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กรดเอเซียติกบรรเทาการบาดเจ็บของไตจากเบาหวานผ่านการยับยั้งภาวะเครียดออกซิเดชันที่ไต

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Asiatic Acid Alleviates Diabetic Kidney Injury Through Suppression of Renal Oxidative Stress

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บทคัดย่อ

หลักการและวัตถุประสงค์: โรคเบาหวานเป็นปัจจัยเสี่ยงที่สำคัญต่อการบาดเจ็บของไต ภาวะกลูโคสในเลือดสูงเรื้อรังส่งผลให้การทำงานของไตลดลง และนำไปสู่การเปลี่ยนแปลงโครงสร้างของไตผ่านภาวะเครียดออกซิเดชัน การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของ กรดเอเซียติก (asiatic acid, AA) ซึ่งเป็นสารต้านอนุมูลอิสระ ต่อการบรรเทาการบาดเจ็บของไตและภาวะเครียดออกซิเดชันในหนูที่เป็นเบาหวาน

วิธีการศึกษา: หนูวิสตาร์เพศผู้อายุ 8 สัปดาห์ซึ่งมีน้ำหนักระหว่าง 180 ถึง 200 กรัม ถูกเหนี่ยวนำให้เป็นโรคเบาหวานประเภท 2 โดยการฉีดสเตรปโตโซโตซิน (STZ; 55 มก./กก.) และนิโคตินาไมด์ (NA; 110 มก./กก.) เข้าสู่ช่องท้องเพียงครั้งเดียว AA ที่ขนาด 10 หรือ 20 มก./กก./วัน ถูกป้อนเข้าสู่กระเพาะอาหารเป็นเวลา 8 สัปดาห์ ระหว่างนี้การเปลี่ยนแปลงทางเมแทบอลิซึมในหนูถูกติดตาม เมื่อสิ้นสุดการทดลอง ทำการเก็บเนื้อเยื่อไตและเลือดจากหนูทุกตัวเพื่อทำการทดลองต่อไป

ผลการศึกษา: หนูที่เป็นเบาหวานมีระดับกลูโคสในเลือดสูง การหลั่งอินซูลินบกพร่อง มีภาวะดื้อต่ออินซูลิน อัตราการผลิตซูเปอร์ออกไซด์ไอออนในไตเพิ่มขึ้น นอกจากนี้ หนูที่เป็นโรคเบาหวานยังพบการหนาตัวขึ้นของโกลเมอรูลัสและการเปลี่ยนแปลงโครงสร้างของไต การให้ AA โดยเฉพาะในขนาด 20 มก./กก./วัน ในหนูเบาหวาน พบว่าสามารถลดภาวะดื้อต่ออินซูลิน และลดระดับมาลอนไดอัลดีไฮด์ที่ไตอย่างมีนัยสำคัญทางสถิติ นอกจากนี้ AA ยังบรรเทาการหนาตัวของโกลเมอรูลัส (glomerular hypertrophy) และการหนาตัวของเนื้อเยื่อกรองที่โกลเมอรูลัส (glomerular basement membrane)

สรุป: AA บรรเทาการบาดเจ็บของไตในหนูที่เป็นเบาหวานนั้นเกี่ยวข้องกับฤทธิ์ในการลดระดับกลูโคสในเลือดและฤทธิ์ต้านอนุมูลอิสระ ซึ่งมีบทบาทสำคัญต่อการลดความเสียหายของไต โดยรวมแล้ว การค้นพบเหล่านี้ชี้ให้เห็นถึงผลประโยชน์ของ AA ในฐานะสารต้านอนุมูลอิสระที่มีศักยภาพ ซึ่งสามารถช่วยป้องกันการดำเนินของภาวะแทรกซ้อนในไตจากเบาหวาน

คำสำคัญ: กรดเอเซียติก, การบาดเจ็บของไตจากเบาหวาน, ภาวะเครียดออกซิเดชันที่ไต

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Abstract

Background and Objective: Diabetes mellitus is a well-established risk factor for kidney injury. Chronic hyperglycemia in diabetes can potentially impair kidney function and structural changes through promotion of oxidative stress. The present study aimed to investigate the effect of asiatic acid (AA), an antioxidant on kidney injury and oxidative stress in diabetic rats.

Methods: Eight-week-old male Wistar rats weighing between 180 to 200 grams were induced to have type 2 diabetes by a single intraperitoneal injection of streptozotocin (STZ; 55 mg/kg) and nicotinamide (NA; 110 mg/kg). AA at doses of 10 or 20 mg/kg/day was administered intragastrically for 8 weeks. Metabolic changes in these rats were monitored during the experimental period. At the end of the experiment, kidney tissues and blood were collected from all rats for further experiments.

