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Effects of Berberine on Bloog Glucose, Glycated Hemoglobin A_1, Serum Insulin, C–Peptide, Insulin Resistance and β– Cell Physiology

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Authors' contributions

This work was carried out in collaboration among all authors. Authors HKK, DMS and GSN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors HKK, TZS and IU managed the biochemical analysis and analyses of the study. Author HKK and AAU managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Objective: Determine effects of berberine (BBR) on bloog glucose, glycated hemoglobin A1, serum insulin, C – peptide, insulin resistance and β– cell physiology.
Study Design: Experimental study
Place and Duration: Department of Physiology Isra University and Animal house of Sindh Agriculture University, Tando Jam from August 2019 to February 2020.

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Methodology: A sample of 100 adult Wistar male rats was selected according to criteria of study and divided into 5 groups through random technique. Group A – negative controls (N/S 0.9%), Group B – positive control (diabetic rats – Alloxan 120 mg/kg) (i.p), Group C – E; Diabetic rat + BBR (50, 100 and 200 mg/kg) respectively. Blood samples were collected from retro-orbital venous plexus by capillary tube. Sera were separated at 3000 rpm (15 minutes). Insulin resistance (HOMA-IR) and β – cell secretory activity (HOMA-β) were calculated by mathematical formula. Statistical analysis was performed on SPSS version 21.0 (IBM, incorporation, USA) at 95% CI (P≤ 0.05).
Results: The present study noted significant improvement of fasting and random blood glucose, glycated HbA1, serum insulin and C –peptide, insulin resistance (HOMA-IR) and β – cell physiology.
Conclusion: Berberine (BBR) ameliorates the bloog glucose, glycated hemoglobin A_1, serum insulin, c – peptide, insulin resistance and β– cell physiology.

Keywords: Blood glucose; HbA1c; insulin resistance; β– cell physiology.

1. INTRODUCTION

Diabetes mellitus (DM) is a glucose metabolic disorder caused primarily by dysfunction of β-cells of endocrine pancreas. DM is an endocrinpathy of chronic hyperglycemia caused by relative and/or absolute insulin deficiency. Currently 415 million of World population is suffering from DM, and is estimated to 624 million by the 2040 year [1]. DM claims 5 million lives yearly, mainly by the coronary artery and chronic renal diseases [1,2]. Diabetic causailities may be reduced by tight glycemic control [3]. Lifestyle modificatin is initial protocol to reach a good glycemic goal, if not then pharmacological interventions with oral hypoglycemic agents (OHAs) and insulin is recommended. However, OHAs and insulin have there own merits and demerits with a number of potential adverse effects. A recent study demonstrated OHAs were efficacious in 41% diaebtics in achieving optimal glycemic control [4]. It is reproted the insulin secretagogues and insulin increases the cancer risk in liver, breast, pancrea and colorectum. Metformin causes gut irritation and diarrhoea [5]. Berberine (BBR) is a herbal agent derived from Chinese rhizomacoptidis of berberis family. BBR is a traditional Chinese medicine extracted from berberis and other plants. BBR is considered the "secret prescription" of China [6]. Previous studies [7,8] compared the BBR and meformin in clinical trials and found BBR efficacy similar to metfromin. Later study found increased insulin receptor expression by BBR [8]. A previous study [8] found insignificant glucose lowering potential of BBR, although cholesterol and sytemic blood pressure were reduced significantly. Numerous studies [9-11] have reproted hypoglycemic efficacy of BBR, but its efficacy has not been confirmed in terms of β– cell physiology and Insulin resistance. Prevalnce and incidence of DM is increasing in the country, and growing interest has been observed in the alternative herbal remedy for the diabetic populations with least side effects. The present experimental study analysed effects of berberine on glucose homeostasis, β– cell secretory function (HOMA-β %) and Insulin resistance (HOMA-IR %) in alloxan induced diabetic rats.

