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Original Article

Role of Novel Polyherbal Formulations in Secondary Complications like Peripheral Neuropathy in Streptozotocin-Induced Diabetic Wistar Rats

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ABSTRACT

Background: Diabetes becomes a chronic disease when glucose constantly remains high and uncontrolled. As time goes off, it may gradually develop acute or chronic associated complications. Diabetic neuropathy is one of the chronic microvascular complications, reported to affect 25% of the diabetic patient and makes patient's life disabled and life-threatening. Hence, the present research study was designed to evaluate a novel antidiabetic polyherbal formulation, for its activity against diabetic neuropathy induced in an animal model by using streptozotocin. **Materials and Methods:** Novel polyherbal formulations were developed and were evaluated against streptozotocin-nicotinamide-induced diabetic neuropathy using 8–10 weeks old Wistar albino rats. Animals with diabetic neuropathy were subjected to standard neuro-protective evaluation parameters, including mechanical, chemical, and thermal tests. **Results:** Streptozotocin-induced hyperglycemia developed chronic neuropathic symptoms in the experimental rats between 25 - 28 days. Treatments with all four tests PHF for 8 weeks significantly improved the thermal sensation, hyperalgesia, muscle grip strength, and locomotor activity. **Conclusion:** By detailed research study it was revealed that out of the four polyherbal formulations studied. PHF 4 having the plant ratio of: *H. sabdariffa* (100): *A. marmelos* (50): *F. religiosa* (75): *A. squamosa* (25), the result was significantly ($p < 0.001$) better than PHF 2, 8 and 10 ($p < 0.01$) in reversing the symptoms of diabetic neuropathy. Thus, it was concluded that PHF 4 is excellent in treating diabetic neuropathy as well have excellent potential to prevent further progression and thus can be used against diabetic peripheral neuropathy.

Keywords: Cold allodynia, Diabetic neuropathy, Diabetic complications, Locomotor activity, Motor co-ordination, Thermal hyperalgesia.

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INTRODUCTION

Diabetes is a chronic metabolic disorder; when remains uncontrolled for the long term, progressively may develop many severe complications that may be disabling or even life-threatening.[1,2] Uncontrolled diabetes may cause several chronic complications, which is classified as microvascular and macrovascular type. Microvascular complications include diabetic nephropathy, neuropathy, and retinopathy, which may develop via the production of advanced glycation end products (AGEs) or by the initiation of oxidative stress.[2] It is reported that diabetic neuropathy has resulted when; excess glucose in blood circulation injures the walls of the tiny blood capillaries that nourish the nerves, especially of the legs. This results in tingling, burning, or pain sensation followed by numbness that slowly spreads upward. If left untreated, all the senses of the affected limbs may be lost. Nerve damage caused in the foot increases the risk of various foot complications, which can further develop into serious infections that may ultimately lead to partially or completely toe, foot, or leg amputation and also increases the risk of early death.[3] It is reported that almost 80% of the diabetic suffers from peripheral diabetic neuropathy (feet) and up to 25% have diabetes with impaired sensation in their feet and may develop a foot ulcer in their lifetime.[4]

In the research study streptozotocin (STZ) which has been used to develop diabetes, is not only diabetogenic but also causes gastric ulceration, hepatopathy, nephropathy, and neuropathy. While developing neuropathy, it acts by decreasing neurotrophic factors, which in turn exaggerate the degeneration of neurons. STZ further acts by slowing down the nerve conduction velocity (reflecting nerve dysfunction severity), generating peripheral neuropathy marked by mechanical and thermal allodynia.[5]

In the present study, four novel polyherbal formulations were developed by mixing 50% hydroalcoholic extracts of four

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different plant parts i.e., leaves of *Annona squamosa*, calyx of *Hibiscus sabdariffa*, leaves of *Aegle marmelos*, and stem bark of *Ficus religiosa*. These selected plants having proven antidiabetic potential,[6] were evaluated for their capacity in improving and protecting neurological (peripheral neuropathy) complications induced by diabetes in an animal model. Polyherbal formulations evaluated against diabetic peripheral neuropathy were named PHF 2 which contained extract in a ratio; as *H. sabdariffa* (100): *A. marmelos* (25): *F. religiosa* (75): *A. squamosa* (50), PHF 4 had a ratio of 100:50:75:25, PHF 8 had 75:25:100:50 and PHF 10 had a ratio of 75:50:100:25, making 250 mg dose.

MATERIALS AND METHODS

Collection of materials

Four plant parts were selected for research study i.e., leaves of *Annona squamosa*, Linn, leaves of *Aegle marmelos*, (L.) Corr, stem bark of *Ficus religiosa* Roxb, and calyx of *Hibiscus sabdariffa*, Linn. (HSC) were collected and authenticated by the botanist Dr. S.N. Dwivedi, Janata PG College, Rewa M.P. India. Herbarium specimens were deposited with voucher specimen number JC/B/PAN 482. 50% hydroalcoholic extracts were developed using the soxhlet apparatus after defatting with petroleum ether. Analytical grade chemicals were used in the research, including streptozotocin (Sigma Chemical Co., Bangalore, India), nicotinamide (Aster Pharmaceuticals, India), and acetone. The types of equipment used were glucometer (Accu-Check active), temperature and precision control water bath, Eddy's hot plate, rota-rod apparatus, actophotometer (Scientec, India), and bent gauge needle.

