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# Berberine Improves Glucose Homeostasis Through Insulin, Nkx – 6.1 and Pdx – 1 Gene Expression in Pancreatic Tissue

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**Abstract:**
The Berberine (BBR), herbal agent, has been used in Chinese traditional medicine. The present experimental study analyzed effects of BBR on glucose homeostasis, Insulin, Nkx 6.1 and Pdx – 1 gene expression in pancreatic tissue in diabetic rats. A sample of 100 adult Wistar male rats was selected according to study criteria and divided into five groups by random technique after induction of Diabetes mellitus by alloxan and BBR was administered orally for six weeks. Blood sera were separated at 3000 rpm (15 minutes) for biochemical analysis. Pancreatic tissue pieces were collected in RNAse free Eppendorf tubes. Gene Primers were purchased from M/s Macrogen. PCR was performed on Thermo Scientific GeneJET RNA Purification Kit (K0731, K0732). Data was analyzed on SPSS version 21.0 (IBM, incorporation, USA) at 95% CI (P≤ 0.05). Blood glucose, A1C, Serum Insulin, C-peptide, Insulin resistance (HOMA-IR) and β – cell function (HOMA- β) were improved significantly (P=0.0001). We observed significant overexpression of Insulin gene, Nkx 6.1 gene and Pdx 1 gene in pancreatic tissue after six weeks berberine therapy (P=0.0001). Quantification shows gene expression was dose dependent. Conclusion: Berberine induces insulin, Nkx – 6.1 and Pdx – 1 gene expression in pancreatic tissue.

*Keywords: Berberine, Insulin gene, Nkx–6.1, Pdx–1, Pancreas*

## INTRODUCTION

Berberine (BBR) is an isoquinolone alkaloid derived from plants of *berberis* family. Various medicinal plants contain berberine alkaloid including *Phellodendron chinense* Schneid, Berberis lycium Royle, *Coptis chinensis* Franch, etc. It has been used in Chinese traditional medicine (CTM) since time immemorial extracted from the Chinese rhizomacoptidis. Manifold biological effects of BBR are known including the anti-diarrheal, anti-tumor, anti-hyperglycemic, antimicrobial.[1,2] It is considered a "secret prescription" recipe of CTM.[2] BBR has been used for treating obesity, hyperlipidemia and diabetes mellitus (DM).[1-3] Positive effects on glucose metabolism and insulin resistance (IR) have been reported possibly mediated through regulation of adenosine monophosphate- activated protein kinase (AMPK), insulin receptor expression and activation of protein tyrosine phosphatase 1 B.[1-5] Many studies[5,6] had found anti-diabetic activity of BBR similar to metformin. Later study[6] found anti-diabetic activity of BBR was mediated through expression of insulin receptor. Various studies[7,8] had reported its hypoglycemic efficacy through decrease in IR and improved β– cell physiology. As the DM is increasing in the modern societies including the developing countries[9], there is need to search for alternative therapy and herbs have

attracted much attention of scientists. Herbal agents have been experimented of their efficacy as anti – diabetic agent including berberine.[10,11] In this context of increasing prevalence of DM there is strong compulsion for analyzing new herbs for their anti –diabetic efficacy. The present study was planned to analyze the dose response effect of berberine therapy on Insulin gene, Nkx 6.1 and Pdx-1 genes in an alloxan induced Wistar male albino rat model.

