Biomedicine & Pharmacotherapy 85 (2017) 725–732

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Protective potential of *Averrhoa bilimbi* fruits in ameliorating the hepatic key enzymes in streptozotocin-induced diabetic rats

Surya B Kurup, Mini S*

Department of Biochemistry, University of Kerala, Kariavattom, Thiruvananthapuram, Kerala, India

ARTICLE INFO

Article history:
Received 18 October 2016
Received in revised form 10 November 2016
Accepted 18 November 2016

Keywords:
Averrhoa bilimbi Linn
Diabetes mellitus
Carbohydrate metabolism
GC–MS analysis
Phytomedicine

ABSTRACT

Back ground: Diabetes is a mutifactorial disease which leads to several complications. Currently available drug regimens for management of diabetes have certain drawbacks. Need for safer and effective medicines from natural sources having potent antidiabetic activity. *Averrhoa bilimbi* Linn. (Oxalidaceae) is a medicinal plant and is reported to possess hypoglycemic activity.
Objective: To investigate the antidiabetic potential of *Averrhoa bilimbi* fruit extract in streptozotocin-induced diabetic rats.
Methods: Diabetes was induced in male Sprague Dawley rats by single intraperitoneal injection of streptozotocin (STZ) (40 mg/kg body weight). The diabetic rats were treated orally with ethyl acetate fraction of *A. bilimbi* fruits (ABE) (25 mg/kg body weight) and metformin (100 mg/kg body weight) by intragastric intubation for 60 days. After 60 days, the rats were sacrificed; blood, liver and pancreas were collected. Several indices such as blood glucose, plasma insulin, toxicity markers and the activities of carbohydrate-metabolizing enzymes were assayed. The phytochemicals present in the ABE was identified by gas chromatography-mass spectrometry analysis.
Results: ABE significantly ($p < 0.05$) reduced the level of blood glucose and hepatic toxicity markers and increased plasma insulin in diabetic rats. ABE modulated the activities of carbohydrate-metabolizing enzymes, significantly increased the activities of hexokinase (59%) and pyruvate kinase (68%) and reduced the activities of glucose-6-phosphatase (32%) and fructose-1, 6-bisphosphatase (20%). The histological studies of the pancreas also supported our findings. The results were compared with metformin, a standard oral hypoglycemic drug. GC–MS analysis of ABE revealed the presence of 11 chemical constituents in the extract.
Conclusions: ABE exerts its antidiabetic effect by promoting glucose metabolism via glycolysis and inhibiting hepatic endogenous glucose production via gluconeogenesis.

1. Introduction

Diabetes mellitus (DM) is a multifaceted metabolic disorder characterized by chronic hyperglycemia ensuing from defects in insulin secretion, action or both [1]. Persistent hyperglycemia is a stage of the increased blood glucose level of circulating blood system that leads to the development of microvascular and macrovascular complications. Diabetes and its complications create a severe health care crisis worldwide. Globally, the prevalence of diabetes is predicted to grow from 366 million in 2011 to 552 million by 2030 [2].

Diabetes results in the derangement of carbohydrate, lipid and protein metabolism [3]. Glucose homeostasis involves the coordinated regulation of pathways that direct glucose production and utilization [4]. In diabetes, the activities of key enzymes of glycolysis and gluconeogenesis are distorted, which leads to chronic hyperglycemia [5]. Effective measures to manage fasting and postprandial glucose levels are crucial in the management of diabetes. Though, numerous oral hypoglycemic drugs are now available, but their extended consumption produces adverse side effects [6]. Hence, there is a need to explore for phytocomponents that regularize hyperglycemia and diabetes-associated complications [7].

Averrhoa bilimbi Linn. (Oxalidaceae), commonly known as cucumber tree or tree sorrel is a widely cultivated plant in India, Indonesia, Sri Lanka, Bangladesh, Myanmar, Malaysia, Central and South America. The whole plant is used for treating coughs, cold,

* Corresponding author at: Department of Biochemistry, University of Kerala, Kariavattom, Thiruvananthapuram 695 581, Kerala, India.
E-mail address: minisaraswathy@gmail.com (M. S).

http://dx.doi.org/10.1016/j.biopha.2016.11.088
0753-3322/

itches, rheumatism, whooping cough, hypertension etc. [8,9]. Traditionally *A. bilimbi* fruits are reported to have antidiabetic activity and no scientific data are available on the antidiabetic properties of the fruits [10,11].

Previous studies showed that the leaf extract of *A. bilimbi* and its semi-purified fractions possesses hypoglycemic and hypolipidemic properties in Type I diabetic rats when treated both intraperitoneally [12] as well as orally [13,14]. Our previous studies revealed the beneficial effect of aqueous extracts of *Averrhoa bilimbi* fruits in controlling blood glucose and lipid metabolism and preventing diabetic complications from lipid peroxidation in Type II diabetic rats [15]. In our preliminary study the ethyl acetate fraction of *Averrhoa bilimbi* fruits was found to be rich in phenolic compounds with superior antioxidant activity [16]. Preliminary *invitro* studies revealed the potency of ABE fraction.

Present study evaluates the antidiabetic potential of ethyl acetate fraction of *A. bilimbi* fruits by monitoring activities of the key enzymes of carbohydrate metabolism in Type II diabetic rats.

2. Materials and methods

2.1. Chemicals

Chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA), Merck Chemical Company (Darmstadt, Germany) and Sisco Research Laboratories (Mumbai, India).

2.2. Extraction of Averrhoa bilimbi fruits

Fresh fruits of *Averrhoa bilimbi* were obtained from Thiruvananthapuram, Kerala, India, during the fruiting season (July–December 2014) and documented by Dr. Valsala Devi, Department of Botany, University of Kerala (Voucher no: KUBH 5865). Care was taken that the fruits, which were whitish-green in color and about 5–7.0 cm in size, were not overripe, spoiled or damaged. Fruits (5 kg) were cut and shade dried at a temperature of 28 °C. Shade dried fruits were ground in a blender to give 500 g of fine powder. The aqueous extract was prepared by cold maceration of 500 g powder in 1000 ml of distilled water and lyophilized (Thermo electron corporation, MODUL YOD-230) (yield 26%).

100 g lyophilized *A. bilimbi* fruit extract was defatted with petroleum ether and the defatted extract was fractionated with ethyl acetate (1:1 v/v) (ABE-yield 5%) was concentrated at room temperature and used for the present study.

2.3. Experimental animals

Two months old male Sprague Dawley rats (200–220 g body weight, 35 animals in total) bred in our Department animal house was used for the study. Animals were housed in polypropylene cages and maintained under standard conditions [12-h light/dark cycles, (25 ± 10 °C)]. All the animal care was taken as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals and the experimental protocol approved by the Institutional Animal Ethics Committee [IAEC-KU-20/2013-14-BC-SM (22)].

2.4. Induction of diabetes

Diabetes was induced in rats by single intraperitoneal injection of freshly prepared streptozotocin (STZ) at a dose of 40 mg/kg body weight in 0.1 M citrate buffer (pH 4.5) [17]. The animals were given 5% glucose in drinking water overnight to overcome the drug-induced hypoglycemia. 48 h after STZ injection, blood glucose levels were estimated and rats with blood glucose ranging between 200 and 400 mg/dl were considered as diabetic and used for the study.

2.5. Experimental design

A dose dependent toxicity study was carried out using ABE at different concentrations (5, 10 and 25 mg/kg body weight). The minimal effective dose was fixed as 25 mg/kg body weight by evaluating the activities of toxicity markers and antioxidant enzymes (results not shown). So for the present study, we have selected ABE at a dose of 25 mg/kg body weight and efficacy of ABE was compared with metformin, a standard oral hypoglycemic drug in streptozotocin induced diabetic rats.

The experimental animals were divided into five groups, comprising of seven rats. ABE and metformin were administered intragastrically for 60 days.

Group I: Normal control rats

Group II: Normal rats treated with ABE (25 mg/kg body weight)

Group III: Diabetic control rats

Group IV: Diabetic rats treated with ABE (25 mg/kg body weight)

Group V: Diabetic rats treated with metformin (100 mg/kg body weight)

During the experimental period, body weight, blood glucose and physical examinations were done at regular intervals. The dosage was adjusted every week, according to changes in body weight to maintain similar dose per kg body weight of rat over the entire period of study. After 60 days, the rats were sacrificed by sodium pentothal injection. Blood, liver and pancreas were collected for various experimental analysis.

2.6. Gas chromatography–mass spectrometry (GC–MS) analysis

The GC–MS analysis of the extract was performed using Agilent CP 3000 GC/Saturn 2200 MS (Agilent, Palo Alto, CA) equipped with ECD, PFPD and MS detectors. For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas was used as the carrier gas at a constant flow rate of 1 ml/min. The inlet temperature was set at 250 °C. The oven temperature was maintained at 100 °C for 1.5 min and gradually increased up to 270 °C at the rate of 5 °C per min 1 ml diluted sample was injected and the scan range was 40–600 m/z.

2.7. Biochemical parameters

The fasting blood glucose was monitored by glucometer (One Touch Horizon, Johnson and Johnson). Insulin was estimated by using an ELISA kit (DRG Diagnostics, USA). Hepatic toxicity markers such as alkaline phosphatase (ALP), acid phosphatase (ACP) and gamma glutamyl transferase (GGT) were measured using commercially available kits (Agappe Diagnostics, Ernakulam, India). Glycolytic enzymes viz, hexokinase (EC 2.7.1.1) [18] and pyruvate kinase (2.7.1.40) [19] were assayed. Activities of hepatic gluconeogenic enzymes, glucose-6-phosphatase (EC 3.1.3.9) [20] and fructose-1, 6-bisphosphatase (EC 3.1.3.11) [21] were assayed.

2.8. Histopathological analysis of pancreas

The pancreatic samples were set for 48 h in 10% neutral formalin, fixative solution was dehydrated by passing effectively in different mixture of ethyl alcohol-water, cleaned with xylene and embedded in paraffin. Sections of pancreas (5 μm thick) were prepared by using a rotary microtome and then stained with hematoxylin and eosin (H & E) dye, which mounted in a neutral deparaffinated xylene medium for microscopic observations [22].