Biomedicine & Pharmacotherapy 106 (2018) 381–389

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Ginger alleviates hyperglycemia-induced oxidative stress, inflammation and apoptosis and protects rats against diabetic nephropathy

Amir M. Al Hroob[a], Mohammad H. Abukhalil[b], Reham D. Alghonmeen[c], Ayman M. Mahmoud[c,*]

[a] Department of Medical Analysis, Princess Aisha Bint Al-Hussein Faculty of Nursing and Health Sciences, Al-Hussein Bin Talal University, Jordan
[b] Zoology Department, Faculty of Science, Mansoura University, Egypt
[c] Physiology Division, Zoology Department, Faculty of Science, Beni-Suef University, Egypt

ARTICLE INFO

Keywords:
Hyperglycemia
Diabetic nephropathy
Ginger
Oxidative stress
Cytokines
Apoptosis

ABSTRACT

Oxidative stress plays a major role in the development and progression of diabetic nephropathy (DN). In this study, the potential protective effect of ginger (*Zingiber officinale*) rhizome extract on hyperglycemia-induced oxidative stress, inflammation and apoptosis was investigated. An experimental diabetic rat model was induced by intraperitoneal injection of streptozotocin. Diabetic rats were treated orally with 400 or 800 mg/kg/day *Z. officinale* extract for six weeks. Diabetic animals exhibited elevated blood glucose levels and glycated hemoglobin (HbA1c) with altered lipid profile. Blood urea nitrogen, serum creatinine and urea, and urine albumin levels were significantly increased in diabetic rats. Treatment with *Z. officinale* ameliorated hyperglycemia, hyperlipidemia and kidney function. In addition, *Z. officinale* minimized the histological alterations in the kidney of diabetic rats. Chronic hyperglycemia resulted in a significant increase in malondialdehyde, protein carbonyl, pro-inflammatory cytokines, cytochrome *c* and caspase-3 in the kidney of rats. *Z. officinale* extract significantly attenuated oxidative stress, inflammation and apoptosis, and enhanced antioxidant defenses in the diabetic kidney. In conclusion, this study strongly suggests that *Z. officinale* rhizome extract exerts a protective role against diabetes-induced renal injury by ameliorating oxidative stress, inflammation and apoptosis.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder associated with long-term damage and failure of various organs [1–3]. Diabetic nephropathy (DN) is one of the major microvascular complications induced by long-standing hyperglycemia through many mechanisms [4]. Oxidative stress provoked by chronic hyperglycemia is a key component in the development of diabetic kidney disease. It results in metabolic and cellular disturbances, including lipid peroxidation, protein oxidation and DNA damage, eventually culminating in cell death [5]. The pathogenesis of DN is likely to be a result of interactions between metabolic and hemodynamic abnormalities. Hyperglycemia-mediated excessive generation of reactive oxygen species (ROS) is the common denominator linking disrupted renal hemodynamics with the metabolic pathways. ROS can activate several cellular responses that play a key role in the pathogenesis of hyperglycemia-induced renal injury [6].

Excessive ROS generation under hyperglycemic conditions stimulates the production of growth factors, cytokines and transcription factors implicated in DN. Therefore, oxidative stress can lead to chronic inflammation, tubulointerstitial fibrosis and renal hypertrophy [7]. Accordingly, several studies have demonstrated the crucial role of pro-inflammatory cytokines in the development and progression of DN [8,9]. Oxidative stress and inflammation work in concert to activate the mitochondria-dependent apoptotic pathway that participates in the pathogenesis of DN [10]. Thus, counteracting oxidative stress might help preventing hyperglycemia-induced kidney injury.

Medicinal plants have been traditionally used for the treatment of DM and multiple studies have demonstrated their beneficial effects on DN in human and experimental animals [11–14]. Since oxidative stress is involved in the development of DM and its complications, medicinal plants with potent antioxidant effects have attracted a great attention for the prevention of these complications, including DN. Ginger (*Zingiber officinale*) is widely used all over the world in medical practices for many purposes [15]. It contains many bioactive constituents, including zingerone, 6-shogaol and 6-gingerol, that have been shown to have a variety of medicinal benefits [16,17]. *Z. officinale* possesses antioxidant, anti-diabetic, anti-inflammatory, anti-cancer and anti-viral efficacies [18–21]. It has been shown that *Z. officinale* alleviated renal injury induced by carbon tetrachloride (CCl_4) [22] and lead [23] in rats

* Corresponding author at: Physiology Division, Zoology Department, Faculty of Science, Beni-Suef University, Salah Salim St., 62514, Beni-Suef, Egypt.
E-mail address: ayman.mahmoud@science.bsu.edu.eg (A.M. Mahmoud).

https://doi.org/10.1016/j.biopha.2018.06.148
Received 11 June 2018; Received in revised form 25 June 2018; Accepted 27 June 2018

through decreasing oxidative stress and inflammatory response. In addition, *Z. officinale* is well-tolerated without side effects in rats [24]. The protective mechanism of *Z. officinale* on DN is not fully understood. Therefore, the present study was conducted to explore the ameliorative effect of *Z. officinale* against diabetes-induced renal injury in rats and to identify the possible underlying mechanism of action.

2. Materials and methods

2.1. Plant preparation and extraction

Fresh ginger rhizomes were purchased from a local market in Amman (Jordan) and were cut into pieces, dried in shade and powdered. The dried rhizome powder was extracted using 80% ethanol in a suitable container at room temperature for 72 h. The extract was filtered through filter paper and evaporated to dryness under reduced pressure in a rotatory vacuum evaporator at temperature not exceeding 45 °C. The extracts were dissolved in saline for oral administration [25].

