Research Article

EFFICACY OF BERBERINE CHLORIDE ON HYPERGLYCEMIA IN STREPTOZOTOCIN INDUCED DIABETIC RATS

Govindasami Chandirasegaran [1], Chakkaravarthy Elanchezhiyan [1]*, Kavisa Ghosh [1], Subramaniyan Sethupathy [2]
[1]Department of Zoology, Annamalai University, Tamil Nadu, India
[2]Department of Biochemistry, Rajah Muthiah Medical College and Hospital, Annamalai University, Tamil Nadu, India
*Corresponding Author Email: chezhiyanzooau@gmail.com

ABSTRACT

The aim of this study to find out the perfect dose of Berberine chloride (BC) in streptozotocin (STZ) induced diabetic rats by observation of effect of BC on blood glucose, plasma insulin, glycated hemoglobin and hemoglobin. Experimental diabetes was induced in rats by a single dose of intraperitonial injection of streptozotocin (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 levels of blood glucose, glycated hemoglobin and renal markers whereas elevated the levels of plasma insulin, hemoglobin and body weight in diabetic rats. 50 mg/kg b.w of BC showed the prominent effect compared to other two doses. From these results clearly shows the antidiabetic activity of BC.

Keywords: Berberine chloride, Streptozotocin, Diabetes, Insulin and Blood glucose

INTRODUCTION

Diabetes mellitus (DM) is characterized by increased blood glucose levels and insufficiency of insulin secretion/action. Such setting causes the impairment of glucose uptake in the peripheral tissues and reduced the glucose consumption for energy purposes[1-2]. 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].

Plants have long been the sources of various medicines for the Indian traditional system of medicine like ayurveda and siddha[5]. BC (Figure 1) is a plant alkaloid, present in many medicinal plants, which have many pharmacology activities. Previous studies have reported that BC has a wide range of pharmacological and biological activities including anti-protozoal functions[6]. Dkhil[7] also reported the anti-malarial and antischistosomal activities of BC as well as its ameliorative effect on the induced liver injury inflicted by *Schistosoma mansoni* infection[8]. In addition[9-10] reported 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[11]. Hence, this study was aimed to investigate the antidiabetic activity of BC in STZ induced diabetic rats.

MATERIALS AND METHODS

Animals

Healthy male albino Wistar rat's weighting180-200g were used for this experiment. During the experimental period, standard pellet diet provided to rats and water *ad libitum*.

This study was approved by the Animal Ethics Committee of Rajah Muthiah Medical College and Hospital, Annamalai University (Reg No 166/1999/CPCSEA, Proposal No. 1085).

Chemicals

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

Induction of diabetes

Diabetes was induced in rats by intraperitonial injection of a freshly prepared solution of STZ (40 mg/kg b.w) in citrate buffer (0.1M; pH 4.5). After the three days STZ induced rats were having fasting blood glucose above 230 mg/dl were considered as diabetic that 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 various 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. Blood samples

were collected on 0, 22nd and 45th day from the tail veins of all rats for blood glucose estimation by the method of Trinder [12].

Biochemical estimation

On the 45th day, the animals were sacrificed by cervical dislocation and the blood samples were collected for analysis of biochemical parameters. 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) [13]. Plasma urea and creatinine were estimated by the diacetylmonoxime method of [14-15] respectively. Uric acid was measured according to kit manufacturer's protocol (Automated Span Diagnostic Reagents, Mumbai, India).

Statistical analysis

Values are given as means ± S.D for six rats in each group. Data were analyzed by one-way analysis of variance followed by Duncan's Multiple Range Test (DMRT) using SPSS version 15 (SPSS, Chicago, IL). The limit of statistical significance was set at $p < 0.05$.

RESULTS

Effect on BC on body weight

Reductions in the body weight of diabetic rats were observed, whereas increases in the body weight were observed in normal control rats. The treatment with three doses of BC and glibenclamide significantly ($p < 0.05$) increased the body weight of diabetic rats, whereas the body weight of diabetic control rats were decreased. Among the three doses of BC 50 mg/kg b.w

was found to be better in comparison with other two doses (25 and 100 mg/kg B.w) (Figure 2).

