ORIGINAL ARTICLE

Diabesity 2021, Volume 7, Number 1: 1-9

# Enhancement of the Neuroprotective Effect of Curcumin in Combination with Piperinein Nicotinamide-Streptozotocin Induced Diabetic Rats

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## ABSTRACT

**Introduction:** Curcumin (CUR) is a hydrophobic molecule with poor bioavailability.Piperine is reported to enhance the bioavailability of drugs by increasing absorption in the small intestine and decreasingglucuronidation in the liver.

**Objective:** The objective of the present investigation was to evaluate theneuroprotectiveeffect of curcumin in combination with piperine innicotinamide- streptozotocin (NIC-STZ) induced diabetic rats.

**Methods:**MaleWistar rats were divided into groups viz.vehicle control, diseasecontrol, glibenclamide(1), glibenclamide+ piperine(1+50), curcumin (50) and curcumin (50) with piperine10, 30 and 50.All the groups except vehicle control were induced diabetes by injecting NIC-STz. The animals received treatment daily for six weeks after the confirmation of diabetic status.Behavioural, biochemical and histological parameters were evaluated to access the neuroprotective effect.

**Results:**Oral administration of curcumin + piperine(50+50 mg/kg) caused a significant fall inblood glucose levels.Disease control showed increased paw withdrawal latency and nociceptive threshold compared with vehicle control. Curcumin + piperine treated animals showed decreased paw withdrawal latency. A combination of curcumin + piperine (50+50 mg/kg, p.o.) showed significant antioxidant property by increasing tissue GSH and SOD and lowering lipid content (MDA) compared to the disease control group. The histopathological study showed reduced damage to the sciatic nerve in curcumin + piperine (50+50 mg/kg, p.o.) treated group.

**Conclusion:** Itis concluded that the curcumin-piperine combination reducedthedegeneration of sciatic nerve by reduction of lipid peroxidation and lowering oxidative stress indicating the neuroprotective effect in diabetic neuropathy.

**Keywords:**Curcumin, diabetic neuropathy, sciatic nerve, piperine, oxidative stress.

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**Cite This Article:** Gurav, R.V., Bodhankar, S.L., Arulmozhi, S., Ghaisas, M.M., Mahadik, K.R. 2021. Enhancement of the Neuroprotective Effect of Curcumin in Combination with Piperinein Nicotinamide-Streptozotocin Induced Diabetic Rats. *Diabesity* 7(1): 1-9. DOI: 10.15562/diabesity.2021.60

## INTRODUCTION

Diabetic neuropathy is a major complication of diabetes, which is observed in both type I and types II diabetes. Hyperglycemia is the main reason behind increased oxidative stress leading to macro-vascular as well as microvascular complications such as neuropathy, nephropathy, retinopathy, strokeandneurodegeneration.[1] Increased oxidative stress leads to increased generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS).[2] Axons are susceptible to mitochondrial degeneration since mitochondria are present at a high concentration in axons of nerve cells.[3] High blood sugar level causes depletion of energy stores in mitochondria, which ultimately resultsin increased generation of free radicals.[3] In patients with diabetic neuropathy, both degeneration and regeneration occur simultaneously, suggesting that the disorder is highly dynamic.[4]

The NIC-STZ model is a well-established and reproduciblelaboratory animal model used to induce diabetes.[5] STZ has diabetogenic action which is related to its selective destruction of pancreatic β-cells, which are the only source of insulin in body.[6]

Nicotinamide (NIC) has neuroprotective and antioxidant functions and is given to animals to partially protect pancreatic β-cells against STZ.[7,8] Single dose of STZ (65mg/kg) and NIC (110 mg/kg) (i.p.) leads to the gradual development of neuropathic pain in rats similar to that seen in patients with painful diabetic neuropathy.[9] Mechanical hyperalgesia and mechanical allody-nia along with thermal hyperalgesiaandthermal allodynia are consequences of NIC-STZ induced diabetic neuropathy in rats.[10] Various drug classes including antidepressants, anti-inflammatory, NSAID's, opioids, selective serotonin reuptake inhibitors (SSRI), serotonin, norepinephrine reuptake inhibitors (SNRI), antioxidants, anti-convulsants and antiepileptics are available to ameliorate the mechanical hyperalgesia indiabetic neuropathy.[10]

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Curcumin (CUR)[(*E*, *E*)-1, 7-bis(4-hydroxy-3 -methoxyphenyl)-1, 6-heptadiene-3, 5-dione], a well-known antioxidant, is present in the rhizomatous herbaceous perennial plant *Curcuma longa*L (Zingiberaceae).[12] In India, these rhizomes have been used for centuries as a spice, also for its various medicinal properties.Curcumin has therapeutic effects such as anti-inflammatory,[13] antioxidant activity,[13] anticancer activity.[14] Evaluation of safety studies have shown that curcumin is well tolerated.[15] Curcumin, when administered orally, gets poorly absorbed in the small intestineandundergoes glucuronidation in the liver.[15] Therefore, only traces of the compound appear in the blood, while most of it is excreted in the feces.[15]

Piperine is the active ingredient of pepper. The seeds of black pepper (*Piper nigrum*L) and long pepper (*Piper longum*L)(Piperaceae) have been in use as spices.[16] Piperineis reported toenhance the bioavailability of drugs by increasing absorption in the small intestine and decreasingglucuronidation in the liver.[17]

The aim of the present investigation was to study the effect of curcumin in combination with piperine in NIC-STZ induced diabetic neuropathy in male Wistar rats. The neuroprotective effect was assessed using behavioral, biochemical and histopathological parameters associated withNIC-STZ induced diabetic neuropathy.

