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Original Article

Averrhoa bilimbi fruits attenuate hyperglycemia-mediated oxidative stress in streptozotocin-induced diabetic rats

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ABSTRACT

Hyperglycemia-mediated oxidative stress plays a major role in the development of diabetic complications. *Averrhoa bilimbi* Linn. (Oxalidaceae) is a medicinal plant with fruits reported to possess antidiabetic activity. This study evaluated the beneficial effects of the ethyl acetate fraction of *A. bilimbi* fruit (ABAEE) on the antioxidant/oxidant status in diabetes mellitus. Diabetic rats were treated orally with the ethyl acetate fraction of *A. bilimbi* fruits at a dose of 25 mg/kg body weight for 60 days. Serum glucose, glycated hemoglobin, plasma insulin, hepatic toxicity markers, antioxidant enzymes, lipid peroxidation products, and liver histopathology were assayed checked after 60 days of extract treatment. Diabetic rats administered ABAEE showed a significant decline in serum glucose, glycated hemoglobin, and also significantly increases the level of plasma insulin, as well as a notable attenuation in thiobarbituric acid-reactive substances, conjugated dienes, and hydroperoxides. ABAEE also modulated hepatic antioxidant potential by significantly increasing the activities of catalase, glutathione peroxidase, glutathione reductase, superoxide dismutase, and reducing glutathione content. The results associated with ABAEE were more significant than those observed following treatment with the standard drug metformin. Histopathological observations showed that ABAEE effectively rescued hepatocytes from oxidative damage without affecting cellular function and structural integrity. High-performance liquid chromatography analysis of ABAEE indicated the presence of phenolic compound, quercetin, indicating that the antidiabetic effect of the extract might be related to quercetin. These results demonstrated the potential beneficial effect of ABAEE on streptozotocin-induced diabetes in rats.

1. Introduction

Diabetes mellitus, a pervasive and multifactorial metabolic syndrome, is characterized by defects in insulin secretion and insulin receptor or postreceptor events, with derangement in carbohydrate, protein, and lipid metabolism resulting in chronic hyperglycemia, a clinical hallmark of diabetes [1]. In 2011, there were 366 million people with diabetes, with this

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number expecting to rise to 552 million by 2030 [2]. Hyperglycemia describes a stage of increased blood glucose levels in circulating blood and generates reactive oxygen species (ROS) through various pathways, including dysregulation of redox equilibrium, augmentation of advanced glycation products, activation of protein kinase C, or overproduction of mitochondrial superoxides that eventually leads to oxidative stress in various tissues [3].

Management of diabetes without side effects remains a challenge. Many synthetic drugs are used in the treatment of diabetes; however, plant-based drugs are often considered less toxic and free of side effects. Indian traditional medicines belong to one of the richest medicinal systems in the world. Therefore, there is a need to search for phytocomponents that normalize hyperglycemia and ameliorate oxidative stress for the prevention or minimization of diabetes-associated complications.

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, and Central and South America. The entire plant is used for treating coughs, colds, itches, rheumatism, whooping cough, and hypertension [4,5]. Traditionally *A. bilimbi* fruits are employed for curing diabetes, although no scientific data are available concerning the antidiabetic properties of the fruits.

Previous studies reported that *A. bilimbi*-leaf extract regulates blood glucose and lipids in streptozotocin (STZ)-induced diabetic rats [6]. Studies on *A. bilimbi* fruits in rats showed that the fruit (125 mg/kg), as well as its water extract (50 mg/kg), was effective in lowering lipids in rats fed a high-fat diet, and can be used as a dietary ingredient to prevent and treat hyperlipidemia [7]. Studies showed that the semipurified fractions of *A. bilimbi* leaves exhibited antidiabetic activity in STZ-induced diabetic rats fed a high-fat diet [8]. Our previous studies revealed the beneficial effect of aqueous extracts of *A. bilimbi* fruit in controlling blood glucose levels, improving lipid metabolism, and preventing diabetic complications from lipid peroxidation in experimental diabetic rats [9]. *In-vitro* antioxidant studies of different solvent fractions (petroleum ether, ethyl acetate, butanol, and water) of *A. bilimbi* fruits revealed the superior effect elicited by the ethyl acetate fraction.

However, no reports are available on the protective effect of the ethyl acetate fraction of *A. bilimbi* fruits (ABAEE) on hyperglycemia-mediated oxidative stress in STZ-induced diabetic rats. Therefore, the present study investigated the ameliorative potential of the ethyl acetate fraction of *A. bilimbi* in STZ-induced diabetic rats, and the efficacy of the ABAEE was compared with that of metformin, a standard oral antidiabetic drug.

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 A. bilimbi fruits

Fresh fruits of *A. bilimbi* were obtained from Thiruvananthapuram, Kerala, India, during the fruiting season (July–December 2014) and identified by Dr. Valsala Devi, Department of Botany, University of Kerala (Voucher number: KUBH 5865). Care was taken that the fruits, which were whitish-green in color and ~5–7.0 cm in size, were not overripe, spoiled, or damaged. Fruits (5 kg) were shade dried at a temperature of 28°C and coarsely powdered.

The crude aqueous extract was defatted with petroleum ether and the remaining defatted extract was fractionated with ethyl acetate (1:1 volume/volume) to obtain the ethyl acetate fraction (ABAEE-yield: 5%), which was concentrated and used for the experimental study.

2.3. Experimental animals

Two-month-old male Sprague Dawley rats (200–220 g body weight; 35 animals in total) bred in our departmental animal house were used for the study. Animals were housed in polypropylene cages and maintained under standard conditions [12-hour light/dark cycles, (25 ± 10°C)]. Animal care was performed 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 Animals Ethics Committee [IAEC-KU- 20/2013-14-BC-SM (22)].

2.4. Induction of experimental diabetes

Rats were rendered diabetic by a single intraperitoneal injection of freshly prepared STZ at a dose of 40 mg/kg body weight in 0.1M citrate buffer (pH 4.5) [10]. The animals were administered a 5% glucose solution by dissolving in drinking water overnight to overcome drug-induced hypoglycemia. Following STZ injection (48 hours), blood glucose levels were measured, and rats with blood glucose levels between 200 mg/dL and 400 mg/dL were considered diabetic and used for the experiment.

2.5. Experimental design

Dose-dependent toxicity studies were previously performed in our laboratory using three different doses of ABAEE (5 mg/kg body weight, 10 mg/kg body weight, and 25 mg/kg body weight), and the extract at an optimal dose of 25 mg/kg body weight was found to be effective and safe compared with the other two doses (5 mg/kg body weight and 10 mg/kg body weight). Therefore, the dose of 25 mg/kg body weight was selected for the present study, and the results were compared with a standard antidiabetic drug metformin.

The experimental animals were divided into five groups, with each group comprising of seven rats. ABAEE and metformin were administered orally using an intragastric tube once daily in the morning after food delivery for 60 days. Metformin is generally recommended as a first-line treatment for diabetes.

Group I: normal control rats.
Group II: normal rats treated with ABAEE (25 mg/kg body weight).