Effect on BC on fasting blood glucose

Table 1 depicts the level of blood glucose in normal and experimental animals. The level of plasma glucose was observed in normal and experimental animals in 0, 22nd and 45th days of treatment. The blood glucose levels were also significantly ($p < 0.05$) declined in BC treated diabetic rats when compared with diabetic control rats and BC 50 and 100 mg/kg b.w produced a conspicuous effect.

Effect of BC on plasma insulin, blood hemoglobin and glycated hemoglobin

Plasma insulin and hemoglobin levels were significantly declined, whereas glycated hemoglobin level was increased in STZ induced diabetic control rats. BC and glibenclamide treated groups exhibited significant ($p < 0.05$) elevation of plasma insulin, hemoglobin and decline of glycated hemoglobin. BC 50 and 100 mg/kg b.w dosage reverted the levels back to normal comparable with glibenclamide (Table 2).

Effect of BC on kidney functional markers

Figure 3 show the levels of urea, uric acid and creatinine, which were significantly, elevated in diabetic control rats. BC at and glibenclamide treated rats exhibited significantly ($p < 0.05$) lesser levels of urea, uric acid and creatinine. Based on our observations, 50 and 100 mg/kg b.w were found to be better effective.

Table 1: The level of blood glucose (mg/dl) in control and experimental rats

Groups	0 day	22 day	45 day
Normal control	83.61 ± 6.37	85.91 ± 6.54	89.81 ± 6.84[a]
Diabetic normal	2.46.48 ± 18.87	262.73 ± 20.11	291.87 ± 22.34[b]
Diabetic + BC (25 mg /kg b.w)	248.02 ± 18.89	190.63 ± 14.52	162.05 ± 12.34[c]
Diabetic + BC (50 mg /kg b.w)	248.32 ± 19.01	171.71± 13.14	120.37 ± 9.21[d]
Diabetic + BC (100 mg /kg b.w)	244.84 ± 18.64	170.04 ± 12.95	119.86 ± 9.13[d]
Diabetic + Glibenclamide (6 mg /kg bw)	253.19 ± 19.38	162.14 ± 12.41	102.51 ± 8.38[ad]

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 $p < 0.05$.

Table 2: The level of plasma insulin hemoglobin and glycated hemoglobin in control and experimental rats