## METHODOLOGY

The present experimental study was conducted at the Faculty of Medicine & Allied Medical Sciences Isra University in collaboration Animal house of Sindh Agriculture University Tando Jam (SAUTA) from August 2019 to February 2023. 100 male Wistar albino rats were purchased from Animal Husbandry & Veterinary Sciences (SAUTA). Adult Wistar male rat of 150 – 200 grams, healthy, eating and moving well was the inclusion criteria. Lazy, sick male rats and female rats were excluded. Animals were lived-in stainless-steel cages with saw dust bedding. Cages were provided with plastic drinkers. Animals were housed according to guidelines as cited.[2] Animal environment was observed strictly according to criteria[2] and dark- light cycles of 12/12 hours were ensured. Feeding and tap water were available *ad libitum*. In phase – 1 the rats were selected according to the inclusion criteria, phase-2 the rats were induced DM with Alloxan (120 mg/kg) and in phase – 3 the diabetic animals were randomly divided into five groups. Group – I, negative control, Group II – positive control (diabetic rats), Group III – VI – diabetic rats + received BBR therapy of 50, 100 200 mg/kg bwt respectively. BBR was given daily for six weeks.[2] DM was defined as blood glucose ≥ 250 mg/dl at 72 hours.[2] BBR was given mixed in diet. After six-week BBR therapy, the rats were anesthetized by Ethylene- ether, blood samples were taken from retro-orbital venous plexus using capillary tube. Animals were sacrificed by cervical dislocation.[2] Pancreatic tissue pieces were in RNAse free Eppendorf tubes, stored at -80°C after frozen in liquid nitrogen for RNA isolation. Blood was centrifuged to get sera for biochemical analysis. Insulin resistance (HOMA-IR) and β – cell function (HOMA-β) was calculated and graded as cited.[12-14] Gene primers used for quantitative polymerase chain reaction (PCR) were purchased from Thermo scientific, Korea (Thermo Scientific GeneJET RNA Purification Kit (K0731, K0732) primers for the PCR were purchased from M/s Macrogen). Gene Primers included; insulin forward → 5- GGATTATTCATACCGTCCCA-3 (20 mer) and insulin reverse → 5- CACCTT TGTGGTCCTCACCT-3 (20 mer), Pdx – 1 forward → 5- GGGACCCCT CAAGTTTGTAA-3 (20 mer) and Pdx – 1 reverse → 5- GGCTTAACCTAAACG CCACA-3 (20 mer), Nkx – 6.1 forward → 5- GGG CTT GTT GTA ATC GTC GT -3 (20 mer) and Nkx – 6.1 reverse → 5- ACT TGG CAG GAC CAG AGA GA-3 (20 mer), 18s RNAr forward → 5- GTAACCCGTTGAACCCCATT-3 (20 mer) and 18s RNAr reverse →5- CCATGGAATCGGTAGTAGCG-3 (20 mer).[14] RNA was extracted according to procedure cited[15] and quantified. Negative control group I genes were taken as 1.0±0.0 for quantitative analysis[16] as ratio for comparison of gene expression in positive control and BBR treated experimental rats. Data was analyzed on SPSS version 21.0 (IBM, incorporation, USA). Analysis of variance and post – hoc Tuckey Cramer test analyzed the continuous variables. Significance of variable analysis results was taken at 95% CI (P≤ 0.05).

## RESULTS

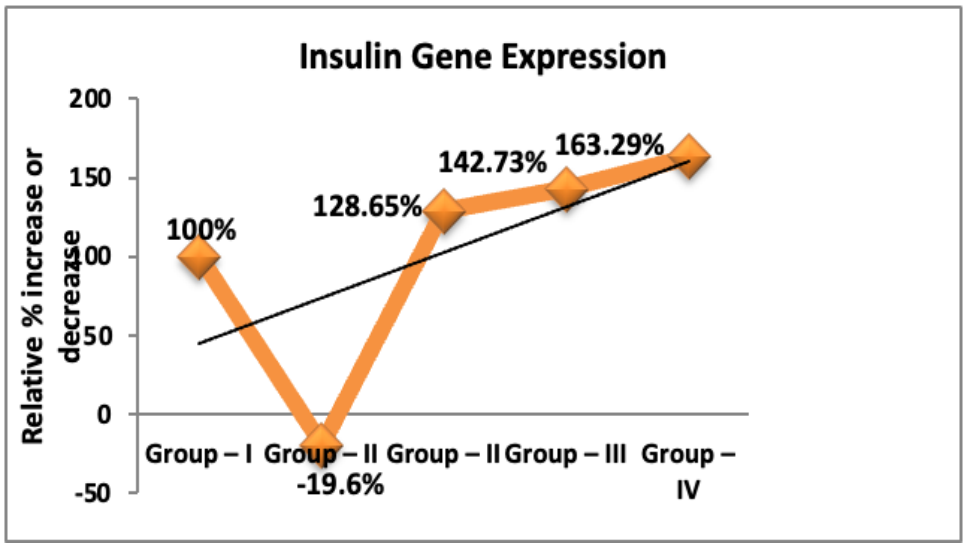
Blood glucose, A1C, C-peptide, Insulin resistance (HOMA-IR) and β – cell functions (HOMA-β) show significant improvement (P=0.0001) (table – 1). We observed significant overexpression of Insulin gene, Nkx 6.1 gene and Pdx 1 gene in pancreatic tissue after six weeks berberine therapy (P=0.0001) (table 2 – 4). Berberine induced gene expression was dose dependent (graphs 1 – 3).

