

## Original Article

# Neuroprotective effects of *Bauhinia variegata* in ameliorating diabetic neuropathy and neurodegeneration

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**Abstract:** Introduction: In diabetic neuropathy with neurodegeneration (DNN), a serious diabetes consequence, extreme hyperglycemia destroys neurons in the brain and limbs. The main therapies for this condition are glucose control and pain management. Phytopharmacology is thought to be more successful in addressing the pain and blood sugar management issues associated with DNN. The objective of this study was to investigate how *Bauhinia variegata* (BV) could offer protection against streptozotocin (STZ)-induced diabetic neuropathy. Methodology: STZ-associated DNN was induced in rats, and these diabetic rats were treated with BV at 200 mg/kg and 400 mg/kg doses for 28 days. Blood glucose (BG), serum nitrite, lipid peroxidation, antioxidants, C-reactive protein, behavioral, and histopathological parameters were assessed. Results: BV dramatically reduced BG and HbA1c levels in diabetic rats, according to the findings. The levels of superoxide dismutase and catalase both rose significantly. Both lipid peroxidation and serum nitrite levels were drastically decreased with BV treatment. In this study, it was found that BV has anti-hyperglycemic and anti-inflammatory effects on DNN. This was shown by a significant drop in C-reactive protein in diabetic rats, which was a key factor in diabetic neuropathy. Thermal hyperalgesia was significantly alleviated after BV therapy, and diabetic rats' pain thresholds improved. Conclusion: Present study concluded that BV treatment has excellent glycemic control, a high antioxidant status, and relevant pain-relieving potential in diabetic neuropathy with neurodegeneration by reversing thermal hyperalgesia and decreasing hyperglycemia in diabetic rats.

**Keywords:** Diabetic neuropathy, *Bauhinia variegata*, HbA1c, lipid peroxidation, C-reactive protein, catalase, thermal hyperalgesia

## Introduction

Diabetes mellitus (DM) includes various forms of neuropathy, such as autonomic, peripheral, proximal, and mononeuropathy, associated with decreased perception of blood sugar, numbness-tingling, extreme pain, and double vision [1]. Neuropathy with type 1 and 2 diabetes was reported as a common and costly complication, with a prevalence rate of 8 percent in newly diagnosed cases and about 50 percent in chronic disease patients [2]. In the United States alone, the cost associated with diabetic neuropathy and its related morbidities was 10.9 billion United States dollars (USD) [3]. In the current trend, more emphasis is placed on neurological comorbidities associated with diabetes, cognitive dysfunction, and dementia [4]. Of primary concern is oxidative stress linked to

hyperglycemia through multifaceted pathways such as redox imbalances, altered protein kinase-C [7, 50], elevated advanced glycation end products [5], nitric oxide, and superoxide overproduction [6, 50]. Oxidative nitric oxide-mediated injury was caused by peroxynitrite, which has degrading effects on the nerve tissue, contributing to neuropathic pain [8]. The human body's antioxidant defenses are superoxide dismutase, catalase, and glutathione peroxidase; inflammation plays a key role in the production of late diabetes complications [9, 10]. An acute-phase reactant C-reactive protein (CRP) level rises dramatically during inflammatory processes [11], and studies have also shown that the CRP level is associated with glycemic control levels of HbA1c [12]. Lipid peroxidation is one of the characteristic features of chronic diabetes, and this increased lipid per-

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oxidation impairs membrane function by decreasing membrane-bound enzymes and receptors, which are harmful to the body's cells [13]. The central nervous system, due to a high level of polyunsaturated lipids in neuronal cell membranes and poor antioxidant defense, is a significant target for lipid peroxidative damage [14]. Thousands of herbal plants and their therapeutic effects have been reported in ayurvedic literature. BV belongs to the Leguminosae family, spread all over India, with high altitudes in the Himalayas; it is planted in tropical and warm regions of the world [15]. BV stem bark produces 5.7 dihydroxy and 5.7 dimethoxy flavanone-4-O-a-L rhamnopyrosyl- $\beta$ -D-glycopyranosides, caempferol-3-glucoside, lupeol, and betasitosterol [16]. Given its biological activities like antioxidant [17, 18], anti-inflammatory [19], hepatoprotective [20], insulin release enhancer, hypoglycaemic and anti-diabetic activity [21, 22], nephroprotective [23], and cardioprotective [24], there was considerable interest in the potential benefit of BV and its active phytochemicals to human health. Due to the significant side effects of traditional antidiabetics and drugs used in diabetic complications, the focus has now shifted to an herbal and medicinal plant-based therapeutic approach, with this objective being investigated in the present study: the protective effects of BV plant extracts in STZ-induced diabetic neuropathy.

