Dose-Response:
An International Journal
July-September 2019:1-15

DOI: 10.1177/1559325819862449
journals.sagepub.com/home/dos
SAGE

Ameliorative Effect of Berberine on Neonatally Induced Type 2 Diabetic Neuropathy via Modulation of BDNF, IGF-1, PPAR-γ, and AMPK Expressions

Guangju Zhou[1], Mingzhu Yan[2], Gang Guo[3], and Nanwei Tong[1]

Abstract

Neonatal-streptozotocin (n-STZ)-induced diabetes mimics most of the clinicopathological symptoms of type 2 diabetes mellitus (T2DM) peripheral neuropathy. Berberine, a plant alkaloid, is reported to have antidiabetic, antioxidant, anti-inflammatory, and neuroprotective potential. The aim of the present study was to investigate the potential of berberine against n-STZ-induced painful diabetic peripheral polyneuropathy by assessing various biochemical, electrophysiological, morphological, and ultrastructural studies. Type 2 diabetes mellitus was produced neonatal at the age of 2 days (10-12 g) by STZ (90 mg/kg intraperitoneal). After confirmation of neuropathy at 6 weeks, rats were treated with berberine (10, 20, and 40 mg/kg). Administration of n-STZ resulted in T2DM-induced neuropathic pain reflected by a significant alterations ($P < .05$) in hyperalgesia, allodynia, and motor as well as sensory nerve conduction velocities whereas berberine (20 and 40 mg/kg) treatment significantly attenuated ($P < .05$) these alterations. Berberine treatment significantly inhibited ($P < .05$) STZ-induced alterations in aldose reductase, glycated hemoglobin, serum insulin, hepatic cholesterol, and triglyceride levels. The elevated oxido-nitrosative stress and decreased Na-K-ATPase and pulse Ox levels were significantly attenuated ($P < .05$) by berberine. It also significantly downregulated ($P < .05$) neural tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6 messenger RNA (mRNA), and protein expressions both. Streptozotocin-induced downregulated mRNA expressions of brain-derived neurotrophic factor (BDNF), insulin-like growth factor (IGF-1), and peroxisome proliferator-activated receptors-γ (PPAR-γ) in sciatic nerve were significantly upregulated ($P < .05$) by berberine. Western blot analysis revealed that STZ-induced alterations in adenosine monophosphate protein kinase (AMPK; Thr-172) and protein phosphatase 2C-α protein expressions in dorsal root ganglia were inhibited by berberine. It also attenuated histological and ultrastructural alterations induced in sciatic nerve by STZ. In conclusion, berberine exerts its neuroprotective effect against n-STZ-induced diabetic peripheral neuropathy via modulation of pro-inflammatory cytokines (TNF α, IL-1β, and IL-6), oxido-nitrosative stress, BDNF, IGF-1, PPAR-γ, and AMPK expression to ameliorate impaired allodynia, hyperalgesia, and nerve conduction velocity during T2DM.

Keywords

5′-adenosine monophosphate-activated protein kinase, brain-derived neurotrophic factor, berberine, diabetic neuropathy, insulin-like growth factor-1, neonatal-streptozotocin, peroxisome proliferator-activated receptors-γ, type 2 diabetes

[1] Department of Endocrinology and Metabolism, West China Hospital of Sichuan University, Chengdu, China
[2] Department of Neurology, Xijing Hospital, Fourth Military Medical University (FMMU), Shaanxi, China
[3] Department of Talent Highland, Department of General Surgery, The First Affiliated Hospital of Xi'an Jiaotong University, Shaanxi, China

Received 18 April 2019; received revised 15 June 2019; accepted 18 June 2019

Corresponding Authors:
Nanwei Tong, Department of Endocrinology and Metabolism, West China Hospital of Sichuan University, 37 Guoxuexiang, Chengdu 610041, China; Gang Guo, Department of Talent Highland, Department of General Surgery, First Affiliated Hospital of Xi'an Jiao Tong University, Xian 710061, China. Emails: tongnw@scu.edu.cn or tn0280@sina.com; boyiiman1988@163.com

Introduction

Diabetic peripheral polyneuropathy (DPN) is one of the most complex, chronic, and diverse metabolic complications of type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM).[1] The clinicopathological features of DPN include allodynia, hyperalgesia, abnormal or loss of sensation of nerve fibers due to segmental demyelination, degeneration of axonal, and loss of nerve fiber.[2] The prevalence of DM is expected to reach 366 million by 2030.[3] Additionally, almost 50% of the patient population with DM suffer from DPN, and it remains undiagnosed, which may result in skin ulceration, lower limbs debilitation, and amputation.[4] Thus, it causes a decrease in quality of life that increases health costs associated with DPN. A report suggested that total annual medical cost for the management of severe painful DPN is $30 755 per patient.[5]

Numerous clinical evidence suggested an array of pathogenic mechanisms of DPN, including increased blood sugar levels, accumulation of advanced glycation end products (AGEs), elevated activity of aldose reductase (AR) and polyol metabolism, increased production of reactive nitrogen species and reactive oxygen species (ROS) including hydrogen peroxide, hydroxyl and superoxide radicals, pro-inflammatory cytokines (such as tumor necrosis factor-α [TNF-α] and interleukins [ILs]), damage to the mitochondria of dorsal root ganglion (DRG), nonenzymatic glycation of proteins, and decreased activity of Na^+K^+-ATPase.[6,7] Recently, various growth factors such as insulin-like growth factor (IGF)-1, nerve growth factor (NGF), and vascular endothelial growth factor and brain-derived neurotrophic factor (BDNF) have been recognized significantly as important pathogenic factors during DPN.[8] Furthermore, activation of peroxisome proliferator-activated receptors (PPARs) and adenosine monophosphate protein kinase (AMPK) have been reported to decrease the production of pro-inflammatory cytokines, which promote pain relief during painful DPN.[9]

An array of researchers has implicated streptozotocin (STZ)-induced neuropathic model in an adult rodent for investigation of the potential of various therapeutic moieties against painful DPN.[10,11] However, intraperitoneal (ip) administration of STZ in adult rats resulted in induction of T1DM with delayed production of hyperalgesia and allodynia at 4 to 6 weeks. Additionally, the high mortality rate limits the findings of T1DM-induced DPN.[12] Thus, the use of an animal model that mimics clinicopathological characteristics of T2DM-induced DPN is needed. The neonatal-STZ (n-STZ)-induced neuropathy is well-studied, established, and validated a model of non–insulin-dependent DM, that is, T2DM-induced DPN.[13,14] Hence, we have used n-STZ-induced T2DM model of neuropathy that exhibited the slower and long-lasting complications, including hyperglycemia, insulin resistance, glycosuria, polyphagia, polyuria, polydipsia, and abnormal glucose tolerance.[13,14]

Berberine (18, 5,6-dihydro-9,10-dimethoxybenzo(g)-1,3-benzodioxolo (5,6-a) quinolizinium), a naturally occurring benzyl tetra isoquinoline alkaloid, is widely present in various medicinal plants including *Berberis aquifolium*, *Berberis vulgaris*, *Berberis sargentiana*, *Coptis chinensis*, and *Hydrastis canadensis*. Berberine has been reported to possess an array of pharmacological potential, including anti-inflammatory, antihypertensive, antihyperlipidemic, antidiabetic, cardioprotective, neuroprotective, antiarthritic, antioxidant, and anticancer activity.[15-18] It has been reported that berberine exerts its beneficial effects via activation of PPAR-γ and AMPK pathways.[15,16] A recent study showed that berberine ameliorates paclitaxel-induced neuropathic pain via activation of Nrf2 messenger RNA (mRNA) expression.[19] Previous study showed that berberine showed its potential against DPN against T1DM-induced model.[20] However, the effect of berberine against n-STZ-induced T2DM neuropathic pain has not been evaluated yet. Therefore, the present study was aimed to investigate the potential of berberine against n-STZ-induced painful DPN by assessing various biochemical, electrophysiological, morphological, and ultrastructural studies.

Materials and Methods

Animals

Adult male and female Sprague-Dawley rats (180-200 g) were maintained at 24°C ± 1°C, with a relative humidity of 45% to 55% and 12-hour:12-hour dark/light cycle. The animals had free access to standard pellet chow (except prior to fasting blood collection) and water throughout the experimental protocol. All experiments were carried out between 0900 and 1700 hours. All procedures involving animals were conducted in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals and were approved by the Animal Ethics and Use.

Chemicals

Streptozotocin and berberine hydrochloride hydrate (purity 99%) were purchased from Sigma Chemical Co (St Louis, Missouri). Insulin injection (Mixtard) was purchased from Novo Nordisk India Limited, India. Cholesterol and triglyceride kit were obtained from Accurex Biomedical Pvt Ltd, India. Further, TNF-α, IL-1β, IL-6, and BDNF enzyme-linked immunosorbent assay (ELISA) kits were obtained from Thermo Scientific (Chengdu, SiChuan). Insulin obtained from Mercodia AB, Uppsala, Sweden, and glycated hemoglobin (Hb) assay kit obtained from Crystal Chem, Inc, Downers Grove, Illinois. The primary antibodies of Thr-172 (phosphorylated AMPK) and protein phosphatase 2C-α (PP2C-α; nonphosphorylated AMPK) were purchased from Abcam (Cambridge, Massachusetts). Total RNA extraction kit and One-step Reverse transcription-polymerase chain reaction (RT-PCR) kit was purchased from MP Biomedicals India Private Limited, India.

Neonatal STZ-Induced Diabetic Neuropathy Model

The vaginal smears of the adult female Sprague-Dawley rats were examined, and the rat in the pro-estrous stage of estrus

cycle was allowed to mate with a male of proven fertility. The presence of spermatozoa in the vaginal discharge was used as the criterion of pregnancy. The pregnant rats were isolated and allowed to go to term. The rat pups of age 2 days (10-12 g) were administered 90 mg/kg ip of STZ prepared in acetate buffer (0.1 M, pH 4.5). Rat pups in nondiabetic control groups were administered only buffer (ip) used for the preparation of STZ solution. After 4 weeks, rats were separated from their mothers. The young rats had free access to rodent chow food and water in an air-conditioned environment (23°C with 55% humidity) under a 12-hour:12-h light/dark cycle. After 6 weeks of age, diabetes was recognized by polyuria, polydipsia and by measuring fasting plasma glucose levels. Rats with glucose levels above 150 mg/dL were incorporated in the study.[13]

Experimental Design and Protocol

The animals were randomly distributed into the following groups, each consisting of 18 rats.

[A] Nondiabetic animals

NC: Nondiabetic control received carboxymethyl cellulose (CMC) 5% (1 mL/kg/d, orally [PO]) for 8 weeks.

[B] Diabetic animals

Group1: n-STZ: n-STZ diabetic control received CMC 5% (1 mL/kg/d, PO) for 8 weeks.

Group2: P (10): n-STZ diabetic rats received pioglitazone (10 mg/kg/d, PO) for 8 weeks.

