Biomedicine & Pharmacotherapy 96 (2017) 30–36

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Original research article

Antioxidant potential of *Xylopia aethiopica* fruit acetone fraction in a type 2 diabetes model of rats

Aminu Mohammed[a,b], Md. Shahidul Islam[a,*]

[a] Department of Biochemistry, School of Life Sciences, University of KwaZulu-Natal (Westville Campus), Durban 4000, South Africa
[b] Department of Biochemistry, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria

ARTICLE INFO

Keywords:
Xylopia aethiopica
Acetone fraction
Antioxidant
Type 2 diabetes
Ethanolic extract

ABSTRACT

The fruit decoction of *Xylopia aethiopica* (Dunal) A. Rich. is widely used for the treatment of diseases associated with oxidative stress such as diabetes, particularly in Africa. The present study was aimed to investigate the effects of *X. aethiopica* fruit acetone (XAFA) fraction in ameliorating oxidative stress in a type 2 diabetes (T2D) model of rats. The crude *X. aethiopica* fruit ethanolic extract was fractionated using solvents with increasing polarity and acetone fraction showed significantly ($p < 0.05$) higher *in vitro* antioxidant potentials which were measured by (1,1-diphenyl-2-picrylhydrazyl radical (DPPH), hydroxyl radical (HRS) and nitric oxide (NO) assays compared to other fractions. It was then subjected to *in vivo* antioxidant study in a T2D rat model. Acetone fraction depicted potent *in vitro* antioxidant actions (IC_{50}: DPPH: 19.82 ± 0.73 µg/mL; HRS: 25.34 ± 6.19 µg/mL; NO: 14.45 ± 2.44 µg/mL) compared to other fractions. Additionally, a significant ($p < 0.05$) and dose-dependent improvement on the *in vivo* antioxidant status was observed in the animals in diabetic treated groups (DXAL, DXAH) compared to the diabetic control (DBC) group. The results of our study suggest that XAFA possesses potent antioxidant potential and could be used to ameliorate oxidative stress associated metabolic complications such as T2D.

1. Introduction

Considerable evidences are available that correlate the cellular injury arising from reactive oxygen species (ROS) in the etiology of metabolic disorders such as obesity, hypertension and type 2 diabetes (T2D) [1,2]. T2D is a heterogeneous disorder characterized by insulin resistance and partial pancreatic β-cell dysfunction leading to hyperglycemia [3]. The mechanisms involve in hyperglycemia-induced cellular and tissue damages have been well documented [4–6]. This condition compromises the *in vivo* antioxidant defense system and increase the reactive oxygen species (ROS) production, a term referred as oxidative stress [7]. Therefore, owing to the diverse consequences linked to T2D, search for molecules that simultaneously ameliorate hyperglycemia and diabetes-induced oxidative stress are of major interest. Moreover, studies available showed a direct correlation between increase intake of exogenous antioxidants and reduction of oxidative stress associated biomarkers in T2D [8–10]. However, currently available synthetic antidiabetic drugs fail to completely ameliorate the diabetes-induced oxidative stress and are associated with unwanted adverse consequences [11,12]. Interestingly, in the last two decades, there has been an upsurge of interest in utilizing plant-derived biomolecules with dual abilities to ameliorate hyperglycemia as well as oxidative complications linked to T2D [13,14]. This has been attributed to the health-protecting and less toxicological effects associated with the usage of plant-derived products [15].