Diabetic neuropathy induction and treatment protocols

By using a single intraperitoneal (i.p.) injection of freshly prepared streptozotocin (STZ) 60 mg/kg b.w., in 0.1 M cold citrate buffer (pH 4.5), diabetes was induced in the overnight fasted rat.[7] After 15 min nicotinamide (NA) 120 mg/kg b.w., dissolved in normal saline was given via the i.p route. Animals were evaluated for blood glucose levels at regular intervals. Animals having fasting blood glucose concentrations of more than 300 - 350 mg/dL were considered permanent and severe diabetic.[8] To prevent STZ-induced hypoglycemia and mortality due to hypoglycaemic shock, 10% dextrose solution was given to rats after 6 hr for the next 24 hr. Blood glucose level was checked by using a glucometer using the tail prick method. Rats without hyperglycemia were rejected and replaced. Animals with constant hyperglycemia were further tested for the symptoms of diabetic neuropathy and were used for the research study.

Preparation of polyherbal formulations test suspension

For the detailed *in-vivo* neuroprotective study four different test suspensions were developed using four polyherbal formulations (PHF 2, 4, 8, and 10). Uniform suspensions for all formulations were prepared by constantly triturating powdered extracts 5 gm/100 ml of 0.5% CMC, which will give a 50 mg/ml concentration.

Experimental design

The effect of polyherbal formulations on diabetic neuropathy was evaluated by using STZ-NA induced diabetes in 8 – 10 weeks old Wistar albino rats of 180-250 g weight. Overnight (12 hr) fasted rats were divided into 6 groups of 6 animals (n=6) each (including both male and female). All the female animals chosen were nulliparous and non-pregnant. Animals' were housed in cages 7 days before, for acclimatization at room temperature 25 ± 2° and relative humidity of 30-60 percent. Animals were subjected to controlled 12 hr light/dark cycle before the study. During experimentation, animals were given standard animal feed and water *ad libitum*. All experimental procedures were approved and were conducted as per the guidelines given by CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Vedica College of B. Pharmacy, RKDF University, Bhopal, India (Registration number: 1693/PO/Re/S/13/CPCSEA). Test samples (formulations) were given orally using an intragastric tube to the fasted animals. Reading was noted on fortnightly basis for up to 8 weeks. Before these studies, animals were acclimated to the environment for at least 10 min.

Animals were grouped as:

Normal control group	Animals received normal saline.
Diabetic control group	Diabetic animals received normal saline
Test group 1	Diabetic rats received with PHF 2 formulations 250 mg/kg b.w.
Test group 2	Diabetic rats received with PHF 4 formulations 250 mg/kg b.w.
Test group 3	Diabetic rats received with PHF 8 formulations 250 mg/kg b.w.
Test group 4	Diabetic rats received with PHF 10 formulations 250 mg/kg b.w.

Experimental protocols for evaluation of polyherbal formulations

A hot water tail immersion test was performed using the temperature and precision control water bath, where the terminal part of the rat tail i.e., 5 cm was immersed in the hot water maintained at 55 ± 1°. The duration (in seconds) in which the rat withdraws its tail was recorded using a digital timer. A decrease

Table 1: Effect of PHF on hot water tail withdrawal latency in diabetic rats.

Treatments	Duration of treatment (in weeks) and Reaction time (seconds)				
	0 Week	2 Week	4 Week	6 Week	8 Week
Group I Normal control	11.27±0.22	10.90±0.55	11.13±0.20	11.28±0.17	11.12±0.30
Group II Diabetic control	5.37±0.18	5.42±0.17	5.37±0.14	5.46±0.12	5.43±0.10
Group III PHF 2 treated	5.31±0.37	5.76±0.36	6.63±0.53	8.17±0.60	10.31±0.50**
Group IV PHF 4 treated	5.23±0.11	6.37±0.09	7.84±0.37	9.89±0.55*	12.88±0.44***
Group V PHF 8 treated	5.27±0.15	5.92±0.29	6.53±0.32	7.80±0.42	9.88±0.73*
Group VI PHF 10 treated	5.30±0.12	5.59±0.52	7.01±0.39	8.46±0.58*	11.11±0.73***

All values are presented as Mean ± SD, (n = 6), $p < 0.05$*, $p < 0.01$**, $p < 0.001$***, When compared to diabetic control.

