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Effects of Berberine chloride on the liver of streptozotocin-induced diabetes in albino Wistar rats

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ABSTRACT

The goal of the present study is to evaluate the effect of Berberine chloride (BC) on the liver of streptozotocin (STZ) induced diabetic rat. Diabetic rats were treated with BC (50 mg/kg b.w) or glibenclamide (6 mg/kg b.w), daily for 45 days. After BC treatment in diabetic rats, there was a significant ($P < 0.05$) decline in the levels of hepatic markers, lipid peroxidation markers such as lipid hydroperoxides (LOOH) and thiobarbituric acid reactive substances (TBARS), and pro-inflammatory mediators like tumor necrosis factor-alpha (TNF-α), phosphorylated nuclear factor kappa-B-p65 (phospho-NF-κB p65), cyclooxygenase (COX-2), nitric oxide synthase (iNOS) as well as pro-apoptotic mediators such as Bax and cytochrome c. A significant ($P < 0.05$) increase in hexokinase, glucose-6-phosphate dehydrogenase, enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), and non-enzymatic antioxidants such as glutathione (GSH), vitamin E and vitamin C, as well as anti-apoptotic protein Bcl-2 were observed in the liver of BC treated diabetic rats. Thus, from these findings, it can be concluded that the administration of BC notably recovered the liver from hyperglycemia induced antioxidant imbalance, inflammation and apoptosis as well as rectified the imbalance in carbohydrate metabolizing enzymes.

1. Introduction

Diabetes mellitus is an endocrine disease characterized by chronic hyperglycemia linked with irregularities in carbohydrate, lipid and protein metabolism caused by insufficiency of insulin secretion and/or insulin action, and usually accompanied by a variety of microvascular, macrovascular, neurologic and infectious complications [1]. Globally, the diabetic population had raised from 108 million in 1980 to 422 million in 2014 in which the age group of above 18 years old, has raised from 4.7% in 1980 to 8.5% in 2014 [2]. According to IDF 2015, the diabetic population is expected to rise to 642 million people in 2040, and every 6 s a person dies due to diabetes. In India alone, 69.2 million people have suffered from diabetes, and this will increase to 123.5 million in 2040 [2].

Physiologically, glucose level is regulated by the equilibrium between hepatic glucose production (gluconeogenesis and glycogenolysis) and utilization of glucose by peripheral tissues [3]. Increased hepatic glucose production due to lack of insulin secretion/action is the major cause of hyperglycemia in diabetes [3]. Hyperglycemia promotes oxidative stress, inflammation and apoptosis in tissues [4,5]. Glucose autooxidation and protein glycosylation [6] are responsible for the production of free radicals in tissues [7] that promote inflammation and apoptosis [8]. Inflammation can also stimulate more oxidative stress in tissues [9].

Berberine is a plant isoquinoline alkaloid that can be found in many herbs like *Hydrastis Canadensis* (goldenseal), *Rhizoma coptidis* (Huanglian), *Cortex phellodendri* (Huangbai), *Coptis chinensis* (Coptis or golden thread), *Berberis vulgaris* (barberry), *Berberis aquifolium* (Oregon grape), and *Berberis aristata* (tree turmeric) [10]. All these herbs have long been used in Chinese and Ayurvedic medicines, and BC is the major phytocompound found in these herbs [11]. BC has been used for its various pharmacological activities like anti-microbial, anti-diarrheal, anti-protozoal, anti-trachoma activity, and anti-schistosomal activity [10,12,13]. Berberine has also been reported to be a promising candidate for the treatment of cardiac disorders, hyperlipoidemia and chronic inflammation diseases [11]. The classical technique utilized for berberine isolation is either extraction by alcohol in a neutral medium or by the addition of acetic acid. Further, the purification of berberine is obtained commonly by precipitation as berberine chloride or hydrosulphate [14]. Earlier Moghaddam et al. [15] and Chandirasegaran et al. [16] reported that BC has an ameliorating effect towards STZ-induced diabetic rats. Therefore, the goal of this study was to evaluate

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the effect of BC on hepatic glucose production, oxidative status, inflammation and apoptosis in the liver of STZ-induced diabetes albino Wistar rats.

2. Materials and methods

2.1. Chemicals

Monoclonal antibodies for pNF-κB p65, iNOS, COX-2, Bcl-2, and cleaved caspase-3 and secondary antibodies were purchased from Cell Signalling Technology, Inc. (CST) (Danvers, Massachusetts). Antibodies for Bax and cytochrome c were purchased from BioLegend Inc. (San Diego, CA) and Abcam, respectively. BC and STZ were procured from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade and were purchased from Himedia, India.

2.2. Animals

Healthy male albino Wistar rats (180–190 g b.w) obtained from the Central Animal House, Annamalai University were housed in polycarbonate cages and maintained under constant 12 h light and dark cycle, and room temperature at 25 ± 2 °C. Rats were fed with standard rodent pellet food (Hindustan Lever Ltd, Mumbai, India) and water was provided *ad libitum*. Before the start of the experiment, animals were acclimatized to laboratory condition for one week. This study was approved by the Animal Ethics Committee of Rajah Muthiah Medical College and Hospital (Reg No 166/1999/CPCSEA, Proposal No. 1085).