Results: Diabetic rats exhibited hyperglycemia, impaired insulin secretion, insulin insensitivity, an increased rate of kidney superoxide anion production. Moreover, diabetic rats also exhibited glomerular hypertrophy and glomerular ultrastructural changes. Treatment with AA, especially at a dose of 20 mg/kg/day, significantly attenuated insulin resistance, and kidney malondialdehyde (MDA) levels. AA also exerted a beneficial effect on renal protection by reducing glomerular hypertrophy and thickening of glomerular basement membranes.

Conclusions: AA alleviates kidney injury in diabetic rats through its glucose-lowering effect and antioxidant activity. These findings suggest the beneficial effects of AA as a potent antioxidant that could help prevent the progression of diabetic kidney complications.

Keywords: asiatic acid, diabetic kidney injury, renal oxidative stress

Introduction

Diabetes mellitus (DM) is a major global public health concern, particularly due to its association with diabetic kidney disease (DKD), a leading cause of death among diabetic patients[1]. Early stages of DKD involve kidney injury, followed by structural changes and declining renal function. The development of DKD is intricately linked to chronic hyperglycemia, potentially inducing kidney injury through multiple pathways, notably the excessive overproduction of reactive oxygen species (ROS) leading to oxidative stress.

To prevent the development progression of DKD, it is crucial to regulate intrarenal metabolic and hemodynamic disturbances[2]. Therefore, the attenuation of hyperglycemia and oxidative stress in diabetic patients is one of the strategies to prevent DKD. Asiatic acid (AA) is an important phenolic acid, a flavonoids bioactive compound enriched in *Centella asiatica* (L.). It has been reported to reduce hyperglycemia in type 1 diabetic rats by increasing the AKT and Bcl-xL expression in the pancreatic islets[3] and also suppress islet fibrosis in Goto-Kakizaki rat, a spontaneous type 2 diabetic animal model[4]. However, the study of the effect of AA on glycemic profiles, kidney function, and oxidative stress under diabetic conditions is still limited.

In this study, we aimed to investigate the effect of AA on kidney injury and oxidative stress in diabetic rats. Experimental diabetes was induced by a single intraperitoneal injection of nicotinamide (NA) at a dose of 110 mg/kg/day, along with streptozotocin (STZ) at a dose of 55 mg/kg/day, and supplementation of their diet with a 25% w/v fructose solution for a duration of 8 weeks. AA was administered concurrently with the STZ-NA injection and continued for 8 weeks.

Methods

Drugs and chemicals

AA (98% purity by HPLC analysis) was purchased from Leap Labchem Co., Ltd. (Hangzhou, China).

Animals

Male Wistar rats, eight weeks old and weigh 180 to 200 grams were acquired from Khon Kaen University's Northeast Laboratory Animal Center, Thailand. At the Northeast Laboratory Animal Center, all animals were housed in an HVAC (Heating, Ventilation, and Air-Conditioning) system with a 12-hour dark/light cycle. All animals had free access to standard water for drinking and normal chow diet (Chareon Pokapan Co. Ltd., Thailand). The Khon Kaen University Institutional Animal Ethics Committee (AEKKU 96/62) examined and approved the experimental protocols.

After acclimatization, rats were divided into 5 groups with 8 animals per each group: nondiabetic groups and diabetic groups as follows Group I: rats + vehicle (2 mL/kg/day; intragastical (i.g.) administration) for 8 weeks, Group II: rats + AA (20 mg/kg/day; i.g.) for 8 weeks, Group III: DM + vehicle (2 mL//day BW; i.g.) for 8 weeks, Group IV: DM + AA (10 mg/kg /day; i.g.) for 8 weeks, Group V: DM + AA (20 mg/kg/day; i.g.) for 8 weeks.

Induction of animal model of type 2 diabetes

Diabetes was induced in overnight fasted rats. Rats were intraperitoneally injected with NA (110 mg/kg/day) followed by peritoneal injection of STZ (55 mg/kg/day). Only rats with fasting plasma glucose higher than 200 mg/dL at 48 hours after STZ-NA injection were used in the following experiments.

Metabolic parameters measurements

Blood glucose levels were determined by a glucometer with a strip (Accu-Chek, Roche). The plasma insulin level was evaluated by using ELISA assay kits (Millipore Corporation, Billerica, MA, USA). A homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as follows. HOMA-IR= fasting blood glucose (mg/dL) × insulin (μIU/mL)/405.