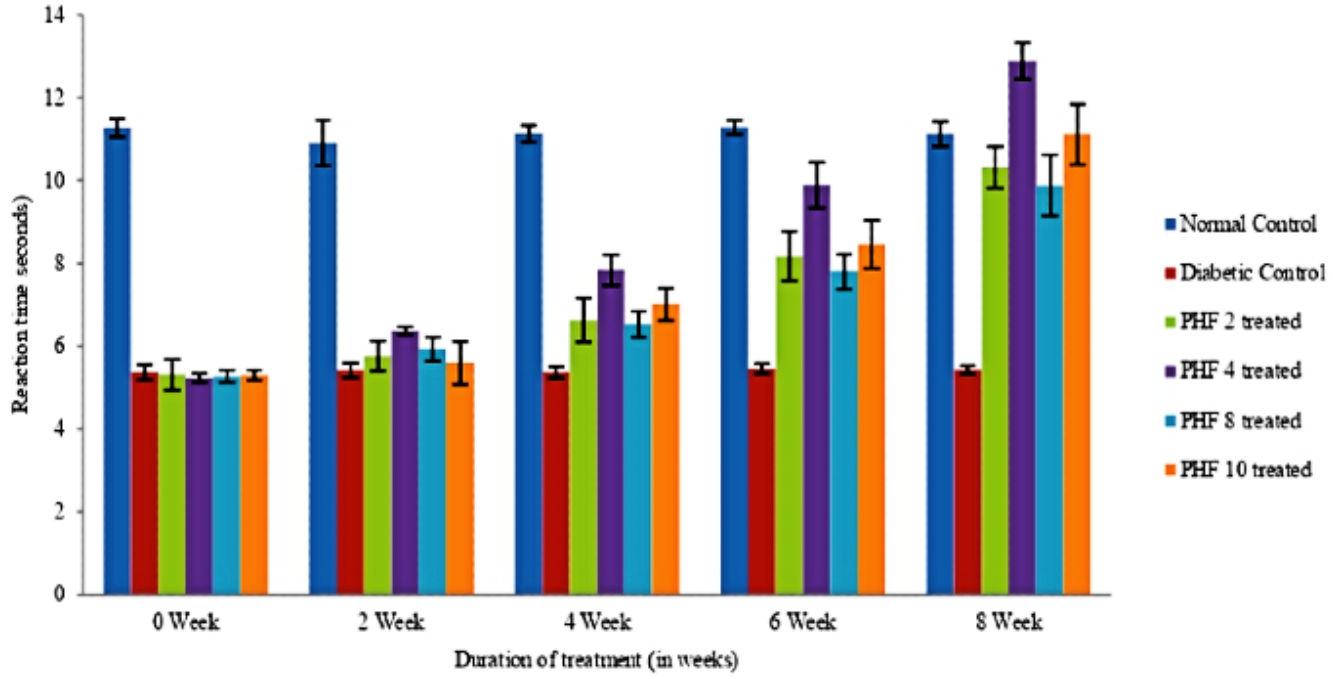

Figure 1: Effect of PHF on hot water tail withdrawal latency in diabetic rats.

in the time of tail withdrawal was an indication of thermal hyperalgesia.[9] The cold-water tail immersion test was performed the same as the hot water tail immersion test, by replacing hot water with cold water, maintained at 10 ± 0.5°C. A decrease in cold water tail contact time points towards nociception, whereas increased contact time was reported as an anti-allodynic effect.[10] A paw heat-hyperalgesia test was performed to evaluate sensory function using Eddy's hot plate. Here, the preheated plate maintained at 55.5 ± 1.0°, was used in the study. Nociceptive threshold was observed and noted in seconds when the rats started licking the hind paw or started jumping. 20 second cut-off time was kept to prevent tissue injury.[10] Cold hyperalgesia or acetone drop test was performed using acetone as described by Yoon *et al.*, (1994) with modification.[11] Here, a freshly prepared acetone drop (50µL) was gently applied to the mid plantar surface of the hind paw. After 2-5 sec of application acetone generated cold chemical sensitivity i.e., the nociceptive response was confirmed in the rats by paw licking, shaking, or rubbing, with brisk foot withdrawal. Each test was repeated three times with 5 min intervals and the mean value was calculated.

Pinprick test was performed by using the point of a gauge needle bent at a 90°C angle was used to touch the surface of the injured hind paw, without piercing deep. This produced a reflex of withdrawal in normal control animals; the duration of time (in seconds) altered in the paw withdrawal was recorded.[10]

Motor coordination or muscle grip strength test was performed to evaluate the neuromuscular function using rota-rod apparatus, which might have got altered due to the presence of a high glucose level or hyperglycemic index. Animals were pretested at 25 rpm revolving rod speed and those rats which confirmed their ability to stay on the revolving rod for at least 1 minute were used in the study. Alteration in the falling time of each rat from the rotating rod was recorded during five minutes period for all the experimental groups.[12,13] To assess the spontaneous motor behavior of the animals the actophotometer was used. Each animal was observed for 5 minutes after placing them in an actophotometer by using digital counter interruptions of photocell beam.[14] To get the mean value all the procedures were repeated three times for each animal for all the experimental groups.

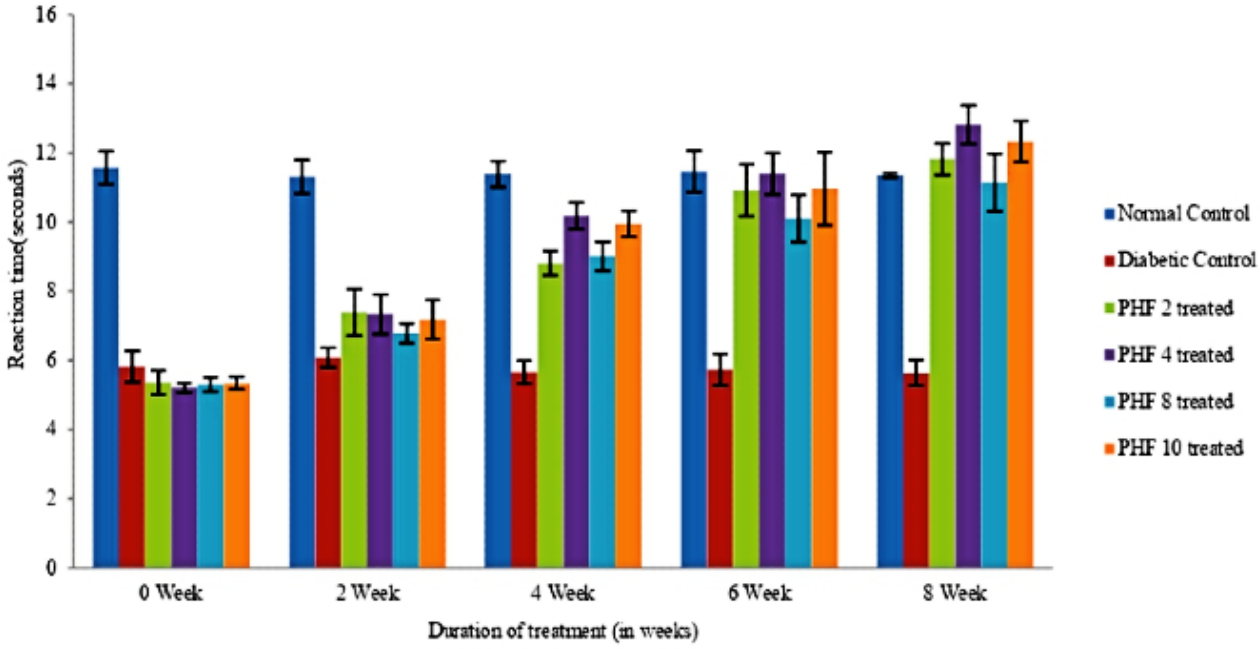

Figure 2: Effect of PHF on cold water tail withdrawal latency in diabetic rats.

