Biomedicine & Pharmacotherapy 103 (2018) 539–545

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Ameliorating effect of berbamine on hepatic key enzymes of carbohydrate metabolism in high-fat diet and streptozotocin induced type 2 diabetic rats

Sankaranarayanan Chandrasekaran*, Ramajayam Nishanthi, Pachaiappan Pugalendi

Department of Biochemistry and Biotechnology, Annamalai University, Annamalainagar, 608002, Tamilnadu, India

ARTICLE INFO

Keywords:
High fat diet
Streptozotocin
Carbohydrate metabolic enzymes
Berbamine
Sprague Dawley rats

ABSTRACT

Background: Aberrations in the activities of key enzymes of carbohydrate metabolism is well documented in diabetes mellitus. Previous studies have shown that active ingredients in the extracts of *Berberis aristata* exhibits diverse pharmacological activities in animal models.

Objective: The present study was undertaken to investigate whether berbamine (BBM), an alkaloid from the roots of *Berberis aristata* can ameliorate the altered activities of carbohydrate metabolic enzymes in high fat diet (HFD)/streptozotocin (STZ) induced diabetic rats.

Results: Supplementation of HFD for 4 weeks followed by intraperitonial administration of single low dose of STZ (40 mg/kg b.w.) to Sprague Dawley rats resulted in significant hyperglycemia with a decline in plasma insulin levels. The rats also exhibited decreased hemoglobin with an increase in glycated hemoglobin levels. The activities of hexokinase, glucose-6-phosphate dehydrogenase were decreased whereas increases in the activities of glucose-6-phosphatase and fructose-1,6-bisphosphatase were observed in the hepatic tissues of diabetic control rats. Glycogen content in the hepatic and skeletal muscle tissues were found to be decreased in diabetic rats. Oral administration of BBM for 56 days, dose dependently (50, 100, 200 mg/kg b.w.) improved insulin secretion in diabetic treated rats. Immunohistochemical studies on pancreas revealed a strong immunoreactivity to insulin in BBM treated rats. At the effective dose of 100 mg/kg b.w., BBM restored the altered activities of carbohydrate metabolic enzymes and also improved glycogen content in insulin dependent tissues.

Conclusion: From the biochemical and histochemical data obtained in this study we conclude that BBM ameliorated the activities of metabolic enzymes and maintained glucose homeostasis in HFD/STZ induced diabetic rats and it can be used as a potential phytomedicine for the management of diabetes mellitus.

1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemic results from the total absence or insufficient secretion/action of insulin. Though the etiology is multifaceted, genetic predisposition associated with an intake of ill-balanced diet play a vital role in the incidence of diabetes. Sedentary lifestyle, increasing availability of energy dense food and lack of exercise have led to dramatic rise in type 2 diabetes mellitus (T2DM). It is estimated that a global prevalence of 382 million people with diabetes in 2013, is expected to double by 2035 [1].

Nutrition plays an important role in the development and also in the prevention of diabetes mellitus. A diet rich in fat can induce metabolic disorders like insulin resistance, dyslipidemia and hypertension in rodents and humans [2]. Administration of high fat diet and streptozotocin (HFD/STZ) to experimental animals lead to β-cell dysfunction, defective insulin function and persistent hyperglycemia that mimics the natural stages of progression of diabetes in humans [3]. An aberration in insulin action causes derangement in carbohydrate metabolism resulting from altered activities of enzymes that control glycolysis, gluconeogenesis in liver and muscle and produces hyperglycemia. Presently available oral hypoglycemic drugs have limitations and hence a search for newer drugs is warranted.

Medicinal plants and their bioactive constituents are commonly used worldwide to control diabetes and its complications due to their non-toxic nature, availability and affordability. Hence WHO has recommended the use of traditional plants for the treatment of chronic and life threatening diseases including diabetes mellitus. *Berberis aristata* is a well known medicinal plant whose fruits, stem, bark and root are used in ayurvedic preparations for their diverse medicinal properties. Berbamine (BBM) is a bis-benzylisoquinoline alkaloid derived from the roots of *Berberis aristata*, possess antioxidant, anti-inflammatory, immunomodulatory and cardiovascular effects [4,5]. However there was no study in the literature on the possible antidiabetic effects of

* Corresponding author.
E-mail address: sankarhari05@gmail.com (C. Sankaranarayanan).

https://doi.org/10.1016/j.biopha.2018.04.066
Received 11 January 2018; Received in revised form 9 April 2018; Accepted 9 April 2018

BBM. In this context, the present study was designed to evaluate the effect of BBM on the activities of carbohydrate metabolic enzymes in HFD/STZ induced diabetic rats.

2. Materials and methods

2.1. Chemicals and drugs

BBM and STZ were purchased from Sigma Aldrich Pvt. Ltd., (St. Louis, MO, USA). Insulin kit was purchased from Bioassay Technology Laboratory (Korain Biotech Co. Ltd, China). Diagnostic kits for glucose, hemoglobin (Hb) and glycated hemoglobin (HbA_{1c}) were purchased from Agappe diagnostics Ltd, Kerala, India. All other chemicals used in this study were of analytical grade and obtained from SD fine and HIMEDIA, India.