Fasting blood glucose levels and intraperitoneal glucose tolerance test

Following the overnight fasting for at least 8 hours, fasting blood glucose was detected by a glucometer with a strip (Accu-Chek, Roche). After that, a glucose load at dose 2 g/kg (25% glucose solution) was intraperitoneally injected into the rats, and blood glucose levels were measured by 15, 30, 60, and 120 minutes after glucose loading for determining glucose tolerance.

Assessment of oxidative stress markers

The levels of renal malondialdehyde (MDA) were measured. MDA levels are often measured as an index of lipid peroxidation. It can react with thiobarbituric acid (TBA) in boiling water temperature to form a colored complex called thiobarbituric acid-reaction substance (TBARS), a colored complex, which can be detected by spectrophotometric assay.

Lucigenin-enhanced chemiluminescence determined superoxide anion production in kidney tissues. Excised renal tissues were swiftly cleaned, incubated in oxygenated Krebs-Ringer bicarbonate solution at 37°C for 30 minutes, and their chemiluminescence signal was measured and averaged over 5 minutes at 15-second intervals. Subsequently, the kidney tissue was dried for 24 hours at 45 °C and weighed.

Histomorphometry analysis of renal morphology

The kidneys were embedded in paraffin block and were cut in 5 μm thickness for each section. The sections were stained with Hematoxylin and Eosin (H&E) (Bio Optica Milano SpA., Milano, Italy) to investigate the general appearance and glomerular volume in the kidney.

Analysis of renal ultrastructural morphology

The renal cortex was partially excised and immersed in a buffer solution for transmission electron microscopy (TEM). The immersed samples were prepared by electron microscopy unit, Department of Anatomy, Faculty of Medicine, Khon Kaen University. The ultrastructure was visualized by transmission electron microscope at Center of Laboratory Instruments, Khon Kaen University. The glomerular basement membrane (GBM) thickness and foot podocyte width were investigated at 15,000X magnification and were analyzed by the ImageJ program (NIH).

Statistical analysis

Results were expressed as mean ± SEM. The differences among various groups were compared by using one-way analysis of variance (ANOVA) followed by a post-hoc Turkey test. A value of $p < 0.05$ was considered statistically significant.

Results

Effect of AA on metabolic parameters in diabetic rats

The induction of type 2 diabetes in rats by the injection of STZ and NA, supplemented with a 25% fructose solution, was successful, as evidenced by significantly elevated plasma glucose ($p<0.05$; 300.93 ± 1.30 vs. 93.74 ± 1.96 mg/dL), insulin resistance ($p<0.05$; HOMA-IR 10.68 ± 0.22 vs. 4.51 ± 0.01), and impaired insulin secretion ($p<0.05$; insulin 13.92 ± 0.51 vs. 15.37 ± 0.59 μU/mL) (Table 1). These results suggest that the administration of STZ-NA partially destroyed pancreatic β-cells, consequently impairing insulin secretion.

Treatment with AA, especially at a dose of 20 mg/kg/day for eight weeks, significantly alleviates impaired glucose regulation, insulin resistance, and dyslipidemia in diabetic rats ($p< 0.05$ vs. diabetic rats (Table1.)

Effect of AA on histomorphologic changes of diabetic glomeruli rats

Kidney tissue sections were stained with hematoxylin and eosin (H&E) to assess glomerular histomorphology (Figure 1A and 1B). Glomeruli in diabetic rats exhibited significant expansion (0.76 ± 0.05 vs. 0.42 ± 0.03 $\mu m^2 x10^4$ (normal controls), $p<0.05$, Figure 1A) and increased glomerular volume (0.43 ± 0.06 vs. 0.21 ± 0.02 $\mu m^2 x10^6$ (normal controls); $p<0.05$, Figure 1B). Administration of AA at a dosage of 20 mg/kg/day reversed these glomerular alterations.

Effect of AA on ultrastructural changes of diabetic glomeruli rats

Diabetic glomeruli showed a marked increase in GBM thickness when compared with non-diabetic glomeruli ($p < 0.05$; 44.39 ± 2.14 and 31.21 ± 1.59 μm, respectively). Furthermore, the width of podocyte foot processes was significantly increased in diabetic glomeruli 60.37 ± 2.45 vs. 40.03 ± 1.77 μm of normal controls, $p<0.05$), suggesting that podocyte detachment occurred in diabetic kidneys. Treatment with AA, particularly at a dosage of 20 mg/kg/day, mitigated these alterations (Figure 2A and B).

