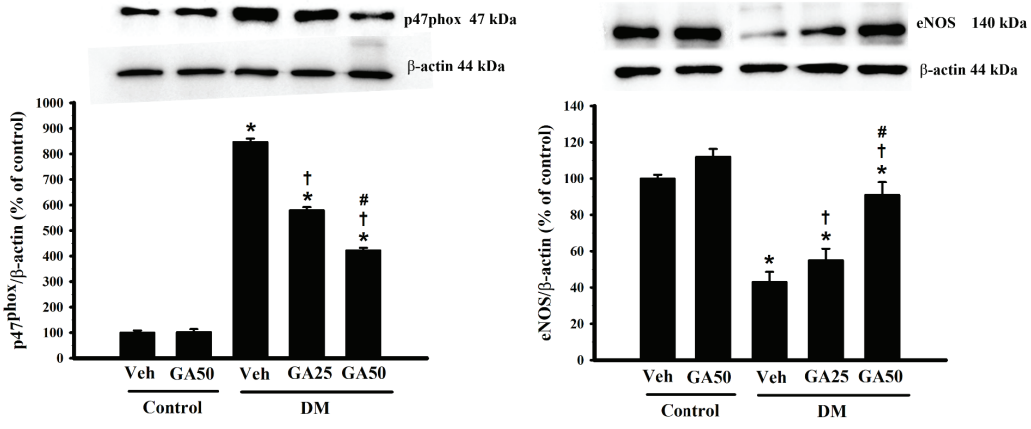

Figure 2 Effect of GA on the protein expression of intrarenal eNOS and p47phox of DM rats. p47phox protein expression (A) and eNOS protein expression (B). β-actin serves as the housekeeping protein for quantifying the expression of the target protein. The data is presented as the mean ± SEM. *$p < 0.05$ vs. control; $^{\dagger}p < 0.05$ vs. DM+Veh; $^{\#}p < 0.05$ vs. DM+GA25. Abbreviations: Vehicle (Veh), Gallic acid 25 mg/kg/day (GA25), Gallic acid 50 mg/kg/day (GA50).

GA attenuated changes of histopathology and ultrastructure of diabetic glomeruli

H&E staining of kidney tissue sections was performed to evaluate glomerular histomorphology (Figure 3A). In diabetic rats, glomeruli displayed expansion, and a mark increased in glomerular volume. However, the administration of GA at a dosage of 50 mg/kg/day reversed these glomerular alterations, indicating that GA effectively prevented glomerular hypertrophy. This protective effect could be attributed to the mitigation of mesangial expansion or extracellular matrix accumulation.

The increase in GBM thickness is a characteristic feature of DN. DM rats displayed elevated GBM thickness (Figure 3D). However, treatment with GA reversed alterations in GBM thickness (Figure 3D). These findings indicate that GA treatment at a dosage of 50 mg/kg/day effectively prevented glomerular hypertrophy and mesangial expansion triggered by diabetic conditions.

GA suppressed the expression of renal fibrosis of DM rats.

The glomeruli of diabetic rats displayed a substantial increase in the accumulation of collagen type IV and fibronectin. Nevertheless, following a 4-week treatment with GA in diabetic rats, there was a noteworthy reduction in the expression of fibronectin and collagen IV, as evidenced by IHC staining (Figure 3B-C). These results strongly indicate that GA, especially at a dose of 50 mg/kg/day, proficiently hinders the accumulation of extracellular matrix (ECM), thereby providing protection against renal fibrosis.

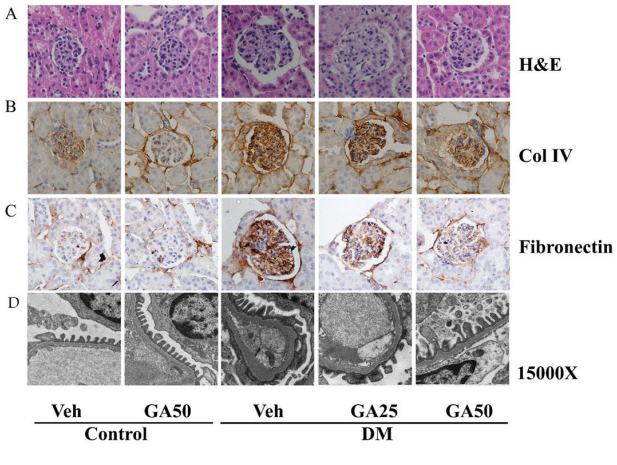

Figure 3 Effect GA on glomerular structure (A), expression of renal collagen type IV (B), fibronectin (C), and glomerular basement membrane thickness (D) of DM rats. Abbreviations: Vehicle (Veh), Gallic acid 25 mg/kg/day (GA25), Gallic acid 50 mg/kg/day (GA50).

Discussion

The present study demonstrated that induction of diabetes using STZ-NA combined with 25% w/v fructose mimicked type 2 diabetes and resulted in hyperglycemia, increased oxidative stress, overexpression of p47phox, downregulation of eNOS, and kidney injury, as evidenced by glomerular structural and functional abnormalities in diabetic rats. These abnormalities were attenuated after administration of GA, especially at 50 mg/kg/day for 4 weeks.