### Materials and methods

Analytical reagent was purchased from SD fine chemicals, Sigma Aldrich, Himedia Laboratory, India.

#### *Plant material and extract preparation*

Authenticated *Bauhinia variegata* bark was obtained from Medicinal and Aromatic Plant Research (DMAPR) in Boriavi, India. The plant material was then dried at  $30^{\circ}\text{C}\pm 3^{\circ}\text{C}$  for 15 days. Alcoholic extract was prepared by the powdered plant reflux process; the principle of ethanol selection was the simple penetration into the cell membrane to extract intracellular ingredients from plant material [25]. Briefly, BV bark powder was extracted with ethanol (2/3 quantity) and a rotary vacuum evaporator ( $60^{\circ}\text{C}$ , 100-110 rotation per minute, 100 psi) using full solvent evaporation, and the dried product was stored in a cool position before use.

*Phytochemical analysis:* Various chemical and reagents were utilized for phytochemical analysis mentioned below: Dragendorff's reagent; Anisaldehyde- $\text{H}_2\text{SO}_4$ ; Mayer's reagent; Wagner's reagent; Hager's reagent; Molisch's reagent; Benedicts reagent; Gelatin solution; Lead acetate; Ninhydrin solution; Natural Product reagent.

#### *Experimental design*

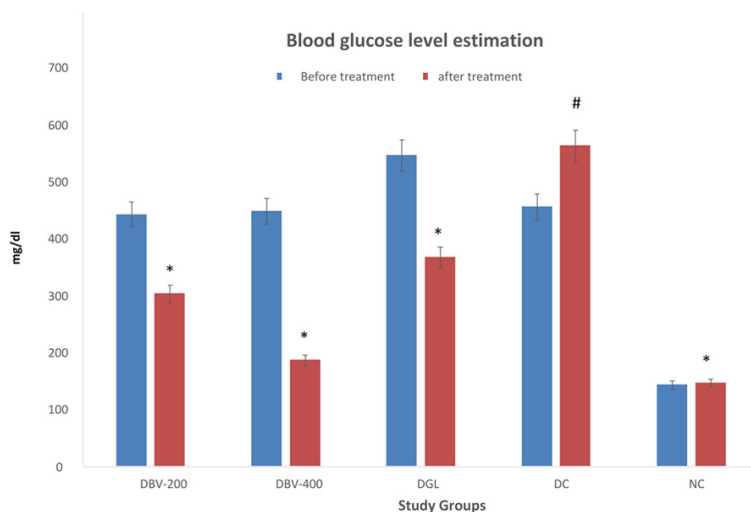
The Institutional Animal Ethics Committee (IAEC) approved the experimental protocol, and animal treatment was carried out in compliance with the guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA), India. The study used male Wistar rats ( $300\pm 50$  gms). Animals were kept in a standard laboratory diet at a temperature of  $23\pm 3^{\circ}\text{C}$  and humidity. The experiment maintained a 12-hour light-dark interval. A single intraperitoneal injection of a freshly formulated streptozotocin solution (60 mg/kg body weight) in citrate buffer (0.1 M, pH 7.4) induced diabetes in rats. After streptozotocin injection diabetes was confirmed by stable hyperglycemia ( $>18$  mmol/L) of the tail vein blood glucose level using a glucometer (Accu-Chek, Johnson & Johnson, India). An acute toxicity study was conducted as per the Organization for Economic Cooperation and Development 423 guidelines using Wistar rats. A single dose level was selected as 2000 mg/kg of body weight of ethanolic extract of BV (EBV). After depriving the animals ( $n = 6$ ) of food overnight, EBV was given orally once, which was dissolved in 3% acacia gum solution.

Animals were divided into five groups: Normal Control (NC); Diabetic control (DC); Diabetic BV ethanolic extract (200 mg/kg) treated; Diabetic BV ethanolic extract (400 mg/kg) treated; Glibenclamide (GL) treated (2.5 mg/kg).

