Research Article **ISSN: 2349 – 7106**

Asian Journal of Research in Chemistry and Pharmaceutical Sciences

Journal home page: www.ajrcps.com

PHARMACOLOGICAL EFFECT OF BERBERINE HYDROCHLORIDE ON STREPTOZOTOCIN INDUCED DIABETIC RATS

N. L. Mahammed[*1], G. Tulja Rani[1], B. Sravani[1], A. Mamatha[1], D. Rupesh[1], V. Asha Ranjani[1], K. Jagadeeshwar[1]

[1*]Malla Reddy Pharmacy College, Maisammaguda, Dhulapally, Secunderabad, Telangana-500014, India.

ABSTRACT

The aim of this study to find out the perfect dose of Berberine Hydrochloride instreptozotocin (STZ) induced diabetic rats by observation of effect of Berberine Hydrochloride on blood glucose, plasma insulin and hemoglobin. Experimental diabetes was induced in rats by a single dose of intraperitonial injection of streptozotocin (40mg/kg b.w). After the 72 hours, diabetic rats treated with Berberine Hydrochloride at different concentrations (25. 50 and 100mg/kg b.w) for 45 days. Berberine Hydrochloride administration significantly declined the levels of blood glucose, glycolated hemoglobin and renal markers whereas elevated the levels of plasma insulin, hemoglobin and body weight in diabetic rats. 50mg/kg b.w of Berberine Hydrochloride showed the prominent effect compared to other two doses. From these results clearly shows the antidiabetic activity of Berberine Hydrochloride.

KEYWORDS

Berberine Hydrochloride, Streptozotocin, Diabetes, Insulin and Blood glucose.

Author for Correspondence:

Mahammed N L,

Malla Reddy Pharmacy College,

Maisammaguda, Dhulapally, Secunderabad,

Telangana-500014, India.

Email: mohammad.lal121@gmail.com

INTRODUCTON

Diabetes mellitus (DM) is distinguished by the increase of blood glucose levels and inadequacy of insulin secretion or action. Such background causes the impairment of glucose uptake in the peripheral tissues and reduces the glucose consumption for energy purposes[1-3]. India has been categorized as second in the world in diabetes prevalence, after China[4]. Plants have long been the origin of different medicines for the Indian traditional system of medicine like ayurveda and siddha[5]. Berberine hydrochloride (BC) is a plant alkaloid, present in numerous medicinal plants, which have countless

pharmacology activities. Many Previous studies have reported that Berberine hydrochloride has a broad range of pharmacological and biological activities including anti- protozoal functions[6]. Dkhil[7] also reported the anti-malarial and antischistosomal activities of Berberine hydrochloride as well as its ameliorative outcome on the induced liver injury inflicted by *Schistosoma mansoni* infection[8]. In addition[9,10] reported the amelioration of synaptic plasticity and decreased blood glucose by Berberine hydrochloride treatment in Streptozotocin (STZ) induced diabetic rats. Many oral antidiabetic agents are available in markets, which are present withsome side effects[11]. Hence, this study was aimed to investigate the antidiabetic activity of Berberine hydrochloride in streptozotocin induced diabeticrats.

MATERIAL AND METHODS

Animals

Healthy, adult albino Wistar rats of both sexes (150-220g) were obtained from Sainath enterprises, Hyderabad, India. The animals were kept in a well ventilated room and the animals were exposed to 12 hrs day and night cycle with a temperature between 20±3ºC. The animals were housed in large spacious, hygienic polypropylene cages during the course of the experimental period. The animals were fed with water and rat feed ad libitum, supplied by this laboratory. The maintenance and the handling of animals were performed according to the rules and regulations of this experiment was approved by the Institutional Animal Ethics Committee (IAEC) (627/02/a/CPCSEA) and the experiments were carried out in accordance with IAEC guidelines on animal experimentation.

Materials

Berberine hydrochloride(assembled as a gift sample from PRS info tech and engineers (herbal division), Adarsh Nagar BLB FBD 121004 (Haryana)), Streptozotocinand Glipizide were purchased from Sigma - Aldrich (St. Louis, MO, USA), GOD-POD diagnostic kit was purchased from Coral diagnostics Ltd., Mumbai 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.

