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Concentrated hot water-infusion of *phragmanthera incana* improves muscle glucose uptake, inhibits carbohydrate digesting enzymes and abates Fe^{2+}-induced oxidative stress in hepatic tissues

Olakunle Sanni[a], Ochuko L. Erukainure[a], Olajumoke A. Oyebode[a], Neil A. Koorbanally[b], Md. Shahidul Islam[a,*]

[a] Department of Biochemistry, School of Life Sciences, University of Kwazulu-Natal (Westville Campus), Durban, 4000, South Africa
[b] School of Chemistry and Physics, University of KwaZulu-Natal, (Westville Campus), Durban, 4000, South Africa

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ABSTRACT

Chronic hyperglycemia has been implicated in the development of oxidative stress and as a major factor in etiology of secondary complication in diabetes. In the present study, the antidiabetic potential of *Phragamenthra incana* (*P. incana*) hot infusion and its possible inhibitory effects on carbohydrate digesting enzymes, promotion of muscle glucose uptake, and the antioxidative potentials in Fe^{2+}-induced oxidative stress in hepatic tissue were investigated. The infusion significantly ($p < 0.05$) scavenged free radicals (DPPH) and displayed favourable ferric reducing antioxidant power (FRAP) with increasing concentrations. It also significantly ameliorated Fe^{2+}-induced oxidative stress in hepatic tissues by increasing superoxide dismutase (SOD) and catalase activities and depleting malondialdehyde (MDA) level. The results further showed that the infusion significantly ($p < 0.05$) inhibited α-amylase and α-glucosidase activity, and enhanced muscle glucose uptake, with and without insulin. Liquid Chromatography–Mass Spectroscopy (LCMS) analysis of the infusion revealed the presence of 2-methoxythiazole; l-cysteine; nicotinic acid; S-methyl-l-cysteine; isoquinoline, 1-methyl-; and 1H-indole-2,3-dione,5-methyl. The results of this study suggest that the observed antidiabetic and antioxidative potentials of *P. incana* could be attributed to its identified phytochemical constituents, however, this supports folkloric medicinal use of this plant.

1. Introduction

Diabetes mellitus (DM) has, over the years, become one of the leading threats to human health [1]. Available data reveal that the prevalence of diabetes has increased from 4.7% in 1980 to 8.5% in 2014 [1,2]. The International Diabetes Federation (IDF) projected a 55% increase of prevalence by 2032, when at least 592 million people will be living with diabetes [3].

Diabetes is a chronic disorder of energy metabolism characterized by hyperglycemia and glucose intolerance due to the deficiency of insulin as seen in type 1 diabetes, or impaired insulin action, or both as seen in type 2 diabetes (T2D) [4]. Among all types of diabetes, T2D is the most common one, owing to urbanization and changes from traditional to westernized diets [2]. Chronic hyperglycemia in T2D has been linked to increase in free radicals or impaired antioxidant defense mechanism in the body, resulting to oxidative stress [5]. Oxidative stress has been implicated in the progression and pathogenesis of T2D, leading to micro- and macro-vascular complications [6].

Most oral synthetic antidiabetic drugs have been targeted towards the control of hyperglycemia, thus, limiting its progression to secondary complications [7]. These drugs exert their actions by improving pancreatic β-cell function, reducing glucotoxicity, decreasing glucose reabsorption in the kidney, increasing insulin sensitivity in the muscle and liver, decreasing hepatic glucose production, and increasing gastric emptying following increasing GLP-1 secretion [8].

Plant-derived natural products are gaining more and more popularity in the management of diabetes not only due to their lower cost, but also for having negligible or no side effects [9]. The ability of polyphenols, terpenoids, alkaloids, saponins, flavonoids, and glycosides from plants to scavenge free radicals or chelate active metals to attenuate their redox action have been beneficial to human health [10]. Some medicinal plants have been employed in the folkloric treatment and management of diabetes and its complications from time immemorial. Their antidiabetic properties have also been demonstrated in

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

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both basic and clinical studies [8]. Amongst such plants is *Phragmenthra incana*.

P. incana is a parasitic plant species of the Loranthaceae family that grows on cocoa (*Theobroma cacao*) and Kolanut (*Cola nitida*) trees. It is commonly known as mistletoe. Being a parasitic plant, it grows on the branches of its host, where it derives its nutrients. It is found in the bushes and jungle areas of Western Africa, Gulf of Guinea and Congo base of Zaire and Angola [11]. Ethnomedicinally, it is used in the treatment of different disease conditions and ailments including hypertension, diabetes, inflammation, cancer and insomnia [10]. In Nigeria, traditional healers boil the leaves to use as a concoction in the treatment of diabetes [10]. However, there are limited scientific validations on the use of this plant in the treatment of diabetes.

Therefore, the objective of this study was to investigate the ameliorative effects of the concentrated hot water infusion of the leaves against Fe^{2+} -induced hepatic oxidative injury; its ability to inhibit key enzymes linked to diabetes as well as its ability to increase muscle glucose uptake, and to possibly identify its bioactive compounds.