2.2. Ethical statement for animal experimentation

Forty two male Sprague-dawley rats with body weight ranging from 160 to 180 g were purchased from National Centre for Laboratory Animal Science, Hyderabad. They were housed in well-ventilated cages (temperature 23 ± 2 °C, humidity 65-70% and 12 h light/dark cycle), fed on pellet diet and water *ad libitum*. Studies were carried out in accordance with Indian National Law on Animal Care and Use and were approved by the Institutional ethical committee (Proposal No.1033) of Rajah Muthiah Medical College and Hospital, Annamalai University, India.

2.3. Development of HFD-fed STZ treated type 2 diabetic rats

Rats divided into seven groups with six in each group were placed under normal pellet diet and water for a week prior to dietary manipulation. Experimental animals in five groups were fed with a high-fat diet (HFD: 40%) for 4 weeks. This was followed by a single intraperitoneal injection of freshly prepared STZ (40 mg/kg b.w.) dissolved in 0.1 M citrate buffer (pH 4.5). After one week, fasting blood glucose levels were determined and rats with glucose levels > 250 mg/dl were considered as diabetic and used in this study.

2.4. Experimental design

Animals were grouped and treated as follows for a period of 56 days.

Group I: Control rats fed with normal pellet diet
Group II: Control rats fed with normal pellet diet and administered p.o with an aqueous solution of BBM (200 mg/kg b.w.)
Group III: Diabetic rats fed with high fat diet
Group IV: Diabetic rats administered p.o with an aqueous solution of BBM (50 mg/kg b.w.)
Group V: Diabetic rats administered p.o with an aqueous solution of BBM (100 mg/kg b.w.)
Group VI: Diabetic rats administered p.o with an aqueous solution of BBM (200 mg/kg b.w.)
Group VII: Diabetic rats administered p.o with an aqueous solution of metformin (200 mg/kg b.w.)

2.5. Tissue preparation

At the end of experimental period, rats were fasted overnight, anaesthetized and sacrificed by cervical decapitation. Blood samples were collected in tubes containing potassium oxalate and sodium fluoride (3:1) mixture for the estimation of plasma glucose and insulin. Hb and HbA_{1c} levels were estimated in whole blood samples. Tissues were dissected out, washed in ice-cold saline and homogenized in Tris-HCl buffer (0.1 M, pH 7.5), centrifuged (3000 rpm/min), and the supernatant was collected. Biochemical estimations were carried out in the homogenates.

2.6. Estimation of glucose, insulin, hemoglobin and glycated hemoglobin

Plasma glucose was estimated by the method of Trinder using commercial kit [6]. Plasma insulin was determined using an ELISA reader according to manufacturer's instruction. Hb and HbA_{1c} were estimated using commercial kits by cyanmethemoglobin method of Drabkin and Austin [7] and by the method of Nayak and Pattabiraman [8] with modifications according to Bannon [9] respectively.

2.7. Assay of carbohydrate metabolic enzymes and hepatic glycogen content

Liver homogenate was used to assay hexokinase [10], glucose 6-phosphate dehydrogenase [11], glucose 6-phosphatase [12] fructose 1,6-bisphosphatase [13] and to measure glycogen [14] content respectively.

2.8. Immunohistochemical analysis

An immunohistochemical experiment was performed as described by Babu et al. [15].

The mean percent of insulin positive cells were calculated using ImageJ (rsb.info.nih.gov/ij/), a freely available image analysis software.

2.9. Statistical analysis

All data were expressed as mean ± SD for six rats in each group. The statistical analysis was done by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) using SPSS software package, version 11. $p < 0.05$ was considered as significant and included in the study [16].

3. Results

3.1. Dose dependent effect of BBM on plasma glucose and insulin levels

An increase in plasma glucose with a significant decrease in insulin levels were observed in diabetic rats (Table 1). Oral administration of BBM at three different doses (50, 100 and 200 mg/kg b.w.) for 56 days resulted in significant improvement in plasma insulin with a fall in glucose levels at the end of the treatment period. There was no significant change in normal rats treated with BBM. The effect of BBM was compared with metformin, a standard drug.

3.2. Effect of BBM on changes in body weight, food and fluid intake of control and diabetic treated rats

Table 2 shows the changes in the body weight of control and

Table 1
Effect of BBM on plasma glucose and insulin in normal control and diabetic treated rats.

Groups	Glucose (mg/dl)	Insulin (μU/ml)
Normal Control	85.02 ± 6.47[a]	16.23 ± 0.66[a]
Normal + Berbamine (200 mg/kg b.w.)	84.24 ± 6.45[a]	16.90 ± 1.29[a]
Diabetic (HFD + STZ)	389.06 ± 29.63[b]	5.78 ± 0.44[b]
Diabetic + Berbamine (50 mg/kg b.w.)	245.33 ± 18.78[c]	8.56 ± 0.66[c]
Diabetic + Berbamine (100 mg/kg b.w.)	133.09 ± 10.13[d]	13.53 ± 1.03[d]
Diabetic + Berbamine (200 mg/kg b.w.)	140.07 ± 10.72[d]	13.08 ± 1.00[d]
Diabetic + Metformin (200 mg/kg b.w.)	115.29 ± 8.82[e]	14.88 ± 1.14[e]

Values are expressed as means ± S.D. for six rats. Values not sharing a common superscript (a–e) differ significantly at $p < 0.05$ (DMRT).