Method Development

Berberine and Glipizide dissolved in PBS buffer (Phosphate buffer saline).

Preparation for Phosphate Buffer Saline (PBS) Buffer

Weigh 8gms of sodium chloride (NaCl) and added to 800ml of distilled water, then add 0.2g of potassium chloride (KCl), 1.44g of disodium phosphate, 0.24gms of potassium dihydrogen phosphate and adjust the Ph to 7.4 with HCl and make up to 1litre with distilled water. Sterile by autoclaving at 121ºC for 20mins and store at room temperature.

Preparation For Krebs Ringer Bi carbonate Solution (KRPB): Weigh 5.6gms of Potassium chloride (KCl) (mol.wt: 74.56) and dissolved on 500ml of distilled water, 3.7gms of magnesium sulphate and add 100ml distilled water, then 1.62gms of calcium chloride and add 100ml distilled water, finally make up to 1litre with 1.5M Sodium chloride.

Preparation of 1.5m of Sodium Chloride: Weigh 58.44gms of Sodium chloride and dissolve on 500ml of distilled water, finally make up to 1000ml of distilled water 5.6.2. Induction and assessment of diabetes.

Induction of Diabetes

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

Assessment of Diabetes

Diabetes was confirmed after 48 hr of streptozotocin injection, the blood samples were collected through retro-orbital puncture and serum glucose levels were estimated by enzymatic GOD-POD diagnostic kit method. The rats having fasting plasma glucose levels more than 250mg/dL were selected and used for the present study.

Experimental Design

A total of 36 rats were used in this experiment and divided into six groups, each group consists of 6

rats. Group 1 is Normal Control rats, Group 2 is Diabetic Control rats, Group 3 is Diabetic + Berberine chloride (25 mg/kg b.w), Group 4 is Diabetic + Berberine chloride (50 mg/kg b.w), Group 5 is Diabetic + Berberine chloride (100 mg/kg b.w) and Group 6 is Diabetic + Glipizide (6 mg/kg b.w) Diabetic rats were treated with various doses of berberine hydrochloride (25, 50 and 100 mg/kg b.w) and Glipizide 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. Parameters (Serum glucose estimation (initial and final) and Body weight of the animals (initial and final)) were observed.

Blood sampling and serum separation: Un-haemolysed sample of blood was collected from the clean tail tips/ orbital sinus in eppendroff tubes from the anaesthetized animals. The blood was allowed to clot at room temperature 37ºC and centrifuge at 3000 rpm for 10 min. To separate the serum and subjected to biochemical estimation. Biochemical parameters (Plasma insulin, Blood hemoglobin, Glycolated Hemoglobin, Urea, Uric Acid, Creatinine) were studied using Merck Micro lab 200 Auto analyzer.

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 berberine hydrochloride 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 Berberine hydrochloride and glipizide 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 Berberine hydrochloride 50mg/kg b.w was found to be better in comparison with other two doses (25 and 100 mg/kg B.w) (Figure No.1).

Effect on berberine hydrochloride on fasting blood glucose

Table No.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 berberine hydrochloride treated diabetic rats when compared with diabetic control rats and berberine hydrochloride 50 and 100 mg/kg b.w produced a conspicuous effect.

Effect of berberine hydrochloride on plasma insulin, blood hemoglobin and glycolated hemoglobin

Plasma insulin and hemoglobin levels were significantly declined, whereas glycolated hemoglobin level was increased in streptozotocin induced diabetic control rats. Berberine hydrochloride and glipizide treated groups exhibited significant ($p < 0.05$) elevation of plasma insulin, hemoglobin and decline of glycolated hemoglobin. Berberine hydrochloride 50 and 100 mg/kg b.w dosage reverted the levels back to normal comparable with glipizide (Table No.2).

Effect of berberine hydrochloride on kidney functional markers

Figure No.2 show the levels of urea, uric acid and creatinine, which were significantly, elevated in diabetic control rats. Berberine hydrochloride at and glipizide 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.