Animals were treated for 28 days. At the end of the 28<sup>th</sup> day of treatment, the following parameters were evaluated: The development of diabetic neuropathy and neurodegeneration was confirmed through histopathological evidence compared with a normal brain and nerve tissue sample (**Figure 3**).

*Blood glucose analysis, HbA1c:* Blood glucose level was measured using a glucose reagent strip with a glucometer, while HbA1c level [26] was assessed using an HPLC-based method.

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**Figure 1.** Effect of BV on Blood glucose level (mg/dl). Values were significantly different from diabetic control (\* $P < 0.01$ ) and significantly different from Normal control (# $P < 0.01$ ). DBV-200 and DBV-400 - diabetic rats treated with BV, DGL - diabetic rats treated with Glibenclamide (GL), DC - diabetic rats, NC - Normal control rats.

**Serum antioxidant catalase (CAT) & dismutase (SOD):** Serum sample CAT activity was expressed in  $\mu\text{mol H}_2\text{O}_2$  used per min/mg protein [27], and serum SOD estimation was expressed as mU SOD/mg protein [28].

**Serum nitrite (SN):** SN was calculated using Greiss reagent as an indicator of nitric oxide production [29].

**Serum C reactive protein (CRP):** SP level was estimated using the method mentioned in a kit (Span Diagnostics Kit, India) [30, 31] expressed as gm/dl. The CRP level was calculated as mg/l in serum by the particle-enhanced immunoturbidimetric process (Span Diagnostic Kit, India) [32, 33].

**Tissue Malondialdehyde (MDA), calcium and protein content:** In cold phosphate buffer, tissue homogeneity was made of isolated rat brain and sciatic nerve. Lipid peroxidation product as MDA level calculated by a reference method [34]. The O-Cresol phthalein complex-one endpoint assay [31, 40] measured calcium in homogenate.

**Hot-plate test:** It is believed that the hyperalgesic reaction to the hot plate results from a mix of central and peripheral processes. Individual animals were placed on a hot plate (Eddy's Hot Plate) at the regulated temperature. As an indicator of the pain threshold, the latency to the

first evidence of paw licking or jumping to avoid the heat was measured; the cutoff period was 10 seconds to prevent paw injury [49].

**Tail immersion (hot water) test:** The tail of the rat was submerged in a bath of hot water ( $55^\circ\text{C} \pm 0.5^\circ\text{C}$ ) until withdrawal (flicking reaction) or indications of struggle were noticed (cutoff 10 seconds). A shortened tail-withdrawal time is indicative of hyperalgesia [49].

### Statistical analysis

Results were expressed as mean  $\pm$  standard error of mean (SEM). Differences between mean groups were estimated

using one-way variance analysis of variance (ANOVA). Paired t-tests were applied for comparison of groups at various intervals.

### Results

#### Phytochemical analysis of ethanolic extract of BV

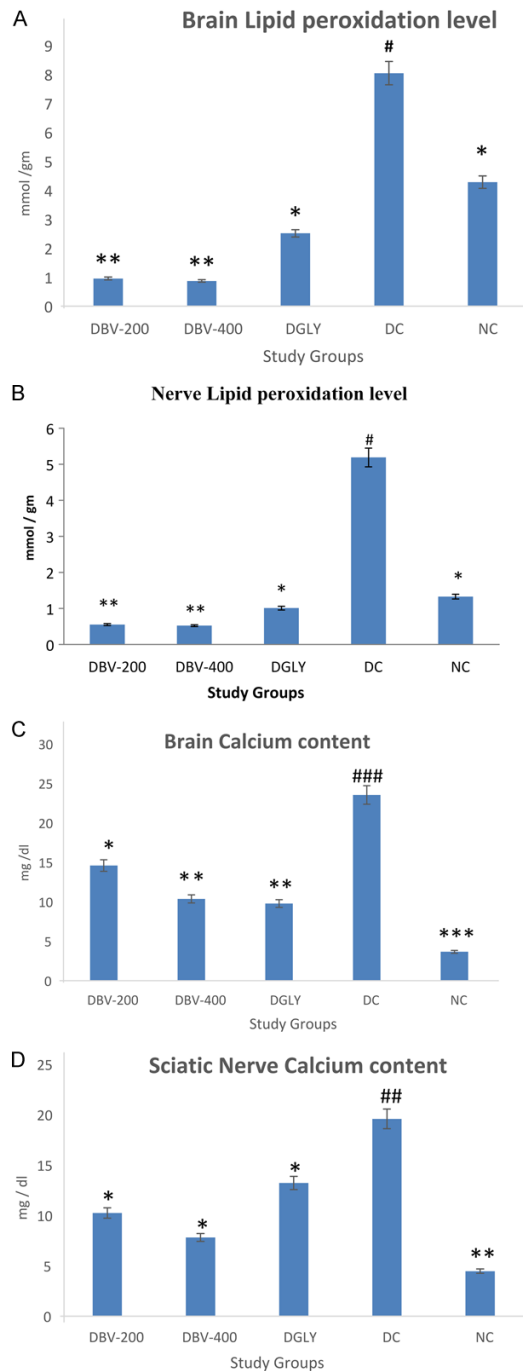
Various phytochemicals like alkaloids, glycosides, phytosterols, saponins, phenols, tannins, and flavonoids were characterized in the current study with inferences (Table 3).