**Table – 1: Laboratory findigns in control and experimental rats**

| | Group I | Group II | Group III | Group IV | Group V | P |
|---|---|---|---|---|---|---|
| Fasting Glucose (mg/dl) | 79.2±11.3 | 290.1±23.4 | 223.1±33.3 | 202.1±41.2 | 188.9±55.3 | 0.0001 |
| Random Glucose (mg/dl) | 123.1±8.7 | 399.0±34.2 | 323.1±9.9 | 320.8±18.8 | 207.6±29.4 | 0.0002 |
| A1C (%) | 5.1±0.6 | 8.66±0.72 | 8.14±0.69 | 7.08±0.78 | 6.48±0.50 | 0.0001 |
| Serum Insulin (µU/L) | 11.27±0.7 | 3.6±0.89 | 4.3±1.1 | 4.8±1.1 | 6.4±0.50 | 0.0003 |
| Serum C-peptide (mg/dl) | 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 |

**Table – 2: Insulin Gene expression in Control and Experimental Rats**

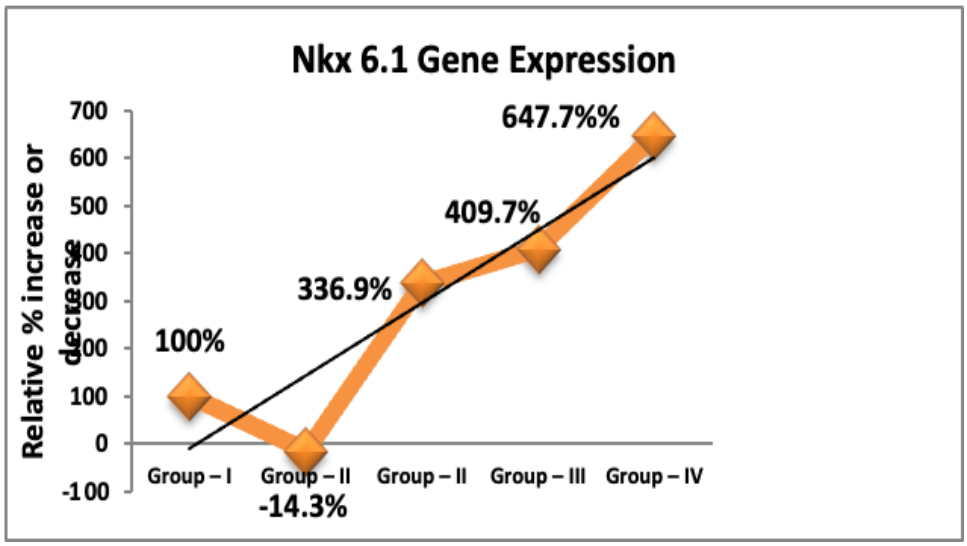
| Groups | Mean | SD | SEM | 95% CI of Mean | | P |
|---|---|---|---|---|---|---|
| | | | | LB | UB | |
| Group – I | 1.000 | 0.000 | 0.000 | 1.000 | 1.000 | 0.0001 |
| Group – II | 0.196 | 0.235 | 0.052 | 0.087 | 0.306 | |
| Group – III | 12.865 | 1.072 | 0.240 | 12.364 | 13.367 | |
| Group – IV | 14.273 | 0.916 | 0.205 | 13.844 | 14.702 | |
| Group – V | 16.329 | 1.237 | 0.277 | 15.750 | 16.908 | |