Group3: I (10): n-STZ diabetic rats received insulin (10 IU/kg/d, SC) for 8 weeks.

Group4: B (10): n-STZ diabetic rats received berberine (10 mg/kg/d, PO) for 8 weeks.

Group5: B (20): n-STZ diabetic rats received berberine (20 mg/kg/d, PO) for 8 weeks.

Group6: B (40): n-STZ diabetic rats received berberine (40 mg/kg/d, PO) for 8 weeks.

After confirmation of neuropathy, the basal readings were taken before initiation of the experimentation and drug treatment, that is, at week 6. The dose of pioglitazone 10 mg/kg and berberine (10, 20, and 40 mg/kg) were selected on the basis of previous study.[21] Pioglitazone and berberine were dissolved in 0.5% Na-CMC. An observer (blind) took readings of the behavioral parameters to the drug administration at weeks 6, 8, 10, 12, and 14. Food intake, water intake, and urine output were measured with the help of a metabolic cage (Metabolic cage, Techniplast, Italy).

Estimation of Plasma Glucose Level

100 μL of blood was collected by retro-orbital puncture of each rat. Blood was centrifuged at 5000 rpm for 5 minutes, and plasma was collected. Then, the plasma glucose levels were estimated using GOD-POD kit according to the manufacturer's protocol.

Estimation of Glycosuria Level

Glycosuria was qualitatively assessed in urine stored for 24 hours using glucose oxidase-peroxidase (GOD-POD, R&D Systems) kit according to the manufacturer's protocol.

Behavioral Tests

Mechanical hyperalgesia (Randall-Selitto paw pressure test), thermal hyperalgesia (Plantar test), mechano-tactile allodynia (Von Frey hair test), thermal hyperalgesia (Tail immersion test), motor nerve conduction velocity (MNCV), and sensory nerve conduction velocity (SNCV) were measured in rats as described elsewhere.[22]

Pulse Ox Tests

To assess peripheral blood oxygen content in vivo, rats were monitored for the percentage of Hb saturated with oxygen (pulse Ox). The rats were anesthetized with ether, and a peripheral pulse Ox sensor (ChoiceMMed, V1.0CF3, MD300CF3, China) was attached to the tail. Pulse Ox readings were taken as the animal regained consciousness.[23]

Determination of Serum Parameters

Determination of serum insulin. The serum was separated by centrifugation using Eppendorf Cryocentrifuge (model No. 5810, Germany), maintained at 4°C, and run at a speed of 5200*g* for 15 minutes. The assay of serum insulin was performed by a rat ELISA kit (Mercodia AB).

Determination of glycated hemoglobin A_{1c}. The quantification of glycated hemoglobin A_{1c} (HbA_{1c}) was performed by a glycated Hb assay kit (Crystal Chem, Inc).

Biochemical Assays

Sciatic nerve homogenate preparation. All animals were sacrificed at the end of the study, that is week 14, and sciatic nerve was immediately isolated. Tissue homogenate was prepared with 0.1 M Tris–HCl buffer (pH 7.4) and supernatant of homogenate was employed to estimate total protein content, superoxide dismutase (SOD), reduced glutathione (GSH), lipid peroxidation (MDA content), nitric oxide (NO) content, membrane-bound enzyme, TNF-α, IL-1β, IL-6, and BDNF levels as described previously.[24]

Estimation of membrane-bound enzyme determination (Na+-K+-ATPase). The membrane-bound enzyme (Na^+-K^+-ATPase) was estimated in sciatic nerve as described previously.[24] The enzyme activity was expressed as μM of inorganic phosphorus liberated/mg protein/min.

Estimation of TNF-α, IL-1β, IL-6, and BDNF levels. The quantifications of TNF-α, IL-1β, IL-6, and BDNF were performed with the help of instructions provided by Thermo Scientific using the Rat TNF-α, IL-1β, IL-6, and BDNF immunoassay kit. The assay

Table 1. Effect of Berberine Treatment on n-STZ-Induced Alterations in Glycosuria, Aldose Reductase, Glycated Hb, Serum Insulin, Hepatic Cholesterol, Hepatic Triglyceride, and Pulse Ox of Rats.[a]

Treatment	Glycosuria (mg/dL)	Aldose Reductase (nM of NADPH Oxidized /min/mg of Protein)	Glycated Hb (%)	Serum Insulin (μg/L)	Hepatic Cholesterol (mg/dL)	Hepatic Triglyceride (mg/dL)	Pulse Ox (% O_2 Saturation)
NC	29.86 ± 8.79	2.15 ± 0.31	3.57 ± 0.51	3.03 ± 0.22	71.95 ± 5.59	54.80 ± 11.32	94.50 ± 0.34
n-STZ	507.30 ± 17.13[b]	8.56 ± 0.47[b]	9.70 ± 0.69[b]	0.50 ± 0.08[b]	234.10 ± 5.64[b]	367.20 ± 13.03[b]	87.17 ± 0.48[b]
P (10)	119.80 ± 5.79[c,d]	3.71 ± 0.32[c,d]	6.23 ± 0.35[c,d]	1.32 ± 0.19[c,d]	102.40 ± 7.86[c,d]	100.40 ± 12.43[c,d]	93.33 ± 0.61[c,d]
I (10)	56.34 ± 12.78[c,d]	3.45 ± 0.44[c,d]	5.15 ± 0.60[c,d]	2.60 ± 0.24[c,d]	108.00 ± 7.81[c,d]	96.11 ± 15.61[c,d]	93.83 ± 0.83[c,d]
B (10)	451.00 ± 23.51	7.69 ± 0.39	8.57 ± 0.55	0.55 ± 0.22	208.10 ± 6.49	335.60 ± 10.18	88.33 ± 0.42
B (20)	377.10 ± 14.25[c,d]	6.21 ± 0.34[c,d]	7.13 ± 0.39[c,d]	1.50 ± 0.25[c,d]	157.30 ± 7.52[c,d]	227.50 ± 9.57[c,d]	90.67 ± 0.33[c,d]
B (40)	212.10 ± 21.06[c,d]	4.79 ± 0.42[c,d]	5.93 ± 0.53[c,d]	2.07 ± 0.25[c,d]	111.70 ± 3.59[c,d]	126.30 ± 12.94[c,d]	92.00 ± 0.37[c,d]

Abbreviations: B (10), berberine (10 mg/kg, PO) treated group; B (20), berberine (20 mg/kg, PO) treated group; B (40), berberine (40 mg/kg, PO) treated group; Hb, hemoglobin; I (10), insulin (10 IU/kg, SC) treated group; NC, nondiabetic control; n-STZ, neonatal STZ control; P (10), pioglitazone (10 mg/kg, PO) treated group.

[a]Data are expressed as mean ± SEM (n = 6) and analyzed by 1-way analysis of variance followed by Tukey multiple range test.

[b]$P < .05$ as compared to nondiabetic control group.

[c]$P < .05$ as compared to n-STZ control group.

[d]$P < .05$ as compared to one another.

employs the sandwich enzyme immunoassay technique. Briefly, 50 μL of pretreated buffer was added to each well. Then, 50 μL of standards, control, and test samples (aliquot of sciatic nerve homogenate) were added into each well and incubated at room temperature (RT). for 1 hour. If, in any rat, IL-1β, IL-6, and BDNF are present, it would have bound to the immobilized antibody. After having washed away any unbound substance, 50 μL of biotinylated antibody reagent was added to each well and incubated at RT for 1 hour. After washing away any unbound substance, 100 μL of streptavidin-HPR reagent was added to each well, which is an enzyme-linked polyclonal antibody specific for rat TNF-α, IL-1β, IL-6, and BDNF. Then, it was followed by washing to remove any unbound antibody–enzyme reagent. Then, 100 μL of TMB, a substrate solution, and consequently an enzyme reaction was added, which made the blue product turn yellow. The intensity of the color was measured at 550 nm and it was in proportion to the amount of rat TNF-α, IL-1β, IL-6, and BDNF bound in the initial steps. The sample values were then read off using the standard curve. Values were expressed as means ± standard error of the mean (SEM).

Determination of Hepatic Cholesterol and Triglyceride

The activities of hepatic cholesterol and triglyceride were measured as described previously.[25]

Reverse Transcriptase PCR

The levels of mRNA were analyzed in sciatic nerve tissue using RT-PCR approach as described previously.[24] Briefly, total RNA was extracted from skin tissues according to the manufacturer's instructions (Biotools B&M Labs, Spain). The PCR mixture was amplified in a DNA thermal cycler (Eppendorf India Ltd, Chennai, India) using gene-specific primers (Supplementary Table 1). The PCR products were run on 1% agarose gel, stained with ethidium bromide. Gene expression was assessed by generating densitometry data for band intensities in different sets of experiments, by analyzing the gel images on the Image J program (version 1.33, Wayne Rasband, National Institutes of Health, Bethesda, Mary land). The band intensities were compared with constitutively expressed β-actin, which served as a control for sample loading and integrity. The intensity of mRNAs was standardized against that of the β-actin mRNA from each sample, and the results were expressed as PCR-product/β-actin mRNA ratio.

Western Blot Procedure

Western blotting was performed according to the protocol described elsewhere.[26] Briefly, the DRG was isolated, the protein was separated by gel electrophoresis (10% gel) and transferred to polyvinylidene fluoridated (PVDF) membranes. The PVDF membranes were incubated with primary antibody Thr-172 (phosphorylated AMPK) and PP2C-α (nonphosphorylated AMPK). Subsequently, the membranes were treated with horseradish peroxidase-conjugated secondary antibodies. Immune complexes were visualized using ECL plus detection reagents.