In the present study, safety aspects of EBV were evaluated by an acute toxicity study in male rats. EBV was found to be nontoxic up to 2000 mg/kg of body weight. No change in body weight or any abnormal behaviors were observed in EBV-treated rats. Thus, 200 mg/kg and 400 mg/kg were selected for efficacy studies.

#### Diabetic neuropathy with neurodegeneration model development

The confirmation of model development for diabetic neuropathy and neurodegeneration was achieved through histopathological evaluation. In this evaluation, STZ diabetic rats exhibited neurodegenerative changes and the formation of plaques in brain tissue, which were not observed in normal control brain tissue.

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**Figure 2.** Effect of BV and GL on Lipid peroxidation (MDA) level (A and B) and Calcium content (C and D) in brain and sciatic nerve respectively. Values were significantly different from diabetic control, \*, \*\* $P < 0.01$  and significantly different from Normal control, # $P < 0.01$ ; DBV-200 and DBV-400 - diabetic rats treated with BV, DGL - diabetic rats treated with Glibenclamide (GL), DC - diabetic control rats, NC - Normal control rats.

Additionally, the nerves of diabetic rats showed damage resulting from oxidative stress, inflam-

mation, and nerve fiber damage, in contrast to the normal control nerve tissue (Figure 3). Behavioural parameters were also provided positive insight during model development.

### Effects of BV treatment on blood glucose and glycosylated hemoglobin (HbA1c)

STZ-induced diabetic rats showed sustained hyperglycemia. Significantly decreased blood glucose levels ( $P < 0.001$ ) in diabetic animals treated with BV were observed compared to DC rats (Figure 1). In diabetic rats HbA1c was significantly raised; while dose dependent reduction in HbA1c (Table 1) and blood glucose levels (Figure 1) were observed with BV treatment.

