Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2017, 9(3):69-73

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Protective Role of Berberine Chloride on Blood Components in Streptozotocin Induced Diabetic Rats

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ABSTRACT

The aim of this study is to observe the hematological alterations in berberine chloride (BC) treated streptozotocin (STZ) induced diabetic rats. Diabetes was experimentally induced in healthy male rats by a single dose of intraperitonial injection of STZ (40 mg/kg b.w). After the 72 hours, diabetic rats treated with BC at different concentrations (25, 50 and 100 mg/kg b.w) for 45 days. BC administration significantly declined the level of blood glucose, white blood cells (WBC) and glycated hemoglobin (HbAlc) whereas elevated the levels of plasma insulin, hemoglobin, red blood cells (RBC), haematocrit (Ht), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) in diabetic rats. 50 and 100 mg/kg b.w of BC showed the prominent effect compared to 25 mg/kg b.w. In which, there is no any significant between dosage 50 and 100 mg/kg b.w. These findings clearly showed that the treatment of BC notably protected the blood components against STZ induced diabetic rats.

Keywords: Berberine chloride; Blood glucose**;** Insulin; Red blood cells; White blood cells

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INTRODUCTION

Diabetes mellitus, a chronic disease, occurs either when the pancreas does not generate adequate insulin, i.e., a hormone that adjusts the blood glucose level, or the body cannot effectively utilizes the insulin it produces [1]. Blood glucose elevation or hyperglycemia is a general effect of uncontrolled diabetes, leading to severe damage to the blood vessels, heart, eyes, kidney as well as nerves [2]. In the past three decades, the incidence of diabetes has switched from being a mild disorder among elderly population into one of the leading causes of morbidity and mortality affecting youth and middle aged population [3]. India has been ranked as second in the world in diabetes prevalence, after China [4]. The haematological studies are essential in finding out toxicity, which is a widely used parameter. The activity of toxic agents, primarily those with mutagenic or cytotoxic potential, limits the use of medicine. Estimation of haematological parameters helps screen the drugs that could stimulate the haemolymphopoetic system [5].

BC is a plant alkaloid, present in many medicinal plants, which has many pharmacology activities. Previous studies Moghaddam *et al*. [6] and Chandirasegaran *et al*. [7] have reported that the amelioration of synaptic plasticity and reduced blood glucose by BC treatment in STZ induced diabetic rats. Many oral diabetic agents are available in markets, which are giving some side effects like increase body weight and hypoglycemia etc. Hence, the intention of this study is to find out hematological alteration in BC in STZ induced diabetic rats.

MATERIALS AND METHODS

Animals

About 180-200g weighting male albino Wistar rats were used for this experiment. During the whole investigational period, standard pellet diet was given to the rats and water *ad libitum*. This present study was accepted by the Animal Ethical Committee, Rajah Muthiah Medical College and Hospital, Annamalai University (Reg No. 166/1999/CPCSEA and Proposal No. 1085).

Chemicals
Berberine Chloride and STZ were purchased from Sigma – Aldrich (St. Louis, MO, USA) and other chemicals were obtained from E. Merck, Himedia (Mumbai, India). All of the chemicals and reagents used in these experiments were analytical grade.

Induction of diabetes
Diabetes was induced in healthy male albino rats by intraperitoneally injecting the freshly prepared solution of STZ (40 mg/kg b.w), which was prepared in citrate buffer (0.1M; pH 4.5). After the three days, STZ injected rats, showing fasting blood glucose values above 230 mg/dl were considered as diabetic, and those animals were used for further study.

Experimental design
A total of 36 rats were used in this experiment and divided into six groups; each group consists of 6 rats. BC and glibenclamide were dissolved in water.

Group 1- Normal Control rats
Group 2- Diabetic Control rats
Group 3- Diabetic + Berberine chloride (25 mg/kg b.w)
Group 4- Diabetic + Berberine chloride (50 mg/kg b.w)
Group 5- Diabetic + Berberine chloride (100 mg/kg b.w)
Group 6- Diabetic + Glibenclamide (6 mg/kg b.w)

Diabetic rats were treated with different doses of BC (25, 50 and 100 mg/kg b.w) and glibenclamide whereas normal and diabetic control rats were fed with distilled water alone. Treatment was given to experiment rats at every day morning for 45 days.

Biochemical estimation
On the 45^{th} day, the animals were sacrificed by cervical dislocation, and the blood samples were collected for analysis of biochemical parameters. Blood glucose was estimated by the method of Trinder, [8]. Plasma insulin was measured by ELISA kit (Boehringer Mannheim kit). Hemoglobin and glycated hemoglobin were estimated using a diagnostic kit (Agape Diagnostic Pvt. Ltd, India) (Bisse and Abragam, [9]. The total amount of red blood cells (RBC), white blood cells (WBC), haematocrit (Ht), mean cell hemoglobin (MCH/MEH) and mean cell hemoglobin concentration (MCHC) were evaluated and calculated by method of Dacie and Lewis, [10].