2. METHODOLOGY

The present experimental study was conducted at the animal house of Sindh Agriculture University, Tando Jam. It was conduced from August 2018 to July 2019 (one year duration). A sample of 100 adult Wistar male rats was selected according to criteria of study. Adult albino Wistar male rats looking healthy, feeding well, and body weight 150- 200 grams qualified for inclusion. Female rats, unhealthy, lazy and sick male rats and rats of different body weight were excluded. Rats were purchased from the Animal Husbandry & Veterinary Sciences Department, Sindh Agriculture` University Tando Jam. Animal housing fulfilled the critera of NIH, Updated National Research Council, USA Committee "Guidelines for the Care and Use of Lab Animals" [12]. Wooden saw dust was used for bedding of animals. Rats were kept in stainless steel cages that were equipped with plastic drinkers. Feed was kept in the steel feed containers. Hygienic standards of animal house were observed strictly. Tap water was availabel *ad libitum*. Dark- light cycles of 12/12 hours was ensured. Animals were divided into five groups by random technique.

2.1 Animal Grouping

Rat groups; Group A – negative controls (N/S 0.9%), Group B – positive control (diabetic experimental group) (Alloxan 120 mg/kg) intra - peritoneal (i.p) (No BBR drug therapy) [13],

Group C – Diabetic rat received BBR (50 mg/kg), Group D – Diabetic rat received BBR (100 mg/kg) and Group E – Diabetic rat received BBR (200 mg/kg) daily for 6 weeks [14].

2.2 Experimental Procedure

Experimental procedure included phase I and II. In phase I, the animals were selected by purposive – sampling technique to fulfil the inlcusion and exclusion criteria.

2.3 Induction of DM

The animals were injected Alloxan 120 mg/kg intra - peritoneal (i.p) to induce diabetes. Alloxan (Sigma - Aldrich, St. Louis, MO, USA) was purchaed from broker (World scientific, Pakistan). It was kept at low temperature (4°C). Alloxan was dissolved in the 0.9% N/S and injected in rats fasting for an overnight. Dose of Alloxan used - 120 mg/kg bwt intraperitoneal [13]. DM was confirmed glucose level ≥ 250 mg/dl at 72 hours. After successful DM induction, the rats were randomly divided into positive control and experimental groups.

2.4 Animal Handling

Institutional ethics of experimental animal handling was adhered strictly. Diet was prepared by commercial feed (~40%), wheat flour (~40%) and dry milk (~20%). BBR was administred mixed in diet at 50 mg, 100 mg and 200 mg/Kg body weight [14].

2.5 Experimental Details

At the end of experiemnt, the rats were anesthetized by Ethylene- ether and blood samples were collected from retro-orbital venous plexus by capillary tube.

2.6 Biochemical Analysis

Samples were collected in EDTA and Plain tubes. Sera were taken by blood centrifuged at 3000 rpm (15 minutes), and stored -20^{o}C. HbA1c was detected by TINIA method. C-peptide and serum insulin levels were detected by Elisa assay kit according to the method mentioned. Insulin resistance (HOMA-IR %) was calculated as Glucose (mmol/L) x Insulin (mU/L)/22.5 [15]. Insulin Resistance (IR) was defined as No IR <1.0, early IR <1.9, moderate IR >1.9 - <2.5 and severe IR >2.5 [15]. HOMA-β% was calculated as Insulin (mU/L) x20/Glucose (mmol/L)-3.5 [15].

2.7 Statistical Analysis

Statistical analysis was performed on SPSS version 21.0 (IBM, incorporation, USA). Analysis of variance (ANOVA) and post – hoc Tuckey Cramer test were used for the continuous variables with normal Gaussian distribution. Significance of analysis was taken at 95% CI (P≤ 0.05).