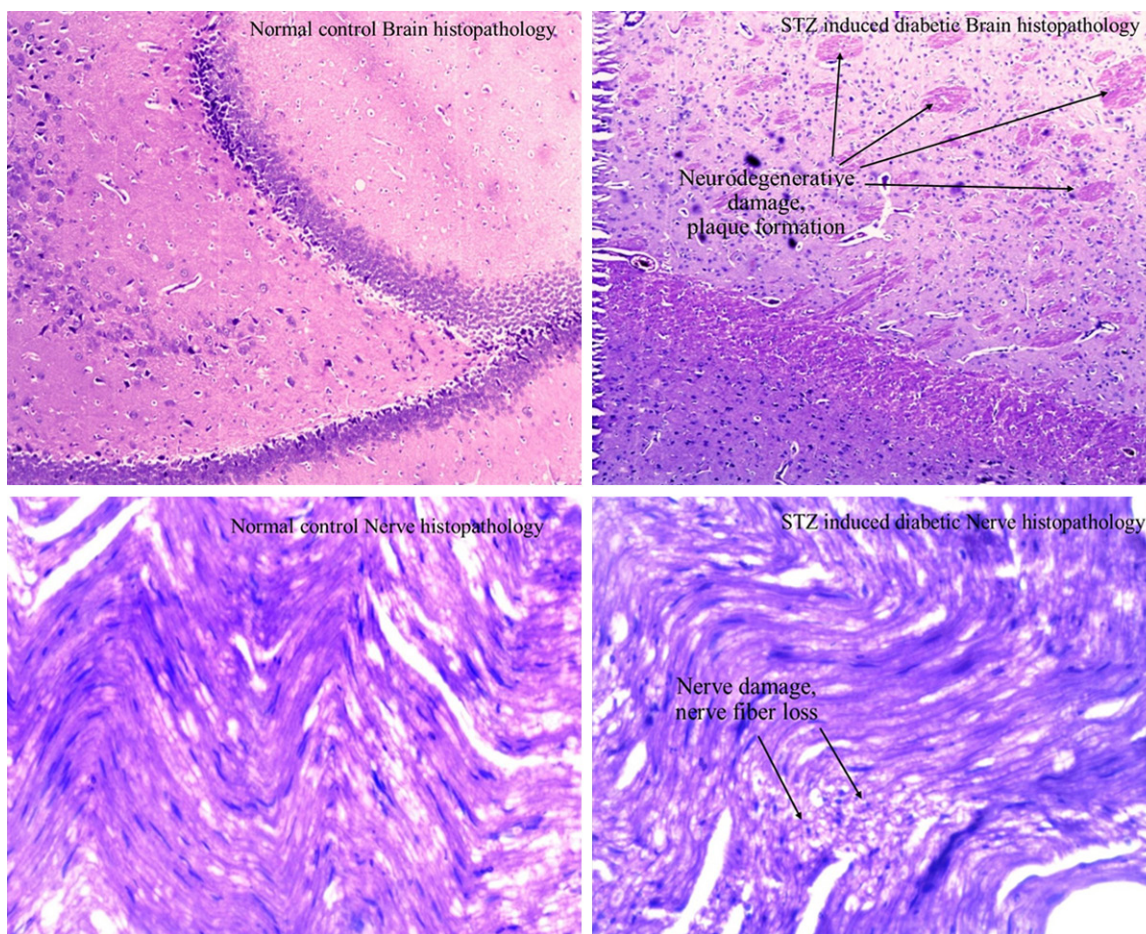
### Effects of BV treatment on serum protein, anti-oxidant, CRP and nitrite

Among diabetic rats, there has been a large rise in total protein compared with normal rats. BV therapy reported a substantial decrease in the amount of serum protein compared with DC rats. GL has shown a positive effect on the protein content reduction in diabetic rats. Diabetic rats, who were reversed via BV therapy, have substantially improved their levels of antioxidants (CAT and SOD). Increased dose dependency has been found in CAT and SOD levels in diabetic rats. Diabetic rats treated with GL displayed elevated levels of CAT and SOD. Because of chronic hyperglycemia and increased oxidative stress, the number of antioxidants in diabetic rats was significantly reduced. In STZ diabetic rats, CRP levels have significantly increased. In comparison to DC rats, BV treatment has significantly reduced CRP levels in diabetic rats. In STZ-treated diabetic rats, nitrite levels were significantly increased relative to a GL treatment, while in serum, BV treatment significantly decreased nitrite levels depending on the dosage. Diabetic rats treated with GL showed a decrease in the nitrite level compared to DC rats, as shown in Table 1.

### Effects of BV treatment on the tissue lipid peroxidation and calcium

Lipid peroxidation was considered to increase dramatically from the diabetic brain and nerve tissue. Treatment with BV substantially decreased levels of MDA in the brain ( $P < 0.01$ ) and sci-

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**Figure 3.** Histopathological estimation of STZ induced diabetic brain and nerve tissue compared with normal brain and nerve tissue (Brain histopathology in 10X and Nerve histopathology in 40X).