Statistical analysis
Values obtained were represented as the means ± S.D. for six rats in each group. All the data were analyzed by one-way analysis of variance, which was followed by Tukey multiple comparison tests, using the SPSS version 15 (SPSS, Chicago, IL). The limit of statistical significance was set at $p < 0.05$.

RESULTS

Effect on BC on fasting blood glucose
Figure 1 depicts the level of blood glucose in normal and experimental rats. The level of blood glucose was found to be high in diabetic control rats when compared to normal control rats. The continuous treatment of different dose of BC (25, 50 and 100 mg/kg b.w) for 45 days to diabetic rats significantly declined the elevated levels of blood glucose in diabetic rats. BC 50 and 100 mg/kg b.w produced conspicuous effects which are similar with effect of glibenclamide.

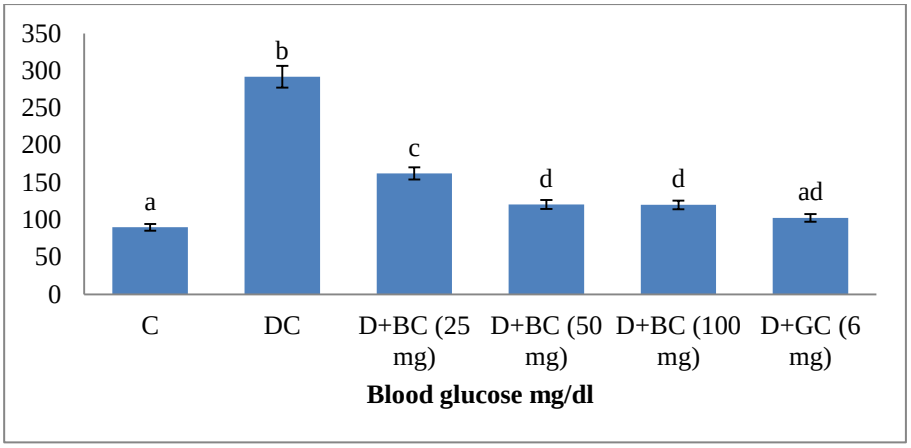

Figure 1 Effect of BC on blood glucose

All the data are expressed as the mean ± S.D. for 6 rats. The results with different superscripts (a,b,c..) in each experiment are significantly different at $p < 0.05$.

Effect of BC on plasma insulin

The level of plasma insulin was significantly declined in STZ induced diabetic control rats as compared to normal control rats.

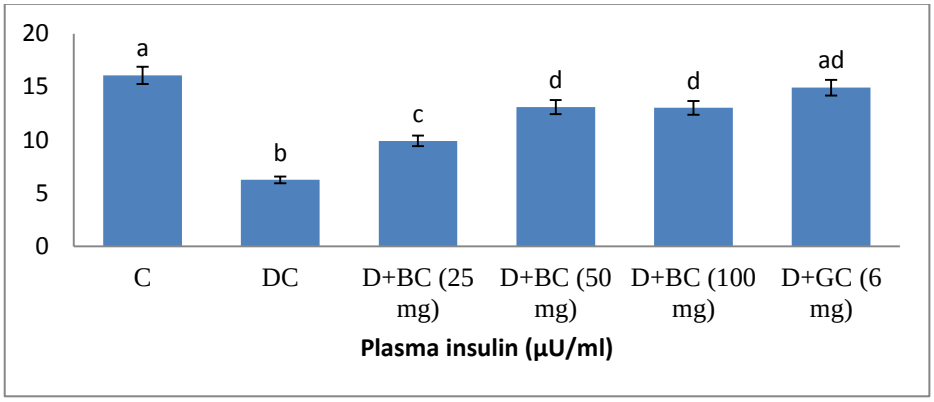

Figure 2: Effect of BC on plasma insulin

All the data are expressed as the mean ± S.D. for 6 rats. The results with different superscripts (a,b,c..) in each experiment are significantly different at $p < 0.05$.

Three doses of BC and glibenclamide treated groups exhibited significant ($p < 0.05$) elevation of plasma insulin in diabetic control rats. From the three doses, BC 50 and 100 mg/kg b.w dosage reverted the levels back to normal comparable with glibenclamide (Figure 2).

Effect of BC on blood components

In diabetic rats, the total level of RBC, MCH, MCHC, haematocrit and WBC were observed, in which RBC, hemoglobin, MCH, MCHC and haematocrit levels significantly declined as well as WBC and glycated hemoglobin levels increased. Three dosages of BC drastically recovered the RBC, hemoglobin, glycated hemoglobin, MCH, MCHC, haematocrit and WBC levels in diabetic rats. From three doses, 50 and 100 mg of BC improved levels nearly found to be as glibenclamide (Table1).

