



Original Research Article

A randomized placebo-compare study on the efficacy of classical ayurvedic pharmaceutical form versus aqueous alcoholic extracts of *Phyllanthus niruri* Linn. Plus *Sida cordifolia* Linn. In patients of diabetic sensory polyneuropathy



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ABSTRACT

Background: In routine, Ayurveda practitioners prefer classical pharmaceutical form of herbal medicines in compare to modern extracts.

Objective: To explore the difference of efficiency between whole drug powder of *Phyllanthus niruri* plus root decoction of *Sida cordifolia* and modern extracts of the same in compared to placebo in patients of diabetic poly-neuropathy.

Material and methods: A randomized, partly-double-blinded, placebo-controlled trial evaluated the efficacy of two different pharmaceutical forms of herbal medicines over placebo in 90 patients (30 in each group) of diabetic sensory polyneuropathy for first three weeks period. After three weeks, active herbal medication groups were continued with their assigned medicaments for next 5 weeks period and all placebo-patients were randomized again into 2 groups of active medication and treated for next 8 weeks. Patients were assessed with Neuropathy Total Symptom Score 6 and sensation thresholds.

Results: Significant effect of both form of herbal medicines over placebo was found in aching pain [F (2, 49) = 6.79, p = 0.002], allodynia [F (2, 59) = 6.74, p = 0.002], burning pain [F (2, 82) = 14.66, p < 0.0001], numbness [F (2, 77) = 16.37, p < 0.0001] and pricking pain [F (2, 50) = 14.23, p < 0.0001]. After the 8 weeks period, no significant difference was identified between the effect of both the herbal treatment on aching pain (U = 220, p = 0.03), allodynia (U = 421.5, p = 0.29), burning pain (U = 881.5, p = 0.846), numbness (U = 778, p = 0.92) and pricking pain (U = 260, p = 0.15).

Conclusion: Both herbal groups have significant effect to reduce NTSS-6 score in compare to placebo. No significant difference found between the effect of two different pharmaceutical forms of *Phyllanthus niruri* and *S. cordifolia*.

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Abbreviations: NTSS-6, Neuropathy total symptoms score-6; OPD, Out-patient department; Sd, Standard deviation; MP, Madhya pradesh; ANOVA, Analysis of variance; HSD, Honest significant difference; FDA, United State Food and drug administration; IEC, Institutional ethics committee; MRD, Mean rank difference.

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1. Introduction

Diabetes mellitus is a chronic metabolic disorder and diabetic patients mostly suffer any one or more of the associated micro-vascular or macrovascular complications. Diabetic poly-neuropathy is one of them which affects the quality of life [1]. Diabetes mellitus is the second most common cause of acute or chronic poly-neuropathy in India [2]. Prevalence of diabetic poly-neuropathy is gradually increasing in India [3–6]. It is also the most common

cause of foot ulcers or diabetic foot. The lifetime incidence of foot ulcers may be about 15% in diabetic patients [7]. More than 40000 amputations are done every year in the USA where diabetic peripheral neuropathy plays a role [8].

Currently, no therapy has been shown in randomized controlled trials which can restore the functions of damaged nerves as well as relieve the signs and symptoms satisfactorily. A traditional ayurvedic herbal combination of *Abutilon indicum* Linn. and *Phyllanthus niruri* Linn. has been observed with a satisfactory result in diabetic sensory poly-neuropathy [9] but it was non-randomized and has not been compared to placebo or modern extract form till now. Therefore, one clear research question based on this scientific finding of whether it is effective in comparison to placebo or not as a treatment of diabetic sensory poly-neuropathy was identified.

Most of the traditional ayurvedic doctors prefer the use of whole drug powders and decoction rather than modern aqueous and alcoholic extracts. Medicinal plant powder contains the full range of explored and unexplored active as well as passive drug agents while extracts contain only soluble agents depending on the extraction procedure standardized by a single or few substances and parameters. However, in modern practice the extracts are widely used because of their extended shelf life and their administration in smaller amount. The crude form of both herbal medicines used in this clinical trial is not easily available outside India. However, their modern aqueous-alcoholic extracts are available in Europe and American countries. This is also the purpose of the study to find out the similarity or difference in the efficacy of these pharmaceutical forms to enable a practitioner in choosing a clinically effective as well as convenient preparation.

Until now only two studies related to this question but on other plants with different purpose are identified. Both traditional and modern extracts have shown similar results, and are better in comparison to the decoction [10,11].

As no prior studies using placebo-controlled standards could be found to prove the efficacy of the combination of *Phyllanthus niruri* Linn. and *S. cordifolia* Linn. on diabetic sensory peripheral polyneuropathy, a placebo control was included in this study to exclude the possibility of the placebo effect of both the forms of administration used in this study. As diabetic polyneuropathy is a slowly progressive chronic condition, patients of the placebo group may not have any harm in only 3 weeks with standard antidiabetic treatment. After finishing part one of the study, they were randomized into herbal treatment which also helped to resolve the ethics related issue.

In this manner the aim of this study was to find out the difference in efficacy between modern extracts and whole drug powder of *Phyllanthus niruri* Linn. plus *S. cordifolia* Linn. root decoction in diabetic neuropathy in comparison to a placebo.

2. Methods

The study protocol was reviewed and approved by the Institutional Ethics Committee for Human Research. IEC approval number is 17/01–2014. The study was designed in January 2014. Enrolling of patients was started in June 2014 and the study was completed in February 2016. It was conducted according to the principles of the declaration of Helsinki. The study was registered on <https://clinicaltrials.gov/> and registration number is [ClinicalTrials.gov](https://clinicaltrials.gov/) - NCT02107469.

2.1. Study design

The whole study was performed in two parts. The study design is a clinical randomized partly double-blinded placebo-control trial (investigator blinded for placebo vs powder/decoction group and

double blinded for placebo vs extracts group) in part I with 30 patients per group for 3 weeks. In part II there was a descriptive observation of the course of symptoms and parameters of both pharmaceutical forms (powder and decoction against extracts). At the end of placebo-controlled Part I, the patients of the placebo group were assigned by randomization into both medicine groups further for 8 weeks. Patients of the medication groups continued their herbal medicine for 8 weeks in total. This part of the study was investigator blinded.