**Table 1.** Effect of BV on HbA1c level and serum protein, CAT, SOD, CRP and nitrite levels

Groups	Biochemical estimations					
	HbA1C level (%)	Protein level (g/l)	CAT (Micromol/mg of protein)	SOD (MilliU/mg protein)	CRP level (mg/L)	Nitrite level (Microgm/ml)
DBV-200	6.96±0.78 <sup>§</sup>	3.807±0.372 <sup>**</sup>	433.15±142.12 <sup>*</sup>	398.6±172.61 <sup>*</sup>	1.98±0.587 <sup>***</sup>	6.61±2.72 <sup>***</sup>
DBV-400	5.06±0.73 <sup>**</sup>	3.42±0.477 <sup>**</sup>	689.4±184.02 <sup>*</sup>	545.3±170.8 <sup>*</sup>	1.66±0.45 <sup>***</sup>	2.93±1.24 <sup>***</sup>
DGL	7.56±0.93 <sup>§</sup>	3.192±0.577 <sup>**</sup>	587.18±201.83 <sup>*</sup>	284.8±88.36 <sup>§</sup>	4.54±1.417 <sup>**</sup>	6.72±2.44 <sup>***</sup>
DC	8.14±0.34 <sup>###</sup>	8.34±1.344 <sup>##</sup>	182.8±25.56 <sup>#</sup>	167±42.42 <sup>#</sup>	8.07±2.806 <sup>##</sup>	22.26±0.29 <sup>###</sup>
NC	3.88±0.08 <sup>***</sup>	3.62±0.653 <sup>**</sup>	758.01±203 <sup>*</sup>	679.1±242.57 <sup>*</sup>	3.9±0.632 <sup>**</sup>	3.53±1.12 <sup>***</sup>

Values were significantly different from diabetic control, \*\*\*P<0.001, \*\*P<0.01, \*P<0.05 and Significantly different from Normal control, ###P<0.001, ##P<0.05, #P<0.05, §non significant difference from DC rats P>0.5; DBV-200 and DBV-400 - diabetic rats treated with BV, DGL - diabetic rats treated with Glibenclamide, DC - diabetic control rats, NC - Normal control rats.

atic nerve (P<0.01) in contrast to rats with DC. Double levels of Brain MDA have been observed in diabetic rats treated with BV compared to NC rats. Glibenclamide also significantly improved brain MDA (P<0.05) and nerve MDA (**Figure 2A and 2B**). Persistent hyperglycemia caused oxidative stress in brain and nerve tissues to

increase in calcium content (**Figure 2C and 2D**). Brain and nerve calcium content were substantially increased with BV treatment (P<0.05) compared with DC rats. GL treated rats reported substantial decreases in brain Ca<sup>2+</sup> content (P<0.01) while nerve Ca<sup>2+</sup> reduction were not substantial (P>0.5), relative to DC rats.

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**Table 2.** Effect of EBV on behavioural methods (Hot plate assay and Tail immersion test)

Behavioural tests	Animal groups				
	Normal control	Disease control	Glibenclamide treated	Ethanollic extract of BV (200 mg/kg)	Ethanollic extract of BV (400 mg/kg)
Hot plate assay [Jump time (seconds)]	4.614±0.836	2.092±0.231*	3.948±0.127*	3.576±0.274*	5.122±0.907**
Tail immersion test	6.56±0.86	3.02±0.10#	5.09±0.48*	6.77±0.63*	6.10±0.42*

\*P<0.05 significant difference of BV and Glibenclamide treated rats compared to diseased control; \*\*P<0.01 highly significant difference of BV treated rats compared to diseased control; #P<0.05 significant difference of disease control rats compared to normal control rats.

**Table 3.** Phytochemical analysis of EBV

Sr. no	Phytochemical analysis with specific test	Observation for test	Inference (presence or absence of phytochemical)
1	Alkaloids		
	A) Dragendorff test	Red precipitates (ppts) formation	-
	B) Mayer's Test	Yellow ppts	+
	C) Wagner's Test	Brownish red ppts	+
	D) Hager's test	Yellow ppts	+
2	Carbohydrates		
	A) Molisch's Test	Purplish ring formation	-
	B) Benedict's Test	Orange red ppts	++
	C) Fehling's Test	Red ppts	+
3	Glycosides		
	A) Modified Borntrager's Test (Anthranol Glycosides)	Formation of rose pink color in ammonia layer	+
	B) Legal Test (Cardiac Glycosides)	Formation of pink to Blood red color	+
4	Phytosterols & Triterpenoids		
	A) Libermanburchard test	Formation of brown ring at junction	+
	B) Reaction with anisaldehyde		+
	C) Salkowski's Test (Triterpenes)	Appearance of golden yellow color of solution	+
5	Saponins		
	A) Froth test	1 cm layer of foam must be formed	-
	B) Foam test	Foam persist for 10 min	-
6	Phenols		
	FeCl <sub>3</sub> Test	Formation of bluish black color	+
7	Tannins		
	Gelatin test	Formation of white ppts	+
8	Flavanoids		
	A) Alkali reagent test	Formation of intense yellow color, on addition of dilute HCL color disappears	+/-
	B) Lead acetate test	Formation of yellow ppts	+
	C) Natural Product reagent test	Orange fluorescence	+
9)	Protein & Aminoacid		
	A) Xanthoproteic test (Protein)	Formation of yellow coloration	+ (delayed)
	B) Ninhydrin test (Amino acid)	Blue coloration of solution	-

+ indicated presence; - indicated absence.