DISCUSSION

In the present study, streptozotocin was used for induction of diabetes because the cytotoxic action of streptozotocin selectively destroys β-cells of the pancreas without affecting other cells by generating excess rosand carbonium ion (ch^{3+}) leading to dna breaks by alkylation dna bases causing oxidative damage[16]. Dose of 40 mg /kg b.wof streptozotocin have the ability to incomplete destruction of β-cells

of pancreas, which considered as type 2 diabetes[17]. Streptozotocin 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. Berberine hydrochloride 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 Berberine hydrochloride.

In streptozotocin 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 berberine hydrochloride and glipizide significantly improved body weight of streptozotocin induced diabetic rats, which could be due to increased insulin secretion. Glycolated 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 glycolation, which is formed between glucose and the n-end of the beta chain of hb, forming glycolated hemoglobin. Further, glucose and dicarbonyl compounds can also react with hemoglobin, forming advanced glycolation end products, which can contribute to the additional development of complicationsin diabetes. The extent of increased glycolated hemoglobin levels is found to be directly proportional to the fasting bloodglucose levels in diabetic patients[21].

In the current study, an elevated level of glycolated hemoglobin was observed in streptozotocin induced diabetic rats due to the increased formations of glycated hemoglobin. Administration of berberine hydrochloride and glipizide notably decreased the level of glycolated 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 berberine hydrochloride inhibited increased concentration of urea and creatinine, which were comparable to the effect observed with glipizide. This signifies the prevention of any considerable kidney change, which may be possible in diabeticrats.

Table No.1: The level of blood glucose (mg/dl) in control and experimental rats

S.No	Groups	0 Day	22 Day	45 Day
1	Normal control	83.61 ± 6.37	85.91 ± 6.54	89.81 ± 6.84
2	Diabetic normal	246.46 ± 18.87	262.73 ± 20.11	291.87 ± 22.34
3	Diabetic + BC (25mg/kg bw)	248.02 ±18.89	190.63 ± 14.52	162.05 ± 12.34
4	Diabetic + BC (50mg/kg bw)	248.32 ± 19.01	171.71 ± 13.14	120.37 ± 9.21
5	Diabetic + BC (100mg/kg bw)	244.84 ± 18.64	170.04 ± 12.95	119.86 ± 9.13
6	Diabetic + glipizide (6mg/kg bw)	253.19 ± 19.38	162.14 ± 12.41	102.51 ± 8.38

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 No.2: The level of plasma insulin hemoglobin and Glycolated hemoglobinin control and experimental rats

S.No	Groups	Insulin (µu/ml)	Hemoglobin (mg/ml)	HB ALC (Ng/ml
1	Normal control	16.08 ± 1.22[a]	13.26 ± 1.01[a]	042 ± 0.03[a]
2	Diabetic normal	6.86 ± 0.53[o]	6.26 ± 0.48[o]	1.12 ± 0.09[o]
3	Diabetic + BC (25mg/kg bw)	9.91 ± 0.75[c]	8.72 ± 0.66[c]	0.82 ± 0.06[c]
4	Diabetic + BC (50mg/kg bw)	13.09 ± 1.00[a]	11.47 ± 0.88[a]	0.61 ± 0.05[a]
5	Diabetic + BC (100mg/kg bw)	13.22 ± 1.01[a]	11.68 ± 0.89[a]	0.60 ± 0.05[a]
6	Diabetic + glipizide (6mg/kg bw)	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 $p < 0.05$.

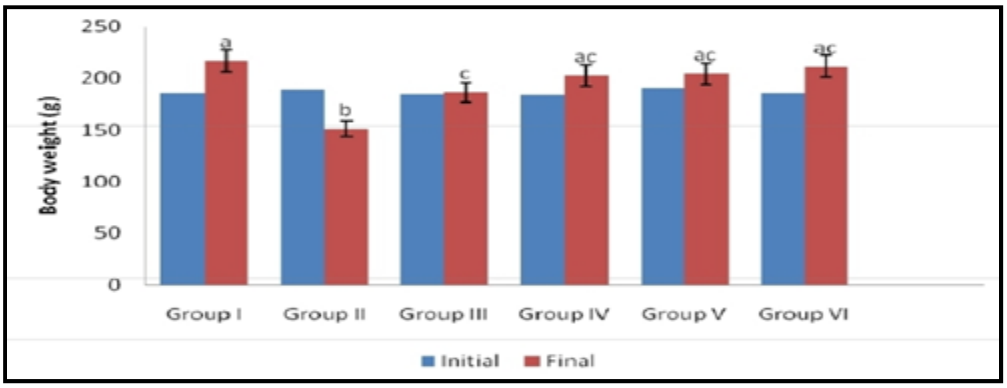

Figure No.1: The level of body weight in control and experimental rats

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 $p < 0.05$.

