Biomedicine & Pharmacotherapy 101 (2018) 447–457

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Centella asiatica ameliorates diabetes-induced stress in rat tissues *via* influences on antioxidants and inflammatory cytokines

Bubuya Masola[a], Oluwafemi O. Oguntibeju[b], Ayodeji B. Oyenihi[a,*]

[a] Discipline of Biochemistry, School of Life Sciences, University of KwaZulu-Natal, Westville Campus, Private Bag X54001, University Road, Durban, 4000, South Africa
[b] Department of Biomedical Sciences, Phytomedicine and Diabetes Research Group, Oxidative Stress Research Centre, Cape Peninsula University of Technology, P.O. Box 1906, Bellville 7535, South Africa

ARTICLE INFO

Keywords:
Apiaceae
Diabetes
Kidney
Brain
Cytokines
Oxidative stress

ABSTRACT

Centella asiatica (L.) Urban (Family: Apiaceae) is a perennial herb that has been used to elevate mood, improve memory, treat wounds and manage kidney-related ailments in African traditional medicine practice. This study evaluated the potential benefits of *C. asiatica* (CA) on diabetes-induced stress in kidney and brain of rats. Following the induction of *diabetes mellitus* (DM), rats were orally treated with vehicle, CA or Metformin daily for 14 days. After treatment, renal and brain levels of inflammatory cytokines, TNF-α, IFN-γ, IL-4, and IL-10 were assessed. Oxidant and antioxidant biomarkers were also evaluated. Phyto-compounds in the crude methanol extract of CA were analyzed by Gas Chromatography–Mass Spectroscopy. Diabetes increased malondialdehyde (MDA) concentration by 39%; elevated levels of TNF-α (44%) and IFN-γ (20%); and reduced the antioxidant status in the kidney in comparison to normal control rats. In the brain, diabetic control rats had significantly greater levels of MDA, TNF-α, and IFN-γ (182%, 40%, and 20%, respectively) in addition to the lowered antioxidant status when compared to normal control rats. However, treatment with CA significantly reduced the renal levels of MDA (33%), TNF-α (78%), and IFN-γ (42%) while that of IL-10 increased by 18% when compared to diabetic control rats. In the brain, CA treatment elicited significant reductions in MDA (37%), TNF-α (30%), and IFN-γ (37%) levels while those of IL-4 and IL-10 increased by 94% and 20% respectively. In addition, the renal and brain antioxidant status was significantly boosted by CA treatment. Several medicinal compounds including ascorbic acid, asiatic acid, oleanolic acid, stevioside, stigmasterol, and α-humulene were identified in the crude extract of CA. Findings from this study suggest CA may protect diabetic tissues from stress *via* antioxidant and anti-inflammatory mechanisms that can be useful in the management of diabetic complications.

1. Introduction

Globally, diabetic nephropathy and neuropathy are significant causes of diabetes-related end-stage renal failure and non-traumatic amputations respectively [1,2]. Therapeutic agents that have the capacity to ameliorate or prevent the deleterious consequences resulting from the prolonged oxidative stress and inflammatory processes in *diabetes mellitus* (DM) may be highly beneficial to diabetic patients [3]. Chronic hyperglycemia, a hallmark of DM, results in increased generation of reactive oxygen species (ROS) through various molecular pathways leading to a decline in the activities of enzymatic antioxidants as well as levels of non-enzymatic cellular antioxidants [4]. The resulting oxidative stress coupled with the over-activation of pro-inflammatory processes within tissues are major recurring themes proposed to be responsible for the progression of DM and associated life-limiting complications [4]. In DM, the elevated ROS results in damage to membrane lipids, proteins, and DNA that terminate in tissue damage with consequent development of diabetic complications such as nephropathy and neuropathy [5].