STZ causes partial β-cell damage, leading to hypoinsulinemia and hyperglycemia, which in turn increases ROS and impairs insulin signaling (e.g., PI3K/Akt), promoting insulin resistance[17]. In this study, GA alleviated insulin resistance, as indicated by improved IPGTT responses and decreased HOMA-IR. Mechanistically, GA's antihyperglycemic action may involve improved insulin sensitivity via reduced oxidative stress and potential modulation of AMPK/PI3K-Akt signaling[18,19], together with direct ROS scavenging of superoxide and peroxyl radicals[18].

Kidney damage in diabetic rats is closely linked to hyperglycemia-driven oxidative and nitrosative stress and altered renal hemodynamics[20]. Hyperglycemia activates multiple pathogenic pathways: (1) mitochondrial overproduction of superoxide; (2) Advanced glycation end products (AGEs) formation with activation of receptor for advanced glycation end products (RAGEs); (3) increased diacylglycerol-protein kinase c (PKC) signaling; (4) flux through the polyol and hexosamine pathways; and (5) activation of the renin-angiotensin-aldosterone system (RAAS)[21]. These processes converge to activate NADPH oxidases (NOX1/NOX2) via p47phox phosphorylation and translocation, stimulate proinflammatory and profibrotic signaling (e.g., NF-κB and TGF-β/Smad)[22,23], and promote glomerular hypertrophy, podocyte injury, and ECM accumulation[24]. In the present study, diabetic kidneys showed increased glomerular area and volume, thickening of the glomerular basement membrane, and foot-process effacement. GA significantly attenuated podocyte fusion and improved these pathological changes. Consistent with reduced fibrosis, immunohistochemistry demonstrated increased fibronectin and type IV collagen (COL IV) deposition in diabetic glomeruli.

To corroborate oxidative stress, we measured lipid peroxidation (MDA), protein carbonyls, and antioxidant status (GSH/GSSG). The results indicate a hallmark oxidative stress phenotype in diabetic rats, plausibly derived from hyperglycemia via mitochondrial ROS overproduction, AGE–RAGE signaling, PKC activation, and NOX-driven superoxide generation[25]. GA significantly reduced MDA and protein carbonyls and improved the GSH/GSSG ratio, supporting a pharmacologic antioxidant effect. Potential mechanisms include direct radical scavenging, metal chelation that limits Fenton chemistry, suppression of NOX activity via downregulation of p47phox expression/phosphorylation, and activation of endogenous antioxidant defenses (e.g., Nrf2–HO-1/SOD/GPx pathways reported in prior studies)[18].

To gain mechanistic insight, we assessed eNOS and p47phox protein expression. Hyperglycemia elevates ROS, which activate NOX1/NOX2; high glucose and AGEs also increase p47phox phosphorylation and assembly of the NOX complex[26, 27]. Excess superoxide reacts with •NO to form peroxynitrite, oxidizing tetrahydrobiopterin (BH4) and causing eNOS uncoupling further amplifying superoxide production and diminishing •NO bioavailability[28,29]. In agreement, diabetic rats showed increased amount of p47phox and reduced eNOS expression. GA reversed these changes, suggesting that it interrupts the NOX–ROS–eNOS uncoupling cycle by suppressing p47phox and restoring eNOS expression and function.

Reduced eNOS-derived •NO lowers renal •NO bioavailability, impairing endothelium-dependent vasodilation and disrupting intrarenal microvascular tone. This facilitates RAAS-mediated vasoconstriction, increases intraglomerular pressure, and promotes albuminuria, mesangial expansion, and ECM synthesis via TGF-β1 signaling. NO deficiency also favors leukocyte adhesion and endothelial inflammation[21],

exacerbating podocyte stress and basement membrane remodeling. By restoring eNOS and •NO bioavailability and lowering ROS, GA likely rebalances glomerular hemodynamics and limits downstream inflammatory and fibrotic cascades.

Collectively, these findings support a dual mechanism by which GA protects the diabetic kidney: (1) improvement in glycemic control that reduces glucose-driven ROS production; and (2) direct antioxidative and NOX-inhibitory actions that suppress p47phox-dependent superoxide formation, recouple eNOS, and blunt profibrotic signaling, culminating in reduced fibronectin and COL IV deposition and improved glomerular structure.

Although supplementation with GA demonstrated significant attenuation of diabetic kidney injury, a key limitation of this study is the four-week treatment duration employed for both GA and metformin administration. Given that diabetic kidney disease represents a chronic and progressive pathological condition, this relatively brief intervention period may be insufficient to determine whether GA provides sustained long-term protection or whether the observed therapeutic improvements represent transient effects that may not persist over extended treatment periods.

Conclusion

In conclusion, GA treatment in diabetic rats attenuates STZ-NA plus fructose–induced kidney injury by suppressing systemic and intrarenal oxidative stress, downregulating p47phox, and restoring eNOS expression, thereby improving •NO bioavailability and glomerular homeostasis. GA also reduces glomerular ECM accumulation (fibronectin and COL IV) and mitigates podocyte injury. The protective actions of GA likely arise from both antihyperglycemic effects and direct antioxidant/NOX-modulatory mechanisms. These results position GA as a potential adjuvant therapy to prevent or slow diabetic kidney disease, warranting further mechanistic and translational studies.

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