Groups	Insulin (µU/ml)	Hemoglobin (mg/mL)	HbAlc (ng/mL)
Normal control	16.08 ± 1.22[a]	13.26 ± 1.01[a]	0.42 ± 0.03[a]
Diabetic control	6.86 ± 0.53[b]	6.26± 0.48[b]	1.12±0.09[b]
Diabetic + BC (25 mg /kg b.w)	9.91 ± 0.75[c]	8.72 ± 0.66[c]	0.82 ± 0.06[c]
Diabetic + BC (50 mg /kg b.w)	13.09 ± 1.00[d]	11.47 ± 0.88[d]	0.61 ± 0.05[d]
Diabetic +BC (100 mg /kg b.w)	13.22 ± 1.01[d]	11.68 ± 0.89[d]	0.60 ± 0.05[d]
Diabetic + Glibenclamide (6 mg/kg b.w)	14.92±1.14[a]	12.57 ± 0.96[ad]	0.48 ± 0.04[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 $p < 0.05$.
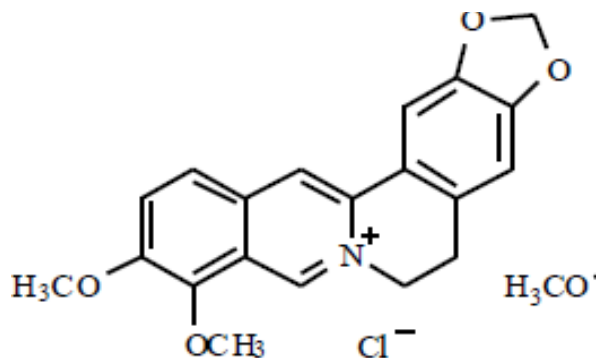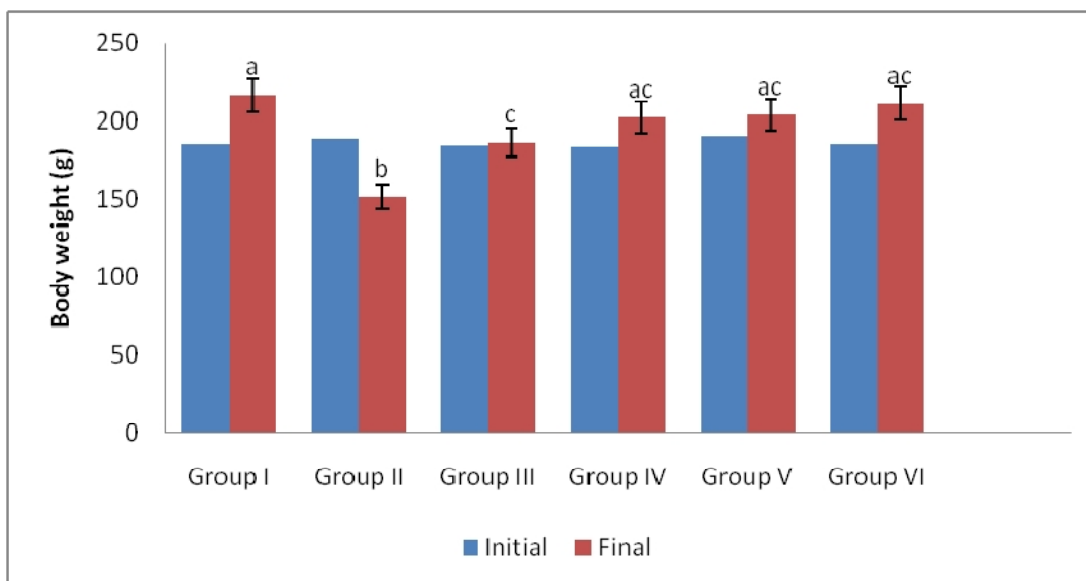
**Figure 1: Structure of Berberine chloride**

**Figure 2: The level of body weight in control and experimental rats**
Group I: Control; Group II: Diabetic control; Group III: Diabetic + BC 25 (mg/kg b.w); Group IV: Diabetic + BC (50 mg/kg b.w); Group V: Diabetic + BC (100 mg/kg b.w); Group VI: Diabetic + Glibenclamide (6 mg/kg b.w). 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 $p < 0.05$.
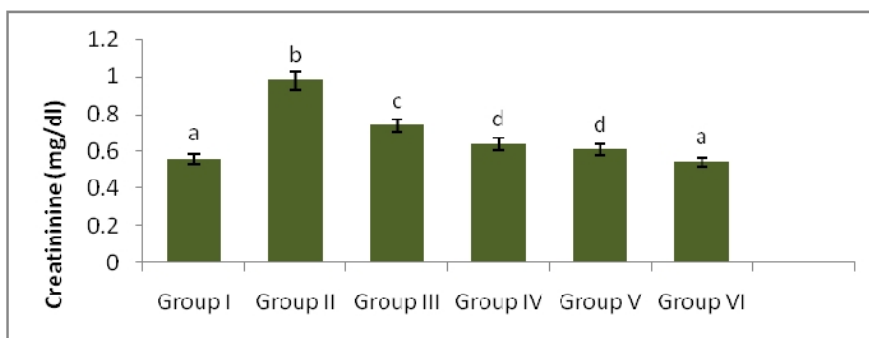
A)
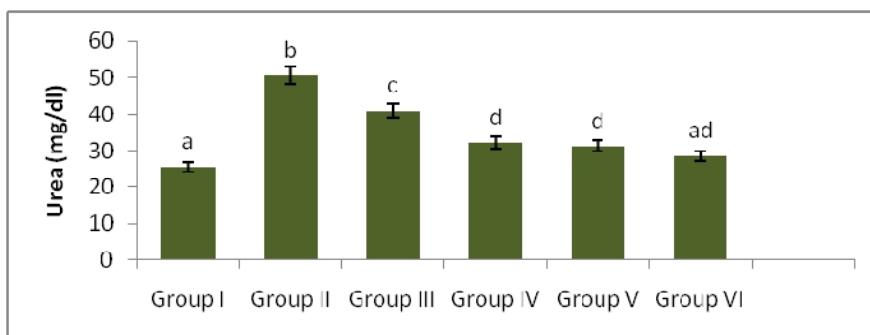
B)
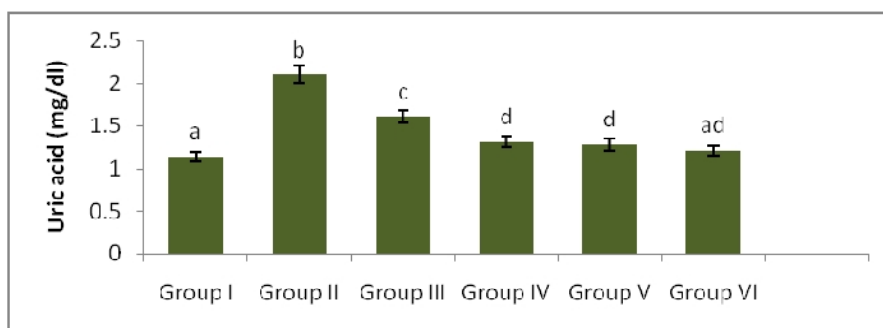
C)