Effect of AA on renal oxidative stress in diabetic rats

The MDA levels were elevated in the diabetic rats compared to the normal controls (p<0.05, 12.27 ± 1.34 and 5.86 ± 0.54 μmol/mg protein, respectively; Figure 3A). After diabetic rats were treated with AA (20 mg/kg/day), there was a significant reduction in MDA levels compared to the untreated diabetic group (p < 0.05; 7.92 ±1.34 and 12.27 ± 1.34 μmol/mg protein, respectively, as shown in Figure 3A. Moreover, the rate of superoxide anion production in kidney tissues was assessed. Diabetic kidneys showed a significant increase in superoxide anion production compared to control rats (Figure 3B). Treatment with AA significantly suppressed superoxide production, particularly when diabetic rats were treated with AA at 20 mg/kg/day.

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

Variable	Control		DM		
	Vehicle	AA20	Vehicle	AA10	AA20
Body weight (g)	481.46 ± 1.65	489.96 ± 1.62	462.70 ± 1.35*	461.21 ±1.47*	472.23 ±1.54*,†
FBG (mg/dL)	93.74 ± 1.96	94.18 ± 2.31	300.93 ± 1.30*	267.32 ± 1.58*,†	221.82 ± 1.25*,†,#
AUC (mg/dL/120 min)	18756.30 ± 54.76	18459.80 ± 32.56	26844.20 ± 34.68*	22630.10 ± 38*,†	20454.50 ± 66.12*,†,#
HOMA-IR	4.51 ± 0.01	4.38 ± 0.05	10.68 ± 0.22*	9.48 ± 0.08*,†	7.32 ± 0.41*,†,#
Insulin (μU/mL)	15.37± 0.59	15.70 ± 0.13	13.21 ± 0.38*	13.92 ± 0.51*	14.97 ± 0.62†,#

Rats received STZ-NA-induced DM with 25% W/V fructose solution and AA 10 or 20 mg/kg/day. Data are expressed as mean ± SEM, n=6-8/group. The data are expressed as the means ± SEM. *p<0.05 vs. control, †p<0.05 vs. DM+Veh, #p<0.05 vs. DM+AA10

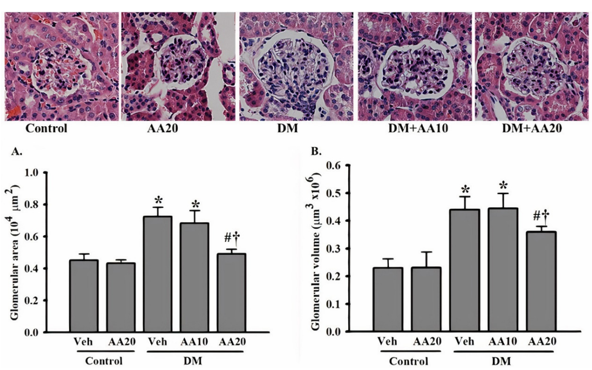

Figure 1 Effect of AA on H&E stained glomeruli in diabetic rats: quantification of glomerular area (A.) and glomerular volume (B.). The data is presented as the means ± SEM. *p < 0.05 vs. control, †p < 0.05 vs. DM+Veh, #p < 0.05 vs. DM+AA10.

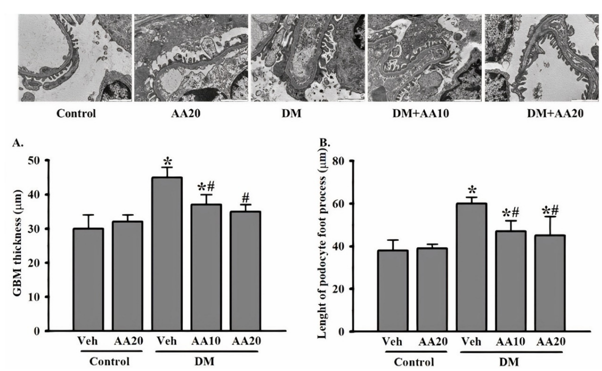

Figure 2 Effect of AA on transmission electron microscope (TEM) for length of glomerular basement membrane (A) and length of foot processes of podocyte (B). The data are expressed as the means ± SEM. *p < 0.05 vs. control, †p < 0.05 vs. DM+Veh, #p < 0.05 vs. DM+AA10.