Table 2: Effect of PHF on cold water tail withdrawal latency in diabetic rats.

Treatments	Duration of treatment (in weeks) and Reaction time (seconds)				
	0 Week	2 Week	4 Week	6 Week	8 Week
Group I Normal control	11.57±0.48	11.31±0.49	11.39±0.38	11.46±0.60	11.35±0.06
Group II Diabetic control	5.82±0.45	6.08±0.29	5.66±0.33	5.73±0.46	5.64±0.37
Group III PHF 2 treated	5.36±0.35	7.39±0.67	8.81±0.35	10.92±0.75**	11.82±0.46***
Group IV PHF 4 treated	5.21±0.13	7.33±0.57	10.18±0.38**	11.40±0.60***	12.82±0.56***
Group V PHF 8 treated	5.30±0.20	6.78±0.28	9.01±0.42	10.10±0.68**	11.14±0.83**
Group VI PHF 10 treated	5.34±0.18	7.18±0.57	9.95±0.37	10.97±1.06**	12.33±0.59***

All values are presented as Mean ± SD, (n = 6), $p < 0.05$*, $p < 0.01$**, $p < 0.001$***, When compared to diabetic control.

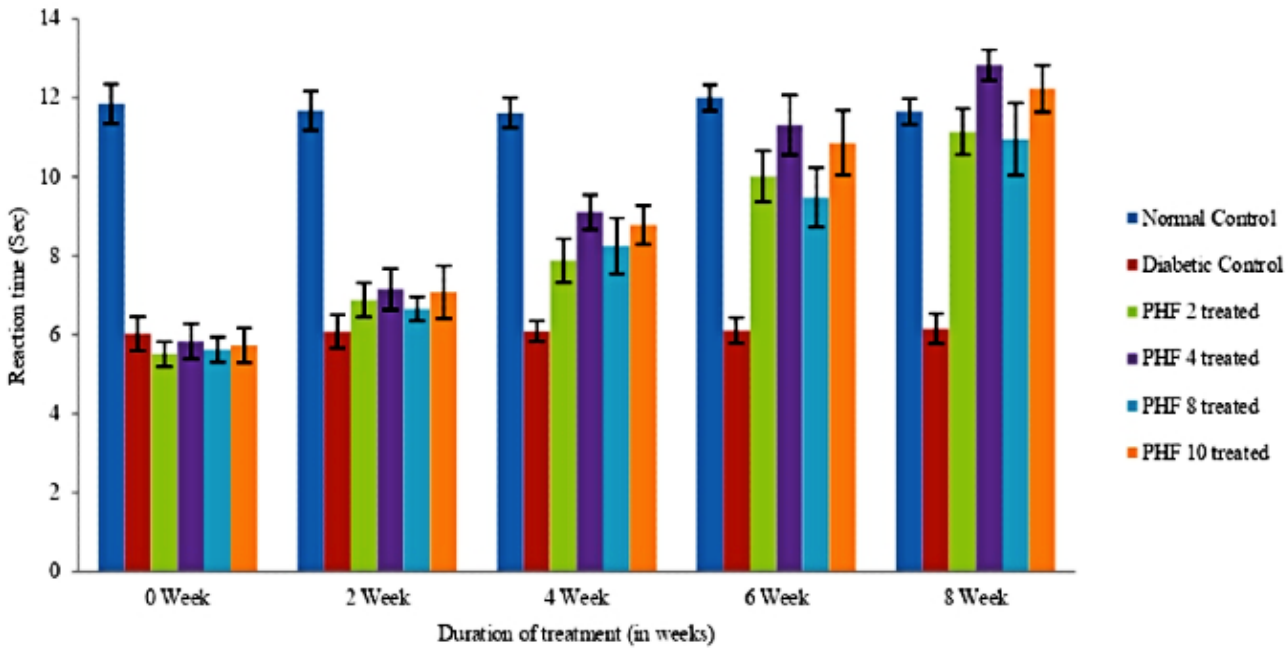

Figure 3: Effect of PHF on paw withdrawal latency (hot plate) in diabetic rats.

Table 3: Effect of PHF on paw withdrawal latency (hot plate) in diabetic rats.

Treatments	Duration of treatment (in weeks) and Reaction time (seconds)				
	0 Week	2 Week	4 Week	6 Week	8 Week
Group I Normal control	11.85±0.50	11.67±0.49	11.62±0.38	12.00±0.33	11.65±0.32
Group II Diabetic control	6.02±0.43	6.08±0.42	6.09±0.26	6.11±0.32	6.15±0.38
Group III PHF 2 treated	5.51±0.31	6.88±0.44	7.88±0.55	10.01±0.65*	11.14±0.58**
Group IV PHF 4 treated	5.83±0.44	7.15±0.52	9.10±0.44*	11.31±0.76**	12.83±0.39***
Group V PHF 8 treated	5.62±0.32	6.65±0.30	8.25±0.71	9.48±0.75*	10.95±0.92**
Group VI PHF 10 treated	5.73±0.44	7.08±0.67	8.78±0.49	10.86±0.82**	12.23±0.59***

All values are presented as Mean ± SD, (n = 6), $p < 0.05$*, $p < 0.01$**, $p < 0.001$***, When compared to diabetic control.

Statistical analysis

All the results obtained were expressed as mean ± SD (n = 6) in each experimental group. Statistically, the data were analysed using Prism Graph pad version 5.0. The data were evaluated using a one-way analysis of variance (ANOVA) followed by Dunnett's test. p-values < 0.05 were considered as statistically significant, p < 0.01 as very significant and p < 0.001 as extremely significant.