## MATERIALS AND METHODS

### Material

Adult male Wistar rats (170–250 g) (9 weeks old) were obtained from the National Institute of Biosciences, Pune (India). The animals were housed under standard laboratory conditions (temperature 24 ± 1°C, the relative humidity of 45–55% and 12h dark and light cycle) and fed commercial rat feed(Baramati Agro Ltd.) and water*adlibitum* throughout the experimental protocol. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Pune. (Registration number: 1703/PO/Re/S/01/CPCSEA dated 17/06/2016) Constituted under Committee for the Purpose for Control and Supervision ofExperiments on Animals (CPCSEA). CPCSEA Number: CPCSEA/PCP/PCL10/2018-19.

### Drugs and chemicals

Streptozotocin (18883-66-4), Nicotinamide (98-92-0), Curcumin (C7727) and Piperine(P49007) were procured from Sigma Aldrich, USA.Glibenclamide (DAONIL)tablets were procured from EmcurePharmaceuticals, Pune. All other chemicals used were of analytical grade. Curcumin was prepared daily as a fresh suspension with 2 % Tween 80 in distilled water.Piperine was combined with curcumin or glibenclamide and fresh suspension was prepared daily with 2 % Tween 80in distilled water. Glibenclamidesuspensionwasprepared by using 2% Tween 80 in distilled water, daily. The doses were administred between 10 am to 11 am once daily.

## METHOD

### Induction of diabetes

Diabetes was induced by a single dose of nicotinamide (110 mg/kg) and streptozotocin (65 mg/kg) (i.p.). Streptozotocin was dissolved in freshly prepared chilled citrate buffer, pH 4.5.Nicotinamidewas dissolved in normal saline solution and injected intraperitoneally (i.p.) 15 minutes before STZ administration. The steady hyperglycemic stage was confirmed on 15$^{th}$ day of NIC-STZ injection. Blood was withdrawn from each rat by retro-orbital puncture (ROP) under anesthesia.Ratsthat showed blood glucose level of≥250 mg/dl were considered diabetic and selected for further study. At this stage, rats were divided into eight groups, each containing eight rats (N=8). It was found that rats developed neuropathic pain on 5$^{th}$ week after induction of diabetes.Treatment was given to all groups except group 1 and 2 for six weeks by the oral route.

Group 1: (VC) Vehicle control (2% Tween 80)

Group 2: (DC)Disease control (2% Tween 80)

Group 3: (S1 GL 1) Glibenclamide (1mg/kg)

Group 4: (S2 GL+ PIP1+ 50) Glibenclamide+ piperine (1+ 50 mg/kg)

Group 5: (T1 CUR 50)Curcumin (50 mg/kg)

Group 6: (T2 CUR + PIP 50+10) Curcumin+ piperine (50+10 mg/kg)

Group 7: (T3 CUR + PIP 50+30)Curcumin+ piperine (50+30 mg/kg)

Group 8: (T4 CUR + PIP 50+50) Curcumin+ piperine (50+50 mg/kg)

### Blood parameters

Blood glucose and triglyceride levels were estimated using GOD-POD and GPO/PAP kit respectively. (Coral Clinical Systems, India).

## BEHAVIOURAL PARAMETERS

### Mechanical hyperalgesia (Randall-Selitto paw pressure test)

Mechanical hyperalgesia was performed as described by Randall and Selitto.[11] Mechanical

nociceptive threshold, an index of mechano-hyperalgesia was quantified using the Randall-Selitto paw pressure device (UGO Basile, Italy). The nociceptive threshold was expressed in grams, as measured by applying increasing pressure to the left and right hind paw. Withdrawal of hind paw was used to assess the nociceptive threshold.

### Mechano-tactile allodynia (von-Frey hair test)

Mechano-tactile allodynia (non-noxious mechanical stimuli) was assessed as described by Chaplan *et al.*[12] von-Frey hairs (ALMEMO, Germany)with calibrated bending forces (in g) of different intensities were used to deliver punctuate mechanical stimuli of varying intensities. The criterion for the threshold value, in grams, was equal to the filament evoking a withdrawal of the paw.

### Thermal hyperalgesia (Hot plate test)

The hot plate test was performed as previously reported by Hargreaves *et al.* using a hot plate apparatus[13] (UGO Basile, Italy).

The latency to the firstsign of paw licking or jump response to avoid heat pain was taken as an index of painthreshold. The cut-off time was kept 10 sec to avoid damage to the paw. Bothhyperalgesia and allodynia were assessed weekly until the end of the study.

### Thermal allodynia (Tail flick test)

The test was performed as previously reported by Hargreaves *et al.*by usingHargreaves' tail-flick apparatus[13] (Ugo Basile, Italy) by placing the tip (last 1-2 cm) of the tail on the radiant heat source. The tail withdrawal from the heat (flicking response) was recorded.A cut off period of 15 secwas observed to avoid damage to the tail.

### Assessment of lipid peroxidation and endogenousantioxidants

At the end of the study, the animals were humanely sacrificed, the sciatic nerve was isolated, weighed on an electronic balance (SCALE-TEK™, India) and quickly transferred to ice-cold tris-hydrochloride buffered saline (pH 7.4). The sciatic nerve sample was homogenized in 10% chilled tris-hydrochloride buffer (10 mM, pH 7.4) by tissue homogenizer (Remi motors, Mumbai, India) and centrifuged at 10000 rpm at 0°C for 15 minutes using Eppendorf 5424-R high speed cooling centrifuge (Beckman Coulter, USA).[14] The clear supernatant was used for the determination of MDA, GSH, SOD, and total protein.