2.2. Sample size

We have chosen a sample size of 30 patients in each group based on the data of the prior study [9]. Considering the results of previous studies, moderate to high effect size f (0.35) was taken to detect the sample size. The required sample size was calculated with the help of G Power statistical software version 3.1.9.7 (available in March 2020 for windows 7). A one-way ANOVA test for the 3 groups parallelly with a 0.05 significance level would have 80% power to detect medium to high effect size f (0.35) when the sample size in each group is 28. Hence, we have chosen to take 30 patients in each group for the study.

2.3. Participants

2.3.1. Recruitment procedure

Patients coming to OPD (out-patient department) of the P.D. Patel Ayurveda Hospital in Nadiad, Gujarat (India) having the signs and symptoms of diabetic sensory poly-neuropathy, were screened for the confirmation of diagnosis and inclusion. The diagnosis was made by investigators (Ayurveda Physician) who assessed the patients later. It was based on the signs and symptoms of the patients who already had diabetes mellitus and with the help of neuropathy analyser machine (Vibrotherm Dx). The informed consent form was filled out for enrolment. The block randomization was done according to usual modern standards [12]. Three block-randomized (BLOCK of 6) groups with equal size of 30 patients in each were randomly assigned by using a randomisation list related to the consecutive ID-numbers. The placebo group was additionally block-randomized (BLOCK of 4) and equally assigned to both herbal treatment groups for further treatment after 3 weeks-assessment using a new ID-number for linking to the second randomisation list. The ID-number (in ascending order of assessment) was given on each of 90 assessment forms and distribution process slips. Only the distribution process slip was forwarded to the distributors and afterwards collected by the investigators. Only the person providing medication to the patients knew a plan of distribution and the patients were instructed not to reveal their therapy regime to the assessing doctor. Distribution took place in a separate room by a trained distributor. After completion of the enrolment of 90 patients, the patients who had discontinued the treatment (dropped out) were replaced with other patients. They were enrolled one by one and on a first come first serve basis with the same ID number. Hence, total 30 numbers of patients in each group were maintained.

2.3.2. Inclusion and exclusion

Patients with a clinically positive history of type 1 or 2 diabetes mellitus having the symptoms of diabetic neuropathy (sensory, peripheral) were selected. Only outpatient setting was included. Patients from 20 to 80 years of age, irrespective of gender with stable medication for diabetes were included. Patients with ≥ 2 symptoms and having at least one symptom with moderate severity in occasional frequency (3 points in NTSS-6) were included

if they additionally showed an impaired vibration detection threshold.

Patients suffering from any other associated clinical condition influencing peripheral nerve function i.e. peripheral vascular disease, vitamin deficiency (B12, E) and other intoxications (alcohol, medicine), cancer, autoimmune disease, hepatitis, vasculitis, amyloidosis, severe kidney failure, pregnancy and disorder of connective tissue were excluded. Steroids taken up to 1 month prior to the study and the likely need for insulin therapy were also excluded.

2.4. Interventions

2.4.1. Method of administration

Patients of group DE received decoction obtained from 7 gm coarse powder of *S. cordifolia* root two times and 3-g powder of *Phyllanthus niruri* whole plant three times with water. Patients of group EX received two capsules of red colour (each capsule containing extract obtained from 3.6-g of coarse powder of *S. cordifolia* root) and two capsules of green colour (each containing extract obtained from 1.5-g fine powder of *Phyllanthus niruri* whole plant) three times with water. Patients of group PL received two red capsules two times and two green capsules three times a day containing the placebo (300 mg maltodextrin powder) with water. Patients of group A have been instructed for preparing the decoction according to the classical method by a trained distributor. The instruction was as follows. 7 g of coarse powder was to be soaked in 100 ml water for 12 h and then boiled to reduce it to one fourth. Then the fluid was extracted by filtering and taken orally. This procedure was to be done twice a day in the morning and evening. Measure glass and manual for preparation of decoction were provided to the patients for accuracy in the measurement of the materials. Medicines were distributed to patients of all groups by a trained distributor.

2.4.2. Procurement, preparation, laboratory tests and authentication of medicines

Phyllanthus niruri whole plant powder was prepared by college pharmacy using the plants collected from medicinal plant farm of this institute. Coarse powder of *S. cordifolia* root and equivalent dosage extracts of both plants and placebo were purchased from Sears Phytochem Pvt Ltd, Indore (MP).

Capsules containing 300 mg aqueous/alcoholic extract of *S. cordifolia* in 12-fold concentration equating to 3.6 g of original herb, were prepared by above mentioned company. Hence, two capsules were equivalent to 7 g of the original herb. Similarly, capsules containing 300 mg of aqueous/alcoholic extract of *Phyllanthus niruri* in 5-fold concentration equating to 1.5 g of original herb. Placebo was prepared by capsuling 300 mg of maltodextrine having same colour and size of the capsules used for extracts for the purpose of double-blinding.

Authentication of coarse and fine powders of *S. cordifolia* root and *Phyllanthus niruri* was done by the *Dravyagunavijnana* department of the institute where the trial was conducted. Other laboratory tests to identify active chemical constituents, heavy metals and microbiological presence were performed by Sears Phytochem Pvt Ltd from where the medicaments were purchased.

2.4.3. Dietary instructions

Diet was prescribed according to Ayurveda classics. Warm freshly cooked meals made from barley, one-year old wheat, light and easy to digest vegetables were recommended. Adequate quantity of sesame oil, ghee (clarified butter) and spices except chilies were allowed for making food. Food such as sugar and fine flour, bakery items, eggs, meat products, heavy and difficultly

digestible food, all sour and fermented food items were restricted. List of recommended and non-recommended food items was given to all the patients with study information leaflet.

2.4.4. Conventional medical care

All the patients were instructed to continue their conventional anti-diabetic medicines as per their schedule.

2.5. Follow-up, efficacy and safety evaluation

2.5.1. Follow-up and duration of treatment

Patients of both herbal medicine groups (DE and EX) were treated for 8 weeks period. Patients of group PL took the placebo for 3 weeks period. After the completion of 3 weeks period, patients of group PL were immediately shifted to herbal medicine groups (either DE or EX as per randomization) for next 8 weeks period. Hence, total study period for group PL was 11 weeks and for group DE or EX was 8 weeks. Patients of herbal groups visited for follow-up every week for the first three weeks period and then after completion of 5th week and 8th weeks of treatment. Follow-up visits for placebo groups were on every week for the first three weeks and then after their inclusion in herbal groups in same manner as instructed for the herbal groups.