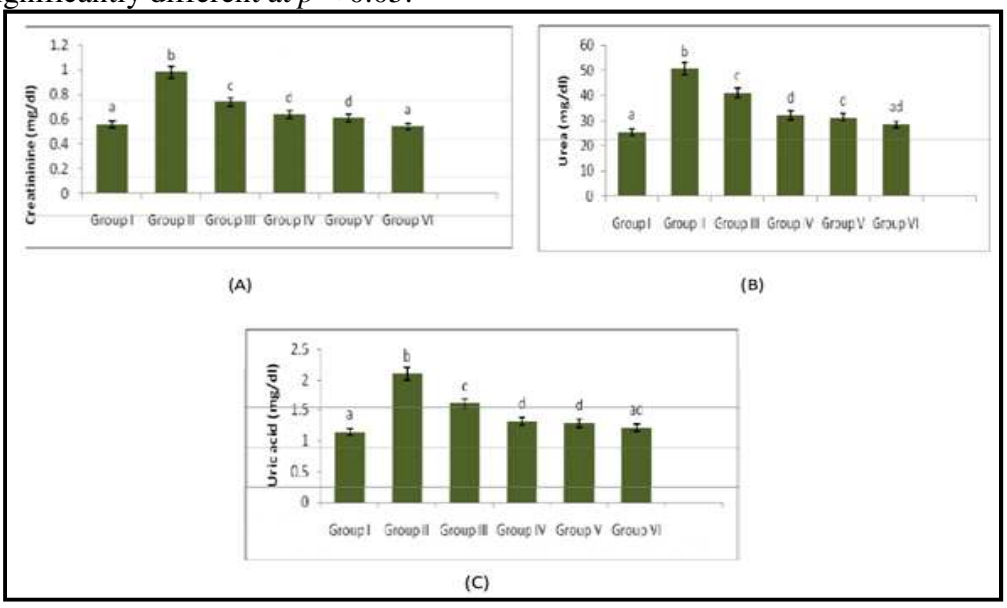

Figure No.2: A), B) and C). Effect of berberine hydrochloride instreptozotocin induced alteration on renal markers of control and experimental rats in each groups.

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 $p < 0.05$.

CONCLUSION

In this experiment, we analyzed the antidiabetic potential of different doses (25, 50 and 100mg/kg b.w) of Berberine hydrochloride instreptozotocin induced diabetic rats. Berberine hydrochloride (50 and 100mg/kg b.w) treatment remarkably increasing the insulin secretion, hemoglobin as well as reduced blood glucose, glycolated hemoglobin and renal markers levels as compared to glipizide. Berberine hydrochloride 50mg/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

The authors thankful to Mr. Ch. Malla Reddy Chairpersons' and Dr. Tulja rani-Principle of Malla Reddy Pharmacy College, Maisammaguda, Secunderabad. For providing necessary facilities and for their guidance and support to carry out this research project.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBILIOGRAPHY

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, *Biochimicaet Biophysica Acta,* 1822(6), 2012, 1051-1061.
2. Sumam Varghese, Narmadha R, Gomathi D, Kalaiselvi M, Devaki K. Evaluation of hypoglycemic effect of ethanolic seed extracts of Citrulluslanatus, *The Journal of Phytopharmacology,* 2(6), 2013, 31-40.
3. Eze E, Mohammed A, Musa K, Tanko Y, Isa A. Effect of ethanolic leaf extract of Mucunapruriens (fabaceae) on lipid profile in alloxan-induced diabetic Wistar rats, *British Journal of Pharmacology and Toxicology,* 3(3), 2012, 102-109.
4. Daivadanam M, Absetz P, Sathish T, Thankappan K R, Fisher E B, Philip N E, Mathews E, Oldenburg B. Lifestyle change in Kerala, India: Need assessment and planning for a community-based diabetes prevention trial, *BMC Public Health,* 13, 2013, 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,* 4(3), 2007, 279-288.
6. Malik T A, Kamili A N, Chishti M Z, Tanveer S, Ahad S, Johri R K. *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 Berberislycium, *Phytomedicine,* 21(5), 2014, 663-669.
7. Dkhil M A. Role of berberinein ameliorating schistosoma mansoni-induced hepatic injury in mice, *Biological Research,* 47(1), 2014, 47-48.
8. Mubaraki M A, Dkhil M A, Al-Shaebi E M, Lubbad, M Y, Ibrahim K E, Al-Quraishy S. The protective effect of pomegranate, Punicagranatum, on murine malaria, *Pakistan Journal Zoology,* 46(5), 2014, 1345-1350.
9. Moghaddam H K, Baluchnejadmojarad T, Roghani M, Goshadrou F, Ronaghi A. Bereberine chloride improved synaptic plasticity in STREPTOZOTOCIN insuced diabetic rats, *Metabolic Brain Disease,* 28(3), 2013, 421-428.
10. Chandirasegaran G, Elanchezhiyan C, Kavisha Ghosh, Sethupathy S. Determination of antidiabetic compound from *Helicteresisora* by oral glucose tolerance test, Journal *of Applied Pharmaceutical Science,* 6(02), 2016, 172-174.
11. Golay A. Metformin and body weight, *International Journal of Obesity,* 32, 2008, 61-72.
12. Szkudelski T. The Mechanism of Alloxanand Streptozotocin Action in B Cells

of the rat Pancreas, *Physiological Research,* 50(6), 2001, 536-546.

13. Balamurugan R, Duraipandiyan V, Ignacimuthu S. Antidiabetic activity of γ-sitosterol isolated from *Lippianodiflora* L. in Streptozotocin induced diabetic rats, *European Journal of Pharmacology,* 667(1-3), 2011, 410-418.
14. Salahuddin M, Jalalpuress S S. Antidiabetic activity of aqueous fruit extract of *cucumistrigonusroxb*in Streptozotocin induced diabetic rats, *Journal of Ethnopharmacology,* 127(2), 2010, 565-567.
15. 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,* 3(10), 2012, 185-188.
16. Kasetti R B, Rajasekhar M D, Kondeti V K, Fatima S S, Kumar E G T, Swapna S, Ramesh B, Rao C A. Antihyperglycemicand antihyperlipidemic activities of methanol: Water (4:1) fraction isolated from aqueous extract of *Syzygiumalternifolium* seeds in streptozotocin induced diabetic rats, *Food and Chemical Toxicology,* 48(4), 2010, 1078-1084.
17. Vijayaragavan K, Iyyam P S, Subramaniyan S P. 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,* 680(1-3), 2012, 122-129.
18. Jaspreet V, Sivakami S, Shahani S, Suthar A C, Banaralikar M M, Biyani M K. Antihyperglycemic effect of three extract from *Monordicacharantia*, *Journal of Ethnopharmacology,* 88(1), 2000, 107-111.
19. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor, *Clinical Biochemistry,* 6(1), 1969, 24-27.
20. Bisse A, Abraham E C. New less temperature sensitive micro chromatographic method for the separation and quantification of glycosylated hemoglobin using a non- cyanide buffer system, *Journal of Chromatography,* 344, 1985, 81-91.
21. Wybenga D R, Giorgio D, Pileggi V J. Manual and automated methods for urea-nitrogen measurement in whole serum, *Clinical Chemistry,* 17(9), 1971, 891-895.
22. Slot C. Plasma creatinine determination, A new and specific Jaffe reaction method, *Scandinavian Journal of Clinical Laboratory Investigation,* 17(4), 1965, 17381-17387.

Please cite this article in press as: Mahammed N L *et al.* Pharmacological effect of Berberine hydrochloride on streptozotocin induced diabetic rats, *Asian Journal of Research in Chemistry and Pharmaceutical Sciences,* 7(2), 2019, 646-652.