Diabetic nephropathy and neuropathy have been considered inflammatory diseases since accumulated basic and clinical data have suggested the important roles played by inflammatory processes such as recruitment of immune cells, expression of pro-inflammatory chemokines, cytokines and cell adhesion molecules in the development of these complications [1,6]. Elevated production of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and interferon (IFN)-γ together with the reduced production of anti-inflammatory cytokines like IL-4 and IL-10, not only enhance systemic stress in diabetics but also worsen the harmful effects of diabetic complications [7]. Moreover, inflammation and oxidative stress have been termed essential partners since the prolongation of inflammatory processes can lead to an increase in the generation of ROS and vice versa [8]. Therefore, the

* Corresponding author. Present address: Department of Physiological Sciences, Faculty of Sciences, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa.
E-mail address: aoyenihi@sun.ac.za (A.B. Oyenihi).

https://doi.org/10.1016/j.biopha.2018.02.115
Received 4 October 2017; Received in revised form 5 February 2018; Accepted 23 February 2018

regulation of excess ROS production may inhibit tissue damage that is initiated by inflammatory processes.

The lack of an ideal cure for DM and reduced effectiveness of current oral medications on disease progression is a cause for concern. While these medications are crucial in the management of DM, the decline in their efficacy in regulating glucose homeostasis over time as well as associated side effects have limited their use [9]. Therefore, the discovery of new interventions that overcome these limitations to achieve durable glycemic control is warranted. In this regard, plant extracts and plant-derived compounds with antioxidant properties may be used as complimentary or alternative treatments to slow and/or prevent the inherent complications of DM [10]. *Centella asiatica* (L.) Urban (CA), also known as "Gotu kola" in India, "Pohekula" in Hawaii, "Indian pennywort" in USA or "Icudwane" in South Africa is a plant with many medicinal properties including those of wound healing and improvement of cognition [11]. It is commonly used in South African traditional medicine to treat different ailments such as wounds and sores, fever, leprosy, syphilis, gastric complaints, *etc*. [12]. It has been suggested that the neuroprotective action of CA in animal models of diseases is due to its antioxidant effects [13]. CA has also demonstrated several pharmacological actions such as anti-hyperglycemic effects in obese diabetic rats [14]; anti-inflammatory and analgesic effects based on chemically-induced animal behavior tests [15]; and restoration of the activities of liver and kidney enzymes involved in glucose and amino acid oxidation following their alteration by diabetes [16]. Recently, it was shown that CA has the beneficial modulation of liver antioxidant status and cytokine activity in type II diabetic rats [17]. There is still limited knowledge of the effects of CA in DM and associated complications. Based on this background, the aim of the present study is to evaluate the potential effects of the crude extract of CA leaves in diabetes-induced oxidative and inflammatory stress in kidney and brain of rats.

2. Materials and methods

2.1. Chemicals

Chemicals and biochemicals of analytical grade were used in this study. They were purchased from Sigma-Aldrich (St. Louis, MO) through Capital Labs, New Germany, South Africa or Merck (Darmstadt, Germany) through Merck, Halfway House, South Africa, except Isofor which was purchased from Safeline Pharmaceuticals (Johannesburg, South Africa) and the Bio-plex Pro-magnetic bead-based Luminex kit which was sourced from BioRad Laboratories (Hercules, CA, USA through BioRad, Johannesburg, South Africa).

2.2. Extraction of plant material

C. asiatica leaves were collected in the Wild specifically within the lush vegetation of Westville Campus of University of KwaZulu-Natal, South Africa and authenticated by Prof. A. Nicholas (Discipline of Biological Sciences) in the same University. A voucher specimen (Dladla 02) was deposited in the Ward Herbarium at University of KwaZulu-Natal. The fresh leaves were rinsed with water and dried at room temperature before being pulverized in an electric grinder. Thereafter, 100 g of the powder was soaked in 1 L of methanol with continuous stirring for 72 h after which the mixture was filtered and the filtrate evaporated to dryness at 60 °C. This gave about 19% yield dry weight which was stored at −20 °C until needed.