Xylopia aethiopica (Dunal) A. Rich. (Annonaceae) also known as Ethiopian pepper is an indigenous spice widely distributed in almost all parts of Western and Central Africa [16]. The fruit is popularly used as spice in food preparations in the different parts of the world [17]. In African tradition, *X. aethiopica* fruit decoction is used locally either alone or as poly-herbal formulation in the treatment of diabetes [18–23] and as an excipient to many other medicines [24]. In some previous studies, extracts derived from the *X. aethiopica* fruit showed antioxidant potentials using various *in vitro* models [25–31]. Adaramoye et al. [32] have shown that supplementation of *X. aethiopica* fruit extract improved the antioxidant defense status of animals exposed to γ-radiation. In another study, oral consumption of seed aqueous extract was reported to exhibit hypolipidemic and antioxidant actions in hypercholesterolemic rats [33]. Woode et al. [34] highlighted that ethanol extract and xylopic acid derived from *X. aethiopica* fruit have shown analgesic property in various pain animal models. Subsequently, hydroethanol extract of *X. aethiopica* was reported to exhibit potent anti-

* Corresponding author.
E-mail address: islamd@ukzn.ac.za (Md. S. Islam).

http://dx.doi.org/10.1016/j.biopha.2017.09.116
Received 4 August 2017; Received in revised form 8 September 2017; Accepted 23 September 2017

anaphylactic and anti-inflammatory actions in mice [35]. Moreover, Biney et al. [36] have recently reported an excellent antidepressant activity of the same extract via interaction with serotonergic neurotransmission in mice. Additionally, our recent study showed that *X. aethiopica* fruit acetone (XAFA) fraction has antihyperglycemic, antihyperlipidemic and pancreatic β-cell ameliorating activities in a T2D model of rats [37].

Therefore, based on the available data, it has been hypothesized that *X. aethiopica* fruit may have beneficial effect in attenuating diabetes-induced oxidative stress. However, information on the antioxidant potential of any parts of *X. aethiopica* in either diabetic animal model or human subjects is still speculative. Hence, our present study was investigated to examine the effects of XAFA in attenuating hyperglycemia-induced alterations on the *in vivo* antioxidant defense system in a T2D rat model.

2. Materials and methods

2.1. Chemicals and reagents

Ascorbic acid, quercetin, Gallic acid, and 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) were purchased from Sigma-Aldrich through Capital Lab Supplies, New Germany, South Africa. Folin Ciocalteau reagent was purchased from Merck Chemical Company, South Africa.

2.2. Plant material

Fruit of *X. aethiopica* was collected in December 2012 from Ibadan, Oyo State, Nigeria. It was identified and authenticated at the herbarium unit of the Biological Science Department, Ahmadu Bello University, Zaria, Nigeria by Mr. Umar Gallah and a voucher specimen number 1026 was deposited accordingly. The sample was immediately washed and shade-dried for two weeks to constant weight. The dried sample was ground to fine powder, and then stored individually in airtight containers to transport to the University of KwaZulu-Natal, Westville Campus, Durban, South Africa for further analysis.

2.3. Preparation of the plant sample

Three (3) kg of the finely powdered fruit was defatted using 10 L hexane (HEX) and then extracted by soaking for 48 h in ethanol. After filtration through Whatmann filter paper (No. 1), the extract was evaporated under vacuum, using a rotary evaporator (Buchi Rotavapor II, Buchi, Switzerland) at 40 °C under reduced pressure and recorded 7.05% yield. Forty (40) grams of the crude ethanolic extract of the fruit was dissolved in 500 mL of distilled water:methanol (9:1) and successively partitioned with 2 × 500 mL of HEX, dichloromethane, ethyl acetate (EtAOc) and acetone. The fractions were evaporated to dryness under vacuum at 40 °C. The dried fractions were transferred to micro tubes and stored at 4 °C until further analysis.

2.4. In vitro studies

2.4.1. DPPH radical scavenging activity

The total free radical scavenging activity of the fractions was determined and compared to that of ascorbic acid by using a slightly modified method as described by Tuba and Gulcin [38]. An aliquot of 500 µL of a 0.3 mM solution of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) in methanol was added to 1 mL of the fraction at different concentrations (30, 60, 120 and 240 µg/mL). These solutions were mixed and incubated at dark for 30 min at room temperature. The absorbance was measured at 517 nm against blank lacking free radical scavenger.