### Assay of Lipid Peroxidation (MDA content)

It was performed using the method described by Slater and Sawyer.[15]

### Estimation of GSH

The assay of GSH was carried out by the method described by Moron *et al.*[6]

### Estimation of SOD activity

The SOD activity was determined by the method of Misera and Fridovich.[17]

### Determination of total proteins

Protein concentrations were determined using the method of Lowry *et al.*[18]

### Histopathology

The sciatic nerve was placed in 10% neutral formalin solution for one week.Fiveμm thickness sections of sciatic nerve samples were stained with Hematoxylin and Eosin (H&E). Sections of the sciatic nerve were observed under a light microscope (10X).[19]

### Statistical Analysis

Data analysis was performed using GraphPad Prism Demo 5.0 software (GraphPad, San Diego, U.S.A.). All values were expressed as mean ± SEM. Data were statistically analyzed using two-way repeated ANOVA using Bonferroni's test, and one-way ANOVA using Dunnett's multiple range test of analysis. A value of $p<0.05$ was considered statistically significant.

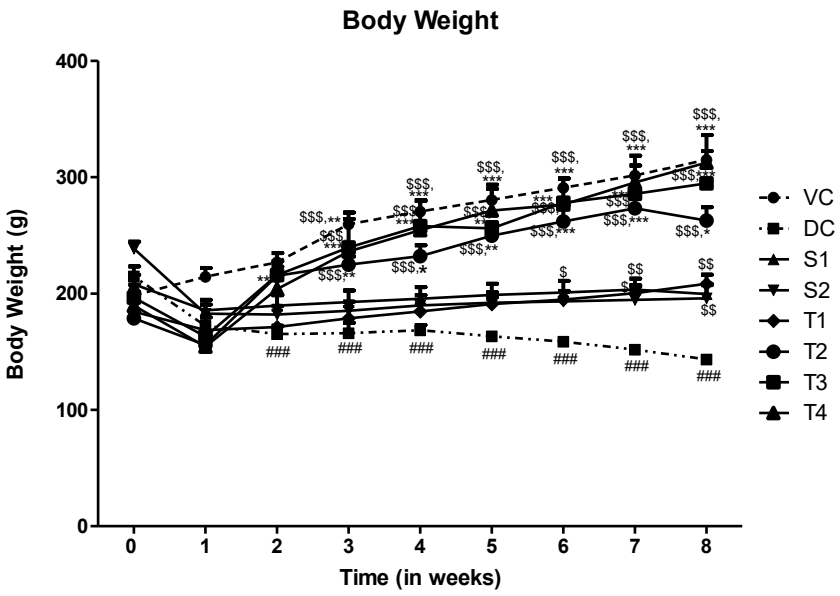
## RESULTS

### Effect of curcumin in combination with piperineon body weight in diabetic rats

Treatment with CUR (50 mg/kg) for six weeks significantly increased ($p<0.01$) the body weight as compared to disease control.Treatment with CUR+PIP (50+10, 50+30 and 50+50 mg/kg) for six weeks significantly increased ($p<0.001$) body weight as compared to disease control. When the diabetic rats were administered CUR+PIP (50+10, 50+30 and 50+50 mg/kg), there was a significant increase ($p<0.001$) in the bodyweight on 8$^{th}$ week as compared to CUR (50 mg/kg) alone (Figure 1).

### Effect of curcumin in combination with piperineonblood glucose level in diabetic rats

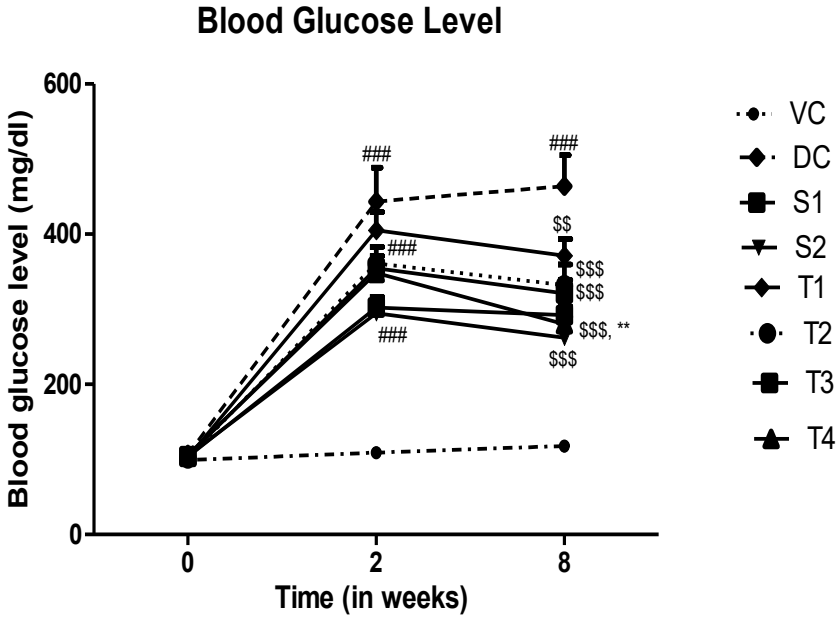
Treatment with CUR (50 mg/kg) for six weeks significantly reduced ($p<0.01$) the increased bloodglucose level as compared to disease control. Treatment with CUR and PIP (50+10, 50+30 and 50+50 mg/kg) for six weeks significantly reduced ($p<0.001$) the blood glucose level as compared to disease control.Rats treated with CUR+PIP (50+ 50 mg/kg) showed a significant decrease ($p<0.01$)




VC: vehicle control; DC: disease control; S1: GL (1 mg/kg); S2: GL+ PIP (1+50 mg/kg); T1: CUR (50 mg/kg); T2: CUR+PIP (50+10 mg/kg); T3:CUR+PIP (50+30 mg/kg); T4: CUR+PIP (50+50 mg/kg); GL: glibenclamide; PIP: piperine; CUR: curcumin

Data were expressed as mean±SEM and analyzed by two-way ANOVA followed by Bonferroni's test. ###p<0.001, #p<0.05 as compared to vehicle control. $^{$$$}$p<0.001, $^{$$}$p<0.01, $^{$}$p<0.05 as compared to disease control, ***p<0.001, **p<0.01, *p<0.05 as compared to T1 group.