2.5.2. Outcomes and their assessment

Primary outcomes of this study were changes observed in signs and symptoms i.e. aching pain, allodynia, burning pain, lancinating pain, numbness and pricking pain which assessed by analysis of Neuropathy Total Symptoms Score (NTSS-6) [13]. Changes observed in sensation thresholds for vibration, cold and hot sensations and laboratory parameters for safety assessment were secondary outcomes of this study. Sensation thresholds were assessed by using Vibrotherm Dx Diabetic Foot Care machine. Severity of grade score of NTSS-6 depended on frequency and intensity of symptoms, hence, frequency and intensity of each symptom was defined accordingly. A questionnaire for defining the symptoms was also adopted [13]. Investigators were trained in a 1-h session, standardized explaining the administration of the questionnaire (See Tables 5 and 6). All investigators were instructed to ask the questions in to the native language of the patients. The investigators were instructed to refrain from leading the patient and from viewing previous evaluations to avoid potential bias. The symptoms were graded on the basis of the experience of the patient during the previous 24 h. Laboratory investigation like haemoglobin, PP₂BS, serum creatinine, blood urea, serum potassium, serum sodium, serum calcium, SGPT, SGOT, serum bilirubin total, urine sugar and urine albumin were performed for safety evaluation.

Patients were assessed with NTSS-6 score and sensation thresholds during every visit. Laboratory investigations were performed initially (first time assessment, before starting the treatment), after 3 weeks, 5 weeks and 8 weeks of treatment period.

2.5.3. Statistical analysis of outcomes

Homogeneity of variance of the data set (observed after the 3 weeks of treatment) for NTSS-6 was checked by the Levene's test which suggests that averages of all the groups were considered to be equal for the observed symptoms (NTSS-6). Single factor ANOVA test was applied for hypothesis testing and post-hoc Tukey HSD test for the comparison of multiple groups. However, the symptoms to be analyzed being ordinal variables come in the category of non-parametric test. Multiple tests for the same data set may give more accurate statement related to results. Hence, Kruskal Wallis with Donn's multiple comparison tests were also performed for this data set. It is more robust for ordinal types of variables. These tests were performed with the help of free version of Graph-pad Prism 8.

Table 1
Baseline characteristics of 98 patients of diabetic sensory polyneuropathy.

Basic characteristics	Mean value ± S.D./number of patients
Age (in Years)	56.32 ± 10.55 (52.26 ± 8.26 of group DE, 59.12 ± 10.62 of EX and 60.34 ± 11.52 of group PL)
Number of patients (Male: Female)	54 : 44 (16 : 11 of group DE, 15 : 18 of group EX and 23 : 15 of PL)
Chronicity of diabetes mellitus (in years)	8.9 ± 3.98 (9.03 ± 4.55 of group DE, 9.43 ± 7.39 of EX and 7.23 ± 3.91 of group PL)
Chronicity of neuropathy symptoms (in months)	11.1 ± 10.44 (11.53 ± 10.95 of group DE, 9.43 ± 7.39 of EX and 11.26 ± 11.59 of group PL)
Average post prandial blood sugar (in mg/dl)	198.71 ± 62.53 (186.9 ± 75.09 of group DE, 198.71 ± 82.53 of EX and 202.53 ± 83.39 of group PL)
Average weight (in kg)	67.13 ± 13.19 (68.74 ± 16.15 of group DE, 67.13 ± 13.19 of group EX and 65.99 ± 11.55 of group PL)
Number of obese patients	12 (5 of group DE, 3 of EX and 4 of PL)
Number of underweight patients	09 (5 of group DE, 1 of EX and 3 of PL)
Number of patients having less than 40 years of age	29 (9 of group DE, 6 of EX and 14 of PL)
Number of patients (excessive hard work: Sedentary lifestyle)	16 : 68 (7 : 21 of group DE, 5 : 18 of EX and 4 : 29 of PL)
Other complications found in patients (retinopathy, vasculopathy, coronary heart disease)	00
Number of patients taking hypoglycaemic agents	83 (29 of group DE, 27 of EX and 27 of PL)
Number of patients taking metformin + insulin	2 (each in group DE & EX)
Number of patients taking metformin only	25 (12 of DE, 4 of EX and 9 of PL)
Number of patients taking metformin + sulfonyleureas	56 (16 of DE, 22 of EX and 18 of group PL)

Table 2
Effect of Treatment DE Vs Treatment EX on NTSS-6 Score (After 8 weeks of herbal treatments).

Signs and symptoms	Number of patients (n)		Difference of Mean ± SD		Sum of Ranks		Mann-Whitney U	P value
	Treat* DE	Treat EX	Treat* DE	Treat EX	Treat DE	Treat EX		
Aching Pain	29	23	1.16 ± 0.68	1.25 ± 0.55	882	496	220	0.030
Allodynia	31	32	1.74 ± 0.70	1.54 ± 0.80	1067	949.5	421.5	0.295
Burning Pain	42	43	2.19 ± 0.55	2.15 ± 0.58	1028	1828	881.5	0.846
Lancinating Pain	7	4	1.09 ± 0.25	1.58 ± 1.13	35	31	7	0.236
Numbness	38	42	1.79 ± 0.67	1.79 ± 0.62	1529	1711	788	0.924
Pricking Pain	28	24	1.86 ± 0.75	2.05 ± 0.60	666	712	260	0.158

Table 3
Effect of Treatment DE Vs Treatment EX on sensation thresholds (After 8 weeks of herbal treatments).

Sensations	Number of patients		Difference of Mean ± SD		Un-paired t value	P value	
	Treat ^a DE	Treat EX	Treat DE	Treat EX			
Vibration (in freq/sec)	Left foot	45	45	11.51 ± 8.82	10.11 ± 6.47	0.8576	>0.05
	Right foot	45	45	11.11 ± 8.47	10.33 ± 7.68	0.4559	>0.05
Hot (in °C)	Left foot	45	45	3.93 ± 5.71	0.330 ± 2.71	0.3727	>0.05
	Right foot	45	45	3.30 ± 2.71	3.54 ± 2.51	0.4290	>0.05
Cold (in °C)	Left foot	45	45	4.73 ± 3.92	4.30 ± 3.54	0.5470	>0.05
	Right foot	45	45	4.48 ± 3.87	4.41 ± 3.51	1.6625	>0.05

^a Treatment, DE – Decoction and powder group, EX – Extract group.

Table 4
Laboratory investigation in 90 patients.