Figure 3: A), B) & C). Effect of BC on STZ induced alteration on renal markers of control and experimental rats in each groups. 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.

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[16]. Dose of 40 mg /kg b.w of STZ have the ability to incomplete destruction of β-cells of pancreas, which considered as type 2 diabetes[17]. 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 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 STZ induced diabetic rats, body weight has been decreased due to lipolysis, muscle destruction or degeneration of structural proteins as a consequence of insulin insufficiency[18-19]. Treatment of BC and glibenclamide significantly improved body weight of STZ induced diabetic rats, which could be due to increased insulin secretion.

Glycated hemoglobin is a very reliable index to monitor glucose lowering therapy and also for long-term blood sugar control[20]. 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[21]. In the current 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 insulin secretion.

Renal maintains optimal chemical composition of body fluid by acidification of urine and removal of metabolic wastes such as urea, uric acid, creatinine and ions. During renal diseases, the concentration of these metabolites increases in blood[22]. In the present study it was observed that, administration of BC inhibited increased concentration of urea and creatinine, which were comparable to the effect observed with glibenclamide. This signifies the prevention of any considerable kidney change, which may be possible in diabetic rats.

CONCLUSION

In this experiment, we analyzed the antidiabetic potential of 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 increasing the insulin secretion, hemoglobin as well as reduced blood glucose, glycated hemoglobin and renal markers levels as compared to glibenclamide. BC 50 mg/kg b.w considered as optimal dose for further research, because there is no significant changes between 50 and 100 mg/kg b.w.

ACKNOWLEDGEMENT

Department of Zoology (UGC – SAP Sponsored), Annamalai University for providing the infrastructure facility and support.