**Graph 1: Relative % increase or decrease in Insulin gene expression**

**Table - 3: Nkx 6.1 gene expression in Control and Experimental Rats**

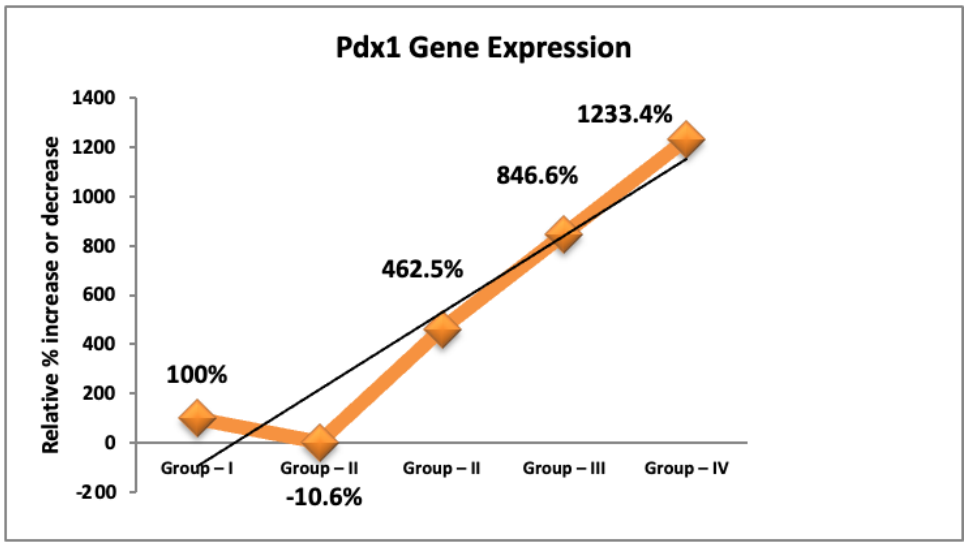
| Groups | Mean | SD | SEM | 95% CI of Mean | | P |
|---|---|---|---|---|---|---|
| | | | | LB | UB | |
| Group – I | 1.000 | 0.000 | 0.000 | 1.000 | 1.000 | 0.0001 |
| Group – II | 0.143 | 0.096 | 0.021 | 0.098 | 0.188 | |
| Group – III | 3.369 | 0.636 | 0.142 | 3.071 | 3.666 | |
| Group – IV | 4.097 | 0.771 | 0.172 | 3.737 | 4.458 | |
| Group – V | 6.477 | 1.546 | 0.346 | 5.753 | 7.200 | |




**Graph 2: Relative % increase or decrease in Nkx –6.1 gene expression**

**Table - 4: Pdx – 1 gene expression in Control and Experimental Rats**

| Groups | Mean | SD | SEM | 95% CI of Mean | | P |
|---|---|---|---|---|---|---|
| | | | | LB | UB | |
| Group – I | 1.000 | 0.000 | 0.000 | 1.000 | 1.000 | 0.0001 |
| Group – II | 0.106 | 0.092 | 0.021 | 0.063 | 0.149 | |
| Group – III | 4.625 | 0.688 | 0.154 | 4.303 | 4.947 | |
| Group – IV | 8.466 | 1.600 | 0.358 | 7.717 | 9.215 | |
| Group – V | 12.334 | 0.881 | 0.197 | 11.921 | 12.746 | |