Investigation	Before treatment		After 3 weeks		After 8 weeks	
	Group DE	Group EX	Group DE	Group EX	Group DE	Group EX
Hemoglobin (gm%)	12.59 ± 1.44	12.29 ± 2.02	12.54 ± 1.51	12.37 ± 1.95	12.70 ± 1.43	12.52 ± 2.01
S. Creatinine (mg/dl)	0.86 ± 0.15	0.87 ± 0.22	0.90 ± 0.18	0.90 ± 0.22	0.87 ± 0.19	0.86 ± 0.20
Blood urea (mg/dl)	31.8 ± 13.0	30.37 ± 11.5	30.24 ± 8.63	29.58 ± 9.14	28.77 ± 6.82	29.36 ± 10.1
SGPT (IU/L)	31.66 ± 12.71	30.54 ± 14.5	31.24 ± 14.6	29.8 ± 13.12	30.17 ± 10.1	29.2 ± 13.12
SGOT (IU/L)	37.46 ± 15.10	35.81 ± 15.9	36.64 ± 15.1	35.66 ± 15.7	36.44 ± 12.9	34.31 ± 12.9
S. Billirubin total (mg/dl)	0.84 ± 0.09	0.84 ± 0.12	0.83 ± 0.07	0.83 ± 0.11	0.81 ± 0.05	0.83 ± 0.12
PP ₂ BS (mg/dl)	189.0 ± 71.0	198.7 ± 82.5	195.1 ± 68.8	196.1 ± 83.0	183.7 ± 68.8	188.5 ± 80.3
Urine Albumin	Nil	Nil	Nil	Nil	Nil	Nil

Quantitative information related to sensation thresholds was also analysed by one-way ANOVA and post-hoc Holm-Sidak test with the help of free version of Graph-pad Prism 8. Holm's method has more power than the Bonferroni or Tukey methods. In contrast, Holm's method can be used to analyze any set of P values, and is not restricted to use as a follow-up test after ANOVA.

After the completion of 8 weeks period of both herbal groups, symptoms (NTSS-6 score) were analysed by Mann-Whitney U test and other quantitative parameters were analysed descriptively. Un-

paired t test was used on secondary endpoint data to find out the difference between the effect of treatment.

3. Results

All the patients have completed the 3 weeks of treatment. After the completion of 3 weeks treatment period, in second part of the study, all the patients of placebo group were randomized again into both herbal groups and started the herbal treatment. Assessment

Table 5
Six questions used in NTSS-6 score for assessment of symptoms.

Symptoms	Questions
Aching pain	"Do you experience a deep, aching, tightness, boring, pulling, or squeezing pain in your feet or legs?"
Allodynia	"Do you experience unusual sensitivity or tenderness when your feet are touched or are used in activities such as walking?"
Burning pain	"Do you experience burning pain in your feet or legs?"
Lancinating pain	"Do you experience sharp, stabbing, or shooting pain, electrical shock-like pain, or surges of pain that last seconds to minutes in your feet or legs?"
Numbness	"Do you experience numbness, lost sensation, or 'dead feeling' like an anaesthetic, without prickling in your feet or legs?"
Prickling sensation	"Do you experience a prickling or tingling feeling, with or without an 'asleep' feeling in your feet or legs?"

after the completion of 3 weeks period (after completion of placebo treatment) of NTSS-6 and sensation thresholds were considered as an initial assessment for these patients. Among them, 8 patients were dropped out (discontinued the study) after the completion of 3, 4 and 6 weeks of treatment due to personal reasons. After the completion of study period, according to study plan, eight new patients were enrolled with same ID number and treated with the same treatment that was discontinued as in dropped out patients. Hence, total 45 patients in each group completed the second part of study. Hence, basic information of 98 patients is presented here (see patients' flow diagram).

3.1. Baseline characteristics of patients

Main characteristics of the patients are mentioned in Table 1. All the patients were taking oral hypoglycaemic agents (OHA). Only two patients were taking OHA along with short-acting insulin before lunch. Metformin or sulfonyleurea or a combination of both the medicines used by the patients before and during the study period (see Table 1).

3.2. Efficacy of both forms of herbal treatment over placebo on primary end-points after 3 weeks of treatment

According to Levene's test for homogeneity, p-values of observed data for all symptoms (NTSS-6) were greater than α and therefore, H_0 was accepted for all. According to one-way ANOVA test, after the completion of 3 weeks treatment, a significant effect of both form of herbal medicines was found at the $p < 0.05$ level for NTSS-6 i.e. aching pain [F (2, 49) = 6.79, $p = 0.002$], allodynia [F (2, 59) = 6.74, $p = 0.002$], burning pain [F (2, 82) = 14.66, $p < 0.0001$], numbness [F (2, 77) = 16.37, $p < 0.0001$] and prickling pain [F (2, 50) = 14.23, $p < 0.0001$]. Post hoc comparisons using the Tukey HSD test indicated that there was a significant difference between the treatment group A and placebo group for NTSS-6 i.e. aching pain [Q = 5.21, $p = 0.0016$], allodynia [Q = 4.94, $p = 0.0025$], burning pain [Q = 5.85, $p = 0.001$], numbness [Q = 5.65, $p = 0.001$] and prickling pain [Q = 6.09, $p = 0.001$]. Moreover, there was also a significant difference between the treatment group B and placebo group for NTSS-6 i.e. allodynia [Q = 4.03, $p = 0.016$], burning pain