**Figure 1** Effect of curcumin in combination with piperine on body weight in diabetic rats




VC: vehicle control; DC: disease control; S1: GL (1 mg/kg); S2: GL+ PIP (1+50 mg/kg); T1: CUR (50 mg/kg); T2: CUR+PIP (50+10 mg/kg); T3:CUR+PIP (50+30 mg/kg); T4: CUR+PIP (50+50 mg/kg); GL: glibenclamide; PIP: piperine; CUR: curcumin

Data were expressed as mean ± SEM and analyzed by two-way ANOVA followed by Bonferroni's test. ###p<0.001 as compared to vehicle control. $^{$$$}$p<0.001, $^{$$}$p<0.01 as compared to disease control, **p<0.01 as compared to T1 group.

**Figure 2** Effect of curcumin in combination with piperine on blood glucose level in diabetic rats.

in blood glucose levels in the8th week as compared to CUR (50 mg/kg) alone (Figure 2).

### Effect of curcumin in combination with piperineon blood triglyceride level in diabetic rats

Rats treated with CUR (50 mg/kg) for six weeks showed a significant reduction (p<0.001) in blood triglyceride levels as compared to disease control. Treatment with CUR and PIP (50+30 and 50+50 mg/kg) for six weeks showed a significant decrease (p<0.05 and p<0.001, respectively) in blood triglyceride levels as compared to disease control. Rats treated with CUR+PIP (50+10 mg/kg) showed a significant decrease (p<0.05) in blood triglyceride level as compared to CUR (50 mg/kg) on 2nd week (Figure 3).

### Effect of curcumin in combination with piperine on thermal hyperalgesia assessed by hot plate test in diabetic rats

The paw withdrawal latency was significantly reduced (p<0.001) after the administration of CUR (50 mg/kg) for six weeks as compared to disease control. When diabetic rats were treated with CUR and PIP (50+10, 50+30, 50+50 mg/kg) for six weeks, there was a significant decrease (p<0.001) in paw withdrawal latency as compared to disease control. Treatment with CUR and PIP (50 + 10 mg/kg) on 5th and 6th week and CUR+PIP (50 + 30 mg/kg) on 5th week showed a significant decrease (p<0.05) in mean paw withdrawal as compared to CUR (50 mg/kg) alone (Table 1).

### Effect of curcumin in combination with piperineon thermal allodynia assessed by tail-flick test in diabetic rats

The tail withdrawal latency was significantly reduced (p<0.001) after theadministration of CUR (50 mg/kg) for six weeks as compared to disease control. When diabetic rats were treated with CUR and PIP (50+10, 50+30, 50+50 mg/kg) for six weeks, showed a significant decrease (p<0.001) in tail withdrawal latency as compared to disease control. Rats treated with CUR and PIP (50+10, 50+30, 50+50 mg/kg) for six weeks didn't show a significant change in tail withdrawal latency compared to CUR(50 mg/kg) alone (Table 2).

### Effect of curcumin in combination with piperine on mechanical hyperalgesia assessed by Randall-Selitto paw pressure test in diabetic rats

Administration of CUR (50 mg/kg) for six weeks resulted in a significant increase (p<0.05) in the paw withdrawal thresholdas compared to disease control. Rats treated with CUR and PIP (50+10, 50+30,

**Table 1** **Effect of curcumin in combination with piperine on thermal hyperalgesia assessed by hot plate test in diabetic rats**

| | Paw Withdrawal Latency (s) | | | | |
|---|---|---|---|---|---|
| | 0 | 35 | 42 | 49 | 56 |
| VC | 3.89±0.29 | 4.37±0.14 | 4.42±0.17 | 3.98±1.58 | 4.50±0.22 |
| DC | 4.49±0.15 | 5.4±0.18$^{\#}$ | 5.96±0.21$^{\#\#\#}$ | 6.17±0.38$^{\#\#\#}$ | 6.13±0.57$^{\#\#\#}$ |
| S1 | 4.20±0.18 | 4.17±0.69 | 5.40±0.34$^{\$}$ | 3.58±0.28$^{\$\$\$}$ | 4.37±0.17$^{\$\$\$}$ |
| S2 | 4.53±0.21 | 5.83±0.33 | 4.83±0.25$^{\$}$ | 4.04±0.17$^{\$\$\$}$ | 4.40±0.25$^{\$\$}$ |
| T1 | 4.03±0.14 | 4.20±0.11$^{\$}$ | 3.88±0.12$^{\$\$\$}$ | 3.62±0.13$^{\$\$\$}$ | 2.85±0.40$^{\$\$\$}$ |
| T2 | 4.21±0.06 | 5.36±0.35$^{*}$ | 5.06±0.01* | 3.34±0.16$^{\$\$\$}$ | 2.95±0.17$^{\$\$\$}$ |
| T3 | 4.30±0.22 | 5.61±0.42$^{*}$ | 3.50±0.05$^{\$\$\$}$ | 3.03±0.27$^{\$\$\$}$ | 2.89±0.33$^{\$\$\$}$ |
| T4 | 4.34±0.30 | 4.92±0.04 | 4.32±0.25$^{\$\$}$ | 3.03±0.24$^{\$\$\$}$ | 2.72±0.10$^{\$\$\$}$ |

VC: vehicle control; DC: disease control; S1: GL (1 mg/kg); S2: GL+ PIP (1+50 mg/kg); T1: CUR (50 mg/kg); T2: CUR+PIP (50+10 mg/kg); T3:CUR+PIP (50+30 mg/kg); T4: CUR+PIP (50+50 mg/kg); GL: glibenclamide; PIP: piperine; CUR: curcumin
Data were expressed as mean ± SEM and analyzed by two-way ANOVA followed by Bonferroni's test. $^{\#\#\#}$p<0.001, $^{\#}$p<0.05 as compared to vehicle control. $^{\$\$\$}$p< 0.001, $^{\$\$}$p< 0.01, $^{\$}$p< 0.05as compared to disease control, *p<0.05 as compared to T1 group.