DISCUSSION

In the present study, STZ was used for induction of diabetes because the cytotoxic action of STZ selectively destroys β-cells of the pancreas without affecting other cells by generating excess ROS and carbonium ion (CH^{3+}) leading to DNA breaks by alkylation DNA bases causing oxidative damage [11]. The dose of 40 mg/kg b.w of STZ has the ability to incomplete destruction of β-cells of pancreas, which considered as type 2 diabetes

[12]. STZ induced diabetic rat showed increased the blood glucose level due to impaired carbohydrate lipid and protein metabolism, which caused by insufficient insulin secretion from the pancreas. BC treated diabetic rats showed notably declined the levels of blood glucose and also increased levels of plasma insulin levels. These results clearly demonstrate the antidiabetic activity of BC. In the diabetic situation, anemia arises due to elevated non-enzymatic protein glycosylation of RBC membrane, which associates with increased blood glucose [13].

Table 1: Effect of BC on Blood Components

Groups/ parameters	RBC x106/mm3	WBCx103/mm3	Hemoglobin (mg/mL)	HbAlc (ng/mL)	Ht%	MCH pg	MCHC%
Normal control	7.12 ± 0.54^{a}	5.49 ± 0.42^{a}	13.26 ± 1.01^{a}	0.42 ± 0.03^{a}	38.42 ± 2.93^{a}	18.62 ± 1.42^{a}	34.51 ± 2.70^{a}
Diabetic control	4.86 ± 0.37^{b}	7.14 ± 0.54^{b}	6.26 ± 0.48^{b}	1.12 ± 0.09^{b}	31.63 ± 2.41^{b}	12.88 ± 0.98^{b}	19.78 ± 1.51^{b}
D + BC (25 mg/kg)	5.82 ± 0.44^{c}	6.59 ± 0.50^{bc}	8.72 ± 0.66^{c}	0.82 ± 0.06^{c}	33.96 ± 2.59^{b}	14.98 ± 1.14^{b}	25.67 ± 1.95^{c}
D + BC 50 (mg/kg)	6.49 ± 0.49^{ac}	5.88 ± 0.45^{ac}	11.47 ± 0.88^{d}	0.61 ± 0.05^{d}	36.48 ± 2.78^{ab}	17.67 ± 1.35^{a}	31.78 ± 2.42^{a}
D + BC 100 (mg/kg)	6.56 ± 0.50^{ac}	5.92 ± 0.45^{ac}	11.68 ± 0.89^{d}	0.60 ± 0.05^{d}	36.48 ± 2.78^{ab}	17.80 ± 1.36^{a}	32.01 ± 2.44^{a}
D + GC (6 mg/kg)	6.76 ± 0.51^{a}	5.61 ± 0.43^{a}	12.57 ± 0.96^{ad}	0.48 ± 0.04^{a}	37.24 ± 2.84^{a}	18.59 ± 1.42^{a}	33.75 ± 2.57^{a}

All the data are expressed as the mean ± S.D. for 6 rats. The results with different superscripts (a,b,c..) in each experiment are significantly different at $p < 0.05$.

Oxidation of RBC membrane and high blood glucose in diabetes result in the elevated production of lipid peroxides that lead to hemolysis of RBCs [14]. However, some phytochemicals, such as alkaloids and flavonoids can trigger the production and secretion of erythropoietin, a glycoprotein hormone, which enhance the production of RBC from stem cells in the bone marrow [15]. In the present study, the results indicate that the abnormal levels of RBCs parameters like Hb, MCHC, MCH and haematocrit levels were found to be improved in BC treated diabetic rats.

White Blood Cells are mostly processing in protecting the body from infection or injury [16]. According to the previous report, the increased levels of WBC indicating the improved level of immune system or pathogenic conditions [13,17]. BC group showing declined levels of WBC, which demonstrating mended injure in the animals.

Glycated hemoglobin is a very reliable index to monitor glucose lowering therapy and also for long-term blood sugar control [18]. In persistent hyperglycemia, there is a raise in non-enzymatic glycation, which is formed between glucose and the N-end of the beta chain of Hb, forming glycated hemoglobin. Further, glucose and dicarbonyl compounds can also react with hemoglobin, forming advanced glycation end products, which can contribute to the additional development of complications in diabetes. The extent of increased glycated hemoglobin levels is found to be directly proportional to the fasting blood glucose levels in diabetic patients [19]. In the present study, an elevated level of glycated hemoglobin was observed in STZ induced diabetic rats due to the increased formations of glycated hemoglobin. Administration of BC and glibenclamide notably decreased the level of glycated hemoglobin as a result of decreased blood glucose level and increased the insulin secretion.

CONCLUSION

The present study, we analyzed the effect different doses (25, 50 and 100 mg/kg b.w) of BC in STZ induced diabetic rats. BC (50 and 100 mg/kg b.w) treatment remarkably increased the insulin secretion, blood components as well as reduced blood glucose levels as nearly to glibenclamide. The improved levels of blood cells were indicating the BC proceeding against diabetes. BC 50 mg/kg b.w considered as the optimal dose for further research, because there are no significant changes between 50 and 100 mg/kg b.w.

ACKNOWLEDGEMENT

The authors wish to acknowledge the University Grants Commission, New Delhi, Project No.41-178/2012/(SR) for funding this work and also extend our thanks to the Department of Zoology (UGC – SAP Sponsored), Annamalai University, for providing the infrastructure facility and support.

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