2. Materials and methods

2.1. Chemicals and reagents

1,1-diphenyl-2-picrylhydrazyl radical (DPPH), p-nitrophenyl-α-D-glucopyranoside (pNPG), p-nitrophenol, acarbose, yeast α-glucosidase, porcine pancreatic amylase, ascorbic acid, gallic acid and potassium ferricyanide were obtained from Sigma-Aldrich through Capital Lab Supplies, Durban, South Africa. Starch, dinitrosalicylic acid (DNS), maltose, absolute ethanol, ethyl acetate, trichloroacetic acid, hydrogen peroxide, ferric chloride, Griess reagent, sodium nitroprusside, thiobarbituric acid, reduced glutathione, Folin ciocalteau reagent, deuterated chloroform and deuterated methanol were purchased from Merck Chemical Company, Durban, South Africa.

2.2. Collection of plant material

Phragmanthera incana plant was collected in March 2016 from Owo, Ondo State, South-Western Nigeria and authenticated at the herbarium unit of the Biological Department, Adekunle Ajasin University, Akungba, Nigeria. It was assigned a voucher specimen number: PSB 175 and deposited at the same herbarium.

2.2.1. Preparation and extraction

The leaves were washed under tap water and air-dried to a constant weight. The dried leaves were blended to fine powder using Thomas-Wiley Laboratory mill, Philadelphia, USA. Fifty grams (50 g) of the blended sample was infused in boiling water and allowed to stand for 20 min. The infusion was then filtered with Whatmann's filter paper No. 1 and concentrated at 50 °C. It was then stored at 4 °C until further use.

2.3. Animals

Five 12-week old male Sprague-Dawley rats were procured from the Biomedical Research Unit of the University of Kwazulu-Natal, Westville, Campus, Durban South Africa. The animals were euthanized with halothane and the psoas muscle were collected and used for glucose uptake study. The liver was also harvested for *ex vivo* oxidative stress biomarker assay.

The animals were maintained under the guidelines of the Animal Ethical Committee of the University of KwaZulu-Natal, Durban 4000, South Africa (Ethical approval number: AREC/003/017D).

2.4. Estimation of total phenolic content

The total phenolic content of the infusion was estimated as gallic acid equivalent using the method described by Antolovich et al. [12]. Briefly, 200 µL of 240 µg/mL of the extract was incubated with 1 mL of diluted Folin-Ciocalteau reagent (diluted with water; 1:10 v/v) and 800 µL of 0.7 M Na_2CO_3 at room temperature for 30 min. Absorbance was measured at 765 nm.

2.5. In vitro antioxidant studies

2.5.1. Free radical scavenging activity

The free radical scavenging activity of the infusion was determined using a slightly modified method as described by Bukhari et al. [13]. Briefly, 500 µL of the DPPH solution (0.3 mM solution of DPPH in methanol) was incubated with 1 mL of the extracts at various concentrations (15–240 µg/mL) in the dark for 30 min at room temperature. The absorbance was read at 517 nm against a blank. The percentage scavenging activity was calculated as follows:

% Scavenging Activity = (Ac - As)/ Ac *100

Ac = Absorbance of the control
As = Absorbance of the sample
Gallic acid was used as standard.

2.5.2. Ferric reducing antioxidant power (FRAP)

FRAP was determined according to the method described by Oyaizu (1986) [14]. Briefly, the infusion was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (1%, 2.5 mL) and incubated at 50 °C for 20 min. Trichloroacetic acid (10%, 2.5 mL) was added to acidify the mixture. Thereafter, 1 mL of the mixture was mixed with 1 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$. The absorbance of the resulting solution was measured at 700 nm in a spectrophotometer. The antioxidant power of the plant extracts was expressed as shown below:

Ferric reducing antioxidant power % = As /A_G *100

As = Absorbance of the sample
A_G = Absorbance of the Gallic acid

2.6. In vitro antidiabetic studies

2.6.1. Determination of α-glucosidase inhibitory activity

α-glucosidase inhibitory activity was measured as described by Ademiluyi and Oboh [19] with slight modifications. Briefly, 250 µL of different concentration (15–240 µg/mL) of the infusion or acarbose was incubated for 15 min with 500 µL of 1.0 U/mL α-glucosidase solution in 100 mM phosphate buffer (pH 6.8). The resulting solution was incubated at 37 °C, with 250 µL of 5 mM pNPG solution in phosphate buffer (100 mM, pH 6.8) for 20 min. The absorbance of p-nitrophenol formed was measured at 405 nm and the inhibitory activity was expressed as follows:

Inhibitory activity % = (Ac - As)/ Ac *100

Ac = Absorbance of the control
As = Absorbance of the sample

2.6.2. Determination of α-amylase inhibitory assay

α-amylase inhibition activity was carried out as described by Shai et al. [20] with slight modifications. Briefly, 250 µL of different concentrations (15–240 µg/mL) of the infusion and acarbose were incubated with 500 µL of porcine pancreatic amylase (2 U/mL) in 100 mM phosphate buffer (pH 6.8) at 37 °C for 20 min. Thereafter, 250 µL of 1% starch (in 100 mM phosphate buffer; pH 6.8) was added to the reaction mixture and incubated at 37 °C for 15 min. 1 mL of dinitrosalicylate (DNS) colour reagent was then added and allowed to boil for 10 min. The absorbance was read at 540 nm and the inhibitory activity was expressed as shown below:

Inhibitory activity % = (Ac - As)/ Ac *100