**Table 2** **Effect of curcumin in combination with piperine on thermal allodynia assessed by tail-flick test in diabetic rats**

| | Tail withdrawal latency (s) | | | | |
|---|---|---|---|---|---|
| **Days** | | | | | |
| **Groups** | 0 | 35 | 42 | 49 | 56 |
| VC | 4.21±0.61 | 6.83±0.85 | 6.86±1.01 | 7.08±0.49 | 6.07±0.46 |
| DC | 5.02±0.43 | 8.91±0.46 | 10.30±0.10$^{\#\#}$ | 13.90±0.85$^{\#\#\#}$ | 14.60±0.36$^{\#\#\#}$ |
| S1 | 4.15±0.21 | 8.27±0.42 | 8.04±0.37 | 6.58±0.61$^{\$\$\$}$ | 5.58±0.59$^{\$\$\$}$ |
| S2 | 4.66±0.14 | 8.85±0.42 | 8.98±0.43 | 7.73±1.91$^{\$\$\$}$ | 6.88±1.62$^{\$\$\$}$ |
| T1 | 6.30±0.20 | 8.20±0.54 | 7.27±0.66$^{\$}$ | 6.00±0.93$^{\$\$\$}$ | 3.92±0.74$^{\$\$\$}$ |
| T2 | 5.25±0.21 | 8.72±0.64 | 8.40±0.64 | 5.68±1.04$^{\$\$\$}$ | 5.76±1.33$^{\$\$\$}$ |
| T3 | 4.48±0.19 | 9.27±1.01 | 8.03±1.11 | 5.70±0.36$^{\$\$\$}$ | 4.53±0.12$^{\$\$\$}$ |
| T4 | 4.33±0.25 | 5.47±0.94$^{\$}$ | 4.87±0.95$^{\$\$\$}$ | 3.65±0.77$^{\$\$\$}$ | 2.95±0.50$^{\$\$\$}$ |

VC: vehicle control; DC: disease control; S1: GL (1 mg/kg); S2: GL+ PIP (1+50 mg/kg); T1: CUR (50 mg/kg); T2: CUR+PIP (50+10 mg/kg); T3:CUR+PIP (50+30 mg/kg); T4: CUR+PIP (50+50 mg/kg); GL: glibenclamide; PIP: piperine; CUR: curcumin
Data were expressed as mean ± SEM and analyzed by two-way ANOVA followed by Bonferroni's test. $^{\#\#\#}$p<0.001, $^{\#\#}$p<0.01 as compared to vehicle control. $^{\$\$\$}$p<0.001, $^{\$}$p<0.05 as compared to disease control.

**Table 3** **Effect of curcumin in combination with piperine on mechanical hyperalgesia assessed by Randall-Selitto paw pressure test in diabetic rats**

| | Paw withdrawal threshold (g) | | | | |
|---|---|---|---|---|---|
| **Days** | | | | | |
| **Groups** | 0 | 35 | 42 | 49 | 56 |
| VC | 131.00±5.30 | 137.00±4.61 | 133.00±3.49 | 131.00±11.70 | 142.00±11.76 |
| DC | 128.00±6.19 | 124.00±6.56 | 111.33±7.74 | 100.00±4.00 | 94.00±3.62$^{\#\#}$ |
| S1 | 148.00±7.65 | 148.57±6.14 | 112.00±7.50 | 122.28±8. 48 | 118.40±13.10 |
| S2 | 141.00±5.31 | 154.66±3.59 | 118.66±9.93 | 119.27±13.36 | 101.81±12.25 |
| T1 | 144.00±7.00 | 154.00±9.04 | 118.00±12.80 | 122.00±11.68 | 134.00±5.18$^{\$}$ |
| T2 | 151.00±7.29 | 141.00±11.88 | 115.00±5.10 | 110.66±6.66 | 116.25±7.25 |
| T3 | 151.00±9.11 | 140.00±5.85 | 125.33±2.66 | 114.66±4.91 | 120.00±6.85 |
| T4 | 155.00±8.60 | 149.33±9.30 | 110.40±13.79 | 120.00±6.76 | 126.00±6.37 |

VC: vehicle control; DC: disease control; S1: GL (1 mg/kg); S2: GL+ PIP (1+50 mg/kg); T1: CUR (50 mg/kg); T2: CUR+PIP (50+10 mg/kg); T3:CUR+PIP (50+30 mg/kg); T4: CUR+PIP (50+50 mg/kg); GL: glibenclamide; PIP: piperine; CUR: curcumin
Data were expressed as mean ± SEM and analyzed by two-way ANOVA followed by Bonferroni's test. $^{\#\#}$p<0.01 as compared to vehicle control. $^{\$}$p<0.05 as compared to disease control.

**Table 4** **Effect of curcumin in combination with piperineon mechanical allodynia assessed by von-Frey hair test in diabetic rats**

| | Paw withdrawal threshold (g) | | | | |
|---|---|---|---|---|---|
| **Days** | | | | | |
| **Groups** | **0** | **35** | **42** | **49** | **56** |
| VC | 4.26±0.15 | 4.23±0.11 | 4.31±0.14 | 4.22±0.12 | 4.29±0.13 |
| DC | 4.22±0.05 | 1.68±0.09$^{###}$ | 1.53±0.11$^{###}$ | 1.21±0.11$^{###}$ | 0.68±0.09$^{###}$ |
| S1 | 4.33±0.13 | 1.81±0.10 | 1.91±0.18 | 2.34±0.20$^{$$$}$ | 2.70±0.17$^{$$**}$ |
| S2 | 4.30±0.17 | 1.83±0.09 | 2.25±0.20$^{$}$ | 2.51±0.13$^{$$$}$ | 3.17±0.23$^{$$**}$ |
| T1 | 3.90±0.24 | 1.68±0.16 | 2.05±0.22 | 2.51±0.22$^{$$$}$ | 3.29±0.16$^{$$$}$ |
| T2 | 4.36±0.17 | 1.53±0.08 | 1.34±0.10 | 2.10±0.30$^{$}$ | 3.47±0.55$^{$$$}$ |
| T3 | 3.76± 0.20 | 1.87± 0.09 | 1.21± 0.34 | 2.26±0.27$^{$}$ | 2.50±0.51$^{$$}$ |
| T4 | 4.44±0.19 | 2.03±0.14 | 1.04±0.24 | 2.64±0.15$^{$$$, **}$ | 3.58±0.18$^{$$**}$ |

VC: vehicle control; DC: disease control; S1: GL (1 mg/kg); S2: GL+ PIP (1+50 mg/kg); T1: CUR (50 mg/kg); T2: CUR+PIP (50+10 mg/kg); T3:CUR+PIP (50+30 mg/kg); T4: CUR+PIP (50+50 mg/kg); GL: glibenclamide; PIP: piperine; CUR: curcumin
Data were expressed as mean ± SEM and analyzed by two-way ANOVA followed by Bonferroni's test. $^{###}$p<0.001 as compared to vehicle control, $^{$$$}$p<0.001, $^{$}$p<0.05 as compared to disease control, **p<0.01 as compared to T1 group.

**Table 5** **Effect of curcumin in combination with piperine on various endogenous biomarkers in diabetic rats**

| Groups | MDA(nM/g of protein) | GSH(µg/g protein) | SOD(mU/gof protein) |
|---|---|---|---|
| VC | 0.045±0.002 | 2.30±0.240 | 100.00± 0.600 |
| DC | 0.098± 0.004$^{###}$ | 0.36± 0.060$^{###}$ | 3.70± 0.570$^{###}$ |
| S1 | 0.076± 0.003$^{$$$}$ | 0.43±0.120 | 6.50± 0.540$^{$$}$ |
| S2 | 0.092±0.004 | 0.22±0.045 | 6.10±0.830$^{$}$ |
| T1 | 0.084±0.004$^{$}$ | 0.94±0.300 | 7.30± 0.360$^{$$$}$ |
| T2 | 0.084±0.005$^{$}$ | 0.62±0.190 | 6.90± 0.510$^{$$}$ |
| T3 | 0.075± 0.003$^{$$$}$ | 0.71±0.240 | 7.50±0.460$^{$$$}$ |
| T4 | 0.071± 0.002$^{$$$}$ | 1.9±0.220$^{$$$**}$ | 8.20± 0.280$^{$$$}$ |

VC: vehicle control; DC: disease control; S1: GL (1 mg/kg); S2: GL+ PIP (1+50 mg/kg); T1: CUR (50 mg/kg); T2: CUR+PIP (50+10 mg/kg); T3:CUR+PIP (50+30 mg/kg); T4: CUR+PIP (50+50 mg/kg); GL: glibenclamide; PIP: piperine; CUR: curcuminData were expressed as mean ± SEM. $^{###}$p<0.001 as compared to vehicle control. $^{$$$}$p<0.001, $^{$$}$p<0.001, $^{$}$p<0.05 as compared to disease control, **p<0.01 as compared to T1 group.

50+50 mg/kg) for six weeks, didn't show a significant change in paw withdrawal threshold as compared to disease control. Rats treated with CUR+ PIP (50+10, 50+30, 50+50 mg/kg) for six weeks didn't show a significant change in paw withdrawal thresholdas compared to CUR (50 mg/kg) alone (Table 3).

### Effect of curcumin in combination with piperineon mechanical allodynia induced by von-Frey hair test in diabetic rats

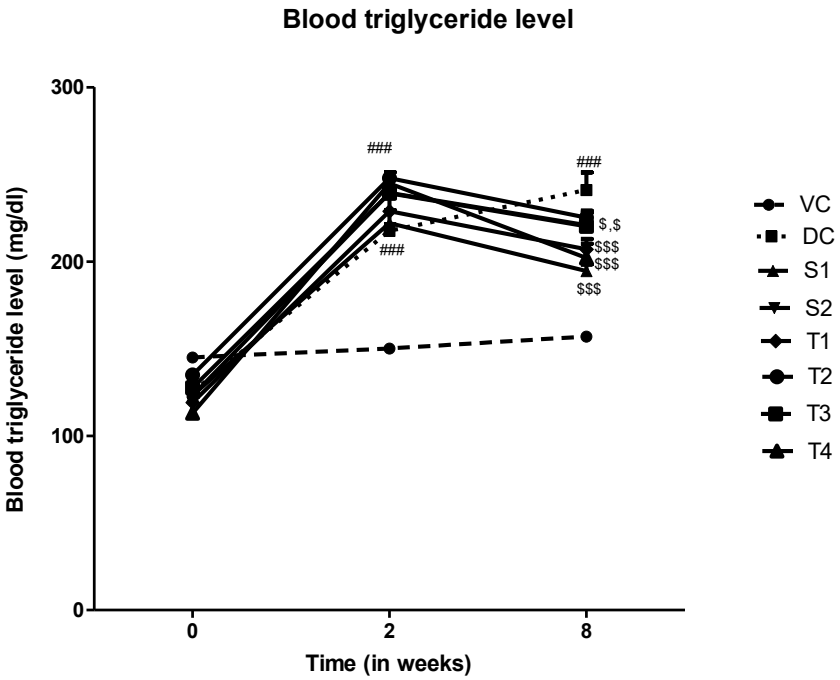
Treatment with CUR (50 mg/kg) for six weeks resulted in a significant increase (p<0.001) in the mean paw withdrawal thresholdas compared to disease control. When rats treated with CUR and PIP (50+10, 50+30, 50+50 mg/kg) for six weeks, showed a significant increase (p<0.001) in mean paw withdrawal threshold as compared to disease control.Rats treated with CUR+ PIP (50+ 50 mg/kg) showed a significant increase (p<0.01) in the mean paw withdrawal threshold compared to CUR (50 mg/kg) alone (Table 4).

### Effect of curcumin in combination with piperineon MDA level in diabetic rats

Intraperitoneal (i.p.) administration of NIC-STZ resulted in a significant increase (p<0.001) in the level of MDA in disease control as compared to vehicle control.whenrats treated with CUR and PIP (50+10, 50+30, 50+50 mg/kg) for six weeks, didn't show a significant change in MDA level as comparedtoCUR (50 mg/kg) alone (Table 5).

### Effect of curcumin in combination with piperineon GSH level in diabetic rats

Intraperitoneal (i.p.) administration of NIC-STZ resulted in a significant decrease (p<0.001) in the GSH levelin disease control as compared to vehicle control. Rats treated with CUR (50 mg/kg) for




VC: vehicle control; DC: disease control; S1: GL (1 mg/kg); S2: GL+ PIP (1+50 mg/kg); T1: CUR (50 mg/kg); T2: CUR+PIP (50+10 mg/kg); T3:CUR+PIP (50+30 mg/kg); T4: CUR+PIP (50+50 mg/kg); GL: glibenclamide; PIP: piperine; CUR: curcumin

Data were expressed as mean ± SEM. $^{###}$p<0.001, $^{##}$p<0.01, $^{#}$p<0.05 as compared to vehicle control, $^{\$\$\$}$p< 0.001, $^{\$}$p<0.05 as compared to disease control, *p<0.05 as compared to T1 group.

**Figure 3** Effect of curcumin in combination with piperine on blood triglyceride level in diabetic rats

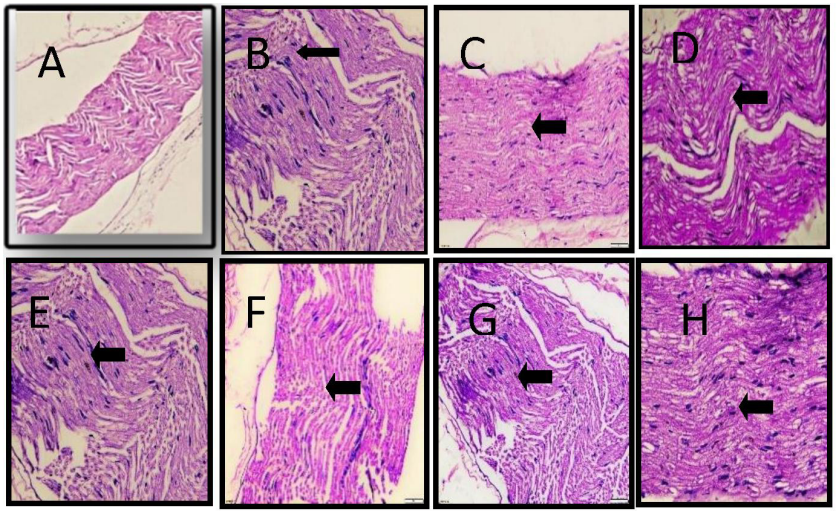



Figure 4. Effect of curcumin in combination with piperine on histopathological analysis of sciatic nerve in diabetic rats. Photomicrographs of sections of the sciatic nerve from rats stained with Hematoxylin & Eosin (H&E). (10X)

(A)Vehicle control (VC), (B) Disease control (DC), (C)S1 GL (1 mg/kg), (D)S2 GL+PIP (1+50 mg/kg), (E)T1 CUR (50 mg/kg), (F)T2 CUR+PIP (50+10 mg/kg), (G)T3 CUR+PIP (50+30 mg/kg), (H)T4 CUR+PIP (50+50 mg/kg). ⬅ Nerve damage

**Figure 4** Histopathology of sciatic nerve

six weeks, showed a significant increase (p<0.001) in the level of GSH compared to disease control. However, when rats were treated with CUR and PIP (50+50 mg/kg) for six weeks, showed a significant increase (p<0.01) in GSH level as compared to CUR (50 mg/kg) alone (Table 5).

### Effect of curcumin in combination with piperineon SOD level in diabetic rats

Intraperitoneal (i.p.) administration of NIC-STZ resulted in a significant decrease (p<0.001) in the SOD level in disease control as compared to vehicle control. Rats treated with CUR (50 mg/kg) for six weeks, showed a significant increase (p<0.001) in the level of SODas compared to disease control. When rats were treated with CUR and PIP (50+10, 50+30, 50+50 mg/kg) for six weeks, they didn't show a significant change in the SODlevelas compared to CUR (50 mg/kg) alone (Table 5).

### Effect of curcumin in combination with piperine on histopathological analysis of sciatic nerve in diabetic rats

Figure 4 Adepicts the normal architecture of the sciatic nerve of vehicle control, as evidenced by the absence of necrosis of the sciatic nerve. Intraperitoneal administration of NIC-STZ resulted in significant histopathological changes in the sciatic nerve of disease control. There was moderate demyelination with bubbled appearance (vacuolization) which resulted in swelling of nonmyelinated and myelinated nerve fibers along with a decrease in a number of myelinated fibers (Figure 4B). Administration of glibenclamide (1 mg/kg) and curcumin (50 mg/kg) for six weeks resulted in inhibition of necrosis and vacuolization in the sciatic nerve and also attenuated the swelling of nonmyelinated and myelinated nerve fibers (Figure 4C and E). Rats treated with glibenclamide+ piperine (1+50 mg/kg) and curcumin+ piperine (50+10 and 50+30 mg/kg) failed to produce the attenuation of the histopathological alteration caused by NIC-STZ administration (Figure 4D, F, G). As shown in Figure 4H curcumin and piperine (50+50 mg/kg) treated rats also showed a decrease in necrosis and vacuolation in the sciatic nerve.

## DISCUSSION

NIC-STZ model of diabetes is a reliable of type 2 diabetes.[5] In the present investigation, after the administration of NIC-STZ, the animals became diabetic as evidenced by hyperglycemia, hypertriglyceridemia, and reduction in body weight.

Curcumin, a known antioxidant reported to be neuroptroective, has tardy intestinal absorption and also undergoes glucuronidation in the liver resulting in reduced bioavailability after oral administration. Therefore, only traces of the compoundgets absorbed in the blood, while most of it is excreted in the feces.[9]

Piperine is reported to increase absorption, decrease glucuronidation and retard oxidative phosphorylation, thereby improves bioavailability of drugs.[10]

The present investigation aims at combining piperine to curcumin for improving the neuroprotective effect of curcumin. In the present study, it was observed treatment of diabetic rats with the curcumin-piperine combination (50+ 50 mg/kg, p.o.) reduced the high blood glucose level as compared to curcumin (50 mg/kg) alone. The bodyweight of the diabetic animals in the curcumin-piperine combination (50+ 50 mg/kg, p.o.) showed a trend towards attaining normal body weight, although complete restoration of body weight was not observed, indicated prevention of muscle wasting due to the curcumin-piperine combination. The animals treated with glibenclamide (1 mg/kg, p.o.) also reduced the blood glucose and triglyceride level.Thus, thecurcumin-piperine combination and glibenclamide had comparable antidiabetic activity. However, the curcumin-piperine combination was more effective in restoring the body weight as compared to glibenclamide (Figure 1).

In the present investigation, the thermal hyperalgesia and thermal allodynia were assessed using the hot plate method and tail flick method in diabetic animals.[22] In thecase of nerve damage, the transmission of an impulse from the paw and tail to the brain is prolonged as the animal does not experience pain; hence, the tail withdrawal latency is increased, indicating neuropathy. Treatment of diabetic animals with curcumin alone and curcumin-piperine combination reduced the tail withdrawal latency compared to diabetic animals showing neuroprotective effects. The mechanical hyperalgesia and mechanical allodynia were assessed using the Randall-Selitto paw pressure test andvon-Frey hairs test in diabetic animals. In the Randall-Selitto paw pressure test, the diabetic animals treated with curcumin alone and curcumin-piperinecombination, showed a significant reduction in reaction latency, indicating a decrease in hyperalgesia due to nerve damage. In thevon-Frey hairs test, the diabetic animals treated with curcumin alone and curcumin-piperinecombination showed a significant increase in paw withdrawal threshold indicating the neuroprotective effect. The mechanical allodynia in curcumin-piperine combination was found to be more effective than curcumin alone which confirms the beneficial effect of piperine.

In diabetic patients, the endogenous antioxidants are reduced mainly due to hyperglycemia.[23] In the present investigation, GSH and SOD levelswereesignificantlydecreased, and MDAlevel was significantly increased in disease control. Administration of curcumin alone and curcumin-piperine combination showed increase in GSH and SOD.

The histopathology of sciatic nerve revealed moderate demyelination with bubbled (vacuole) appearance in disease control. The peroxidation reaction is believed to be responsible for causing damage to the myelin sheath.[24] In the diabetic rats treated with curcumin and curcumin-piperinecombination, the extent of damage was found to be reduced compared to diabetic control.

Piperine is reported to inhibit CYP-3A activity in gut epithelial cells, a potent inhibitor of glucuronidation in rat liver thereby decreasing the metabolism of curcumin. It has also been reported that piperine acts as a modulator of cell membrane dynamics and helps transport of drugs across their barrier.[21] The observed neuroprotective effect is not due to accumulation of curcumin in cellular levels to produce a traditional antioxidant-like impact, but the effect may be due to transient stimulation of cell-signaling pathways relevant to intermediary metabolism and glucose homeostasis.

With respect to increasing the bioavailability of curcumin, thedose-response effect of piperine (PIP) (10, 30 and 50 mg/kg) indicated that theoptimum dose of piperine was 50 mg/kg when concomitantly administered with curcumin. It appears that piperine increased the bioavailability of curcumin.

## CONCLUSION

It is concluded that curcumin-piperine combination reduced the degeneration of sciatic nerve by reduction of lipid peroxidation and lowering oxidative stress indicating the neuroprotective effect in diabetic neuropathy.

## ACKNOWLEDGMENT

The authors would like to acknowledge Dr. S. S. Kadam, Chancellor, BharatiVidyapeeth (Deemed to be University), Pune, India, for providing the necessary facilities to carry out the study.

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