RESULTS

The effect of polyherbal formulations on neurons of STZ-induced diabetic rats was studied by using various standard experimental designs. It was observed that STZ-induced hyperglycemia progressively developed heat and cold hyperalgesia in experimental rats after 28 days, which was demonstrated by changes in the time of tail withdrawal latency compared to normal rats. In the study, STZ-induced diabetes resulted in a decrease in hot water tolerance by 58.81%, which on treatment with PHF for

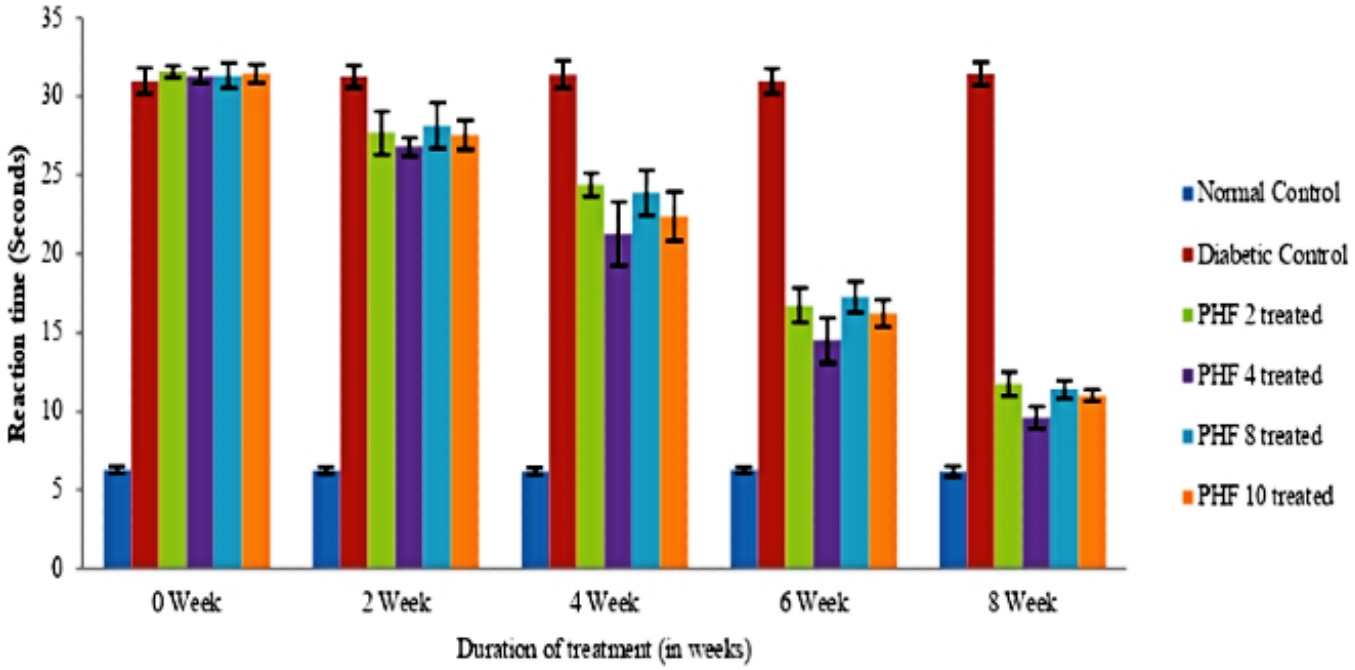

Figure 4: Effect of PHF on paw withdrawal latency (acetone drop) diabetic rats.

Table 4: Effect of PHF on paw withdrawal latency (acetone drop) diabetic rats.

Treatments	Duration of treatment (in weeks) and Reaction time (seconds)				
	0 Week	2 Week	4 Week	6 Week	8 Week
Group I Normal control	6.30±0.21	6.26±0.21	6.21±0.23	6.29±0.19	6.18±0.32
Group II Diabetic Control	30.98±0.81	31.25±0.72	31.39±0.89	30.96±0.79	31.42±0.77
Group III PHF 2 treated	31.58±0.36	27.68±1.37	24.41±0.74	16.74±1.11	11.75±0.74*
Group IV PHF 4 treated	31.31±0.46	26.80±0.59	21.27±2.03	14.50±1.42	9.60±0.70**
Group V PHF 8 treated	31.32 ±0.82	28.14 ±1.45	23.88±1.44	17.24±0.99	11.38±0.57*
Group VI PHF 10 treated	31.45±0.59	27.56±0.93	22.38±1.56	16.20±0.87	11.00±0.38*

All values are presented as Mean ± SD, (n = 6), $p < 0.05$*, $p < 0.01$**, $p < 0.001$***, When compared to diabetic control.

8 weeks was significantly improved, summarized in Table 1 and Figure 1. It was noted that PHF 2 improved thermal tolerance by 94.19%, PHF 4 improved it by 146.29%, PHF 8 by 87.54%, and PHF 10 improved by 109.59%.

In the cold water tail immersion test, all PHF significantly ($p < 0.001$) improved the cold tolerance to near normal. PHF 2 treatment improved cold water tail withdrawal tolerance by 120.52%, PHF 4 improved by 145.84%, PHF 8 by 110.09%, and PHF 10 improved by 131.15%, which has been depicted in Table 2 and Figure 2.

In Eddy's hot plate test, STZ-induced diabetic rats showed a reduction in paw withdrawal latency compared to normal rats. Reaction time that decreased by 48.22% due to STZ-induced diabetes, was significantly reversed to normal. It was noted that the reaction time increased by 102.15% by PHF 2, PHF 4 showed an increase of 120.06%, PHF 8 increased by 94.81%, and PHF 10 by 113.23%, which was depicted in Table 3 and Figure 3.