Histopathological Analysis of Sciatic Nerve

Another portion of the sciatic nerve was stored in 10% formalin for 24 hours. The specimen was dehydrated and placed in xylene for 1 hour (3 times) and later in ethyl alcohol (70%, 90%, and 100%) for 2 hours, respectively. The infiltration and impregnation were carried out by treating with paraffin wax twice, each time for 1 hour. Tissue specimens were cut into sections of 3 to 5 μm thickness and were stained with hematoxylin and eosin. The specimen was mounted on a slide by the use of distrene, with dibutyl phthalate and xylene as mounting

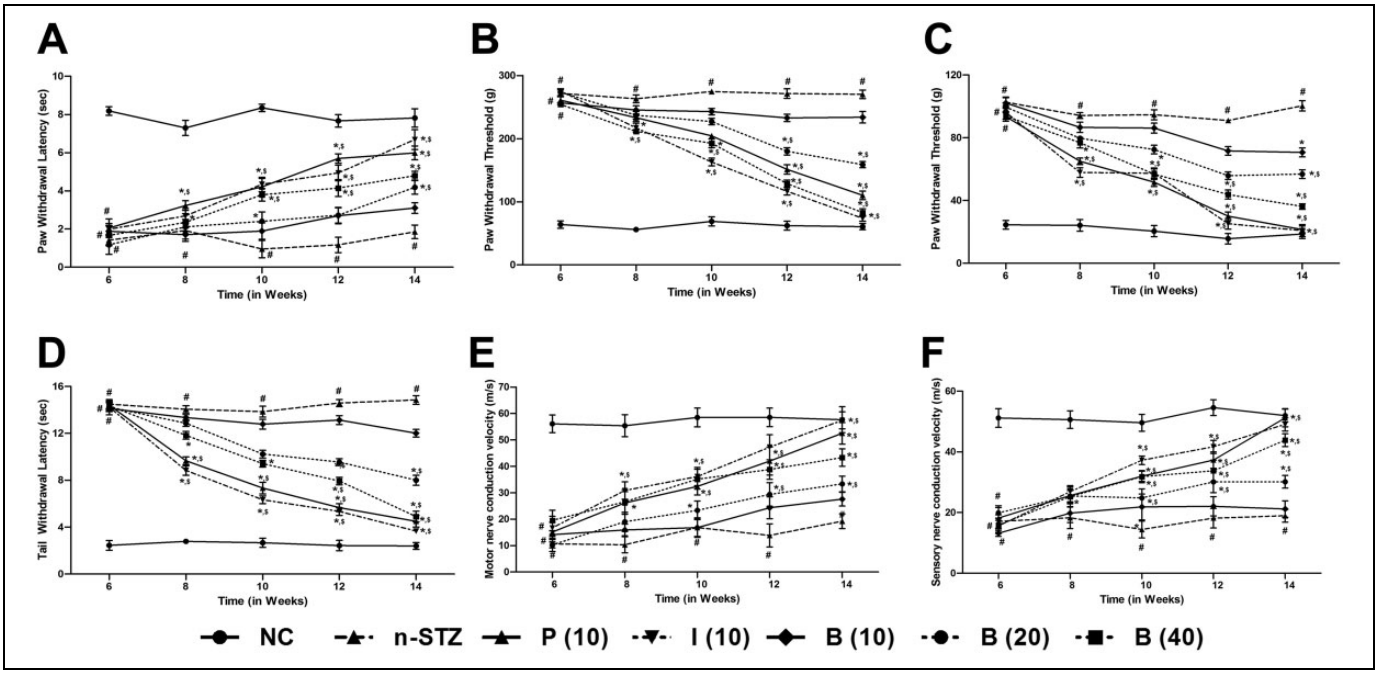

Figure 1. Effect of berberine treatment on n-STZ induced alterations in thermal hyperalgesia in plantar test (A), mechanical hyperalgesia in paw pressure test (B), mechanical allodynia in Von-Frey hair test (C), thermal hyperalgesia in tail immersion test (D), motor nerve conduction velocity (E), and sensory nerve conduction velocity (F) in rats. Data are expressed as mean ± SEM (n = 6) and analyzed by 2-way analysis of variance followed by Tukey multiple range test. $^{*}P < .05$ as compared to n-STZ control group, $^{\#}P < .05$ as compared to nondiabetic control group, and $^{\$}P < .05$ as compared to one another. NC indicates nondiabetic control; n-STZ, neonatal STZ control; SEM, standard error of the mean; P (10), pioglitazone (10 mg/kg, PO) treated group; I (10), insulin (10 IU/kg, SC) treated group; B (10), berberine (10 mg/kg, PO) treated group; B (20), berberine (20 mg/kg, PO) treated group; B (40), berberine (40 mg/kg, PO) treated group.

medium. Sections were examined under a light microscope (Digital Upright Microscope, Motic Asia, Hong Kong) to obtain a general impression of the histopathology features of specimen and infiltration of cells. The images were analyzed using Image J program version 1.33. The various changes in histological features were graded as grade 0 (not present); grade 1 (mild); grade 2 (moderate); and grade 3 (severe), as described previously.[13]

Electron Microscopic Analysis

For ultrastructural studies, sciatic nerve samples were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 18 hours. The tissue sample was dissected into small pieces and postfixed for 1.5 hours in 1% osmium tetroxide dissolved in 0.1 M phosphate buffer (pH 7.4), then dehydrated through a series of graded ethanol solutions and embedded in Araldite (epoxy resin). Ultrathin sections were cut, stained with uranyl acetate and lead nitrate, mounted on copper grids, and examined under a transmission electron microscope (H-7000 Hitachi).

Statistical Analysis

Data are expressed as mean ± SEM. Data analysis was performed using Graph Pad Prism 5.0 software (Graph Pad, San Diego, California). Data were analyzed using 1-way and 2-way analysis of variance, and Tukey multiple range test was applied for post hoc analysis. A value of $P < .05$ was considered statistically significant.

Results

Effect of berberine Treatment on n-STZ-Induced Alterations in Body Weight, Serum Glucose Level, Food Intake, Water Intake, and Urine Output of Rats

Intraperitoneal administration of STZ in neonatal rats resulted in significant decrease ($P < .05$) in body weight and significant increase ($P < .05$) in serum glucose level, food intake, water intake, and urine output in n-STZ rats as compared to nondiabetic rats at weeks 6 and 14. However, 8 weeks treatment with pioglitazone (10 mg/kg) and insulin (10 IU/kg) significantly attenuated ($P < .05$) these STZ-induced alterations in body weight, serum glucose level, food intake, water intake, and urine output as compared to n-STZ control rats. Berberine (20 and 40 mg/kg) treatment also significantly increased ($P < .05$) body weight and significantly decreased ($P < .05$) serum glucose level, food intake, water intake, and urine output as compared to n-STZ control rats. However, inhibition of STZ-induced alterations in body weight, serum glucose level, and intakes (food and water) were more significant ($P < .05$) in pioglitazone (10 mg/kg) and insulin (10 IU/kg) as compared to berberine treatment at week 14 (Supplementary Figure 1).

Effect of Berberine Treatment on n-STZ-Induced Alterations in Hyperalgesia and Allodynia of Rats

There was a significant decrease ($P < .05$) in thermal hyperalgesia and significant increase ($P < .05$) in mechanical hyperalgesia as well as allodynia in n-STZ rats at week 6 after ip administration of STZ as compared to nondiabetic rats. Treatment with pioglitazone (10 mg/kg) and insulin (10 IU/kg) for 8 weeks significantly ameliorated ($P < .05$) STZ-induced decrease in thermal hyperalgesia and increase in mechanical hyperalgesia and allodynia as compared to n-STZ control rats. When compared with n-STZ control rats, berberine (20 and 40 mg/kg) treatment also showed a significant increase ($P < .05$) in thermal hyperalgesia and a significant decrease ($P < .05$) in mechanical hyperalgesia as well as mechanical allodynia. However, pioglitazone (10 mg/kg) and insulin (10 IU/kg) treatment showed more significant ($P < .05$) attenuation in thermal hyperalgesia and mechanical allodynia as compared to berberine treatment (Figure 1).

Effect of Berberine Treatment on n-STZ-Induced Alterations in MNCV and SNCV of Rats

Both MNCV and SNCV were decreased significantly ($P < .05$) in n-STZ rats after 6 weeks of STZ administration as compared to nondiabetic rats. When compared with n-STZ control rats, pioglitazone (10 mg/kg) and insulin (10 IU/kg) treatment significantly increased ($P < .05$) MNCV and SNCV. Berberine (20 and 40 mg/kg) treatment also significantly inhibited ($P < .05$) STZ-induced decrease in MNCV and SNCV as compared to n-STZ control rats. However, both MNCV and SNCV were more significantly ($P < .05$) ameliorated by pioglitazone (10 mg/kg) and insulin (10 IU/kg) treatment as compared to berberine treatment (Figure 1).

Effect of Berberine Treatment on n-STZ-Induced Alterations in Glycosuria, AR, Glycated Hb, and Serum Insulin of Rats

Intraperitoneal administration of STZ caused a significant increase ($P < .05$) in glycosuria, AR, and glycated Hb levels whereas significant decrease ($P < .05$) in serum insulin in n-STZ rats as compared to nondiabetic rats. Treatment with pioglitazone (10 mg/kg) and insulin (10 IU/kg) significantly decreased ($P < .05$) glycosuria, AR, and glycated Hb levels as well as significantly increased ($P < .05$) serum insulin as compared to n-STZ control rats. The increased levels of glycosuria, AR, and glycated Hb were significantly decreased ($P < .05$) by berberine (20 and 40 mg/kg) treatment, whereas it also significantly increased ($P < .05$) serum insulin compared with n-STZ control rats. However, berberine (40 mg/kg) treatment more significantly ($P < .05$) inhibited STZ-induced alterations in glycated Hb and serum insulin levels as compared to pioglitazone (10 mg/kg) treatment. Moreover, insulin (10 IU/kg) treatment showed more significant ($P < .05$) decrease in glycosuria, AR, and glycated Hb levels as well as more significant increase ($P < .05$) in serum insulin as compared to berberine and pioglitazone (10 mg/kg) treatment (Table 1).

Effect of Berberine Treatment on n-STZ-Induced Alterations in Hepatic Cholesterol, Hepatic Triglyceride, and Pulse Ox of Rats

There was a significant increase ($P < .05$) in hepatic cholesterol and triglyceride levels whereas significant decrease ($P < .05$) in pulse Ox levels after ip administration of STZ in n-STZ rats as compared to nondiabetic rats. Pioglitazone (10 mg/kg) and insulin (10 IU/kg) treatment significantly inhibited ($P < .05$) STZ-induced alterations in hepatic cholesterol, hepatic triglyceride, and Pulse Ox as compared to n-STZ control rats. Berberine (20 and 40 mg/kg) treatment also significantly decreased ($P < .05$) hepatic cholesterol and triglyceride whereas significantly increased ($P < .05$) pulse Ox levels as compared to n-STZ control rats. This STZ-induced alteration in hepatic cholesterol, hepatic triglyceride, and pulse Ox was more significantly ($P < .05$) attenuated by pioglitazone (10 mg/kg) and insulin (10 IU/kg) as compared to berberine treatment (Table 1).

Effect of Berberine Treatment on n-STZ-Induced Alterations in Oxido-Nitrosative Stress and Na-K-ATPase Levels of Rats

The levels of oxido-nitrosative stress (ie, SOD, GSH, MDA, and NO), as well as Na-K-ATPase, was significantly altered ($P < .05$) in n-STZ rats after ip administration of STZ as compared to nondiabetic rats. Administration of pioglitazone (10 mg/kg) and insulin (10 IU/kg) for 8 weeks significantly increased ($P < .05$) the levels of SOD, GSH, and Na-K-ATPase whereas significantly decreased ($P < .05$) the levels of MDA and NO as compared to n-STZ control rats. Berberine (20 and 40 mg/kg) treatment also significantly inhibited ($P < .05$) STZ-induced alterations in levels of SOD, GSH, MDA, NO, and Na-K-ATPase as compared to n-STZ control rats. However, berberine (40 mg/kg) treatment showed more significant ($P < .05$) increase in SOD and Na-K-ATPase level as well as more significant ($P < .05$) decrease in MDA and NO levels as compared to pioglitazone (10 mg/kg) treatment (Table 2).