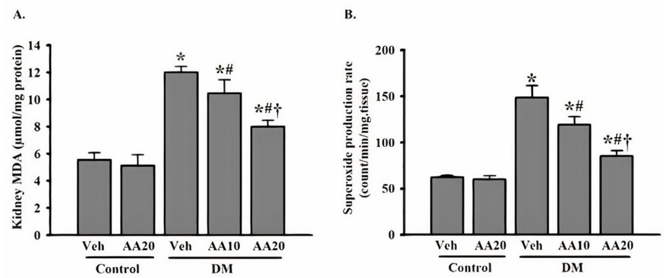

Figure 3 Effect of AA on kidney MDA level (A) and superoxide anion production in kidney tissue (B). The data are expressed as the means ± SEM. $^{*}p < 0.05$ vs. control, $^{\dagger}p < 0.05$ vs. DM+Veh, $^{\#}p < 0.05$ vs. DM+AA10.

Discussion

In this study, we demonstrated that diabetes mellitus induced by STZ/NA lead to increased glucose levels, elevated oxidative stress, and kidney damage in rats. However, these abnormalities were mitigated by an 8-week treatment with AA (20 mg/kg/day). STZ induces damage to pancreatic β-cells, resulting in insulin deficiency. STZ is transported into β-cells by GLUT-2 and causes β-cell toxicity[5]. Our findings confirmed decreased insulin levels in diabetic rats, signifying the successful induction of diabetes and subsequent hyperglycemia. This hyperglycemia potentially activates ROS, leading to the inhibition of Akt/PKB, a pivotal molecule in the insulin signaling cascade, commonly observed in hyperglycemia-induced insulin resistance[6, 7].

Furthermore, our study revealed that AA particularly 20 mg/kg/day attenuated insulin resistance in diabetic rats. This result aligns with a previous study demonstrating the protective effects of AA in a spontaneous type 2 diabetic mouse model[3, 8, 9]. The potential mechanism behind AA's reduction of insulin resistance may be attributed to its antioxidant properties, specifically its ability to scavenge superoxide anions[10].

Kidney injury in diabetic rats has been extensively documented in various studies[11, 12], often linked to heightened production of ROS and disruptions in renal hemodynamics[13]. Elevated ROS levels can potentially trigger processes such as fibrosis, apoptosis, and inflammation. These factors collectively contribute to structural changes in the kidneys, ultimately leading to a decline in renal function[14]. In a diabetic model, hyperglycemia can trigger renal hemodynamic alterations, activate the renin-angiotensin-aldosterone system (RAAS), induce ischemia and inflammation, and promote oxidative stress[15]. These processes involve mediators or enzymes such as ROS, angiotensin II (Ang II), and Advanced glycation end products (AGEs), which collectively contribute to glomerular hypertrophy, podocyte injury, and the accumulation of extracellular matrix (ECM)[14]. The kidney damage observed in diabetic rats in this study was substantiated by kidney morphology, which revealed increases in glomerular area, glomerular volume, length of the glomerular basement membrane, and the length of podocyte foot processes. These findings are consistent with previous study reported that glomerular hypertrophy, mesangial cell proliferation, and ECM accumulation were found in the renal tissues of diabetic rats[16, 17]. Additionally, they observed podocyte foot process effacement in diabetic rats. After being treated with AA significantly attenuated the foot process of podocytes fusion and alleviated abnormal pathological findings in kidney tissues. The mechanism may be associated with antioxidant property and blood glucose lowering effect of AA.

The potential mechanisms through which AA may reduce high blood glucose involve several aspects. A crucial element is the neutralization of free radicals, highly reactive molecules that can trigger oxidative stress. Oxidative stress has been linked to the development of insulin resistance[6]. Our study demonstrated that AA treatment potentially suppressed superoxide production rate in the kidneys and reduced renal lipid peroxidation. By mitigating oxidative stress, AA holds the potential to improve insulin sensitivity. Moreover, AA could influence various cellular signaling pathways associated with insulin sensitivity. Some studies suggest that antioxidants like AA may modulate the activity of protein kinases and phosphatases, crucial players in insulin signaling[18].

As mentioned, oxidative stress also plays a dominant role in the pathogenesis of kidney injury, the primary cause of structural changes and declined kidney functions. AA, by protecting against oxidative stress, could exert beneficial effects on the alleviation of kidney injury.

Conclusions

The information suggests that AA's ability to alleviate kidney oxidative stress and structural changes in the glomeruli of diabetic rats is linked to its capacity to lower glucose levels and exhibit antioxidant properties. These combined mechanisms are crucial in diminishing kidney damage.

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