Application of the acetone drop on the plantar surface of the diabetic rats resulted in cold allodynia, which was indicated by the raised paw withdrawal duration by 399.2%. After treatment

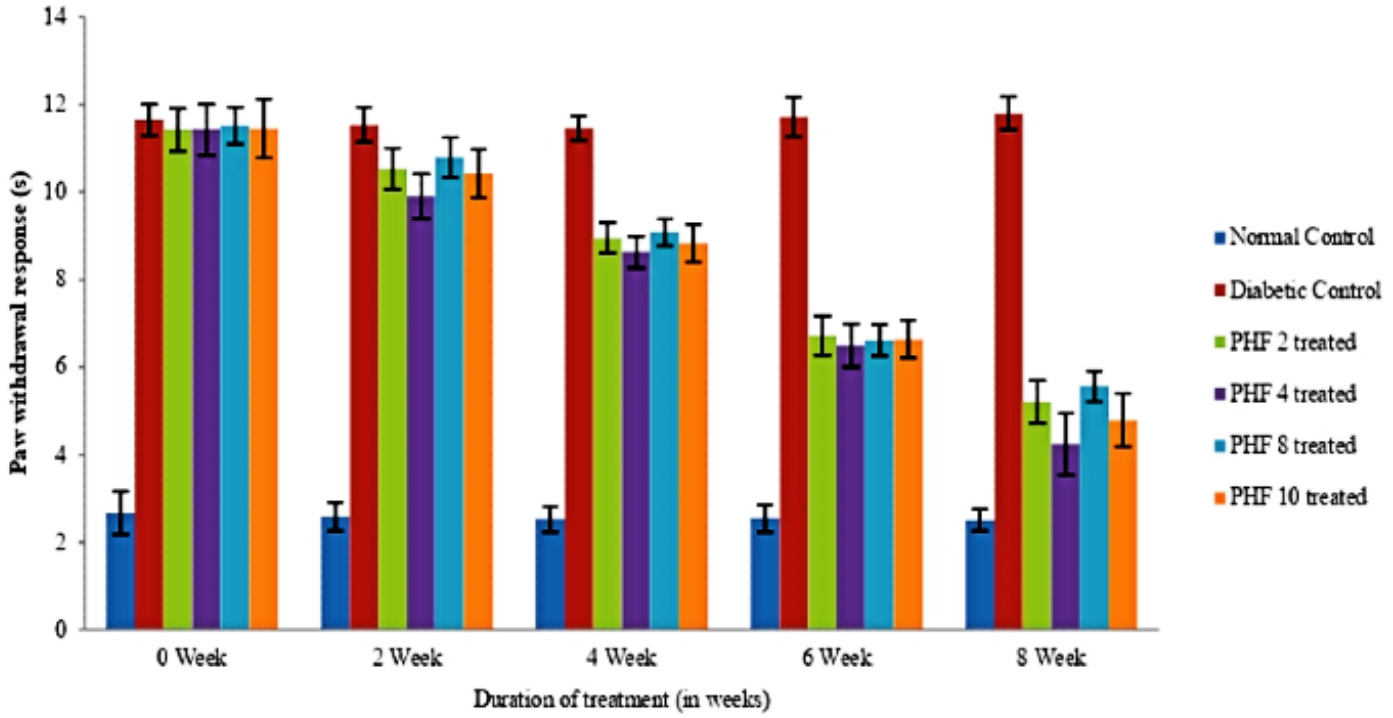

Figure 5: Effect of PHF on paw withdrawal latency (pin prick) diabetic rats.

Table 5: Effect of PHF on paw withdrawal latency (pin prick) diabetic rats.

Treatments	Duration of treatment (in weeks) and Reaction time (seconds)				
	0 Week	2 Week	4 Week	6 Week	8 Week
Group I Normal control	2.67±0.49	2.58±0.33	2.52±0.29	2.54±0.31	2.50±0.25
Group II Diabetic control	11.65±0.36	11.54±0.40	11.46±0.28	11.72±0.45	11.80±0.38
Group III PHF 2 treated	11.42±0.49	10.53±0.47	8.95±0.35	6.72±0.45	5.21±0.49*
Group IV PHF 4 treated	11.43±0.58	9.91±0.51	8.63±0.36	6.49±0.49	4.24±0.71**
Group V PHF 8 treated	11.52 ±0.42	10.80±0.46	9.08±0.31	6.61±0.36	5.56± 0.35*
Group VI PHF 10 treated	11.45±0.67	10.42±0.55	8.83±0.43	6.64±0.43	4.79±0.61**

All values are presented as Mean ± SD, (n = 6), $p < 0.05$*, $p < 0.01$**, $p < 0.001$***, When compared to diabetic control.

with PHF significant time reduction in paw withdrawal was observed, which can be depicted in Table 4 and Figure 4. PHF 2 decreased the reaction time by 62.78%, PHF 4 decreased reaction time by 69.32%, PHF 8 by 63.68%, and PHF 10 by 65.04%.

In the pinprick test, hyper-responsiveness to a noxious stimulus was significantly raised by 365.20%, in STZ-induced diabetic control. That treatment with different PHF caused a significant reduction in paw withdrawal latency, which can be depicted in Table 5 and Figure 5. Overall PHF 2 decreased the response time by 54.35%, PHF 4 decreased by 62.89%, PHF 8 decreased by 51.79%, and PHF 10 by 58.17% of the increased response time.

In the rota-rod test, the reaction time which was decreased by 57.42% due to STZ-induced diabetes was improved to near normal on treatment with PHF. PHF 2 improvised the reaction time by 94.11%, PHF 4 improved by 115.62%, PHF 8 by 85.06%, and PHF 10 by 103.46% which were found to be significant, as depicted by the Table 6 and Figure 6.

In the actophotometer test, locomotor activity reaction time decreased due to diabetes by 57.06%, which was significantly improvised during the treatment with PHF for 8 weeks. Reaction time improved to near normal significantly in a time-dependent manner, as depicted by Tables 7 and Figure 7. Treatment with PHF 2 improvised the reaction time by 90.35%, PHF 4 by 116.41%, PHF 8 by 86.86%, and PHF 10 by 102.56%.