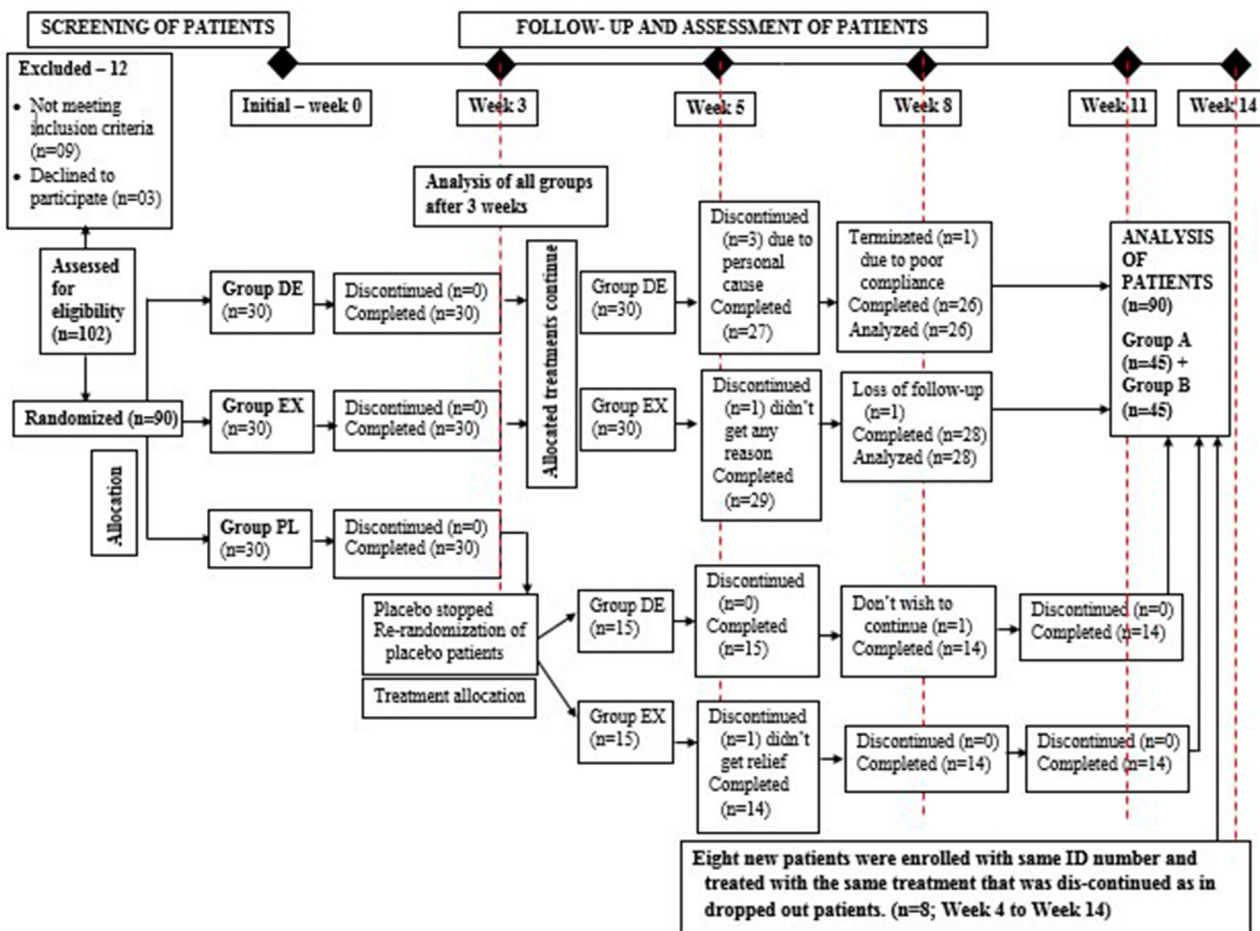


Table 6
Assessment of quantity of the 6 symptoms in NTSS-6 mentioned above.

Symptom frequency	Symptom Intensity			
	Not found	Mild	Moderate	Severe
Never or occasional (normal amount)	0.00	0.00	0.00	0.00
Occasional but abnormal (less than 1/3 of time)	0.00	1.00	2.00	3.00
Often (1/3 to 2/3 of time)	0.00	1.33	2.33	3.33
Almost continuous (more than 2/3 of time)	0.00	1.66	2.66	3.66

[$Q = 7.85$, $p = 0.001$], numbness [$Q = 7.86$, $p = 0.001$] and pricking pain [$Q = 6.78$, $p = 0.001$].

Mean scores of NTSS-6 in classical form of herbal treatment (group DE) were significantly different from the placebo treatment where $p < 0.01$ for all symptoms. Moreover, the mean scores of NTSS-6 in extract form of herbal medicines (group EX) were also significantly different from the placebo treatment group where $p < 0.01$ for all symptoms.

A Kruskal-Wallis H test showed that there was statistically significant difference in NTSS-6 among all the groups after the completion of 3 weeks treatment i.e. aching pain [H (2, 52) = 8.59, $p = 0.014$, with a mean rank of 34.08 for group DE, 19.65 for placebo and 26.53 for group EX]; allodynia [H (2, 62) = 10.59, $p = 0.005$, with a mean rank of 37.8 for group DE, 20.0 for placebo and 34.87 for group EX]; burning pain [H (2, 85) = 23.94, $p < 0.0001$, with a mean rank of 49.21 for group DL, 24.60 for placebo and 54.82 for group EX]; numbness [H (2, 80) = 23.12, $p < 0.0001$, with a mean rank of 52.17 for group EX, 45.46 for group DE and 22.94 for placebo] and pricking pain [H (2, 53) = 19.01, $p < 0.0001$, with a mean rank of 35.4 for group EX, 32.63 for group DE and 14.71 for placebo]. Post-hoc Dunn's test for pair wise comparison revealed that there was a significant difference between the treatment group DE and placebo group for all the symptoms except aching pain. However, in aching pain, there was no significant difference found between group EX and placebo.

3.3. Efficiency of both forms of herbal treatment over placebo on secondary end points after 3 weeks of treatment

According to one-way ANOVA test, after the completion of 3 weeks treatment, a significant difference found between the treatment groups for sensation thresholds i.e. vibration in left foot [F (2, 87) = 12.93, $p < 0.0001$], vibration in right foot [F (2, 87) = 18.03, $p < 0.0001$], hot in right foot [F (2, 87) = 13.27, $p < 0.0001$], cold in left foot [F (2, 87) = 10.39, $p < 0.0001$] and cold in right foot [F (2, 87) = 8.49, $p = 0.0004$]. However, they were not significantly different for hot sensation in left foot [F (2, 87) = 1.57, $p = 0.224$].

Post hoc Holm-Sidak's multiple comparison test indicated that there was a significant difference between the treatment group DE and placebo group for all sensation thresholds except hot sensation in left foot i.e. vibration in left foot [mean difference = 6.76, $t(87) = 4.995$, $p < 0.0001$], vibration in right foot [mean difference = 6.43, $t(87) = 5.423$, $p < 0.0001$], hot in right foot [mean difference = 1.32, $t(87) = 4.399$, $p < 0.0001$], cold in left foot [mean difference = 3.03, $t(87) = 4.162$, $p = 0.0001$] and cold in right foot [mean difference = 1.93, $t(87) = 2.755$, $p = 0.007$]. Moreover, there was also a significant difference between the treatment EX and placebo (PL) for all sensation thresholds except hot sensation in left foot i.e. vibration in left foot [mean difference = 4.5, $t(87) = 3.322$, $p = 0.0013$], vibration in right foot [mean difference = 5.86, $t(87) = 4.945$, $p < 0.0001$], hot in right foot [mean difference = 1.36, $t(87) = 4.52$, $p < 0.0001$], cold in left foot [mean difference = 2.68, $t(87) = 3.69$, $p = 0.0004$] and cold in right foot [mean difference = 2.83, $t(87) = 4.033$, $p = 0.0002$].