### *Effects of BV treatment on the behavioural parameters or tests*

In the hot plate assay at the end of the study, diabetic animals exhibited a significantly re-

duced pain threshold from noxious stimuli (2.092±0.231; P<0.05) as compared to normal control rats (4.614±0.836). EBV treatment showed a significant rise in pain threshold level at the end of the study compared to diabetic

control animals ( $P < 0.01$ ) in this assay (**Table 2**). Diabetic animals showed thermal hyperalgesia, as evidenced by a significant ( $P < 0.05$ ) reduction in tail flick latency in 90% of diabetic animals in comparison to normal animals in the hot water tail immersion test. In diabetic rats, tail flick latency was  $3.02 \pm 0.10$  in the 8<sup>th</sup> week of diabetes induction, and these animals showed more hyperalgesia. Treatment with EBV showed a significant increase in tail flick latency that was dose-dependent at the end of treatment, while treatment with glibenclamide also showed a significant reduction in tail flick latency in diabetic rats. EBV-high-dose (400 mg/kg)-treated diabetic rats produced a significant reversal of thermal hyperalgesia (**Table 2**).

### Discussion

The present study observed no specific toxic effects of EBV, supported by the acute toxicity of BV on aqueous extract. Observational, biochemical, and structural data indicated that the oral toxicity of the BV did not create substantial toxic effects in rats [36]. In recent times, hyperglycemia and diabetic inflammation-related complications have been of prime concern [37]. Diabetes caused by the STZ causes direct damage to the beta-cell pancreatic islet, contributing to the literature-backed hyperglycaemic condition [49, 65]. Our study showed substantial decreases in blood glucose levels with regard to diabetic control rats when treated with BV, as confirmed by earlier scientific studies [22, 49, 65]. The highest levels of HbA1C in diabetic rats have been identified because of the reaction of excess blood glucose to hemoglobin, considered to be a reference to the amount of glucose in the blood [38]. Since there is a lack of scientific evidence on the impact of BV constituents at the HbA1c level, our study found that the glycemic regulation and control of blood glucose and glycemic hemoglobin levels of BV in diabetic rats were significantly successful, supported by earlier scientific work [49]. Oxidative stress was the primary cause of DN pathogenesis. Long-term oxidative stress hyperglycemia defines a uniform mechanism for tissue damage [39, 50]. The decrease in the activity of superoxide dismutase in the hyperglycemic rats could be triggered by oxidative stress inactivation [42]. Here SOD catalyzes the dismutation of superoxide ( $O_2^-$ ) since hydrogen peroxide ( $H_2O_2$ ),

which binds with catalase, has been generated in the process of dismutation to prevent cell damage by  $H_2O_2$  [40]. Catalase, on the other hand, detoxifies  $H_2O_2$  into water and thus needs to be present while SOD is active [41]. Phytoconstitutes present in BV, such as alkaloids, glycosides, sterols, flavanoids, proteins, saponin, phenol, alcohol-flavored tannins, and flavonoids, the most commonly studied and with the greatest amount of polyphenols, are attracted to antioxidants due to their strong capacity to disperse free radicals [43]. A study performed by other scientists concluded that the presence of varieties of phytochemicals in *Ficus racemosa* provided strong antidiabetic potential [35], which supports our study results for BV. A common occurrence of diabetes was hyperglycemia, which interfered with the defenders against antioxidants and altered the function of antioxidant enzymes. The study reported an improvement in the number of antioxidant enzymes in diabetic rats treated with BV. In certain trials, erythrocyte catalase activity in diabetic rats increased [44], while erythrocyte CAT activity showed the opposite results [45]. In one animal study, antioxidant markers such as GSH, SOD, and CAT activity were significantly ( $P < 0.05$ ) reduced in disease control rats compared to normal control rats [57], which supports our study results.