Effect of Berberine Treatment on n-STZ-Induced Alterations in Neural TNF-α, IL-1β, IL-6, and BDNF Protein Levels of Rats

Intraperitoneal administration of STZ resulted in significant increase ($P < .05$) of neural TNF-α, IL-1β, and IL-6 protein levels as well as a significant decrease ($P < .05$) of neural BDNF protein levels in n-STZ rats as compared to nondiabetic rats. Pioglitazone (10 mg/kg) treatment significantly inhibited ($P < .05$) STZ-induced alterations in neural TNF-α, IL-1β, IL-6, and BDNF protein levels as compared to n-STZ control rats. When compared with n-STZ control rats, insulin (10 IU/kg)

Table 2. Effect of Berberine Treatment on n-STZ-Induced Alterations in Oxido-Nitrosative Stress and Na-K-ATPase Level in Rats.[a]

Treatment	SOD (U/mg of Protein)	GSH (μg/mg Protein)	MDA (nM/mg of Protein)	NO (μg/mL)	Na-K-ATPase (μmol/mg of Protein)
NC	23.38 ± 1.14	1.57 ± 0.16	3.26 ± 0.25	122.30 ± 27.70	9.34 ± 0.52
n-STZ	11.36 ± 1.07[b]	0.59 ± 0.14[b]	9.42 ± 0.41[b]	465.70 ± 23.92[b]	2.88 ± 0.81[b]
P (10)	18.68 ± 1.32[c,d]	1.44 ± 0.13[c,d]	3.79 ± 0.43[c,d]	344.20 ± 18.18[c,d]	5.01 ± 0.62
I (10)	17.51 ± 1.48[c,d]	1.48 ± 0.11[c,d]	4.92 ± 0.38[c,d]	275.40 ± 15.47[c,d]	4.28 ± 0.69
B (10)	12.17 ± 1.74	0.63 ± 0.05	8.28 ± 0.33	447.40 ± 22.00	4.12 ± 0.71
B (20)	15.55 ± 1.52[c,d]	0.97 ± 0.10[c,d]	7.07 ± 0.33[c,d]	352.20 ± 16.97[c,d]	5.74 ± 0.74[c,d]
B (40)	20.93 ± 1.39[c,d]	1.10 ± 0.13[c,d]	4.70 ± 0.27[c,d]	229.30 ± 22.39[c,d]	7.97 ± 0.74[c,d]

Abbreviations: B (10), berberine (10 mg/kg, PO) treated group; B (20), berberine (20 mg/kg, PO) treated group; B (40), berberine (40 mg/kg, PO) treated group; GSH, glutathione; I (10), insulin (10 IU/kg, SC) treated group; MDA, malondialdehyde; NC, nondiabetic control; NO, nitric oxide; n-STZ, neonatal STZ control; P (10), pioglitazone (10 mg/kg, PO) treated group; SOD, superoxide dismutase.
[a]Data are expressed as mean ± SEM (n = 6) and analyzed by 1-way analysis of variance followed by Tukey multiple range test.
[b]$P < .05$ as compared to nondiabetic control group.
[c]$P < .05$ as compared to n-STZ control group.
[d]$P < .05$ as compared to one another.

Table 3. Effect of Berberine Treatment on n-STZ-Induced Alterations in Neural TNF-α, IL-1β, IL-6, and BDNF Levels in Rats.[a]

Treatment	TNF-α (pg/mL)	IL-1β (pg/mL)	IL-6 (pg/mL)	BDNF (pg/mL)
NC	54.87 ± 11.27	8.72 ± 1.41	26.91 ± 3.43	20.19 ± 1.24
n-STZ	152.80 ± 7.35[b]	24.35 ± 1.10[b]	56.19 ± 5.00[b]	8.73 ± 1.58[b]
P (10)	78.60 ± 9.20[c,d]	12.63 ± 1.60[c,d]	40.44 ± 2.34[c,d]	15.77 ± 1.68[c,d]
I (10)	64.80 ± 7.16[c,d]	14.62 ± 2.00[c,d]	33.31 ± 1.95[c,d]	10.40 ± 1.60
B (10)	143.60 ± 7.94	21.85 ± 2.07	50.43 ± 4.31	10.01 ± 1.29
B (20)	111.00 ± 12.80[c,d]	17.90 ± 1.81[c,d]	47.20 ± 3.48[c,d]	14.35 ± 1.91[c,d]
B (40)	91.55 ± 12.19[c,d]	15.21 ± 0.50[c,d]	30.74 ± 4.37[c,d]	17.40 ± 1.32[c,d]

Abbreviations: B (10), berberine (10 mg/kg, PO) treated group; B (20), berberine (20 mg/kg, PO) treated group; B (40), berberine (40 mg/kg, PO) treated group; BDNF, brain-derived neurotrophic factor; IL: interleukin; I (10), insulin (10 IU/kg, SC) treated group; NC, nondiabetic control; n-STZ, neonatal STZ control; P (10), pioglitazone (10 mg/kg, PO) treated group; TNF-α: tumor necrosis factor-α.
[a]Data are expressed as mean ± SEM (n = 6) and analyzed by 1-way analysis of variance followed by Tukey multiple range test.
[b]$P < .05$ as compared to nondiabetic control group.
[c]$P < .05$ as compared to n-STZ control group.
[d]$P < .05$ as compared to one another.

treatment significantly decreased ($P < .05$) elevated neural TNF-α, IL-1β, and IL-6 protein levels; however, it failed to produce any significant increase in neural BDNF protein level as compared to n-STZ control rats. Berberine (20 and 40 mg/kg) treatment significantly increased ($P < .05$) neural TNF-α, IL-1β, and IL-6 protein levels as well as significantly increased ($P < .05$) neural BDNF protein level as compared to n-STZ control rats (Table 3).

Effect of Berberine Treatment on n-STZ-Induced Alterations in mRNA Expressions of BDNF, IGF-1, and PPAR-γ in Sciatic Nerve of Rats

The neural mRNA expressions of BDNF, IGF-1, and PPAR-γ were decreased significantly ($P < .05$) in n-STZ rats after ip administration of STZ as compared to nondiabetic rats. The 8-week treatment of pioglitazone (10 mg/kg) significantly upregulated ($P < .05$) BDNF and PPAR-γ mRNA expressions in sciatic nerve; however, it failed to produce any significant upregulation in neural IGF-1 mRNA expressions as compared to n-STZ control rats. Whereas insulin (10 IU/kg) treatment also significantly upregulated ($P < .05$) neural IGF-1 and PPAR-γ mRNA expressions; however, it did not show any significant upregulation in neural BDNF mRNA expressions as compared to n-STZ control rats. Treatment with berberine (20 and 40 mg/kg) treatment significantly increased ($P < .05$) mRNA expression of BDNF, IGF-1, and PPAR-γ in sciatic nerve as compared to n-STZ control rats. Pioglitazone (10 mg/kg) treatment showed more significant ($P < .05$) upregulation ($P < .05$) in neural PPAR-γ mRNA expression as compared to berberine and insulin (10 IU/kg) treatment (Figure 2).

Effect of Berberine Treatment on n-STZ-Induced Alterations in mRNA Expressions of IL-1β, IL-6, and TNF-α in Sciatic Nerve of Rats

Intraperitoneal administration of STZ resulted in significant upregulation ($P < .05$) of neural IL-1β, IL-6, and TNF-α mRNA expressions in n-STZ rats as compared to nondiabetic rats. Treatment with pioglitazone (10 mg/kg) and insulin (10 IU/kg) significantly downregulated ($P < .05$) IL-1β, IL-6, and TNF-α mRNA expressions in sciatic nerve as compared to n-STZ control rats. Berberine (20 and 40 mg/kg) treatment also significantly inhibited ($P < .05$) STZ-induced alteration in

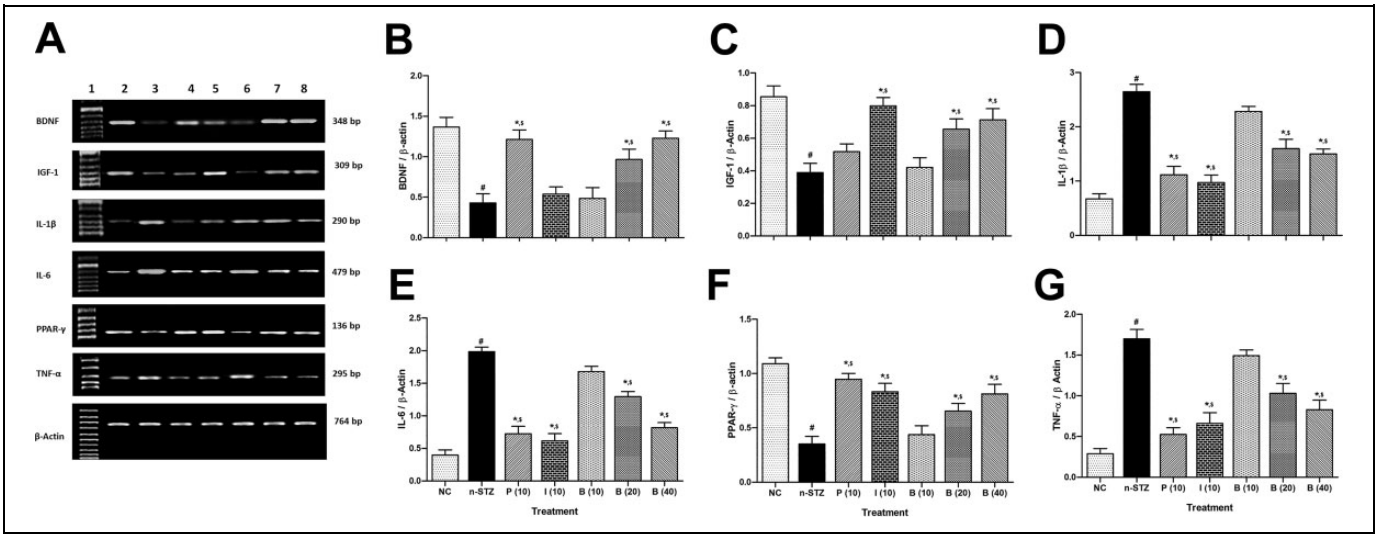

Figure 2. Effect of berberine treatment on n-STZ induced alterations in mRNA expression of BDNF, IGF-1, IL-1β, IL-6, PPAR-γ, and TNF-α in sciatic nerve as determined with relative quantification by reverse transcriptase polymerase chain reaction analysis (A) in rats. Quantitative representation of mRNA expression of BDNF (B), IGF-1 (C), IL-1β (D), IL-6 (E), PPAR-γ (F), and TNF-α (G). Data are expressed as mean ± SEM (n = 4) and analyzed by 1-way analysis of variance followed by Tukey multiple range test. $^{*}P < .05$ as compared to n-STZ control group, $^{\#}P < .05$ as compared to nondiabetic control group, and $^{\$}P < 0.05$ as compared to one another. Lane 1: Ladder 1000 bp; lane 2: mRNA expression of NC rats; lane 3: mRNA expression of n-STZ-treated rats; lane 4: mRNA expression of P (10)-treated rats; lane 5: mRNA expression of I (10)-treated rats; lane 6: mRNA expression of B (10)-treated rats; lane 7: mRNA expression of B (20)-treated rats; and lane 8: mRNA expression of B (40)-treated rats. BDNF indicates brain-derived neurotrophic factor; IGF-1, insulin-like growth factor-1; IL-1β, interleukin-1β; IL-6, interleukin-6; mRNA, messenger RNA; NC, nondiabetic control; n-STZ, neonatal STZ control; PPAR-γ: peroxisome proliferator-activated receptor gamma; SEM, standard error of the mean; TNF-α, tumor necrosis factor-α; P (10), pioglitazone (10 mg/kg, PO) treated group; I (10), insulin (10 IU/kg, SC) treated group; B (10), berberine (10 mg/kg, PO) treated group; B (20), berberine (20 mg/kg, PO) treated group; B (40), berberine (40 mg/kg, PO) treated group.

neural IL-1β, IL-6, and TNF-α mRNA expressions as compared to n-STZ control rats. However, pioglitazone (10 mg/kg) and insulin (10 IU/kg) treatment more significantly downregulated ($P < .05$) neural IL-1β, IL-6, and TNF-α mRNA expressions as compared to berberine treatment (Figure 2).