2.3. Induction of diabetes in male albino Wistar rats

Diabetes was induced in rats by administrating a single dose of intraperitoneal injection of STZ (40 mg/kg b.w) in a buffer (0.1 M citrate buffer, pH 4.5) [17,18]. The STZ treated rats were allowed to drink 5% glucose solution for preventing the drug-induced hypoglycemia. After 3 days of STZ injection, blood was collected from experimental animals from the tail vein, and the blood glucose level was measured. STZ treated rats with fasting blood glucose level of above 230 mg/dl were considered diabetic and used for further study [18].

2.4. Experimental design

A total of 24 rats were divided into four groups of six animals each (6 normal rats and 18 diabetic rats). The optimal dosage of BC 50 mg/kg b.w was fixed based on previous experiments by Chandirasegaran et al. [19].

Group 1- Normal control rats

Group 2- Diabetic control rats

Group 3- Diabetic + BC (50 mg/kg b.w) treated rats

Group 4- Diabetic + Glibenclamide (Reference drug) (6 mg/kg b.w) treated rats [20]

BC or glibenclamide were dissolved in distilled water and administered to STZ induced diabetic rats orally by intragastric intubation, daily for 45 days. The experimental design was based on the previous work by Ramachandran et al. [21].

2.5. Biochemical analysis

On the morning of 46th day, all the experimental rats were sacrificed by cervical decapitation. The blood was collected from experimental animals by cardiac puncturing and serum was separated for analyzing hepatic marker enzymes. The liver was harvested from all experimental rats, washed with ice-cold saline. The harvested liver was used for histopathology and estimation of lipid peroxidation and antioxidant status, as well as the levels of carbohydrate metabolizing enzymes.

2.5.1. Estimation of liver glycogen

Liver glycogen was evaluated by the method of Shirwaikar et al. [22].

2.5.2. Assay of hepatic marker enzymes

The hepatic marker enzymes like aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in serum were estimated using diagnostic kits (Span Diagnostics Ltd, Surat, Gujarat, India).

2.5.3. Determination of carbohydrate metabolizing enzymes

Activities of hepatic hexokinase, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase and fructose-1, 6-bisphosphatase were measured by the methods of Brandstrup et al. [23], Ellis and Kirkman [24], Koide and Oda [25] and Gancedo and Gancedo [26], respectively.

2.5.4. Estimation of lipid peroxidation and antioxidants

Levels of TBARS and LOOH in the liver were measured by the method of Ohkawa et al. [27]. The activity of SOD was evaluated by the method of Kakkar et al. [28]. The activity of CAT enzyme was measured by the method given by Sinha [29]. GPx was evaluated by the method given by Rotruck et al. [30]. The activity of GSH was assessed by the method of Ellman [31]. Vitamin C and E were evaluated by Omaye et al. [32] and Baker and Frank [33] respectively.

2.6. Histopathology

The liver tissue was fixed in 10% formalin for 48 h. It was then followed by dehydration by passing through a series of graded alcohol, beginning with 50% alcohol and progressing in graded step to 100% (absolute) alcohol, and was finally embedded in paraffin. Sections of the liver (5–6 μm thick) were developed using semi-automated rotator microtome, stained with Hematoxylin and Eosin dye and observed microscopically.

2.7. Western blotting analysis

Total protein from the liver tissue was extracted by homogenizing tissue with 1 ml of a buffer containing 10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 0.5 mM PMSF and 10 μl protease inhibitor cocktail. The homogenate was then centrifuged at 8000 × g for 2 min at 4 °C, the supernatant was collected and stored at −80 °C. The protein concentration of the supernatant was determined by the method of Lowry et al. [34]. SDS-PAGE was performed using equivalent protein extracts (50 μg) from each sample. The resolved proteins obtained were then electrophoretically transferred to poly vinylidene difluoride membranes. The blots were incubated in 1 × PBS containing 5% non-fat dry milk for 2 h to block nonspecific binding sites. The blots were further incubated with 1:200 dilution of primary antibodies overnight at 4 °C. After washing, the blots were with 1:1000 dilution of horseradish peroxidase-conjugated secondary antibody for 45 min at room temperature. After continuous washes with high and low salt buffers, the immunoreactive proteins were visualized using enhanced chemiluminescence detection reagents (Sigma-Aldrich). Densitometry was performed on IISP flat bed scanner and quantitated with Total Lab 1.11 software.

2.8. Immunohistochemistry

Immunohistochemistry was performed by using super-sensitive polymer-HRP detection system kit, from Biogenex, USA. Briefly, the tissue sections (pancreas) of 4–5 μm were mounted on poly-L-lysine-coated glass slides. They were deparaffinized by placing the slides in an oven at 60 °C for 10 min and then rinsed twice in xylene for 10 min each. The slides were then hydrated in series of graded ethanol (60, 80