Excessive peroxynitrite output in the sciatic nerve is related to modified vaso-relaxation [46]. Superoxide reduces vascular reactivity and is associated with a nutrient supply impediment to the sciatic nerve. Scientists have seen the excessive proliferation of deoxyribonucleic acid in diabetic animal nerves as oxidative stress [47, 48]. Oxidative stress has been shown to cause hyperglycemia through redox imbalances, an increased advanced glycaemic index [5], and superoxide overproduction. Superoxide combination with nitric oxide, which has a detrimental effect on the nerve tissue, contributes to neuropathic pain [8]. Significant oxidative stress and serum nitrite increases were observed in the present study by diabetic animals, which showed nitrogen stress [51] and impaired neurochemical-structural abnormalities leading to neuronal damage or degeneration in diabetic rats [50]. In this study, nitrite levels have been significantly reduced when treated with BV, which explains its protective role in nitrosative stress and neuropathic pain.

A highly sensitive marker of inflammation was acute-phase C-reactive protein (CRP). During inflammatory processes, its level increases dramatically [11]. CRP has been shown to be associated with diseases such as DM, cardiovascular disorders, metabolic syndrome, and renal insufficiency [52-54]. Serum CRP sensitivity was higher than normal in patients with type 2 diabetes and is critical for the development and progression of type 2 DM [55]. The level of this inflammation marker has also been shown to be associated with glycemic regulation, such as glycemic hemoglobin A1C (HbA1c) [12]. Nevertheless, there was little knowledge on the relationship between CRP and HbA1c in diabetic neuropathy and neurodegeneration. This research showed that the amount of CRP in STZ was substantially higher. Diabetic rats treated that can be a significant factor in the destruction and neurodegeneration of diabetic neuropathy. Inflammation caused by CRP increases blood brain barrier penetration, impaired endothelial function, and high intracellular calcium [56]. Increased lipid peroxidation decreases membrane function by decreasing enzymes and receptors at the membrane boundary [13]. In this study, the community of diabetic controls showed a substantial increase in brain and nerve lipid peroxidation. BV and glibenclamide therapy demonstrated a substantial decrease in the amount of tissue MDA. Due to high levels of polyunsaturated lipids in neuronal cell membranes, the high metabolic rate of transitional metals and poor antioxidative defense of the central nervous system made it a highly sensitive target for lipid peroxidative damage [58]. Low glucose use was found in the brain of people with diabetes [58]. In the brains of type 1 diabetic rats [59] and type 2 diabetic mice [60], scientists have found that lipid peroxidation products have increased. Increased MDA serum levels indicated oxidative stress development in rats treated with STZ. Mechanisms involved in the increase of oxidative stress in diabetes include non-enzymatic glycemia and auto-oxidation, as well as improvements in the tissue quality and function of antioxidant defense systems [61-64]. Lipid peroxidation has the net effect of reducing the membrane fluidity, deformity, and viscoelasticity of the tissues. Treatment with BV demonstrates enhanced resistance against antioxidants, lipid peroxidation, increased membrane fluidity, deformation, viscoelasticity, and the life span of nervous tissue.

### Conclusion

The results of this study showed that *Bauhinia variegata* (BV) contains antioxidative alkaloids, flavonoids, and phenolic substances that are mainly responsible for the defense mechanism against STZ-induced diabetic neuropathy. In diabetic rats, BV therapy reduced oxidative stress, inflammation, intracellular calcium, MDA, protein level, and increased antioxidant status. This led to overall neuroprotective rewards that depended on the dose. Reversal of thermal hyperalgesia and increased pain threshold were observed in BV treated (400 mg/kg) diabetic rats. The current study demonstrated that the neuroprotective potential of BV has resulted in considerable advances in the treatment of diabetic neuropathy.

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### Disclosure of conflict of interest

None.

### Abbreviations

DM, Diabetes mellitus; DNN, diabetic neuropathy with neurodegeneration; BV, *Bauhinia variegata*; STZ, streptozotocin; BG, Blood glucose; USD, united states dollar; CRP, C-reactive protein; IAEC, Institutional Animal Ethics Committee; CCSEA, Committee for the Control and Supervision of Experiments on Animals; EBV, Ethanolic extract of BV; NC, Normal Control; DC, Diabetic control; GL, Glibenclamide; HbA-1c, glycemic hemoglobin A1C; GSH, Glutathione; CAT, catalase; SOD, dismutase; MDA, Malondialdehyde; ANOVA, One-way Variance Analysis of variance; SEM, standard error of mean; ppts, precipitates.

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