Effect of Berberine Treatment on n-STZ-Induced Alterations in Threonine Residue of Phosphorylated on AMPK (Thr-172) and PP2C-α Protein Expression in DRG of Rats

There was significant downregulation ($P < .05$) in residue of phosphorylated on AMPK, that is, Thr-172 protein expression, and significant upregulation ($P < .05$) in PP2C-α protein expression in DRG after ip administration of STZ in n-STZ rats as compared to nondiabetic rats. Pioglitazone (10 mg/kg) treatment significantly attenuated ($P < .05$) STZ-induced alterations in DRG Thr-172 and PP2C-α protein expressions as compared to n-STZ control rats. However, insulin (10 IU/kg) treatment failed to produce any significant effect on altered DRG Thr-172 and PP2C-α protein expressions as compared to n-STZ control rats. Treatment with berberine (20 and 40 mg/kg) significantly upregulated ($P < .05$) Thr-172 protein expression and significantly downregulated ($P < .05$) PP2C-α protein expression in DRG as compared to n-STZ control rats. Furthermore, upregulation in Thr-172 protein expression was more significant ($P < .05$) in berberine (40 mg/kg)) treatment as compared to pioglitazone (10 mg/kg) and insulin (10 IU/kg) treatment (Figure 3).

Effect of Berberine Treatment on n-STZ-Induced Histological Alterations in Sciatic Nerve of Rats

Figure 4A shows the normal architecture of the sciatic nerve from nondiabetic rats without any necrosis and edema; however, it depicts the presence of mild inflammatory cells (neutrophils as well as macrophages) and congestion. There was a significant increase ($P < .05$) in necrosis, edema, inflammatory infiltration, and congestion after ip administration of STZ in sciatic nerve of n-STZ rats as compared to nondiabetic rats (Figure 4B). Pioglitazone (10 mg/kg) and insulin (10 IU/kg) treatment significantly attenuated ($P < .05$) STZ-induced histological alterations in sciatic nerve as compared to n-STZ control rats (Figure 4C and D). Berberine (10 mg/kg) treatment failed to produce any significant effect of attenuation on STZ-induced histological alterations in sciatic nerve compared with n-STZ control rats. However, berberine (20 and 40 mg/kg) treatment significantly decreased ($P < .05$) STZ-induced necrosis, edema, infiltration of inflammatory cells, and congestion in sciatic nerve as compared to n-STZ control rats (Figure 4E-G).

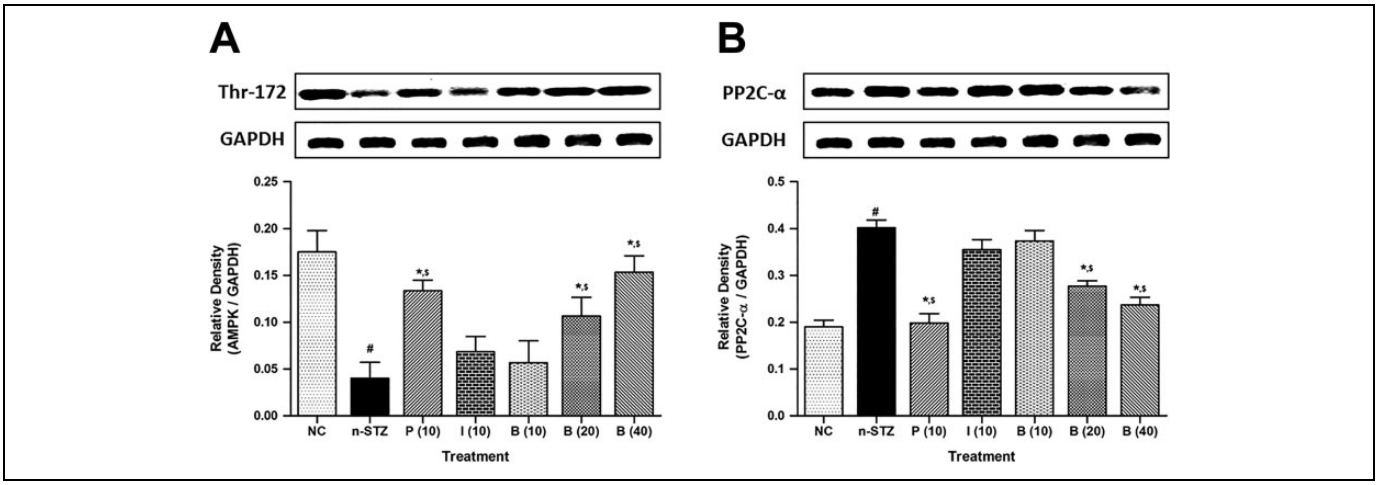

Figure 3. Effect of berberine treatment on n-STZ-induced alterations in Thr-172 (A) and PP2C-α (B) protein expression in DRG of rats. Data are expressed as mean ± SEM (n = 4) and analyzed by 1-way analysis of variance followed by Tukey multiple range test. *$P < .05$ as compared to n-STZ control group, #$P < .05$ as compared to nondiabetic control group, and $$P < .05$ as compared to one another. AMPK indicates 5′ adenosine monophosphate-activated protein kinase; DRG, dorsal root ganglia; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; NC, nondiabetic control; n-STZ, neonatal STZ control; PP2C-α, protein phosphatase 2C-α; SEM, standard error of the mean; P (10), pioglitazone (10 mg/kg, PO) treated group; I (10), insulin (10 IU/kg, SC) treated group; B (10), berberine (10 mg/kg, PO) treated group; B (20), berberine (20 mg/kg, PO) treated group; B (40), berberine (40 mg/kg, PO) treated group. Thr-172, threonine-172 within the catalytic subunit (α) of AMPK; .

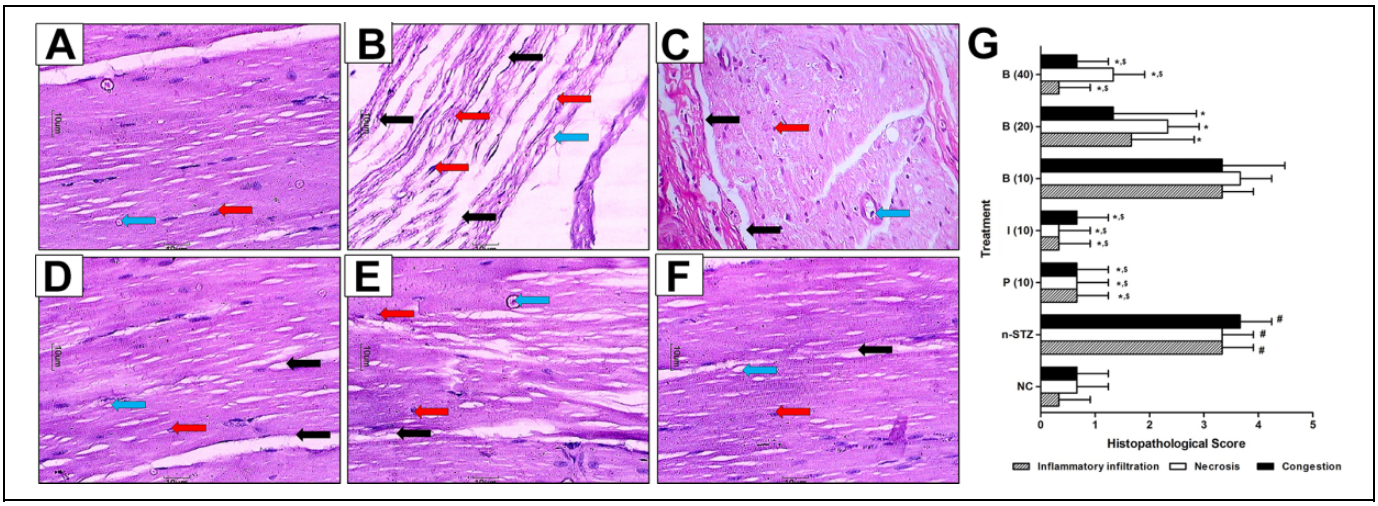

Figure 4. Effect of berberine treatment on n-STZ-induced histological alterations in the sciatic nerve. Photomicrographs of sections of sciatic nerve with hematoxylin & eosin stained from nondiabetic control (A), n-STZ control (B), pioglitazone (10 mg/kg, PO) treated group (C), insulin (10 IU/kg, SC) treated group (D), berberine (20 mg/kg, PO) treated group (E) and berberine (40 mg/kg, PO) treated group (F). Effect of berberine treatment on histological alterations sciatic nerve (G). Data are expressed as mean ± SEM (n = 3) and analyzed by 1-way analysis of variance followed by Tukey multiple range test. *$P < .05$ as compared to n-STZ control group, #$P < .05$ as compared to nondiabetic control group, and $$P < .05$ as compared to one another. Congestion (blue arrow), necrosis (black arrow), and inflammatory infiltration (red arrow). Images (×100 magnification) are typical and are representative of each study group. n-STZ indicates neonatal STZ control; PO, orally; SEM, standard error of the mean; SC, subcutaneously.