2.3. Animal handling and induction of DM

Male Sprague-Dawley rats (150–180 g) were obtained from the Biomedical Resource Unit (BRU) at Westville campus of the University of KwaZulu-Natal, South Africa. The animals were enclosed two rats per cage in a temperature and humidity controlled room (23 ± 1 °C, 40–60% humidity) with a set 12 h light-dark cycle and fed standard rat chow diet (Meadows, Pietermaritzburg, South Africa) and water *ad libitum* for the duration of the study. This study was approved by the University of KwaZulu-Natal Animal Ethics Committee (Reference 024/15/Animal) and animals were handled humanely throughout the experimental period adhering to the guidelines of Laboratory Animal Care of the National Society of Medical Research and the National Institutes of Health Guide for the Care and Use of Laboratory Animals of the National Academy of Sciences (National Institutes of Health (NIH) publication no. 85-23, revised in 1985).

The animals were acclimatized for 7 days following which DM was induced by having them drink 10% fructose solution *ad libitum* for 14 days followed by a single intraperitoneal (*ip*) injection of streptozotocin (STZ, 40 mg/kg) dissolved in freshly prepared 0.1M citrate buffer (pH 4.5). This model of DM induction in rats has been demonstrated to induce insulin resistance coupled with insufficient insulin secretion thus closely mimicking the characteristics of type II DM observed in human diabetic patients [18]. Diabetes was confirmed in rats 7 days after STZ injection by obtaining blood through a once-off tail prick and glucose levels determined using Accu-Chek Glucometer (Roche, Basel, Switzerland). Only diabetic rats having a fasting blood glucose values between 12– 18 mM were used for the study.

2.4. Experimental design

Experimental animals were divided randomly into 5 treatment groups containing 6 rats each as designated as follows: NC: Normal control rats treated with distilled water, DC: Diabetic control rats treated with distilled water, DCA and D1000: Diabetic rats treated with *C. asiatica* extract (500 and 1000 mg/kg body weight respectively) dissolved in distilled water and DME: Diabetic rats treated with the standard antidiabetic drug, Metformin (300 mg/kg body weight) dissolved in distilled water. The NC group was given water *ad libitum* for 14 days prior to a single *ip* injection of freshly prepared citrate buffer. All treatment regimens started 8 days after injection with STZ and continued daily for 14 days. The dose of CA (500 mg/kg body weight) was chosen based on its pharmacological activities reported previously in the literature [19]. The authors used doses of 250, 500 and 1000 mg/kg of an ethanolic extract of CA and the most effective doses in lowering blood glucose and glucose absorption from the intestine were the latter two doses. In our model, the blood glucose lowering effect was evaluated at 500 and 1000 mg/kg. Our result (Fig. 1) showed that the 500 mg/kg dose was more effective in lowering fasting blood glucose in

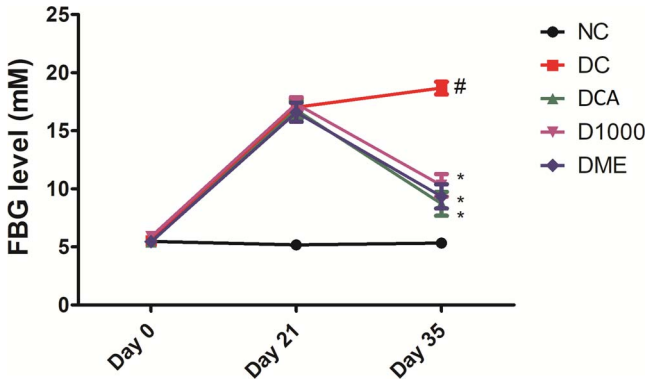

Fig. 1. Effect of *C. asiatica* on fasting blood glucose in diabetic rats. The blood glucose levels were determined using an Accu-Chek® glucometer to evaluate the effects of treatments on hyperglycemia. Day 0 = Start of study; Day 21 = Confirmation of diabetes; Day 35 = After 14 days of treatment; FBG = Fasting blood glucose. NC (Normal Control); DC (Diabetic Control); DCA and D1000 (Diabetic rats treated with *C. asiatica* extract at doses of 500 and 1000 mg/kg body weight, respectively); DME (Diabetic rats treated with Metformin at 300 mg/kg body weight). Data are presented as mean ± standard error of mean; n = 6. $^{\#}p < 0.05$ *versus* NC and $^{*}p < 0.05$ *versus* DC.