3. RESULTS

Findings of fasting blod glucose (FBG), random blood glucose (RBG), HbA1c, Insulin, C-peptide, Insulin resistance (HOMA-IR) and β – cell function (HOMA – β) revealed significant improvement after 6 weeks BBR therapy (Table-1). FBG was reduced by -23%, -30% and -35% in BBR treated experimental groups C, D and E (P=0.0001). RBG showed reduction by -23%, -30% and -35% in BBR treated experimental groups C, D and E (P=0.0001). HbA_1c was decreased by -25%, -18% and -6% in BBR treated experimental groups C, D and E (P=0.0001). C –peptide shows increase of 213%, 180% and 61% in BBR treated experimental groups C, D and E (P=0.0001). Insulin resistance (HOMA-IR %) and β – cell physiology (HOMA-β %) shows significant improvement as shown in Fig. 1.

4. DISCUSSION

The present research observed the physiological efficacy of Berberine (BBR) for its glucose homeostasis, β-cell physiology and Insulin resistance in alloxan induced diabetic male Wistar rat model. FBG was reduced by -23%, -30% and -35% (P=0.0001) and RBG reduced by -23%, -30% and -35% (P=0.0001). Glucose lowering potential of BBR is in agreement with previous studies [16,17]. Hypoglycemic effects are exerted through liver gluconeogenesis inhibitin [18], enhanced glucokinase activity [19] and increased insulin secretion through Islets of Langerhans regeneration [20]. Reduction in insulin resistance (HOMA-IR) is in argreement with previous study [21]. Previous studies [22, 23] found insignificant hypoglycemic activity of BBR that is in contrast to present and previous studies [17,18]. Conflicting result of above studies [22,23] are most probably because of different doses and duration of BBR therapy and research *bias*. In present study, the hypoglycemic effect was prominent after 6 weeks BBR therapy. Another reason of conflicting results is hypoglycemic activity of BBR is dose dependent [23]. In present study, the significant

reduction was noted in HbA1c of BBR treated experimental groups C, D and E. Findings are in accordance to previous studies [16-18]. A recent study [19] reviewed BBR therapy and life style modification were significantly effective (P=0.001) in improving the HbA1c compared to placebo alone. Our finding of improved glycemic control is consistent to previous studies [24,16,17]. A recent 3T3-L1 adipocyte model [25] witnessed the glucose and HbA1c lowering potential of BBR therapy. Another study [26] noted the anti hyperglycemic activity of BBR in diabetic *db/db* mice was mediated through enhanced liver glucokinase (GK) expression. In present study, the HbA1c was reduced by -6% group C, -18% in group D and -25% in group E in comparison to positive control group B (P=0.0001). Finding is consistent with previous studies [26,27]. Huang et al [28] noted 11.1% HbA1c reduction from baseline after 12 weeks of *Rhizoma coptis* (BBR). In present study, the serum insulin and Insulin resistance (HOMA-IR %) were reduced and β – cell physiology (HOMA-β %) was improved. Findings are in agreement with previous studies [16-18]. In present study, the insulin resistance (HOMA-IR) shows reduction of 24% in group C, -60% group D and -70% group E (P=0.0001) after 6 weeks BBR therapy. The β- cell activity (HOMA-β) improved by +2%, +18% and +82% in BBR treated groups C, D and E respectively (P=0.0001). Finidngs are in agreement with previous studies [27-29]. Previous studies [16,18,26] noticed glucose and fasting insulin reduction and improved β- cell physiology and insulin sensitivity in (RIR) rat model. Reduction in insulin resistance is in agreement with a previous study [29] that noted improved insulin resistance, fasting and random blood glucose and serum insulin levels at 3 months of BBR therapy in type 2 diabetics. The findings of present study are also supported by recent studies [16,19,29]. In present study, the fasting plasma insulin (FPI) and insulin resistance (HOMA-IR) were reduced by -14.1% and – 27.3% respectivley this is in agreement with previous study [29]. Amelioration of HOMA-IR of present study is in agreement with previuos studies [27-30]. The β – cell physiology (HOMA-β) of present study shows improvement of +2%, +18% and +82% in BBR