Thus, the basis of the overall effect of PHF on the improvement in the symptoms of diabetic neuropathy can be arranged from highest improvement to lowest improvement, as PHF 4 > PHF 10 > PHF 8 > PHF 2.

DISCUSSION

STZ-induced chronic diabetes mellitus resulted in diabetic neuropathy in experimental rats, having symptoms of sensation, cold and heat algesia i.e., progressive heat hyperalgesia and cold allodynia, alteration in muscles grip strength, and perception of pain and harm along with loss of motor coordination or

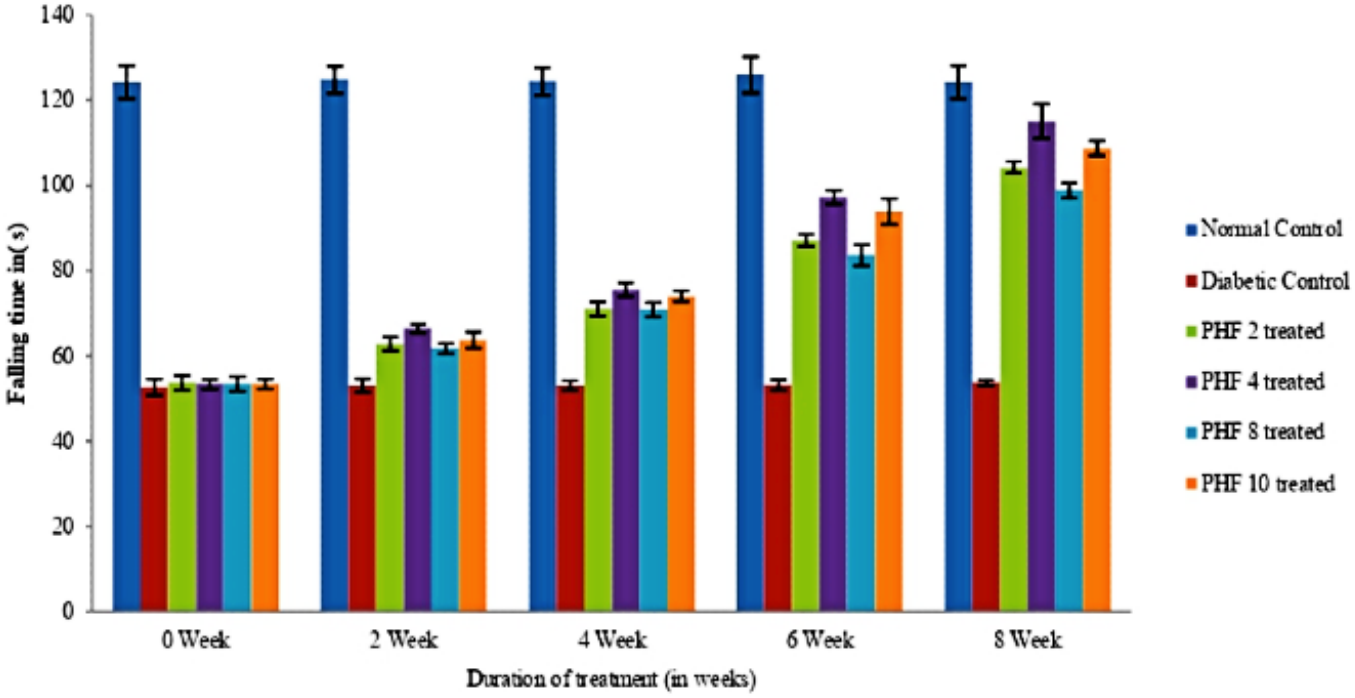

Figure 6: Effect of PHF on muscle grip strength (rota-rod) in diabetic rats.

Table 6: Effect of PHF on muscle grip strength (rota-rod) in diabetic rats.

Treatments	Duration of treatment (in weeks) and reaction time (seconds)				
	0 Week	2 Week	4 Week	6 Week	8 Week
Group I Normal control	124.25±3.89	124.85±3.16	124.43±3.20	125.97±4.25	124.25±3.89
Group II Diabetic Control	52.68±1.92	53.07±1.58	53.08±1.12	53.20±1.22	53.73±0.61
Group III PHF 2 treated	53.68±1.67	62.85±1.63	71.02±1.64	87.11±1.44	104.20±1.33*
Group IV PHF 4 treated	53.37±1.11	66.44±1.00	75.52±1.58	97.22±1.59	115.08±4.05**
Group V PHF 8 treated	53.41 ±1.78	61.78 ±1.21	70.85 ±1.62	83.59 ±2.47	98.84 ±1.76*
Group VI PHF 10 treated	53.45±1.17	63.72±1.92	73.99±1.23	93.85±2.96	108.74±1.76**

All values are presented as Mean ± SD, (n = 6), $p < 0.05$*, $p < 0.01$**, $p < 0.001$***, When compared to diabetic control.

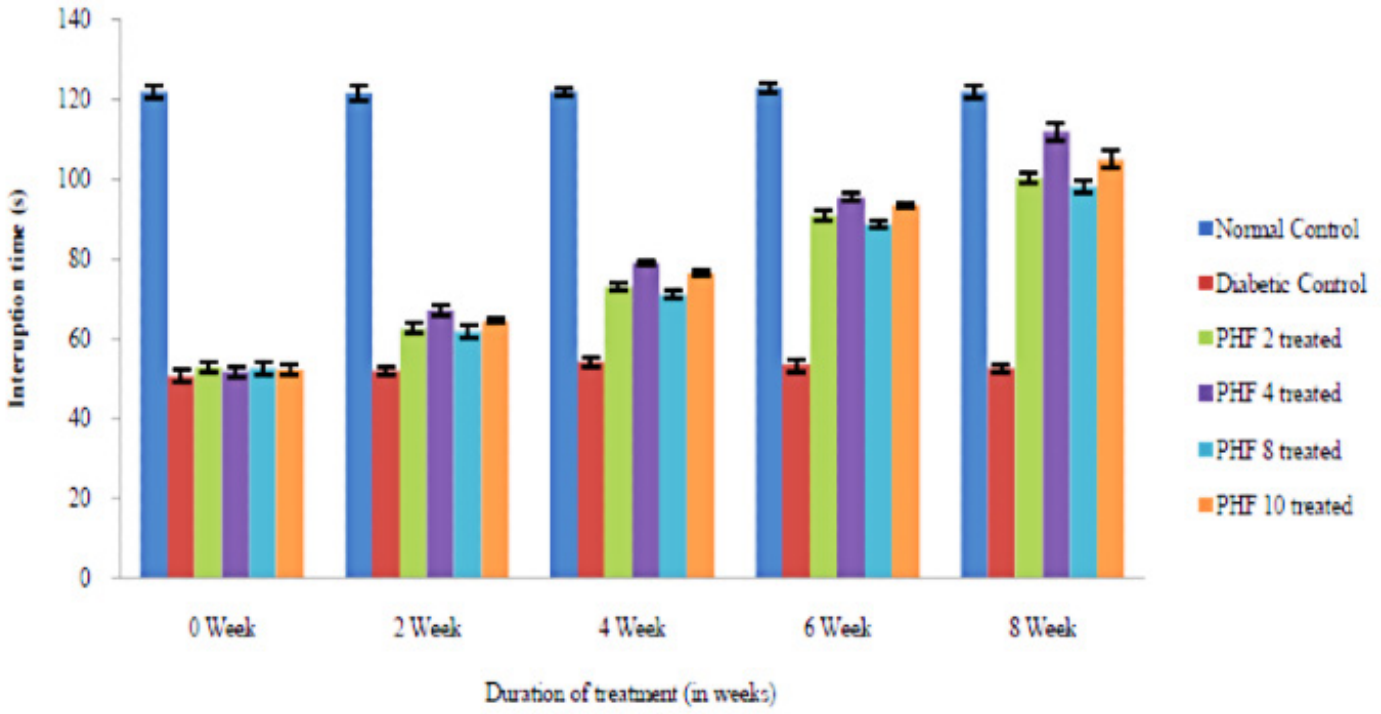

Figure 7: Effect of PHF on locomotor activity in diabetic rats.

Table 7: Effect of PHF on locomotor activity in diabetic rats.

Treatments	Duration of treatment (in weeks) and reaction time (seconds)				
	0 Week	2 Week	4 Week	6 Week	8 Week
Group I Normal control	122.04±1.72	121.49±1.68	121.85±1.07	122.95±1.12	121.91±1.71
Group II Diabetic Control	50.53±1.60	51.83±0.90	53.98±1.07	53.21±1.50	52.45±0.75
Group III PHF 2 treated	52.68±1.48	62.51±1.20	73.05±1.06	90.94±1.29	100.28±1.13*
Group IV PHF 4 treated	51.70±1.21	67.04±1.06	79.18±0.65	95.51±0.72	111.87±2.27**
Group V PHF 8 treated	52.54±1.48	61.86±1.60	70.94±0.96	88.58±1.01	98.18±1.40*
Group VI PHF 10 treated	51.89±1.23	64.38±0.60	76.36±0.80	93.40±0.93	105.10±2.14**

All values are presented as Mean ± SD, (n = 6), $p < 0.05$*, $p < 0.01$**, $p < 0.001$***, When compared to diabetic control.

locomotor activity.[15] These all demonstrated symptoms are signs of neuronal damage, which may be a result of oxidative stress in animals.[16,17] In experimental animals, changes in sensitivity to touch and tenderness, mechanical allodynia, and mechanical hyperalgesia were observed in the animals after 28 days of inducing diabetes.[18] These changes in hyperalgesia may have occurred due to resulting changes in neurotransmitters. It is also reported that; the occurrence of diabetic neuropathy is associated with loss of myelin sheath, atrophy, and deterioration of nerve fibers. In the current research, 4 weeks after diabetes confirmation, animals showed a significant alteration in hyperalgesia, muscle grip, and locomotor activity.[19] This was significantly reversed to normal by the daily treatment with PHF for 8 weeks, where improvement started from the 4th week itself with PHF 4, whereas PHF 2, 8, and 10 started showing improvement from the 6th week onwards. It was envisaged that treatment with PHF might have worked by reversing the deterioration of sensory-motor reflux and preventing the further deterioration of nerve fibers, which would have markedly reversed the algesia and reversed the withdrawal latency.[20] Hence,

in the study, it was revealed that diabetes caused a decrease in tolerance against hot and cold, increased reaction time to cold allodynia, decreased experimental animal hyper-responsiveness to a noxious stimulus, thus increasing the reaction time, reduced the muscle grip strength and locomotor activity was significantly improved after treatment with PHF 4 formulations. PHF 4 might have done this by significantly decreasing blood sugar concentration and neuroprotective activity might have resulted due to the presence of reported phytochemicals with antioxidant potential like phenolic and flavonoids compounds and others including alkaloids, phenols, terpenes, saponins.[21,22]

CONCLUSION

By research study, it was finally concluded that PHF 4 is capable of improving and protecting animals from neurological (peripheral neuropathy) complications induced by diabetes. Polyherbal formulations (PHF 4) which contained extract in a ratio; as *H. sabdariffa* (100): *A. marmelos* (50): *F. religiosa* (75): *A. squamosa* (25) was found to be most effective as neuroprotective among other 3 formulations. Thus it was concluded that PHF is capable of reversing diabetic neuropathy and thus has potential to prevent further progression of neuro complications.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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