3.4. Efficacy of herbal treatment DE versus treatment EX on primary end points (NTSS-6)

After the 8 weeks of the treatment, according to Mann-Whitney U test, no significant difference was found between the effect of both the herbal treatments. The effect of both treatments was nearly equal (see Table 2) on NTSS-6 where p is greater than 0.05 for all the symptoms.

3.5. Efficacy of herbal treatment DL versus treatment EX on secondary end points (sensation thresholds) after 8 weeks period

Table 3 indicates the mean difference of sensation thresholds in 90 patients (45 in each group) of diabetic sensory polyneuropathy treated with both forms of herbal treatment. There was no significant difference in effect of vibration sensation of left foot [$t(81) = 0.85$, $p = 0.39$] for treatment group DE ($M \pm SD = 11.51 \pm 8.82$) and treatment group EX ($M \pm SD = 10.11 \pm 6.47$); vibration of right foot [$t(87) = 0.45$, $p = 0.64$] for treatment group DE ($M \pm SD = 11.11 \pm 8.47$) and treatment group EX ($M \pm SD = 10.33 \pm 7.68$); cold sensations [$t(87) = 0.54$, $p = 0.58$] of left foot for treatment group DE ($M \pm SD = 4.73 \pm 3.92$) and treatment group EX ($M \pm SD = 4.30 \pm 3.54$); cold sensation of right foot [$t(87) = 0.09$ and $p = 0.92$] for treatment group DE ($M \pm SD = 4.48 \pm 3.87$) and treatment group EX ($M \pm SD = 4.41 \pm 3.51$); hot sensation of left foot [$t(79) = 0.37$ and $p = 0.71$] for treatment group DE ($M \pm SD = 3.93 \pm 5.31$) and treatment group EX ($M \pm SD = 3.57 \pm 3.71$); and hot sensation of right foot [$t(88) = 0.42$ and $p = 0.66$] for treatment group DE ($M \pm SD = 3.30 \pm 2.71$) and treatment group EX ($M \pm SD = 3.54 \pm 2.59$).

3.6. Results related to the safety of herbal medicaments

Patients were assessed with renal function and liver function laboratory tests initially, after 3 weeks and after 8 weeks of treatment for safety. No adverse effect was found in any patient during the entire period of treatment. Renal functions and liver functions laboratory tests were also found normal (see Table 4) in all the patients at all three time points.

4. Discussion

According to basic characteristics, twelve patients were identified as having less than 3 years of chronicity of diabetes mellitus. It suggests that this complication may also occur earlier in diabetic patients. Most of the patients had a sedentary lifestyle which is considered as one of the causes of diabetes mellitus in Ayurveda as well as modern medical science. More than 65% (64 patients) had taken conventional medicines and multi-vitamin with antioxidants for sensory polyneuropathy, but hardly 20% (11 patients) among them got satisfactory relief and also only during its administering period. In first part of the study period (after 3 weeks period), all the symptoms and signs were improved in both herbal treatment groups, while in placebo group they remained either unchanged or worsened than the initial. Significant improvement was noticed in the NTSS-6 score (primary end point) for both the

herbal treatment in comparison to placebo. After the completion of 8 weeks of herbal treatment, no significant difference was found between the effect of both forms of herbal treatments.

Glycemic control is the most effective measure to prevent or delay the onset as well as progression of diabetic poly-neuropathy. It is considered as the most reliable disease modifying treatment in this condition. In patients with diabetic neuropathy, intensive glycaemic control significantly reduced the progression of neuropathy [14–16]. However, during the study, means of post prandial blood sugar estimation before and after the treatment were found to be unchanged and the conventional anti-diabetic medicaments were continued as per the schedule without any change. Hence, the effect is not only because of glycaemic control as the means of blood-sugar estimation were unchanged. As the main focus has been on the short term effects against neuro-degenerative changes of the two herbs and even the normalization of HbA1c would not be expected to cause nerves to regenerate so fast, it may have been interesting to know but did not be necessary to evaluate the effect of herbs on tissues.

According to Ayurveda, the fractions of all the *dhatu*s (body tissues) are excreted out through urine in *prameha* (various clinical entities characterized by excessive urine with altered composition) and *madhumeha* (commonly considered as diabetes mellitus). This leads to a state of *dhatukshaya* (decrease in the *dhatu*s quantitatively and qualitatively), which ultimately results in provocation of the *vata dosha* (one of the three *doshas* which are responsible for maintaining the body functions in their normalcy). Modern science gives several theories for causation of pain in the disease. However, all consider microangiopathy as a cause leading to damage to the nerves and developing neuropathic pain. According to Ayurveda, this condition can be correlated with provocation of *vata dosha* due to *dhatukshaya*. The burning sensation is due to *ushna guna* (hot properties) of *pitta dosha*. It cannot be possible without aggravation of *pitta dosha*, but here *pitta dosha* is directed by aggravated *vata dosha* and accumulated in feet which is mentioned as *ashayapakarsha* theory in Ayurveda classics. The burning sensation is also included in complications of *prameha*. The tingling sensation (*harsha*) and numbness (*supti*) are included among the disorders of *vata dosha*. Hence, ultimately provoked *vata dosha* is main responsible principle in this clinical entity.

S. cordifolia Linn. (*bala*, according to Ayurveda) has *balakrita* (strengthening) actions and it mitigates *vata* and *pitta dosha*. Hence, it may be able to pacify *vata* and *pitta dosha* and may relieve the symptoms of sensory poly-neuropathy. *Phyllanthus niruri* Linn. (*bhumyamalaki*, according to Ayurveda) has *pitta-kapha* pacifying actions. Its anti-inflammatory, anti-oxidant and anti-diabetic actions have been proved in-vivo and in-vitro research [17–19]. *Rasayana* (rejuvenating) effect of both medicines fulfills the *dhatu* (body tissues) and removes the effect of *dhatukshaya* (main pathophysiology of disease). Oxidative stress is probably a responsible factor for the pathophysiology of diabetic sensory polyneuropathy. Pharmacological actions of *S. cordifolia* Linn. like diuretic and oxidative stress relieving activities have been proved. *Phyllanthus niruri* Linn. (*bhumyamalaki*) has anti-diabetic actions and also relieves the burning sensation.