Effect of Berberine Treatment on n-STZ-Induced Alterations in Sciatic Nerve Ultrastructure of Rats

Ultrastructural findings of sciatic nerve from a nondiabetic rat are in line with the light microscopic investigations. It showed normal and uniform morphological structure of myelin with the presence of normal axonal mitochondria (Figure 5A). However, STZ administration caused an alteration in the structural integrity of the myelin sheath with evidence of split and disintegrated neurofilaments. It showed the presence of swelling in axonal mitochondria (Figure 5B). Administration of pioglitazone (10 mg/kg) and insulin (10 IU/kg) showed preservation of axonal structures. They showed the presence of myelinated axons with few unmyelinated fibers (Figure 5C and D). Berberine (20 and 40 mg/kg) treatment also decreased STZ-induced atrophy in myelinated axons and mitochondrial

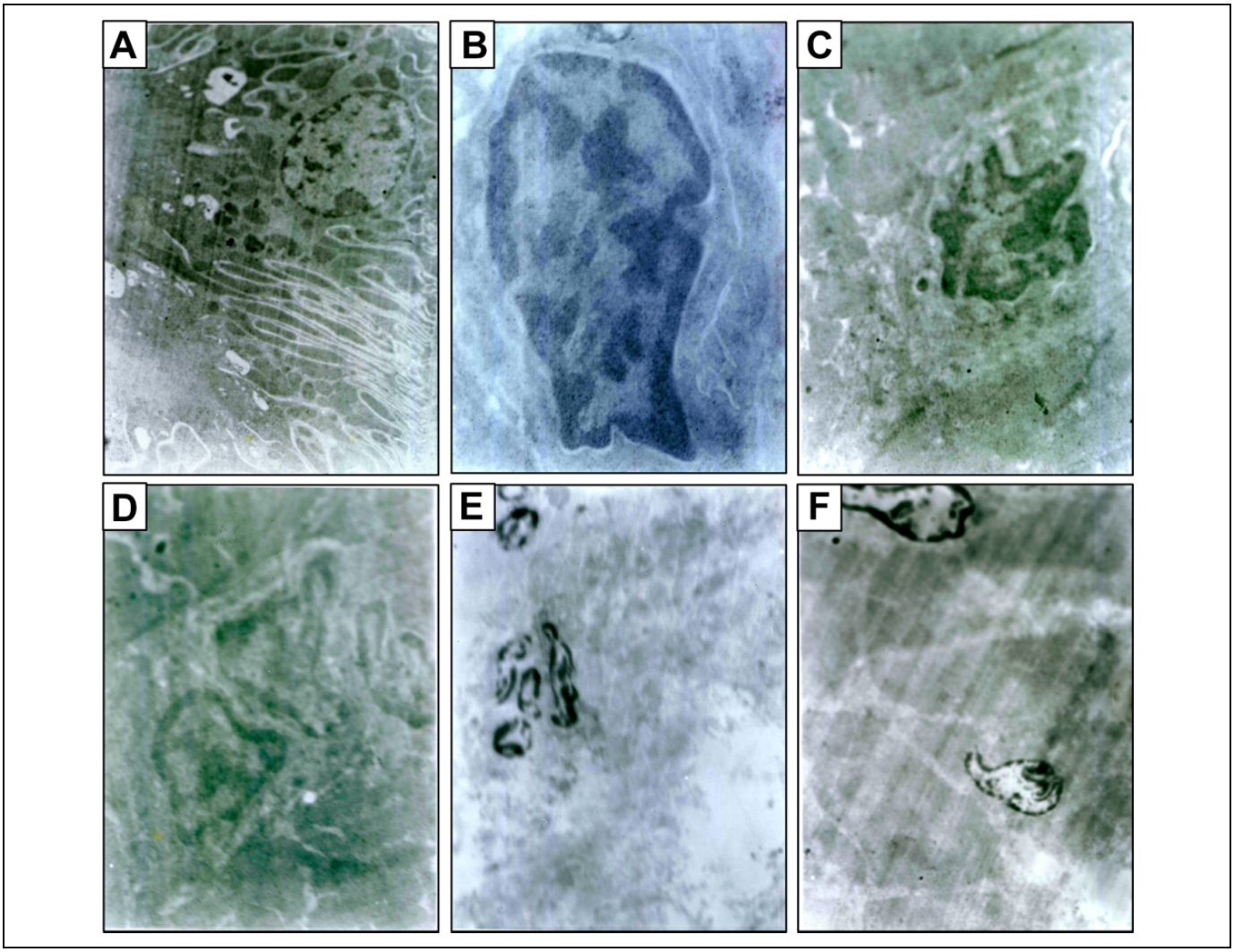

Figure 5. Effect of berberine treatment on n-STZ-induced alterations in sciatic nerve ultrastructure of rats (n = 2). Photomicrographs of sections of sciatic nerve from nondiabetic control (6265X) (A), n-STZ control (8950 X) (B), pioglitazone (10 mg/kg, PO) treated group (14320 X) (C), insulin (10 IU/kg, SC) treated group (14320 X) (D), berberine (20 mg/kg, PO) treated group (5370 X) (E), and berberine (40 mg/kg, PO) (26850 X) treated group (F). n-STZ indicates neonatal STZ control; PO, orally; SC, subcutaneously.

alterations. The myelin degeneration and unmyelinated fibers were less in berberine treatment as compared to n-STZ control rats (Figure 5E and F).

Discussion

Diabetic neuropathy is a complex and diverse group of diseases characterized by loss of sensory and autonomic neural sensation, alterations of electrophysiological function which is reflected by impairment in the conduction of nerve fiber. The elevated blood sugar levels, insulin resistance, and diminished glucose tolerance have been suggested as important pathogenic mechanisms for the progression of DPN.[27] Among the various models of DPN, n-STZ-induced T2DM neuropathy is frequently used and well-established animal model that mimics pathological features, including insulin resistance and impaired glucose tolerance.[14,27] In the present investigation, we have investigated the potential and possible mechanism of action of berberine against n-STZ-induced neuropathic pain. Results of present investigation suggest that berberine ameliorated diabetes-induced alterations in allodynia and hyperalgesia via inhibition of elevated hyperglycemia, AR, oxido-nitrosative stress and pro-inflammatory mediators (TNF-α and IL-1β), activation of PPAR-γ and AMPK pathways, modulation of growth factor (BDNF and IGF-I) and inhibition of apoptosis to improve electrophysiological (nerve conduction velocity [NCV]) functions.

Studies well documented that polyphagia, polydipsia, polyuria, hyperglycemia, and decreased body weight are the characteristic features of diabetes. Evidence suggested that a decrease in body weight is associated with increased wasting of muscles, which may be due to excess tissue protein loss and unavailability of carbohydrate for energy production.[28,29] Furthermore, hypoinsulinemia, glucose intolerance, and

alteration in glucose homeostasis have been reported as strongest indicators of n-STZ-induced diabetes.[14] In the present investigation, administration of STZ to the neonatal rats at day 2 provides the various advantages including a decrease in the mortality, higher rate of induction of diabetes, and sustained hyperglycemia as compared to T1DM. Also, the findings of the present investigation showed that n-STZ-administered rat exhibits decreased serum insulin, increased food and water intake, increased urine excretion, and decreased body weight. Oral administration of berberine showed significant improvements in glucose homeostasis reflected by increased serum insulin, decreased HBA1c, and serum glucose, which may modify activities of enzymes responsible for carbohydrate metabolism and thus halted alterations in body weight, food and water intake, and urine excretion.

Hyperglycemia plays a central role in the induction of T2DM-induced peripheral neuropathic pain. Numerous researchers have well documented that elevated glucose level in blood activated ROS that induce oxidative stress.[6,10] The mechanism responsible for elevated oxidative stress includes alteration in activity of protein kinase C, enhanced activity of AR, decreased Na^+K^+-ATPase activity, imbalance in prostanoid, accumulation of AGEs, and overproduction of mitochondrial superoxide.[30,31] Additionally, HbA_{1c} reflects the elevated levels of blood sugar, which further reacts with Hb and decreases its binding affinity for oxygen, resulting in neural hypoxia. Thus, determination of HbA1c is considered a hallmark of elevated blood sugar level. Clinically, the elevated levels of HbA1c have been directly linked with fasting blood glucose levels.[6] Thus, the researcher has well established the relations between elevated blood glucose levels with nerve injury.[14] Our results showed that berberine treatment significantly inhibited elevated blood glucose and HbA1C levels, which in turn improved neural hypoxia condition determined by pulse Ox, that is, the percentage of Hb saturated with oxygen, and thus ameliorated alterations in motor sensations.

Induction of T2DM in n-STZ caused significant hypoinsulinemia that results in activation of circulatory lipase, which in turn increases mobilization of fatty acid. It has been well documented that the liver is a vital organ that regulates the concentration of plasma glucose via gluconeogenesis and glycolysis.[28] However, increased fatty acid metabolism causes enhanced de novo lipogenesis, leading to accumulation of fat and lipid in hepatic tissue.[32] This vicious cycle resulted in insulin resistance and thus inhibited the activity of lipoprotein esterase, an enzyme responsible for triglyceride hydrolysis. These consequences result in the deposition of cholesterol and triglycerides in the blood, which may lead to cardiovascular complications.[33] Thus, hyperlipidemia is considered an important risk factor in the development of diabetes and its related complications such as neuropathy and cardiomyopathy.[34] In the present investigation, n-STZ control rats showed elevated hepatic lipid levels whereas berberine treatment inhibits the accumulation of liver lipids, which is in line with the findings of previous investigators.[17] Furthermore, a decrease in the levels of hepatic lipid may be attributed to improved insulin sensitivity by berberine treatment. It also contributes to improved body weight by controlling muscle wasting via inhibition of gluconeogenesis.

Various molecular, cellular, and intracellular studies have established that neuropathic pain is the most common impediment associated with DPN.[14] Thus, measurement of allodynia and hyperalgesia in response to mechanical stimuli is considered an important, reliable, reproducible, and sensitive index for neuropathic pain during DPN.[14] Study has reported that ip administration of STZ caused significant alterations in C- and Ad-fibers.[35] Alterations in these motor and sensory fibers caused a reduction in nociceptive threshold toward the mechanical and thermal receptors. The similar alterations in the hyperalgesia and allodynia have been reported during the chronic neuropathic pain in diabetic people.[30] An array of investigators have implicated the various tools such as plantar test, tail immersion test, von Frey hair test, and Randall-Selitto for the assessment of mechanical and thermal stimuli during experimental DPN.[10,11,14] In the present investigation, decreased response to the stimuli against abovementioned test confirmed the induction of neuropathic pain whereas berberine treatment ameliorates the n-STZ-induced painful diabetic neuropathy. The results of the present investigations are consistent with the finding of the previous researcher, where berberine treatment improves the response to mechanical and thermal stimuli in experimental DPN.[18]

Abnormalities in MNCV and SNCV are the essential diagnostic tools of peripheral neuropathy in diabetic patients.[36] Clinically, the abnormalities in MNCV and SNCV are confirmed by reduction in amplitude of sensory nerve action potentials, sensory compound nerve action potentials, and compound muscle action potentials. A strong body of literature suggests that elevated levels of glucose accelerate the polyol pathway where sorbitol converts from glucose in the presence of AR using cofactor nicotinamide adenine dinucleotide phosphate.[8] Thus, accumulated polyol causes inactivation of membrane-bound enzyme (ie, Na^+K^+-ATPase) activity. This Na^+K^+-ATPase has been documented as a vital enzyme in membrane repolarization and thus for the normal functioning of peripheral nerve.[14] Thus, decreased activity of Na^+K^+-ATPase results in a decrease in the action potential and reduced NCV. Previous studies documented the reduction of MNCV and SNCV after administration of STZ[31,37]; however, there are conflicting evidence suggesting that magnitude of nerve conduction varies with multiple factors during T1DM.[38,39] However, our results show that n-STZ induces sustained hyperglycemia, which results in decreased NCVs in C- and Ad-fibers from week 6 onward in T2DM. The results of the present investigation corroborate with the findings of the previous study where T2DM-induced n-STZ DPN was associated with decreased NCV of C fibers.[11] Interestingly, administration of berberine caused inhibition of n-STZ induced decrease in Na^+K^+-ATPase activity, thus improving MNCV and SNCV. This may be attributed to its inhibitory potential against elevated levels of glucose and AR.