**Graph 3: Relative % increase or decrease in Pdx –1 gene expression**

## DISCUSSION

The present study is the first experimental study analyzing the dose response effect of Berberine therapy on glycemic control, Insulin Gene, Nkx 6.1 gene and Pdx – 1 gene expression. Experimental rats showed significant improvement of blood glucose, A1C, serum insulin, C-peptide, insulin resistance (HOMA-IR) and β – cell function (HOMA- β) after 6-week BBR therapy (P=0.0001). These findigns are consistent with previous studies.[1-5] However; novel finding of present study is the increased expression of insulin gene, Nkx – 6.1 gene and Pdx – 1 gene. In

present study, the Insulin gene, Nkx – 6.1 gene and Pdx – 1 gene expression was found high compared to negative control (group I) and positive control (group II) (table – 2 to 4). Insulin gene, Nkx 6.1 and Pdx 1 gene show statistically significant gene expression in BBR treated experimental groups (III – VI) compared to controls (P=0.0001). In present study, the insulin gene expression in positive control (group II) was 0.196± 0.235 (-19.6%) that was found over expressed in BBR treated groups. The BBR treated experimental groups III, IV and V revealed insulin gene expression ratio of 12.865±1.072 (128.6%), 14.273±0.916 (142.7%) and 16.329±1.237 (163.2%) respectively (P=0.0001) (table 2). The findigns are consistent with previous studies.[17,18] A previous study[17] demonstrated the effects of BBR therapy in mouse model and reported improved glycemic indices, insulin resistance and β-cells functioning. They further added the Insulin 2 gene promoter increases the AMP – PK activity that may be exploited for better diabetic therapy. In present study, the Insl Gene, Nkx 6.1 and Pdx1 expression were found elevated after BBR therapy hence the finding of gene over expression is in agreement with above study. A previous study[18] treated Zucker Diabetic Obese Rats with BBR for 12 weeks and demonstrated up – regulation of 91 gene and down – regulation of 63 genes in the livers of rats. Increased gene expression in liver is consistent finding with over – expression of Insl Gene, Nkx 6.1 and Pdx1 genes in pancreatic tissue of present study. Study by Wang et al[19] demonstrated reduced galectin-3 (Gal-3 mRNA) gene expression and suppression of obesity and concluded BBR may prove beneficial anti – obesity agent for obesity and diabetes mellitus. Similar to above findigns the over expression of Insl Gene, Nkx 6.1 and Pdx1 genes by BBR therapy may be exploited for improvement of glycemic control in diabetic subjects. In present study, the Nkx – 6.1 gene expressions in negative control were 1.00, and 0.143±0.096 (-14.3%) in positive control while berberine treated experimental groups III, IV and V show very high gene ratio of 3.369±0.636 (336.9%), 4.097±0.771 (409.7%) and 6.477±1.546 (647.7%) respectively (P=0.0001) (table 3). While Pdx1- m gene was expressed i.e., 4.625±0.688 (462.5%), 8.466±1.60 (864.6%) and 12.334±0.88 (1233.4%) in experimental groups III – VI (P=0.0001) (table 4) that also proved very high. Relative % increase or decrease of insulin gene, Nkx 6.1 gene and Pdx – 1 gene expression is shown in graphs I – III. Zhang et al[20] demonstrated up – regulation of MAPK (mitogen activated protein kinase) gene expression of MAPK8 and MAPK14 by berberine therapy, both genes augment insulin gene expression. This is similar to Nkx – 6.1 and Pdx – 1 of present study as both genes stimulate insulin gene expression. Our findigns of increased Insulin gene, Nkx – 6.1 gene and Pdx –1 gene expression is supported by above studies. Xia et al[21] concluded the BBR exerts hypoglycemic effect through gene inhibition of gluconeogenesis enzyme in the liver. They demonstrated the genes of 2 glucogenic enzymes; the Glucose-6-phosphatase (G6Pase) and mitochondrial enzyme Phospho-enol-pyruvate carboxykinase (PEPCK) were down regulated in the liver. Over expression β-cell gene expression of insulin and its transcriptor factors genes of Nkx – 6.1 and Pdx – 1 in pancreatic tissue is another novel pathway of glycemic control that needs further gene studies.

## CONCLUSION

In conclusion, the present study observed berberine induces gene expression of insulin and its transcriptor factor genes Nkx-6.1 and Pdx-1 in pancreatic tissue in dose dependent fashion. Further studies, both animal and human are recommended to validate findings of present study and making berberine an alternative anti – diabetic therapy through gene stimulation mechanism.

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