Life Sciences 151 (2016) 41–49

Contents lists available at ScienceDirect

Life Sciences

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

Mechanisms of echinochrome potency in modulating diabetic complications in liver

Ayman Saber Mohamed *, Amel Mahmoud Soliman, Mohamed Assem Said Marie

Zoology Department, Faculty of Science, Cairo University, 12613 Giza, Egypt

ARTICLE INFO

Article history:
Received 8 January 2016
Received in revised form 1 March 2016
Accepted 2 March 2016
Available online 3 March 2016

Keywords:
Diabetes
Echinochrome
Oxidative stress
Pancreas
Liver
Histopathology

ABSTRACT

Background: Diabetes mellitus is one of the most public metabolic disorders. It is mainly classified into type 1 and type 2. Echinochrome is a pigment from sea urchins that has antioxidant, anti-microbial, anti-inflammatory and chelating abilities.
Aims: The present study aimed to investigate the anti-diabetic mechanisms of echinochrome pigment in streptozotocin-induced diabetic rats.
Main methods: Thirty six male Wistar albino rats were divided into two main groups, type 1 diabetes and type 2 diabetes groups. Each group was divided into 3 subgroups (6 rats/subgroup); control, diabetic and echinochrome groups. Diabetic model was induced by a single dose of streptozotocin (60 mg/kg, i.p) for type 1 diabetes and by a high fat diet for 4 weeks before the injection with streptozotocin (30 mg/kg, i.p) for type 2 diabetes. Diabetic groups were treated orally with echinochrome extract (1 mg/kg body weight in 10% DMSO) daily for 4 weeks.
Key findings: Echinochrome groups showed a reduction in the concentrations of glucose, MDA and the activities of arginase, AST, ALT, ALP and GGT. While it caused general increase in the levels of insulin, TB, DB, IB, NO and the activities of G6PD, GST, GPx, SOD and GSH. The histopathological investigation showed partial restoration of pancreatic islet cells and clear improvement in the hepatic architecture.
Significance: The suggested mechanism of Ech action in the reduction of diabetic complications in liver involved two pathways; through the hypoglycemic activity and the antioxidant role of Ech.

1. Introduction

Diabetes mellitus (DM) is considered one of the most dangerous metabolic disorders in the world [1]. DM generally classified into type 1 (T1DM) and type 2 (T2DM) diabetes mellitus. T1DM is an autoimmune disease characterized by a loss of insulin secretion due to pancreatic β-cell degeneration and dependence on exogenous insulin for survival [2]. It is characterized by destruction of insulin-producing cells which usually occurs after mononuclear infiltration of the pancreatic islet cells [3]. Mainly, 90–95% of diabetes is diagnosed as T2DM [4]. T2DM is increasing in prevalence worldwide [5], and it is strongly associated with obesity and insulin resistance [6], as well as defects in pancreatic β-cells function and mass [7]. T2DM is a multifactorial disease, where the pathophysiology of which involves not only the pancreas, but also the liver, gastrointestinal tract, adipose tissue and brain [8]. The β cells normally compensate insulin resistance through more insulin production to keep the glucose near the normal level [9]. Reduced sensitivity to insulin in liver, muscle, and fatty tissue lead to impaired insulin secretion and hyperglycemic condition which consider one of the most characteristic feature of T2DM [8]. Oxidative stress has been strongly implicated in the development of diabetes and diabetic complications [10,11]. Metabolic disorders assist the increased reactive oxygen species (ROS) production in the physiological system such as obesity, insulin resistance and diabetes mellitus [12]. Excess production of ROS can overcome the antioxidant system of the body, leading to oxidative stress [13]. Hyperglycemic condition can induce oxidative stress through many mechanisms such as glucose autoxidation, polyol pathway, advanced glycation endproduct (AGE) formation and protein kinase C (PKC) β1/2 kinase [14]. Streptozotocin (STZ) is an antibiotic that was first isolated from the bacterium *Streptomyces achromogens* [15]. The toxic mechanism of STZ is mediated by ROS [16]. Single dose of STZ can induce T1DM in rats [17], while T2DM can be induced by STZ injection after high fat diet (HFD) feeding [18]. In HFD/STZ rat model, HFD induces insulin resistance and low doses of intraperitoneal STZ induce moderate impairment of insulin secretion [18]. Sea urchin (*Paracentrotus lividus*) is a widespread species in the Atlantic and the Mediterranean coasts [19]. It has special substances, such as quinonoid pigments which called spinochromes [20,21]. Echinochrome (Ech) which is found in shells, spines, and eggs of the sea urchins possesses high antioxidant activity [22]. It is a water insoluble compound that possesses strong antioxidant activity and is the active gradient in the

* Corresponding author.
E-mail addresses: ayman81125@hotmail.com, ayman81125@cu.edu.eg (A.S. Mohamed), soliman.amel5@gmail.com (A.M. Soliman), massemmarie@yahoo.com (M.A.S. Marie).

http://dx.doi.org/10.1016/j.lfs.2016.03.007
0024-3205/

Histochrome drug [23]. Ech can act through many antioxidant mechanisms; include reduction of oxidative stress [24], interaction with lipoperoxide radicals [25], chelation of metal ions [26], inhibition of lipid peroxidation [27], and regulation of the cell redox potential [28]. In addition, Popov and Krivoshapko recorded the ability of Ech to reduce blood glucose level in diabetic mice [29]. The present study carried out to evaluate the anti-diabetic efficacy of echinochrome pigment representing its mechanism in streptozotocin-induced types I and II diabetic rats.