The elevated response of pro-inflammatory cytokine has been suggested as an important etiology of DPN.[22] The physical (chronic constriction or partial ligation) or chemical (STZ, alloxan) injury to peripheral nerve results in rapid release of pro-inflammatory cytokine (such as TNF-α and ILs) from Schwann cells.[22] A further influx of this cytokine activates macrophages at the site of injury, which results in Wallerian degeneration and production of mechano-allodynia and heat hyperalgesia.[40] Tumor necrosis factor-α also provokes apoptosis, which is toxic to oligodendrocytes in vitro and affects neuronal function. Along with TNF-α, IL-1β is also known to contribute significantly to the initiation and maintenance of painful diabetic neuropathy.[22] It has been demonstrated that IL-1β may contribute to the central sensitization associated with chronic neuropathic pain.[41] Additionally, patients with T2DM exhibit elevated serum levels of these pro-inflammatory cytokines.[42] Thus, efforts have been taken clinically to ameliorate the development of chronic diabetic complications by implicating the agents with an ability to inhibit the production of TNF-α.[42] The findings of the present investigation also showed that berberine treatment significantly inhibits the n-STZ-induced elevated TNF-α, IL-1β, and IL-6 mRNA and protein levels both. The result of the present study is in line with the findings of the previous investigator where berberine treatment ameliorates allodynia and hyperalgesia via inhibition of release of pro-inflammatory cytokine.[18]

Brain-derived neurotrophic factor, a member from a family NGF, plays a vital role in the neuronal transmissions, neuronal repair, and synapse function; thus, it's an essential protein in the regulation of neuronal functions.[43,44] It has been reported that continued administration of exogenous BDNF significantly ameliorated mechanical and thermal nociception and thus reduced the neuropathic pain via modulation of hyperexcitability in DRG neurons.[43] Furthermore, report suggests that the upregulated expression of BDNF correlates with the improved MNCV via activation of intracellular signaling cascades.[45] Thus, BDNF is referred to as the "neuromodulator of nociception." In the present investigation, administration of STZ induces a decrease in protein and mRNA expression of BDNF in the sciatic nerve, which is in accordance with the findings of previous investigator.[43-45] Recently Shen et al reported that berberine upregulates the BDNF expression to ameliorate neural disorders.[44] In our study also, the administration of berberine significantly upregulated mRNA expression in neural tissue, which might have, in turn, modulated nociception to pain threshold, thus reducing the neuropathic pain.

Insulin-like growth factors, a family of insulin and related proteins (IGF-I and IGF-II), play an essential role in neural growth and differentiation. Evidence suggests that neural tissue is the secondary source of IGFs production besides the liver.[35] While choroid plexus and leptomeninges are responsible for the production and release of circulating IGF during the growth phase in postnatal animals. Thus, researchers have suggested that IGF accelerates the growth of motor and sensory neurons during neonatal development.[46] Moreover, systemic administration of IGF-I in diabetic rats showed significant improvement in the rate of sensory nerve regeneration. Recent clinical reports showed that diabetic patients are associated with decreased levels of serum IGF-I as compared to nondiabetic patients.[35] The experimental study suggested that STZ administration caused significant downregulation in both IGF-I mRNAs and protein expression,[47] whereas treatment with insulin significantly upregulated IGF-I mRNA in the nerve.[48] In the present investigation, administration of berberine during the neonatal development phase may enhance the growth of motor and sensory neurons reflected by an increase in NCV. This neuroprotective potential of berberine may be attributed to its inhibitory activity against n-STZ-induced decrease in sciatic nerve IGF-I mRNA levels.

Studies documented that activation of PPAR regulate the various genes involved in cell growth and differentiation, inflammatory pathways, insulin sensitivity, angiogenesis, lipid, and glucose metabolism.[49] Peroxisome proliferator-activated receptors-γ is also reported to inhibit the release of pro-inflammatory cytokines such as TNF-α and ILs from macrophages, thereby inducing pain relief.[9] Whereas inhibition of PPAR-γ causes nuclear factor-κB activation, leading to the release of various pro-inflammatory cytokines (TNF-α and IL-1β), which further stimulates the pain pathway. Thus, activation of PPAR-γ produces anti-inflammatory and anti-nociceptive effects during neuropathic pain.[9] In this view, PPAR-γ has been well accepted as a pleiotropic transcription factor. In the present investigation, STZ administration resulted in significant downregulation in PPAR-γ expression compared with normal nondiabetic animals. Evidence has determined that activation of PPAR-γ by berberine treatment is the central mechanism behind amelioration of diabetic complications and various disease states.[16] In the present study, we also reported that administration of berberine significantly upregulated PPAR-γ mRNA expression, which is in accordance with findings of previous researcher.[16]

A growing body of evidence reported that activation of AMPK reduces inflammation via inhibition of release of inflammatory cytokines. It has also been documented that activation of AMPK causes reduction in blood glucose level via inhibition of glucose uptake and attenuation of gluconeogenesis.[50] The indirect association of the inhibition of AMPK activity with DPN incidence has been reported in diabetic people clinically.[51] Additionally, direct binding of AMPK to the various ion channels results in phosphorylation of channels, which enhance their activities. This also reduces the cellular stress induced in membrane ion channels and initiates the signaling mechanism.[52] Furthermore, PP2C, a potent inhibitor of AMPK, causes the release of inflammatory cytokines via inhibition of AMPK activity.[53] Thus, malfunctioning of AMPK results in decrease of neuronal pain sensation and modulation of sensory function, which in turn induces diabetic neuropathy. Reports from clinical studies have also established a link between impaired AMPK levels with DPN.[54] Western blot analysis in the present investigation also showed that n-STZ rats exhibit downregulated expression of AMPK whereas

berberine treatment significantly ameliorated it. Recently, Yerra et al also reported that berberine ameliorates neuropathic pain via modulation of AMPK/PPAR-γ in T1DM and the results of the present investigation are in line with findings of the previous investigator.[18]

The recent treatment regimen for management of DPN includes amitriptyline, duloxetine, venlafaxine, gabapentin, and so on which focus on various goals such as pain reduction, decreasing the disease progression, functional restoration, and complications management. However, these treatments are associated with an array of adverse effects such as hepatotoxicity, complications of the cardiovascular system, bone loss, and fluid retention, which restricted their clinical applications. Various antioxidant moieties such as α-lipoic acid and quercetin have proven their potential during the management of diabetic neuropathy clinically.[55,56] A report from a meta-analysis suggested that administration of α-lipoic acid (600 mg/d, 3 weeks) showed significant improvement in DPN symptoms.[56] Interestingly, berberine has been reported as a potent oral hypoglycemic agent in patients with T2DM.[57] Thus, berberine can also be contemplated for clinical trials as a treatment alternative in the management of neonatal diabetic neuropathy in patients with T2DM.

Conclusion

The results of the present investigation suggest that berberine exerts its neuroprotective effect against n-STZ-induced diabetic neuropathy. This beneficial effect is executed via inhibition of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) and oxido-nitrosative stress as well as upregulation of BDNF, IGF-1, PPAR-γ, and AMPK expression to ameliorate impaired allodynia, hyperalgesia, and NCV during T2DM.

Authors' Note

Guangju Zhou and Mingzhu Yan equally contributed to this article.

Acknowledgments

We thanks Dr. Li for her experimental advice. We also thanks Mr. GG for his animal technical support.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iD

Nanwei Tong https://orcid.org/0000-0001-7072-3480

Supplemental Material

Supplemental material for this article is available online.

References

1. Vinik AI, Park TS, Stansberry KB, Pittenger GL. Diabetic neuropathies. *Diabetologia*. 2000;43(8):957-973.
2. Lee YH, Kim KP, Kim YG, et al. Clinicopathological features of diabetic and nondiabetic renal diseases in type 2 diabetic patients with nephrotic-range proteinuria. *Medicine*. 2017;96(36):e8047.
3. Juster-Switlyk K, Smith AG. Updates in diabetic peripheral neuropathy. *F1000Res*. 2016;5:F1000 Faculty Rev-1738.
4. Tesfaye S, Boulton AJ, Dickenson AH. Mechanisms and management of diabetic painful distal symmetrical polyneuropathy. *Diabetes Care*. 2013;36(9):2456-2465.
5. Sadosky A, Mardekian J, Parsons B, Hopps M, Bienen EJ, Markman J. Healthcare utilization and costs in diabetes relative to the clinical spectrum of painful diabetic peripheral neuropathy. *J Diabetes Complicat*. 2015;29(2):212-217.
6. Kandhare AD, Rais N, Moulick ND, Deshpande A, Thakurdesai P, Bhaskaran S. Efficacy and safety of herbal formulation rich in standardized fenugreek seed extract as add-on supplementation in patients with type 2 diabetes mellitus on sulphonylurea therapy: a 12-week, randomized, double-blind, placebo-controlled, multicenter study. *Pharmacogn Mag*. 2018;14(57):393-402.
7. Li W, Kandhare AD, Mukherjee AA, Bodhankar SL. Hesperidin, a plant flavonoid accelerated the cutaneous wound healing in streptozotocin-induced diabetic rats: role of TGF-ß/Smads and Ang-1/Tie2 signaling pathways. *EXCLI J*. 2018;17:399-419.
8. Mizukami H, Yagihashi S. Exploring a new therapy for diabetic polyneuropathy – the application of stem cell transplantation. *Front Endocrinol*. 2014;5:45.
9. Freitag CM, Miller RJ. Peroxisome proliferator-activated receptor agonists modulate neuropathic pain: a link to chemokines? *Front Cell Neurosci*. 2014;8:238.
10. Kandhare AD, Raygude KS, Ghosh P, Ghule AE, Bodhankar SL. Neuroprotective effect of naringin by modulation of endogenous biomarkers in streptozotocin induced painful diabetic neuropathy. *Fitoterapia*. 2012;83(4):650-659.
11. Visnagri A, Kandhare AD, Chakravarty S, Ghosh P, Bodhankar SL. Hesperidin, a flavanoglycone attenuates experimental diabetic neuropathy via modulation of cellular and biochemical marker to improve nerve functions. *Pharm Biol*. 2014;52(7):814-828.
12. Wei M, Ong L, Smith MT, et al. The streptozotocin-diabetic rat as a model of the chronic complications of human diabetes. *Heart Lung Circ*. 2003;12(1):44-50.
13. Kandhare AD, Raygude KS, Kumar VS, et al. Ameliorative effects quercetin against impaired motor nerve function, inflammatory mediators and apoptosis in neonatal streptozotocin-induced diabetic neuropathy in rats. *Biomed Aging Pathol*. 2012;2(4):173-186.
14. Arulmozhi D, Veeranjaneyulu A, Bodhankar S. Neonatal streptozotocin-induced rat model of type 2 diabetes mellitus: a glance. *Indian J Pharmacol*. 2004;36(4):217-221.
15. Jang J, Jung Y, Seo SJ, et al. Berberine activates AMPK to suppress proteolytic processing, nuclear translocation and target DNA binding of SREBP-1c in 3T3-L1 adipocytes. *Mol Med Rep*. 2017;15(6):4139-4147.