A review of the published scientific literature reveals that management of painful diabetic neuropathy is challenging. The alpha lipoic acid was found effective over placebo as disease modifying treatment in various randomized controlled trials but contradictory conclusive trials were also found against this [20]. Many other medicaments and anti-oxidants have been experimented for treatment of diabetic poly-neuropathy but all could not prove their efficacy and none of them has been approved by FDA [21,22]. Disease modifying treatment may not widely used for pain

related symptoms and the treatment remains largely symptomatic for diabetic neuropathy patients [23,24]. Pregabalin or gabapentin and tricyclic anti-depressants are being considered as the first line symptomatic conventional therapy in main five of the major published guidelines [25,26]. However, their effect was also found dose-dependent or symptomatic and was also carrying the intolerable side-effects [27]. This trial is helpful to find out safe and effective alternative for the treatment of diabetic sensory poly-neuropathy. However, more randomized controlled studies with long term use of this therapy are required for its anti-pathogenic or disease modifying effect on diabetic sensory poly-neuropathy. Many well controlled clinical studies were identified on Chinese herbal medicines for the management of diabetic poly-neuropathy [28]. However, there are very few studies on Ayurvedic herbal medicines and also not well planned. Most of them were animal experiments. This is the first clinical randomized placebo controlled partly blinded study conducted on herbal medicaments (combination of *Phyllanthus niruri* Linn. and *S. cordifolia* Linn.) in diabetic sensory peripheral polyneuropathy. Both medicines are also available in Europe but mainly in modern extract form. As clinical trials made with the available Ayurvedic drugs are rare in Europe, this might be a forward step for the acknowledgement of Ayurveda in Europe, especially in the management of diabetic sensory poly-neuropathy. This trial may also be helpful for the patients to choose the alternative extract who do not like to consume powder or a decoction.

As a combination of two ayurvedic herbs was used, it was not possible to distinguish between the proportion of effectiveness of each single herb as mono therapy and also it was not possible to predict how the therapeutic effect would change by using only one of the herbs.

Effect of chosen herbs and type of preparation depends on the constitutional type of patient and further aspects of disease where three main types (*dosha*) are to be distinguished. In this study whole drug powder plus decoction is compared to modern extracts equally on every patient with diabetic polyneuropathy. Hence, prakriti, *dosha*, *srotas* etc. factors were not included in the allocation of treatment. This may slightly influence the outcome and violate the Ayurveda intention to do whole system research but help to simplify the study design and study process.

5. Conclusion

Patients receiving both forms of herbal medicaments were more likely to achieve clinical response than those receiving placebo. Moreover, no significant difference was found in the effect of both the forms of herbal treatment after the 8 weeks of treatment. No adverse events were identified in any patient. Laboratory parameters of renal and liver functions were also within the normal limits initially and also after the completion of treatment. Hence, this may be a safe and effective alternative for the treatment of diabetic sensory polyneuropathy. However, the results may be further authenticated by conducting more randomized controlled trials with a greater number of patients and long-term use of the treatment.

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Authors' statement

The study was conceptualized by DS and designed by DS, SNG, KBP, MVP and MM. Data collection and its analysis were performed by MVP and MMP. The first draft of the manuscript was prepared by MVP and MMP, which was carefully discussed and revised by KBP, SNG, DS, PVC and MM. All authors had read and approved the final manuscript.

Data availability

The data that support the findings of this study will be available from the authors upon reasonable request.

Declaration of competing interest

The authors declare no conflict of interest.