2. Materials and methods

2.1. Chemicals and reagents

Streptozotocin, dimethyl sulfoxide (DMSO) and insulin kits were purchased from Sigma-Aldrich (St. Louis, MO, USA). γ-Glutamyltransferase (GGT) kit was purchased from Spectrum Diagnostics Company (Obour City, EGY), while the other kits were purchased from the Biodiagnostic Company (El Moror St, Dokki, EGY).

2.2. Sea urchin collection

Sea urchins (*P. lividus*) were collected from the Mediterranean coast of Alexandria (Egypt) and transported to the laboratory packed in ice. The samples were thoroughly washed with sea water to remove sand and overgrowing organisms at the collection site and transported to the laboratory. The collected specimens were identified by the standard literature of taxonomic guide [30]. The collected specimens were immediately shade dried.

2.3. Echinochrome (Ech) extraction

Pigments in the shells and spines were isolated by the Amarowicz method with slight modifications [31,32]. After removal of the internal organs, the shells and spines were washed with a stream of cold water, air-dried at 4 °C for 2 days in the dark and then were grounded. The powders (5 g) were dissolved by gradually adding 10 ml of 6 M HCl. The pigments in the solution were extracted 3 times with the same volume of diethyl ether. The ether layer collected was washed with 5% NaCl until the acid was almost removed. The ether solution including the pigments was dried over anhydrous sodium sulfate and the solvent was evaporated under reduced pressure. The extract including the polyhydroxylated naphthoquinone pigment was stored at −30 °C in the dark.

2.4. Experimental animals

Male albino Wistar rats (*Rattus norvegicus*) weighing 140 ± 10 g for T1DM and 80 ± 10 g for T2DM were used in this study. The rats were obtained from the National Research Center (NRC, Dokki, Giza). Rats were housed in a temperature and humidity controlled environment and given food and water ad libitum.

2.5. Ethical consideration and field study

Experimental protocols and procedures used in this study were approved by the Cairo University, Faculty of Science, Institutional Animal Care and Use Committee (IACUC) (Egypt) (CUFS/F/33/14). All the experimental procedures were carried out in accordance with international guidelines for the care and use of laboratory animals.

2.6. Induction of type 1 diabetes mellitus (T1DM)

All rats were starved for 12 h before the experiment, but were allowed free access to water. T1DM was induced by intraperitoneal injection of 60 mg/kg of streptozotocin (STZ) dissolved in 0.1 mol/l sodium citrate buffer at pH 4.5. Blood glucose levels were measured 72 h after injection of STZ. Rats were starved, but had access to drinking water for 6 h before blood glucose measurement. Fasting plasma glucose concentrations ≥300 mg/100 ml were considered diabetic type 1 in this experiment [33].

2.7. Induction of type 2 diabetes mellitus (T2DM)

The rats were fed a high fat diet with an energy of 5.3 kcal/g, comprising 60% calories from fat, 35% from protein and 5% from carbohydrate, according to a modification of the protocols of Reed et al. [34]. After 4 weeks the rats were injected intraperitoneally by a single dose of prepared solution of STZ (30 mg/kg dissolved in 0.1 mol/l sodium citrate buffer at pH 4.5). After 72 h, fasting plasma glucose concentrations ≥300 mg/100 ml were considered diabetic type 2 in this experiment [35].

2.8. Experimental design

After one week of acclimatization, 36 rats were assigned into two main groups; T1DM group (18 rats) and T2DM group (18 rats).

T1DM group was divided into 3 subgroups (6 rats/subgroup):

Control subgroup: after a single dose of citrate buffer (0.1 mol/l, i.p), the rats received 1 ml (10% DMSO, orally) daily for 4 weeks.
Diabetic subgroup: after a single dose of STZ (60 mg/kg, i.p), the rats received 1 ml (10% DMSO, orally) daily for 4 weeks.
Ech subgroup: after a single dose of STZ (60 mg/kg, i.p), the rats received 1 ml Ech (1 mg/kg body weight in 10% DMSO, orally) [36] daily for 4 weeks.

T2DM group was divided also into 3 subgroups (6 rats/subgroup):

Control subgroup: after 4 weeks of normal diets feeding, the rats injected with a single dose of citrate buffer (0.1 mol/l, i.p) then received 1 ml of (10% DMSO, orally) daily for 4 weeks.
Diabetic subgroup: after 4 weeks of HFD feeding, the rats were injected with a single dose of STZ (30 mg/kg, i.p) then received 1 ml of (10% DMSO, orally) daily for 4 weeks.
Ech subgroup: after 4 weeks of HFD feeding, the rats were injected with a single dose of STZ (30 mg/kg, i.p) then received 1 ml Ech (1 mg/kg in 10% DMSO, orally) daily for 4 weeks.

2.9. Animal handling and specimen collection

After the end of all experiments, the rats were fully anesthetized with 3% sodium pentobarbital, and the chest was opened. A needle was inserted through the diaphragm and into the heart. Negative pressure was gently applied once the heart had been punctured, and the needle was repositioned as required until blood flowed into the syringe. The blood collected from the rats was separated by centrifugation (3000 rpm, 15 min) to obtain sera which were stored at −80 °C for the biochemical measurements.

Liver and pancreas were removed and were immediately blotted using a filter paper to remove traces of blood. Part of the liver was stored at −80 °C for biochemical analysis. The pancreas and the other part of the liver were suspended in 10% formal saline for fixation preparatory to histopathological examination.

2.10. Liver homogenate preparation

Liver tissue was homogenized (10% w/v) in ice-cold 0.1 M Tris–HCl buffers (pH 7.4). The homogenate was centrifuged at 860 ×*g* for 15 min. at 4 °C and the resultant supernatant was used for the biochemical analyses.