2.2. Experimental animals

Male albino Wistar rats, weighing 230–250 g, obtained from the animal house of Jordan University (Amman, Jordan) were included in this study. All animals were acclimatized for one week before experimentation. The rats were housed at normal atmospheric temperature (23 ± 2 °C) and relative humidity of 50–60% on a 12 h light/dark cycle. All the animals received a standard diet and water ad libitum. The study protocol was performed in accordance with the National Institutes of Health (NIH) guidelines and was approved by the local animal care review committee.

2.3. Induction of experimental diabetes

Type 1 diabetes was induced in overnight fasted rats by a single intraperitoneal (i.p.) injection of 50 mg/kg body weight freshly prepared streptozotocin (STZ; Sigma, St Louis, MO, USA) dissolved in 0.1 M citrate buffer (pH 4.5) [26]. One week later, blood glucose levels were assayed using a blood glucose monitor provided by Roche Diagnostics (Mannheim, Germany). Animals that had fasting blood glucose levels more than 250 mg/dl were included in further experiments.

2.4. Experimental protocol

Six normal and 24 diabetic rats were divided into five groups, each comprising 6 rats, as following:

Group I (Control): Normal rats received a single i.p. injection of 0.1 M citrate buffer (pH 4.5) and normal saline daily by oral gavage for six weeks.

Group II (Diabetic): Diabetic rats received normal saline daily by oral gavage for six weeks.

Group III (Diabetic + 400 mg/kg *Z. officinale*): Diabetic rats received 400 mg/kg body weight *Z. officinale* extract [19] by oral gavage daily for six weeks.

Group IV (Diabetic + 800 mg/kg *Z. officinale*): Diabetic rats received 800 mg/kg body weight *Z. officinale* extract [19] by oral gavage daily for six weeks.

Group V (Diabetic + Gliclazide): Diabetic rats received 5 mg/kg gliclazide (Diamicron®, Servier Industries, France) daily by oral gavage for six weeks [26].

2.5. Samples collection and preparation

One day before the end of the study, 24 h urine was collected and pooled to assay albumin levels. At the end of the study, overnight fasted rats were anaesthetized, and the blood was collected by cardiac puncture. The blood was left to coagulate, and serum was prepared for biochemical assays. A small amount of blood was collected in EDTA tubes to assay glycated hemoglobin (HbA1c). The rats were then sacrificed, and the kidneys were immediately excised, cleaned and rinsed thoroughly with ice-cold saline. Kidney homogenate (10% w/v) was prepared in 10 mM ice-cold Tris–HCl buffer (pH 7.4), and the homogenate was then centrifuged at 12,000 rpm for 10 min. The clear supernatant was collected and stored at −80 °C. Other kidney samples were collected on 10% neutral buffered formalin for histological processing.

2.6. Biochemical assays

2.6.1. Determination of serum glucose and blood HbA1c

Serum glucose levels were determined according to the method of Trinder [27] using a colorimetric assay kits purchased from Spinreact (Girona, Spain) in accordance with manufacturer's instructions. Blood HbA1c% was assessed using a commercially available kit purchased from Biosystems (Barcelona, Spain) in accordance with manufacturer's instructions.

2.6.2. Determination of kidney function markers

Serum creatinine and urea were assayed using kits purchased from Diamond Diagnostics (Holliston, MA, USA), following the methods of Larsen [28] and Coulombe and Favreau [29] respectively. Blood urea nitrogen (BUN) was calculated according to the following formula: BUN (mg/dl) = urea (mg/dl)/2.14. Urine albumin was determined using a commercially available kit supplied by Biosystems (Barcelona, Spain) in accordance with manufacturer's protocol.

2.6.3. Determination of serum lipids

Serum total cholesterol [30], HDL-cholesterol [31] and triglycerides [32] were assayed using reagent kits purchased from Spinreact (Girona, Spain). Low-density lipoprotein (LDL)-cholesterol level was calculated using the formula:

$$\text{LDL.}cholesterol = Total\ cholesterol - \left(\left[\frac{\text{Triglycerides}}{5} \right] + \text{HDL. } cholesterol \right)$$

2.6.4. Determination of malondialdehyde (MDA), protein carbonyl and antioxidants

The lipid peroxidation end product MDA was assayed in the kidney homogenate according to the method of Ohkawa et al [33]. Protein carbonyl content was estimated as protein hydrazone derivatives using 2,4-dinitrophenyle hydrazine as previously described [34]. Reduced glutathione (GSH) content was estimated according to the method of Sedlak and Lindsay [35]. The activity of superoxide dismutase (SOD) and catalase (CAT) was estimated according to the methods of Nishikimi et al [36] and Aebi [37], respectively. The protein content in kidney homogenate was determined as previously described [38].

2.6.5. Determination of pro-inflammatory cytokines, cytochrome c and caspase-3

Tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β and IL-6 levels in the kidney homogenate were estimated by enzyme-linked immunosorbent assay (ELISA) kits provided by ALPCO (Salem, NH, USA), following the manufacturer's recommendations. Cytochrome *c* and caspase-3 were estimated in the kidney homogenate using ELISA kits provided by CUSABIO (Baltimore, MD, USA) following the manufacturer's instructions.

2.7. Histological study

Immediately after sacrifice, samples from the kidneys of control and