2.4.2. Hydroxyl radical scavenging (HRS) activity (deoxyribose assay)

The HRS activity was determined by analysing the competition between deoxyribose and the fractions for hydroxyl radical generated by the ascorbate/EDTA/H_2O_2 system (Fenton reaction) as described by Hinnerburg et al. [39]. The assay was performed by adding 200 µL of premixed 100 µmol/L $FeCl_3$ and 100 µmol/L EDTA (1:1, V/V) solution, 100 µL of 10 mmol/L H_2O_2, 360 µL of 10 mmol/L 2-deoxy-D-ribose, 1 mL of different fractions dissolved in 10% DMSO (concentration 30–240 µg/mL), 400 µL of 50 mmol/L sodium phosphate buffer (pH 7.4) and 100 µL of 1 mmol/L ascorbic acid as per above-mentioned sequence. The mixture was incubated at 50 °C for 2 h. Thereafter, 1 mL of 2.8% TCA and 1 mL of 1.0% thiobarbituric acid (in 0.025 mmol/L NaOH) were added to each tube. The samples were further incubated in a water bath at 50 °C for 30 min to develop the pink chromogen. The extent of oxidation was estimated from the absorbance of the solution at 532 nm.

2.4.3. Nitric oxide (NO) radical scavenging assay

Sodium nitroprusside in an aqueous solution at physiological pH spontaneously generates NO and interacts with oxygen to produce nitrite ions, which can be estimated using Griess reagent. Scavengers of NO compete with oxygen, leading to reduce the production of NO [40]. The assay was carried out by incubating 500 µL of 10 mmol/L sodium nitroprusside in phosphate buffer (pH 7.4) and 500 µL of different fractions dissolved in 10% DMSO (concentration 30–240 µg/mL) at 37 °C for 2 h. The reaction mixture was then mixed with 500 µL of Griess reagent. The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was read at 546 nm. The percentage inhibition of NO generated was measured in comparison with the absorbance value of the control (10 mmol/L sodium nitroprusside in phosphate buffer).

Concentrations of fractions resulting in 50% inhibition of enzyme activity (IC_{50}) for DPPH, HRS and NO were calculated from the data.

2.5. In vivo studies

2.5.1. Experimental animals

Six-week-old male Sprague-Dawley (SD) rats were obtained from the Biomedical Resource Unit (BRU) located at the University of KwaZulu-Natal (Westville Campus), South Africa with initial mean body weight (bw) 163.25 ± 13.67 g. Animals were housed as two in one medium size poly-carbonated cage in a temperature and humidity controlled room with a 12-h light-dark cycle. A standard rat pellet diet was supplied *ad libitum* to all animals during the entire experimental period. Animals were maintained according to the rules and regulations of the Experimental Animal Research Ethics Committee of the University of KwaZulu-Natal, South Africa (Ethical approval number: 018/14/Animal).

2.5.2. Animal grouping

Animals were randomly divided into six (6) groups of 5 (non-diabetic groups) or 8 (diabetic groups) animals namely; NC: Normal Control, DBC: Diabetic Control, DXAL: Diabetic + low dose (150 mg/kg bw) of XAFA, DXAH: Diabetic + high dose (300 mg/kg bw) of XAFA, DMF: Diabetic + metformin (300 mg/kg bw), NXAH: Non-diabetic + high dose (300 mg/kg bw) of XAFA. The animals were allowed to acclimatize for one week before starting the experiment.

2.5.3. Induction of type 2 diabetes (T2D)

To induce the two major pathogeneses of T2D, insulin resistance and partial pancreatic β-cell dysfunction, during the first two weeks of the experiment, the animals in the DBC, DXAL, DXAH and DMF groups were supplied with 10% fructose solution *ad libitum* for the induction of insulin resistance when the animals in the NC and NXAH groups were supplied with normal drinking water. After this period, a low dose of STZ (40 mg/kg bw) dissolved in citrate buffer (pH 4.5) was intraperitoneally injected to the animals in the DBC, DXAL, DXAH and DMF groups to induce partial pancreatic β-cell dysfunction, whereas the