REFERENCES

1. Prasannarong M, Vichaiwong K, Saengsirisuwan V. Calorie restriction prevents the development of insulin resistance and impaired insulin signaling in skeletal muscle of ovariectomized rats. Biochimica et Biophysica Acta 2012; 1822 (6): 1051–1061.
2. Sumam Varghese, Narmadha R, Gomathi D, Kalaiselvi M, Devaki K. Evaluation of hypoglycemic effect of ethanolic seed extracts of Citrullus lanatus. The Journal of Phytopharmacology 2013; 2(6): 31-40
3. Eze E, Mohammed A, Musa K, Tanko Y, Isa A. Effect of ethanolic leaf extract of Mucuna pruriens (fabaceae) on lipid profile in alloxan-induced diabetic Wistar rats. British Journal of Pharmacology and Toxicology 2012; 3(3); 102–109.
4. Daivadanam M, Absetz P, Sathish T, Thankappan KR, Fisher EB, Philip NE, Mathews E, Oldenburg B. Lifestyle change in Kerala, India: Need assessment and planning for a community-based diabetes prevention trial. BMC Public Health 2013; 13: 95–110.
5. Sophia D, Manoharan S, Hypolipidemic Activities of Ficus Racemosa Linn. Bark in Alloxan Induced Diabetic Rats. African journal of traditional Complementary and alternative medicines 2007; 4(3): 279–288.
6. Malik TA, Kamili AN, Chishti MZ, Tanveer S, Ahad S, Johri RK. In vivo anticoccidial activity of berberine [18, 5,6-dihydro-9,10-dimethoxybenzo(g)-1,3-benzodioxolo(5,6-a) quinolizinium] – an isoquinoline alkaloid present in the root bark of Berberis lycium. Phytomedicine 2014; 21 (5); 663–669.
7. Dkhil MA. Role of berberine in ameliorating schistosoma mansoni-induced hepatic injury in mice. Biological research 2014:47-8.
8. Mubaraki MA, Dkhil MA, Al-Shaebi EM, Lubbad, MY, Ibrahim KE, Al-Quraishy S. The protective effect of pomegranate, Punica granatum, on murine malaria. Pakistan Journal Zoology. 2014; 46(5): 1345–1350.
9. Moghaddam HK, Baluchnejadmojarad T, Roghani M, Goshadrou F, Ronaghi A. Bereberine chloride improved synaptic plasticity in STZ insuced diabetic rats. Metabolic Brain Disease 2013; 28: 421-428.
10. Chandirasegaran G, Elanchezhiyan C, Kavisha Ghosh, Sethupathy S. Determination of antidiabetic compound from Helicteres isora by oral glucose tolerance test. Journal of Applied Pharmaceutical Science 2016; 6(02): 172-174.
11. Golay A, Metformin and body weight. International Journal of Obesity (2008) 32, 61–72
12. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Clinical Biochemistry 1969; 6: 24-27.
13. Bisse A, Abraham EC. New less temperature sensitive micro chromatographic method for the separation and quantification of glycosylated hemoglobin using a non-cyanide buffer system. Journal of Chromatography 1985; 344:81–91.
14. Wybenga DR, Giorgio D, Pileggi VJ. Manual and automated methods for urea-nitrogen measurement in whole serum. Clinical Chemistry. 1971; 17(9):891-895.
15. Slot C. Plasma creatinine determination. A new and specific Jaffe reaction method. Scandinavian Journal of Clinical Laboratory Investigation 1965; 17(4):17381–387.
16. Szkudelski T. The Mechanism of Alloxan and Streptozotocin Action in B Cells of the rat Pancreas. Physiological Research 2001; 50(6):536-546.
17. Balamurugan R, Duraipandiyan V, Ignacimuthu S. Antidiabetic activity of γ-sitosterol isolated from Lippia nodiflora L. in Streptozotocin induced diabetic rats. European journal of pharmacology 2011; 667(1-3): 410-418.
18. Salahuddin M, Jalalpuress SS. Antidiabetic activity of aqueous fruit extract of cucumis trigonus roxb in Streptozotocin induced diabetic rats. Journal of Ethnopharmacology 2010; 127(2):565-567.
19. Shanaz Banu, Arunachalam G, Jayaveera K.N, Asoka Babu V.L, Vimal Kumar. Antidiabetic effect of two species of Barleria in Streptozotocin induced type-2 diabetic rats. Int. Res. J. Pharm. 2012; 3(10): 185-188.
20. Kasetti RB, Rajasekhar MD, Kondeti VK, Fatima SS, Kumar EGT, Swapna S, Ramesh B, Rao CA. Antihyperglycemic and antihyperlipidemic activities of methanol:Water (4:1) fraction isolated from aqueous extract of Syzygium alternifolium seeds in streptozotocin induced diabetic rats. Food and Chemical Toxicology 2010; 48(4):1078–1084.
21. Vijayaragavan K, Iyyam PS, Subramaniyan SP. Design synthesis and characterization of Zinc-3 hydroxy flavones, a novel Zinc mettalo complex for the treatment of experimental diabetes in rats. European journal of pharmacology 2012; 680(1-3):122-129.
22. Jaspreet V, Sivakami S, Shahani S, Suthar AC, Banaralikar M.M, Biyani MK. Antihyperglycemic effect of three extract from Monordica charantia. Journal of Ethnopharmacology 2000; 88: 107-111.

Cite this article as: