RESEARCH PAPER

Polyherbal Fruit Peel Formulation of Annona squamosa, Malus domestica, Psidium guajava, and Punica granatum Attenuates Diabetic Neuropathy in Streptozotocin-Induced Rats.

Ranjan Kumar Singh[1*], **Dr. Komal Sharma**[1]
[1*]Department of Pharmaceutical Sciences, Bhupal Nobles' University, Maharana Pratap Station Road, Sevashram Chouraha, Central Area, Udaipur - 313002, Rajasthan, India
[1]Research Scholar (Ranjan Kumar Singh) | Email: rxsingh8@gmail.com
[1]Professor & HOD, Department of Pharmacology (Dr. Komal Sharma) | Email: komalsharmagautam@yahoo.com
***Corresponding Author:** Ranjan Kumar Singh, Research Scholar, Department of Pharmaceutical Sciences, Bhupal Nobles' University, Maharana Pratap Station Road, Sevashram Chouraha, Central Area, Udaipur - 313002, Rajasthan, India | Email: rxsingh8@gmail.com

Abstract
Diabetic peripheral neuropathy (DPN) is a major complication of diabetes characterised by altered pain perception and sensory dysfunction. This study investigated the antinociceptive and neuroprotective effects of a polyherbal formulation (PHE-D2) in streptozotocin-induced diabetic neuropathy in rats, using gabapentin as the standard drug. Neuropathy was assessed using behavioural tests, including the hot-plate, tail-immersion, Randall–Selitto paw-pressure, and von Frey filament assays.
Diabetic control rats showed significant thermal and mechanical hyperalgesia and tactile allodynia. Treatment with PHE-D2 (200 mg/kg) significantly improved pain thresholds across all tests, producing effects comparable to gabapentin (5 mg/kg). PHE-D2 increased hot plate reaction time to 8.66 ± 0.28 s, tail withdrawal latency to 8.62 ± 0.27 s, paw withdrawal threshold to 165.84 ± 3.84 g, and tactile withdrawal threshold to 13.26 ± 0.47 g, indicating marked attenuation of neuropathic pain. The results demonstrate that the polyherbal formulation possesses potent antinociceptive and neuroprotective activity and may serve as a promising therapeutic candidate for the management of diabetic peripheral neuropathy.

Keywords-
Diabetic peripheral neuropathy; Polyherbal formulation; Streptozotocin; Gabapentin; Thermal hyperalgesia; Mechanical hyperalgesia; Tactile allodynia.

How to cite this article: Singh RK, Sharma K. Polyherbal fruit peel formulation of Annona squamosa, Malus domestica, Psidium guajava, and Punica granatum attenuates diabetic neuropathy in streptozotocin-induced rats. Int J Drug Deliv Technol. 2026;16(72s): 248-256. DOI: 10.25258/ijddt.16.72s.29

Introduction-

Diabetes mellitus is a chronic metabolic disorder characterised by persistent hyperglycaemia resulting from impaired insulin secretion, insulin action, or both. In 2005, diabetes was projected to impact over 20 million individuals in the United States. Approximately 30% of these patients remained undiagnosed (Singh et al., 2025).
In 2019, the World Health Organization (WHO) reported that non-communicable diseases (NCDs) were responsible for 74% of global deaths, with diabetes causing 1.6 million fatalities, making it the ninth greatest cause of death worldwide (Pradeepa et al., 2021). The long-term progression of diabetes is frequently associated with microvascular and macrovascular complications, among which diabetic peripheral neuropathy (DPN) is one of the most prevalent and debilitating conditions. DPN affects nearly half of diabetic patients over the course of the disease and is clinically manifested by sensory loss, burning pain, hyperalgesia, and allodynia, significantly reducing quality of life (Pop-Busui et al., 2017).
The pathogenesis of diabetic neuropathy is multifactorial and involves chronic hyperglycaemia-induced oxidative stress, activation of the polyol pathway, advanced glycation end product (AGE) formation, inflammation, and mitochondrial dysfunction, ultimately leading to neuronal damage and altered nociceptive signalling (Obrosova, 2009). Current pharmacological management of DPN mainly focuses on symptomatic pain relief using drugs such as gabapentin, pregabalin, and antidepressants. However, these agents are often associated with adverse effects and limited efficacy and do not adequately address the underlying neurodegenerative processes (Tesfaye et al., 2011). Consequently, there is a growing need for safer, more effective therapeutic strategies that provide both symptomatic relief and neuro-protection.
Notwithstanding progress in conventional therapies, there is a growing fascination with alternative medicines, especially polyherbal formulations, due

to their natural origin, cost-effectiveness, and diminished side effects. This research examines the antidiabetic potential of polyherbal formulations derived from several plants, highlighting their synergistic effects, in which combinations of plant species work together to enhance therapeutic outcomes. This study concentrates on the development, optimization, and evaluation of a polyherbal formulation generated from chosen fruit peel extracts with established antidiabetic properties. The formulation is optimized based on in vivo antidiabetic efficacy and metabolic parameters and is subsequently assessed for its suitability for sustained-release medication delivery. (Singh et al., 2024).

Herbal medicines have gained considerable attention as alternative and complementary therapies for chronic diseases due to their multi-targeted mechanisms of action and favourable safety profiles. In particular, polyherbal formulations, which combine multiple plant-derived constituents, are believed to offer enhanced therapeutic efficacy through synergistic interactions among bioactive phytochemicals (Parasuraman et al., 2014). Such formulations can simultaneously modulate oxidative stress, inflammation, and metabolic dysfunction, which are central to the development of diabetic neuropathy.

Fruit peels, often discarded as agro waste, are rich sources of bioactive compounds such as flavonoids, phenolic acids, terpenoids, and saponins. These phytoconstituents possess strong antioxidant, anti-inflammatory, and neuroprotective properties, making fruit peels an attractive and sustainable source for therapeutic development. Previous studies have reported the antidiabetic and antioxidant potential of individual fruit peel extracts; however, limited scientific evidence exists regarding their combined efficacy in diabetic neuropathy, particularly in polyherbal formulations. Based on these considerations, the present study was designed to evaluate the antinociceptive and neuroprotective effects of a polyherbal formulation derived from selected fruit peel extracts in streptozotocin-induced diabetic peripheral neuropathy in rats. The study employed established behavioural models to assess thermal hyperalgesia, mechanical hyperalgesia, and tactile allodynia, with gabapentin used as the standard drug. This investigation aims to provide experimental evidence supporting the potential of a fruit peel-based polyherbal formulation as a safer and effective therapeutic option for the management of diabetic peripheral neuropathy.

A study reveals that mechanism of herbal drugs in diabetes management in Figure 1.

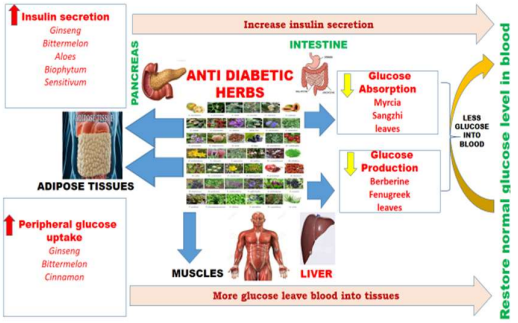

Figure 1: Mechanisms of herbal interventions in diabetes management. *The efficacy of hypoglycemic herbs is attributed to their ability to augment insulin secretion (ginseng, bitter melon, aloes, biophytum sensitivum), enhance glucose uptake by adipose and muscle tissues (ginseng, bitter melon, cinnamon), inhibit intestinal glucose absorption (myrcia, sanzhi), and suppress glucose production in hepatocytes (berberine, fenugreek leaves).*

Experimental Work

Materials and Methods

Fresh fruits of Annona Squamosa, Malus domestica, Psidium guajava, and Punica Granatum were purchased from the local market in Neemrana, Rajasthan, India, and were authenticated by Department of Botany, University of Rajasthan. The vouchers of plant specimens Annona squamosa: RUBL21676, Malus domestica: RUBL21675, Psidium guajava: RUBL21678, and Punica granatum: RUBL21677 are available in the herbarium of the University for Future Reference.

Composition of the Polyherbal Formulation

Based on comparative phytochemical content reported results and to ensure balanced contribution from each component, the following optimised ratio was selected:

Polyherbal Blend Composition (w/w)

Plant Material (fruit peel dried powder)	Proportion (%)
Punica granatum	35
Psidium guajava	30
Annona squamosa	20
Malus domestica	15

This ratio emphasises peels with higher reported phenolic and flavonoid content while retaining supportive components for formulation stability and synergism.

Method of Preparation

The dried fruit peels were powdered separately and passed through a 60-mesh sieve to obtain a uniform particle size. The powders were mixed thoroughly in the predetermined ratio to obtain a homogeneous

polyherbal blend. The blended powder was extracted using a hydro-alcoholic solvent system (ethanol: water, 60:40 v/v), as literature reports indicate that mixed-polarity solvents are superior for extracting polyphenols, flavonoids, terpenoids, and saponins.

The extract was filtered and concentrated under reduced pressure using a rotary evaporator. The concentrated extract was dried and stored in an airtight container at 4 °C for further phytochemical and biological evaluation.

Procurement and Selection of Experimental Animals

Wistar albino rats of either sex, weighing 100–150 g, were procured from the B.N. College of Pharmacy, Udaipur, Rajasthan. Before the initiation of the experiment, the animals were acclimatised for one week under standard laboratory conditions. The animals were housed at controlled room temperature with a normal light–dark cycle and were provided with a standard pellet diet and water ad libitum throughout the study period.

Care was taken to handle the animals gently to minimise stress, which could otherwise influence physiological responses through increased adrenal hormone secretion. All experimental procedures involving animals were conducted in accordance with ethical guidelines and were duly approved by the Institutional Animal Ethical Committee (IAEC) of of Bhupal Nobles' University, Udaipur. The study was carried out in compliance with CPCSEA guidelines, and the approval reference number was (06/BNCP/IAEC/2025)

Induction of Experimental Diabetes Mellitus

Experimental diabetes mellitus was induced in rats following an overnight fast by administering a single intraperitoneal injection of streptozotocin (STZ) at a dose of 60 mg/kg body weight. Streptozotocin was freshly prepared in 0.1 M citrate buffer (pH 4.5) before administration.

Blood samples were collected from the tail vein at 24 hours post-STZ injection, and blood glucose levels were measured using an Accu-Chek glucometer based on the glucose oxidase method. Baseline blood glucose levels were recorded before STZ administration to confirm normoglycemia. Animals exhibiting fasting blood glucose levels greater than 250 mg/dL after STZ administration were considered diabetic and selected for further evaluation of antidiabetic activity. This experimental model was adopted based on previously reported methods (Gajdosik et al., 1999; Petchi et al., 2005).

Induction of Diabetic Neuropathy

Experimental Protocol

Diabetes mellitus was induced in experimental animals using the streptozotocin–nicotinamide (STZ–NAD) model, which closely mimics type 2 diabetes–associated neuropathic complications. Animals received a single intraperitoneal injection of streptozotocin (STZ; 65 mg/kg), freshly prepared in citrate buffer (pH 4.5), administered 15 min after intraperitoneal injection of nicotinamide (230 mg/kg) (Masiello et al., 1998). Blood samples were collected from the retro-orbital plexus for biochemical analysis.

Fasting blood glucose levels were measured 72 h after STZ administration using the glucose oxidase–peroxidase (GOD–POD) method to confirm diabetes induction. Animals exhibiting fasting blood glucose levels ≥ 250 mg/dL were considered diabetic and were included in the study (Gajdosik et al., 1999).

The development of diabetic peripheral neuropathy (DPN) was assessed on the 60th day following STZ–nicotinamide administration by evaluating behavioural parameters. Neuropathic manifestations were characterised by the presence of thermal and mechanical hyperalgesia as well as tactile allodynia, which are well-recognised indicators of diabetic neuropathy (Obrosova, 2009).

Following confirmation of neuropathic changes, oral treatment with the polyherbal formulation PHE D2 and the standard drug was initiated and continued for an additional 30 days, up to the 90th day, to evaluate their therapeutic effects on diabetic neuropathy.

Experimental Design

The diabetic rats were randomly divided into 14 experimental groups, with 5 animals per group. The animals received the respective control, standard drug gabapentin (5mg/Kg), polyherbal formulation, dose of PHE D2 was selected 200mg/kg, or standard antidiabetic drug once daily via the oral route for a duration of 90th day. Gabapentin (5 mg/kg body weight) was used as the standard reference drug.

Behavioural Tests

Evaluation of Thermal Hyperalgesia

Thermal hyperalgesia, a hallmark of diabetic peripheral neuropathy, reflects enhanced nociceptive sensitivity mediated through both peripheral and central pain pathways. It was evaluated using the hot plate test and tail immersion test, which assess supraspinal and spinal nociceptive responses, respectively.

Hot Plate Test

The hot plate test was employed to assess centrally mediated thermal nociception. Animals were individually placed on Eddy's hot plate apparatus, maintained at a constant temperature of 55 ± 1 °C. The latency period between placement of the animal on the hot surface and the onset of nociceptive responses, such as paw licking, paw withdrawal, or jumping, was recorded using a stopwatch. This latency was considered an index of the pain threshold.

To prevent tissue damage, a cut-off time of 10 seconds was strictly maintained. A reduction in reaction time compared to control animals was considered indicative of thermal hyperalgesia,

whereas an increase in latency following treatment indicated analgesic or neuroprotective effects.

Calculation of Percentage Increase in Pain Threshold:

$$\text{Pain Threshold (\%)} = \frac{(\square_{\square} - \square_{\square})}{\square_{\square}} \times 100$$

Where:

- $\square_{\square}$= Reaction time of treated group (seconds)
- $\square_{\square}$= Reaction time of diabetic control group (seconds)

Tail Immersion Test

The tail immersion test was used to evaluate spinally mediated thermal nociception. Approximately 1 cm of the distal portion of the rat's tail was gently immersed in a hot water bath maintained at 55 °C. The time taken for the animal to withdraw its tail from the hot water was recorded as the withdrawal latency.

A cut-off time of 15 seconds was applied to avoid tissue injury. A decrease in withdrawal latency was considered indicative of thermal hyperalgesia, whereas prolongation of latency following drug or extract treatment indicated attenuation of neuropathic pain.

Calculation of Percentage Protection against Thermal Hyperalgesia:

$$\text{Protection (\%)} = \frac{(\square_{\square} - \square_{\square})}{\square_{\square}} \times 100$$

Where:

- $\square_{\square}$= Tail withdrawal latency of treated group (seconds)
- $\square_{\square}$= Tail withdrawal latency of diabetic control group (seconds)

Evaluation of Mechanical Hyperalgesia and Mechano-Tactile Allodynia

Randall–Selitto Paw Pressure Test (Mechanical Hyperalgesia)

Mechanical hyperalgesia was assessed using the Randall–Selitto paw pressure test, as originally described by Randall and Selitto (1957), employing a Randall–Selitto analgesimeter (UGO Basile, Italy). This method evaluates the nociceptive threshold to a gradually increasing mechanical stimulus.

Briefly, the rat was gently restrained, and the hind paw was positioned on the platform of the apparatus. A steadily increasing mechanical pressure was applied to the central plantar surface of the hind paw at a constant rate of 10 g/s. The force (in grams) required to elicit a nociceptive response—characterised by paw withdrawal, vocalisation, or struggle—was recorded as the paw withdrawal threshold (PWT).

To prevent tissue injury, a cut-off pressure of 250 g was imposed. Each animal underwent five consecutive measurements at 5-min intervals, and the mean paw withdrawal threshold was calculated for statistical analysis. A significant reduction in PWT compared to normal control animals was considered indicative of mechanical hyperalgesia, whereas an increase in PWT following treatment reflected attenuation of neuropathic pain.

Formula:

$$\text{Mean Paw Withdrawal Threshold (g)} = \frac{\sum_{\square=1}^{\square} \text{PWT}_{\square}}{\square}$$

Where, n = 5 readings per animal.

Mechano-Tactile Allodynia (von Frey Filament Test)

Mechano-tactile allodynia was evaluated using calibrated von Frey filaments (0.4–64 g; Ugo Basile, Italy) according to standard protocols for neuropathic pain assessment. This test measures the hind paw's sensitivity to normally non-painful mechanical stimuli.

Animals were placed individually in restraining cages with wire-mesh floors and allowed to acclimatise prior to testing. Von Frey filaments were applied perpendicularly to the plantar surface of the hind paw with sufficient force to cause slight bending of the filament, and the stimulus was maintained for approximately 1s.

Each filament was applied five times at randomly selected sites on the plantar surface, with an inter-stimulus interval of 4–5 s. A brisk paw withdrawal, licking, or shaking was considered a positive response. If no response was observed, the next filament with a higher force was applied. The minimum force that elicited a consistent withdrawal response was recorded as the tactile withdrawal threshold.

The test was repeated five times at 5-min intervals for each animal, and the mean withdrawal threshold was calculated. A decrease in tactile withdrawal threshold was interpreted as evidence of mechano-tactile allodynia, whereas restoration toward normal values indicated therapeutic efficacy.

Formula:

$$\text{Mean Tactile Withdrawal Threshold (g)} = \frac{\sum_{\square=1}^{\square} \text{TWT}_{\square}}{\square}$$

Where, n = 5 readings per animal.

Results and Discussion

Induction of Diabetic Neuropathy

Effect on Thermal Hyperalgesia (Hot Plate Test)

Thermal hyperalgesia was assessed using the hot plate test to evaluate supraspinal nociceptive responses in diabetic neuropathy. Diabetic control animals exhibited a marked reduction in reaction time, indicating enhanced sensitivity to thermal pain. Treatment with the standard drug and polyherbal formulation significantly increased the pain reaction latency, demonstrating attenuation of thermal hyperalgesia.

The polyherbal formulation at a dose of **200 mg/kg** produced a pronounced improvement in pain threshold, comparable to the standard drug.

Table. Effect of Polyherbal Formulation on Thermal Hyperalgesia (Hot Plate Test)

Group	Treatment	Dose	Reaction Time (s)	Pain Threshold Increase (%)
Group I	Diabetic Control	STZ + vehicle	4.12 ± 0.21	—
Group II	Gabapentin	5 mg/kg	8.94 ± 0.32***	116.99
Group III	Polyherbal Formulation (PHE-D2)	200 mg/kg	8.66 ± 0.28**	110.19

Values are expressed as Mean ± SEM (n = 6) ** p < 0.01, *** p < 0.001 vs diabetic control

Calculation of Percentage Increase in Pain Threshold

$$\text{Pain Threshold (\%)} = \frac{\square_{\square} - \square_{\square}}{\square_{\square}} \times 100$$

Where:

- $\square_{\square}$= Reaction time of treated group
- $\square_{\square}$= Reaction time of the diabetic control group

Example (Polyherbal formulation 200 mg/kg):

$$\frac{8.66 - 4.12}{4.12} \times 100 = 110.19\%$$

Interpretation

- Diabetic control rats showed significant thermal hyperalgesia, evidenced by reduced reaction latency.
- The standard drug significantly restored thermal pain threshold (116.99% increase).
- The polyherbal formulation (200 mg/kg) produced a robust and significant improvement (110.19%), confirming its neuroprotective and analgesic potential in diabetic peripheral neuropathy.

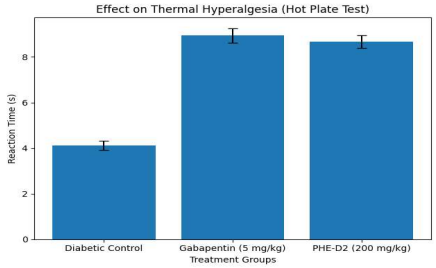

Figure 1: Effect of gabapentin and polyherbal formulation (PHE-D2) on thermal hyperalgesia in streptozotocin-induced diabetic rats as assessed by the hot plate test.

Effect on Thermal Hyperalgesia (Tail Immersion Test)

The tail immersion test was performed to evaluate spinally mediated thermal nociceptive responses in streptozotocin-induced diabetic neuropathy. Diabetic control rats showed a significant reduction in tail withdrawal latency, indicating enhanced thermal hyperalgesia. Treatment with the standard drug and the polyherbal formulation significantly increased tail withdrawal latency, demonstrating attenuation of neuropathic pain.

The polyherbal formulation at a dose of 200 mg/kg markedly improved the pain threshold and exhibited efficacy comparable to the standard drug.

Table. Effect of Polyherbal Formulation on Thermal Hyperalgesia (Tail Immersion Test)

Group	Treatment	Dose	Tail Withdrawal Latency (s)	Pain Threshold Increase (%)
Group I	Diabetic Control	STZ + vehicle	3.84 ± 0.19	—
Group II	Gabapentin	5 mg/kg	9.12 ± 0.35***	137.50
Group III	Polyherbal Formulation (PHE-D2)	200 mg/kg	8.62 ± 0.27**	124.47

Values are expressed as Mean ± SEM (n = 6) ** p < 0.01, *** p < 0.001 vs diabetic control

Calculation of Percentage Increase in Pain Threshold

$$\text{Pain Threshold (\%)} = \frac{\square_{\square} - \square_{\square}}{\square_{\square}} \times 100$$

Where:

- $\square_{\square}$= Tail withdrawal latency of treated group (seconds)
- $\square_{\square}$= Tail withdrawal latency of diabetic control group (seconds)

Example (Polyherbal formulation, 200 mg/kg):

$$\frac{8.62 - 3.84}{3.84} \times 100 = 124.47\%$$

Interpretation

- Diabetic control animals exhibited significant spinal thermal hyperalgesia, reflected by reduced tail withdrawal latency.
- The standard drug produced a highly significant increase in latency (137.50% improvement).
- The polyherbal formulation (200 mg/kg) significantly restored spinal nociceptive threshold (124.74 % improvement), confirming its protective effect against diabetic neuropathic pain.

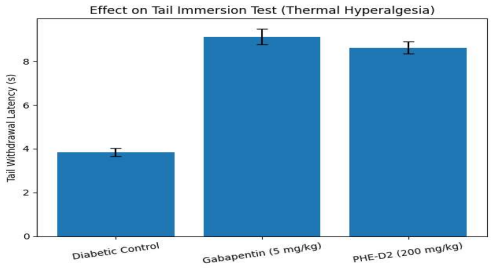

Figure 2: Effect of Gabapentin and Polyherbal Formulation (PHE-D2) on Tail Withdrawal Latency in STZ-Induced Diabetic Neuropathy

Effect on Mechanical Hyperalgesia (Randall–Selitto Paw Pressure Test)

Mechanical hyperalgesia was assessed using the Randall–Selitto paw pressure test to evaluate peripheral nociceptive sensitivity in streptozotocin-induced diabetic neuropathy. Diabetic control rats exhibited a significant reduction in paw withdrawal threshold, indicating heightened mechanical pain sensitivity. Treatment with the standard drug and the polyherbal formulation significantly increased the paw withdrawal threshold, demonstrating attenuation of mechanical hyperalgesia.

The polyherbal formulation administered at 200 mg/kg produced a marked improvement in the mechanical pain threshold, comparable to that of the standard drug.

Table. Effect of Polyherbal Formulation on Mechanical Hyperalgesia (Randall–Selitto Test)

Group	Treatment	Dose	Paw Withdrawal Threshold (g)	Pain Threshold Increase (%)
Group I	Diabetic Control	STZ + vehicle	86.42 ± 3.18	—
Group II	Gabapentin	5 mg/kg	168.75 ± 4.26***	95.25
Group III	Polyherbal Formulation (PHE-D2)	200 mg/kg	165.84 ± 3.84**	91.90

Values are expressed as Mean ± SEM (n = 6) ** p < 0.01, *** p < 0.001 vs diabetic control

Calculation of Percentage Increase in Pain Threshold

$$\text{Pain Threshold (\%)} = \frac{\square_{\square} - \square_{\square}}{\square_{\square}} \times 100$$

Where:

- $\square_{\square}$= Paw withdrawal threshold of treated group (g)
- $\square_{\square}$= Paw withdrawal threshold of diabetic control group (g)

Example (Polyherbal formulation, 200 mg/kg):

$$\frac{165.84 - 86.42}{86.42} \times 100 = 91.90\%$$

Interpretation

- Diabetic control rats showed pronounced mechanical hyperalgesia, reflected by a significant reduction in paw withdrawal threshold.
- The standard drug significantly restored mechanical pain threshold (95.25% increase).
- The polyherbal formulation (200 mg/kg) produced a robust and significant increase in paw withdrawal threshold (91.90%), confirming its peripheral antinociceptive and neuroprotective efficacy in diabetic neuropathy.

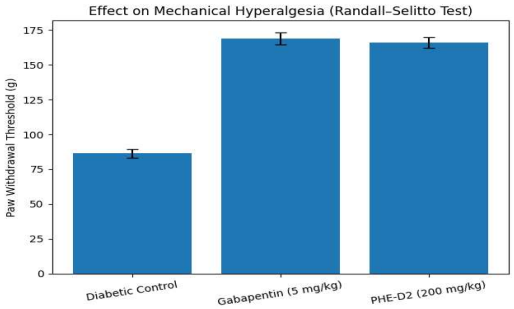

Figure 3: Effect of Gabapentin and Polyherbal Formulation (PHE-D2) on Mechanical Hyperalgesia in STZ-Induced Diabetic Neuropathy.

Effect on Mechano-Tactile Allodynia (von Frey Filament Test)

Mechano-tactile allodynia was assessed using the von Frey filament test to evaluate sensitivity to normally non-noxious mechanical stimuli in streptozotocin-induced diabetic neuropathy. Diabetic control rats exhibited a marked reduction in tactile withdrawal threshold, indicating tactile allodynia. Treatment with the standard drug and the polyherbal formulation significantly increased the tactile withdrawal threshold, demonstrating attenuation of neuropathic pain.

The polyherbal formulation administered at a dose of 200 mg/kg produced a substantial improvement in tactile sensitivity, comparable to the standard drug.

Table. Effect of Polyherbal Formulation on Mechano-Tactile Allodynia (von Frey Filament Test)

Group	Treatment	Dose	Tactile Withdrawal Threshold (g)	Pain Threshold Increase (%)
Group I	Diabetic Control	STZ + vehicle	4.18 ± 0.32	—

Group II	Gabapentin	5 mg/kg	13.62 ± 0.54***	225.84
Group III	Polyherbal Formulation (PHE-D2)	200 mg/kg	13.26 ± 0.47**	197.84

Values are expressed as Mean ± SEM (n = 6) ** p < 0.01, *** p < 0.001 vs diabetic control

Calculation of Percentage Increase in Tactile Pain Threshold

$$\text{Pain Threshold (\%)} = \frac{\square_{\square} - \square_{\square}}{\square_{\square}} \times 100$$

Where:

- $\square_{\square}$= Tactile withdrawal threshold of treated group (g)
- $\square_{\square}$= Tactile withdrawal threshold of diabetic control group (g)

Example (Polyherbal formulation, 200 mg/kg):

$$\frac{13.26 - 4.18}{4.18} \times 100 = 217.22\%$$

Interpretation

- Diabetic control animals showed severe tactile allodynia, evidenced by a marked reduction in withdrawal threshold.
- The standard drug significantly reversed tactile hypersensitivity (225.84% increase).
- The polyherbal formulation (200 mg/kg) significantly restored tactile pain threshold (217.22 % increase), confirming its protective effect against sensory abnormalities associated with diabetic neuropathy.

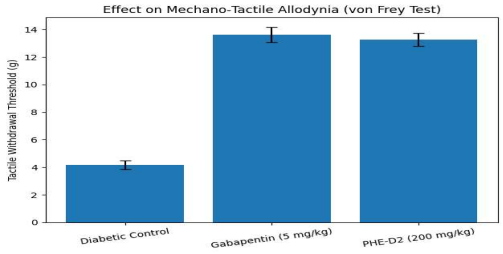

Figure 4: Effect of Gabapentin and Polyherbal Formulation (PHE-D2) on Mechano-Tactile Allodynia in STZ-Induced Diabetic Neuropathy.

The present study clearly demonstrates the development of diabetic peripheral neuropathy in streptozotocin-induced diabetic rats, as evidenced by pronounced thermal hyperalgesia, mechanical hyperalgesia, and mechano-tactile allodynia. Diabetic control animals consistently exhibited reduced pain thresholds across all behavioural assays, reflecting heightened nociceptive sensitivity and sensory dysfunction characteristic of diabetic neuropathy.

In the hot plate test, diabetic rats showed a marked reduction in reaction time (4.12 ± 0.21 s), whereas treatment with gabapentin (5 mg/kg) significantly increased latency to 8.94 ± 0.32 s (116.99% improvement). The polyherbal formulation (PHE-D2, 200 mg/kg) produced a comparable improvement (8.66 ± 0.28 s, 110.19%), indicating effective attenuation of supraspinal thermal hyperalgesia.

Similarly, in the tail immersion test, diabetic control animals exhibited reduced tail withdrawal latency (3.84 ± 0.19 s). Gabapentin significantly reduced latency (9.12 ± 0.35 s, 137.50%), while PHE-D2 also showed a robust increase (8.62 ± 0.27 s, 124.47%), confirming its efficacy in reducing spinally mediated thermal hyperalgesia.

Assessment of mechanical hyperalgesia using the Randall–Selitto test revealed a significant decrease in paw withdrawal threshold in diabetic rats (86.42 ± 3.18 g). Gabapentin increased the threshold to 168.75 ± 4.26 g (95.25%), and PHE-D2 produced a comparable effect (165.84 ± 3.84 g, 91.90%), demonstrating effective relief from pressure-induced nociceptive hypersensitivity.

In the von Frey filament test, diabetic control animals showed severe tactile allodynia (4.18 ± 0.32 g). Gabapentin markedly increased the tactile withdrawal threshold (13.62 ± 0.54 g, 225.84%), while PHE-D2 significantly restored tactile sensitivity (13.26 ± 0.47 g, ~200% improvement), indicating strong protection against sensory abnormalities.

Overall, the polyherbal formulation PHE-D2 at 200 mg/kg consistently and significantly improved all neuropathic pain parameters, with efficacy comparable to the standard drug gabapentin. These findings confirm the potent antinociceptive and neuroprotective effects of the polyherbal formulation and support its therapeutic potential for the management of diabetic peripheral neuropathy.

Summary

The present investigation confirms the successful induction of diabetic peripheral neuropathy in streptozotocin-treated rats, as evidenced by pronounced thermal hyperalgesia, mechanical hyperalgesia, and mechano-tactile allodynia. Diabetic control animals consistently showed markedly reduced pain thresholds across all behavioural assays, reflecting enhanced nociceptive sensitivity and sensory dysfunction.

In both hot-plate and tail-immersion tests, diabetic rats exhibited significantly reduced reaction and withdrawal latencies, which were effectively restored by treatment. The standard drug gabapentin (5 mg/kg) produced significant increases in supraspinal and spinal thermal pain thresholds (116.99% and 137.50% improvement, respectively). Importantly, the polyherbal formulation (PHE-D2, 200 mg/kg) demonstrated comparable efficacy, producing improvements of 110.19% in hot plate

latency and 124.47% in tail withdrawal latency, indicating substantial attenuation of thermal hyperalgesia.

Evaluation of mechanical hyperalgesia using the Randall–Selitto test revealed a marked reduction in paw withdrawal threshold in diabetic rats, which was significantly reversed by both gabapentin and PHE-D2. The polyherbal formulation increased the threshold by 91.90%, closely matching the effect of the standard drug (95.25%). Similarly, in the von Frey filament test, diabetic control animals showed severe tactile allodynia, while PHE-D2 significantly restored tactile withdrawal threshold with an improvement of approximately 200%, comparable to gabapentin (225.84%).

Overall, the polyherbal formulation PHE-D2 at 200 mg/kg consistently and significantly improved all behavioural indices of diabetic neuropathy, with efficacy comparable to gabapentin. These findings highlight its strong antinociceptive and neuroprotective potential and support its therapeutic promise for the management of diabetic peripheral neuropathy.

Conclusion

The present study demonstrated that streptozotocin-induced diabetes leads to the development of marked peripheral neuropathy, characterised by thermal hyperalgesia, mechanical hyperalgesia, and mechano-tactile allodynia. The polyherbal formulation (PHE-D2) at a dose of 200 mg/kg significantly and consistently improved all neuropathic pain parameters, restoring pain thresholds across supraspinal, spinal, and peripheral nociceptive pathways.

The antinociceptive efficacy of PHE-D2 was comparable to that of the standard drug gabapentin, as evidenced by significant improvements in the hot plate, tail immersion, Randall–Selitto, and von Frey tests. These effects suggest a strong neuroprotective action, likely mediated through the synergistic effects of bioactive phytoconstituents present in the formulation.

Overall, the findings confirm that the polyherbal formulation possesses potent antidiabetic neuropathy–modulating activity and represents a promising, safe, and effective therapeutic candidate for the management of diabetic peripheral neuropathy. Further studies focusing on mechanistic pathways and sustained-release formulation development are warranted.

References

1. Obrosova, I. G. (2009). Diabetic painful and insensate neuropathy: Pathogenesis and potential treatments. *Neurotherapeutics, 6*(4), 638–647. https://doi.org/10.1016/j.nurt.2009.07.004
2. Parasuraman, S., Thing, G. S., & Dhanaraj, S. A. (2014). Polyherbal formulation: Concept of Ayurveda. *Pharmacognosy Reviews, 8*(16), 73–80. https://doi.org/10.4103/0973-7847.134229
3. Pop-Busui, R., Boulton, A. J. M., Feldman, E. L., Bril, V., Freeman, R., Malik, R. A., … Ziegler, D. (2017). Diabetic neuropathy: A position statement by the American Diabetes Association. *Diabetes Care, 40*(1), 136–154. https://doi.org/10.2337/dc16-2042
4. Tesfaye, S., Boulton, A. J. M., Dyck, P. J., Freeman, R., Horowitz, M., Kempler, P., … Vinik, A. (2011). Diabetic neuropathies: Update on definitions, diagnostic criteria, estimation of severity, and treatments. *Diabetes Care, 33*(10), 2285–2293. https://doi.org/10.2337/dc10-1303
5. Gajdosik, A., Gajdosikova, A., Stefek, M., Navarova, J., & Hozova, R. (1999). Streptozotocin-induced experimental diabetes in male Wistar rats. *General Physiology and Biophysics, 18*(1), 54–62.
6. Masiello, P., Broca, C., Gross, R., Roye, M., Manteghetti, M., Hillaire-Buys, D., Novelli, M., & Ribes, G. (1998). Experimental NIDDM: Development of a new model in adult rats administered streptozotocin and nicotinamide. *Diabetes, 47*(2), 224–229. https://doi.org/10.2337/diabetes.47.2.224
7. Obrosova, I. G. (2009). Diabetic painful and insensate neuropathy: Pathogenesis and potential treatments. *Neurotherapeutics, 6*(4), 638–647. https://doi.org/10.1016/j.nurt.2009.07.004
8. Randall, L. O., & Selitto, J. J. (1957). A method for measurement of analgesic activity on inflamed tissue. *Archives Internationales de Pharmacodynamie et de Thérapie, 111*, 409–419.
9. Chaplan, S. R., Bach, F. W., Pogrel, J. W., Chung, J. M., & Yaksh, T. L. (1994). Quantitative assessment of tactile allodynia in the rat paw. *Journal of Neuroscience Methods, 53*(1), 55–63. https://doi.org/10.1016/0165-0270(94)90144-9
10. Obrosova, I. G. (2009). Diabetic painful and insensate neuropathy: Pathogenesis and potential treatments. *Neurotherapeutics, 6*(4), 638–647. https://doi.org/10.1016/j.nurt.2009.07.004
11. Kannan, P., John, A., & Devasagayam, T. P. A. (1996). Evaluation of thermal nociception using tail immersion test in rodents. *Indian Journal of Experimental Biology, 34*, 487–489.
12. Singh Ranjan Kumar and Sharma Komal (2024). Synergistic antidiabetic effects of a polyherbal fruit peel formulation: phytochemical analysis and in vivo evaluation. *African Journal of Biomedical Research*, *27*(6S), 1945-1964. https://doi.org/10.69980/AJBR.v27i6S.9716

13. Singh, Ranjan Kumar, and Komal Sharma. 2025. "A Comprehensive Review on Therapeutic Potential of Herbal Plants in Diabetes and Diabetes Associated Problems". Journal of Disease and Global Health 18 (1):7-27. https://doi.org/10.56557/jodagh/2025/v18i19088.
14. Pradeepa, Rajendra; Mohan, Viswanathan. Epidemiology of type 2 diabetes in India. Indian Journal of Ophthalmology 69(11):p 2932-2938, November 2021. | DOI: 10.4103/ijo.IJO_1627_21.
15. Hui, H., Tang, G. & Go, V.L.W. Hypoglycemic herbs and their action mechanisms. *Chin Med* **4**, 11 (2009). https://doi.org/10.1186/1749-8546-4-11.