Table 1. Biochemical findings in controls and experimental rats

	Group A	Group B	Group C	Group D	Group E	P
FBG	79.2±11.3	290.1±23.4	223.1±33.3	202.1±41.2	188.9±55.3	0.0001
RBG	123.1±8.7	399.0±34.2	323.1±9.9	320.8±18.8	207.6±29.4	0.0002
HbA1c	5.1±0.6	8.66±0.72	8.14±0.69	7.08±0.78	6.48±0.50	0.0001
Insulin	11.27±0.7	3.6±0.89	4.3±1.1	4.8±1.1	6.4±0.50	0.0003
C-peptide	1.74±0.6	0.40±0.27	1.01±0.15	1.13±0.1	1.27±0.20	0.0005
HOMA-IR	0.64±0.31	4.55±1.07	3.44±0.38	1.82±0.70	1.35±0.20	0.0001
HOMA-β	85.80±14.7	27.90±16.8	27.23±7.6	30.24±9.3	50.8±4.4	0.0001

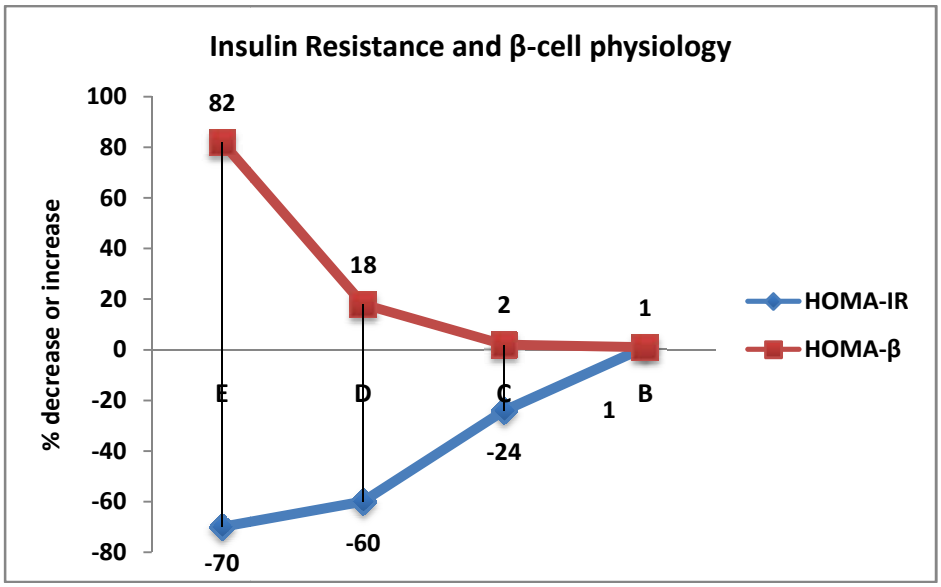

Fig. 1. Relative % increase/decrease in Insulin resistance (HOMA-IR) and β – cell physiology (HOMA-β)

treated groups C, D and E respectively, the finding is consistnet with prevoius studies [30,31]. In the present study, serum C –peptide shows increase of 213%, 180% and 61% in low to high dose BBR treated experimental rats respectivley. Finding is in agreement with previous studies [32,33]. The findigns of present study are in favor of berberine exerting glucose lowering effects through improved insulin resistance and β – cell physiology.

5. CONCLUSION

In conclusion, the present study shows berberine (BBR) ameliorates bloog glucose, glycated hemoglobin A1, serum insulin, insulin resistance and β– cell physiology. Further experimental and clinical studies are recommended. Berberine shows promising results for a more natural type of diabetes therapy through β– cell stimulation.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical and Research Committee of Isra university (Letter # IU/RR-10/D(M& 27/2016/1573).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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