16. Li H, He C, Wang J, et al. Berberine activates peroxisome proliferator-activated receptor gamma to increase atherosclerotic plaque stability in Apoe(−/−) mice with hyperhomocysteinemia. *J Diabetes Investig*. 2016;7(6):824-832.
17. Liang H, Wang Y. Berberine alleviates hepatic lipid accumulation by increasing ABCA1 through the protein kinase C delta pathway. *Biochem Biophys Res Commun*. 2018;498(3):473-480.
18. Yerra VG, Kalvala AK, Sherkhane B, Areti A, Kumar A. Adenosine monophosphate-activated protein kinase modulation by berberine attenuates mitochondrial deficits and redox imbalance in experimental diabetic neuropathy. *Neuropharmacology*. 2018; 131:256-270.
19. Chakrabarti A, Singh J, Saha L, et al. Evaluation of berberine – a Nrf2 Activator, on paclitaxel-induced neuropathic pain model in rat (P2.104). *Neurology*. 2018;90(suppl 15):P2.104.
20. Zan Y, Kuai CX, Qiu ZX, Huang F. Berberine ameliorates diabetic neuropathy: TRPV1 modulation by PKC pathway. *Am J Chin Med*. 2017;45(8):1709-1723.
21. Adil M, Mansoori MN, Singh D, Kandhare AD, Sharma M. Pioglitazone-induced bone loss in diabetic rats and its amelioration by berberine: a portrait of molecular crosstalk. *Biomed Pharmacother*. 2017;94:1010-1019.
22. Cui J, Wang G, Kandhare AD, Mukherjee-Kandhare AA, Bodhankar SL. Neuroprotective effect of naringin, a flavone glycoside in quinolinic acid-induced neurotoxicity: possible role of PPAR-gamma, Bax/Bcl-2, and caspase-3. *Food Chem Toxicol*. 2018;121:95-108.
23. Kandhare AD, Bodhankar SL, Mohan V, Thakurdesai PA. Effect of glycosides based standardized fenugreek seed extract in bleomycin-induced pulmonary fibrosis in rats: decisive role of Bax, Nrf2, NF-κB, Muc5ac, TNF-α and IL-1β. *Chem Biol Interact*. 2015;237:151-165.
24. Mukherjee A, Zhang J, Kandhare AD, Bodhankar SL. Curcumin ameliorates vitamin A deficiency-induced urolithiasis in neonatal rats via inhibition of KIM-1/NGAL, Nrf2, and iNOs pathways. *Lat Am J Pharm*. 2018;37(12):2502-2511.
25. Siques P, Brito J, Naveas N, , et al. Plasma and liver lipid profiles in rats exposed to chronic hypobaric hypoxia: changes in metabolic pathways. *High Alt Med Biol*. 2014;15(3):388-395.
26. Eslami A, Lujan J. Western blotting: sample preparation to detection. *J Vis Exp*. 2010;(44):2359.
27. Zimmet P, Thomas CR. Genotype, obesity and cardiovascular disease – has technical and social advancement outstripped evolution? *J Intern Med*. 2003;254(2):114-125.
28. Newsholme EA, Dimitriadis G. Integration of biochemical and physiologic effects of insulin on glucose metabolism. *Exp Clin Endocrinol Diabetes*. 2001;109(suppl 2):S122-S134.
29. Zhou JY, Zhou SW. Effect of berberine on PPARalpha/delta/gamma expression in type 2 diabetic rat retinae. *Yao Xue Xue Bao*. 2007;42(12):1243-1249.
30. Obrosova IG. Diabetic painful and insensate neuropathy: pathogenesis and potential treatments. *Neurotherapeutics*. 2009;6(4): 638-647.
31. Obrosova IG, Li F, Abatan OI, et al. Role of poly(ADP-ribose) polymerase activation in diabetic neuropathy. *Diabetes*. 2004; 53(3):711-720.
32. Yu AS, Keeffe EB. Nonalcoholic fatty liver disease. *Rev Gastroenterol Disord*. 2002;2(1):11-19.
33. Xu J, Liu T, Li Y, et al. Hypoglycemic and hypolipidemic effects of triterpenoid-enriched jamun (*Eugenia jambolana* Lam.) fruit extract in streptozotocin-induced type 1 diabetic mice. *Food Funct*. 2018;9(6):3330-3337.
34. Ozaki K, Terayama Y, Matsuura T, Narama I. Effect of combined dyslipidemia and hyperglycemia on diabetic peripheral neuropathy in alloxan-induced diabetic WBN/Kob rats. *J Toxicol Pathol*. 2018;31(2):125-133.
35. Rauskolb S, Dombert B, Sendtner M. Insulin-like growth factor 1 in diabetic neuropathy and amyotrophic lateral sclerosis. *Neurobiol Dis*. 2017;97(pt B):103-113.
36. Dyck PJ, Kratz KM, Karnes JL, et al. The prevalence by staged severity of various types of diabetic neuropathy, retinopathy, and nephropathy in a population-based cohort: the Rochester Diabetic Neuropathy Study. *Neurology*. 1993;43(4):817-824.
37. Kellogg AP, Wiggin TD, Larkin DD, Hayes JM, Stevens MJ, Pop-Busui R. Protective effects of cyclooxygenase-2 gene inactivation against peripheral nerve dysfunction and intraepidermal nerve fiber loss in experimental diabetes. *Diabetes*. 2007;56(12): 2997-3005.
38. Sullivan KA, Hayes JM, Wiggin TD, et al. Mouse models of diabetic neuropathy. *Neurobiol Dis*. 2007;28(3):276-285.
39. Kennedy JM, Zochodne DW. Experimental diabetic neuropathy with spontaneous recovery: is there irreparable damage? *Diabetes*. 2005;54(3):830-837.
40. Lees JG, Duffy SS, Moalem-Taylor G. Immunotherapy targeting cytokines in neuropathic pain. *Front Pharmacol*. 2013;4:142.
41. Gustafson-Vickers SL, Lu VB, Lai AY, Todd KG, Ballanyi K, Smith PA. Long-term actions of interleukin-1beta on delay and tonic firing neurons in rat superficial dorsal horn and their relevance to central sensitization. *Mol Pain*. 2008;4:63.
42. Lechleitner M, Koch T, Herold M, Dzien A, Hoppichler F. Tumour necrosis factor-alpha plasma level in patients with type 1 diabetes mellitus and its association with glycaemic control and cardiovascular risk factors. *J Intern Med*. 2000;248(1): 67-76.
43. Li L, Yu T, Yu L, Li H, Liu Y, Wang D. Exogenous brain-derived neurotrophic factor relieves pain symptoms of diabetic rats by reducing excitability of dorsal root ganglion neurons. *Int J Neurosci*. 2016;126(8):749-758.
44. Shen JD, Ma LG, Hu CY, et al. Berberine up-regulates the BDNF expression in hippocampus and attenuates corticosterone-induced depressive-like behavior in mice. *Neurosci Lett*. 2016;614:77-82.
45. Mizisin AP, Bache M, DiStefano PS, Acheson A, Lindsay RM, Calcutt NA. BDNF attenuates functional and structural disorders in nerves of galactose-fed rats. *J Neuropathol Exp Neurol*. 1997; 56(12):1290-1301.
46. Fernyhough P. Mitochondrial dysfunction in diabetic neuropathy: a series of unfortunate metabolic events. *Curr Diab Rep*. 2015; 15(11):89.
47. Bitar MS, Pilcher CW, Khan I, Waldbillig RJ. Diabetes-induced suppression of IGF-1 and its receptor mRNA levels in rat superior cervical ganglia. *Diabetes Res Clin Pract*. 1997;38(2):73-80.

48. Wuarin L, Guertin DM, Ishii DN. Early reduction in insulin-like growth factor gene expression in diabetic nerve. *Exp Neurol*. 1994;130(1):106-114.
49. Mukherjee AA, Kandhare AD, Bodhankar SL. Elucidation of protective efficacy of pentahydroxy flavone isolated from *Madhuca indica* against arsenite-induced cardiomyopathy: role of Nrf-2, PPAR-gamma, c-fos and c-jun. *Environ Toxicol Pharmacol*. 2017;56:172-185.
50. Novikova DS, Garabadzhiu AV, Melino G, Barlev NA, Tribulovich VG. AMP-activated protein kinase: structure, function, and role in pathological processes. *Biochemistry*. 2015;80(2): 127-144.
51. Gerich J, Raskin P, Jean-Louis L, Purkayastha D, Baron MA. PRESERVE-beta: two-year efficacy and safety of initial combination therapy with nateglinide or glyburide plus metformin. *Diabetes Care*. 2005;28(9):2093-2099.
52. Dermaku-Sopjani M, Abazi S, Faggio C, Sopjani M. AMPK-sensitive cellular transport. *J Biochem*. 2014;155(3):147-158.
53. Steinberg GR, Michell BJ, van Denderen BJ, et al. Tumor necrosis factor alpha-induced skeletal muscle insulin resistance involves suppression of AMP-kinase signaling. *Cell Metab*. 2006;4(6):465-474.
54. Roy Chowdhury SK, Smith DR, Saleh A, et al. Impaired adenosine monophosphate-activated protein kinase signalling in dorsal root ganglia neurons is linked to mitochondrial dysfunction and peripheral neuropathy in diabetes. *Brain*. 2012;135(pt 6):1751-1766.
55. Valensi P, Le Devehat C, Richard JL, et al. A multicenter, double-blind, safety study of QR-333 for the treatment of symptomatic diabetic peripheral neuropathy. A preliminary report. *J Diabetes Complicat*. 2005;19(5):247-253.
56. Ziegler D, Nowak H, Kempler P, Vargha P, Low PA. Treatment of symptomatic diabetic polyneuropathy with the antioxidant alpha-lipoic acid: a meta-analysis. *Diabet Med*. 2004;21(2): 114-121.
57. Yin J, Xing H, Ye J. Efficacy of berberine in patients with type 2 diabetes mellitus. *Metabolism*. 2008;57(5):712-717.