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References

- [1] Mohan V, Shah S, Saboo B. Current glycemc status and diabetes related complications among type 2 diabetes patients in India: data from the A1chieve study. *J Assoc Phys India* 2013 Jan;61(1 Suppl):12–5. PMID: 24482981.
- [2] Ghosh B, Sengupta S, Bhattacharjee R, Pal S, Saha SP, Ganguly G, et al. Spectrum of peripheral neuropathy in eastern India. *J Indian Med Assoc* 2006 Apr;104(4):168, 170–3. PMID: 16910321.
- [3] Gill HK, Yadav SB, Ramesh V, Bhatia E. A prospective study of prevalence and association of peripheral neuropathy in Indian patients with newly diagnosed type 2 diabetes mellitus. *J Postgrad Med* 2014 Jul-Sep;60(3):270–5. <https://doi.org/10.4103/0022-3859.138750>. PMID: 25121366.
- [4] Rani PK, Raman R, Rachapalli SR, Pal SS, Kulothungan V, Sharma T. Prevalence and risk factors for severity of diabetic neuropathy in type 2 diabetes mellitus. *Indian J Med Sci* 2010 Feb;64(2):51–7. PMID: 22466493.
- [5] Gaster B, Hirsch IB. The effects of improved glycemc control on complications in type 2 diabetes. *Arch Intern Med* 1998 Jan 26;158(2):134–40. <https://doi.org/10.1001/archinte.158.2.134>. PMID: 9448551.
- [6] Ziegler D. Treatment of diabetic neuropathy and neuropathic pain: how far have we come? *Diabetes Care* 2008 Feb;31(Suppl 2):S255–61. <https://doi.org/10.2337/dc08-s263>. Retraction in: *Diabetes Care*. 2012 Feb;35(2):456. PMID: 18227494.
- [7] Boulton AJ. The diabetic foot: from art to science. The 18th Camillo Golgi lecture. *Diabetologia* 2004 Aug;47(8):1343–53. <https://doi.org/10.1007/s00125-004-1463-y>. Epub 2004 Jul 28. PMID: 15309286.
- [8] Smieja M, Hunt DL, Edelman D, Etchells E, Cornuz J, Simel DL. Clinical examination for the detection of protective sensation in the feet of diabetic patients. International Cooperative Group for Clinical Examination Research. *J Gen Intern Med* 1999 Jul;14(7):418–24. <https://doi.org/10.1046/j.1525-1497.1999.05208.x>. PMID: 10417599; PMCID: PMC1496604.
- [9] Patel K, Patel M, Gupta SN. Effect of Atibalamula and Bhumiymalaki on thirty-three patients of diabetic neuropathy. *Ayu* 2011 Jul;32(3):353–6. <https://doi.org/10.4103/0974-8520.93913>. PMID: 22529650; PMCID: PMC3326881.
- [10] Kudom AA, Mensah BA, Botchey MA. Aqueous neem extract versus neem powder on *Culex quinquefasciatus*: implications for control in anthropogenic habitats. *J Insect Sci* 2011;11:142. <https://doi.org/10.1673/031.011.14201>. PMID: 22233153; PMCID: PMC3281371.
- [11] Bharti U, Patgiri BK, Prajapati PK, Ravishankar B. A comparative pharmaceutical study of Guduchi Ghana prepared by Kwatha and aqueous extract. *AYU* 2009;30(1):77–82.
- [12] Dettori J. The random allocation process: two things you need to know. *Evid Base Spine Care J* 2010 Dec;1(3):7–9. <https://doi.org/10.1055/s-0030-1267062>. PMID: 22956922; PMCID: PMC3427961.
- [13] Bastyr 3rd EJ, Price KL, Bril V, Study Group MBBQ. Development and validity testing of the neuropathy total symptom score-6: questionnaire for the study of sensory symptoms of diabetic peripheral neuropathy. *Clin Therapeut* 2005 Aug;27(8):1278–94. <https://doi.org/10.1016/j.clinthera.2005.08.002>. PMID: 16199253.
- [14] Albers JW, Herman WH, Pop-Busui R, Feldman EL, Martin CL, Cleary PA, et al. Diabetes control and complications trial/epidemiology of diabetes interventions and complications research group. Effect of prior intensive insulin treatment during the diabetes control and complications trial (DCCT) on peripheral neuropathy in type 1 diabetes during the epidemiology of diabetes interventions and complications (EDIC) study. *Diabetes Care* 2010 May;33(5):1090–6. <https://doi.org/10.2337/dc09-1941>. Epub 2010 Feb 11. PMID: 20150297; PMCID: PMC2858182.
- [15] Diabetes Control and Complications Trial Research Group, Nathan DM, Genuth S, Lachin J, Cleary P, Crofford O, Davis M, et al. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 1993 Sep 30;329(14):977–86. <https://doi.org/10.1056/NEJM199309303291401>. PMID: 8366922.
- [16] Diabetes Control and Complications Trial Research Group. The effect of intensive diabetes therapy on the development and progression of neuropathy. *Ann Intern Med* 1995 Apr 15;122(8):561–8. <https://doi.org/10.7326/0003-4819-122-8-199504150-00001>. PMID: 7887548.
- [17] Okoli CO, Obidike IC, Ezike AC, Akah PA, Salawu OA. Studies on the possible mechanisms of antidiabetic activity of extract of aerial parts of *Phyllanthus niruri*. *Pharm Biol* 2011 Mar;49(3):248–55. <https://doi.org/10.3109/13880209.2010.501456>. PMID: 21323477.
- [18] Colpo E, Vilanova CD, Pereira RP, Reetz LG, Oliveira L, Farias IL, et al. Antioxidant effects of *Phyllanthus niruri* tea on healthy subjects. *Asian Pac J Trop Med* 2014 Feb;7(2):113–8. [https://doi.org/10.1016/S1995-7645\(14\)60005-5](https://doi.org/10.1016/S1995-7645(14)60005-5). PMID: 24461523.
- [19] Obidike IC, Salawu OA, Ndukuba M, Okoli CO, Osunkwo UA. The anti-inflammatory and antinociceptive properties of the chloroform fraction from *Phyllanthus niruri* plant is mediated via the peripheral nervous system. *J Diet Suppl* 2010 Dec;7(4):341–50. <https://doi.org/10.3109/19390211.2010.522553>. Epub 2010 Oct 11. PMID: 22432563.
- [20] Ziegler D, Hanefeld M, Ruhnau KJ, Hasche H, Lobisch M, Schütte K, et al. Treatment of symptomatic diabetic polyneuropathy with the antioxidant alpha-lipoic acid: a 7-month multicenter randomized controlled trial (ALADIN III Study). ALADIN III Study Group. *Alpha-Lipoic Acid in Diabetic Neuropathy*. *Diabetes Care* 1999 Aug;22(8):1296–301. <https://doi.org/10.2337/diacare.22.8.1296>. PMID: 10480774.
- [21] Javed S, Alam U, Malik RA. Treating diabetic neuropathy: present strategies and emerging solutions. *Spring-Summer Rev Diabet Stud* 2015;12(1–2):63–83. <https://doi.org/10.1900/RDS.2015.12.63>. Epub 2015 Aug 10. PMID: 26676662; PMCID: PMC5397984.
- [22] Malik RA. Why are there no good treatments for diabetic neuropathy? *Lancet Diabetes Endocrinol* 2014 Aug;2(8):607–9. [https://doi.org/10.1016/S2213-8587\(14\)70067-1](https://doi.org/10.1016/S2213-8587(14)70067-1). Epub 2014 Apr 17. PMID: 24746878.
- [23] Tesfaye S, Boulton AJ, Dickenson AH. Mechanisms and management of diabetic painful distal symmetrical polyneuropathy. *Diabetes Care* 2013 Sep;36(9):2456–65. <https://doi.org/10.2337/dc12-1964>. PMID: 23970715; PMCID: PMC3747929.
- [24] Shillo P, Sloan G, Greig M, Hunt L, Selvarajah D, Elliott J, et al. Painful and painless diabetic neuropathies: what is the difference? *Curr Diabetes Rep* 2019 May 7;19(6):32. <https://doi.org/10.1007/s11892-019-1150-5>. PMID: 31065863; PMCID: PMC6505492.
- [25] Javed S, Petropoulos IN, Alam U, Malik RA. Treatment of painful diabetic neuropathy. *Ther Adv Chronic Dis* 2015 Jan;6(1):15–28. <https://doi.org/10.1177/2040622314552071>. PMID: 25553239; PMCID: PMC4269610.
- [26] Bril V, England J, Franklin GM, Backonja M, Cohen J, Del Toro D, et al. Evidence-based guideline: treatment of painful diabetic neuropathy: report of the American Academy of neurology, the American association of neuromuscular and electrodiagnostic medicine, and the American Academy of physical medicine and Rehabilitation. *Neurology* 2011 May 17;76(20):1758–65. <https://doi.org/10.1212/WNL.0b013e3182166e6e>. Epub 2011 Apr 11. Erratum in: *Neurology*. 2011 Aug 9;77(6):603. Dosage error in article text. PMID: 21482920; PMCID: PMC3100130.
- [27] Backonja M, Beydoun A, Edwards KR, Schwartz SL, Fonseca V, Hes M, et al. Gabapentin for the symptomatic treatment of painful neuropathy in patients with diabetes mellitus: a randomized controlled trial. *J Am Med Assoc* 1998 Dec 2;280(21):1831–6. <https://doi.org/10.1001/jama.280.21.1831>. PMID: 9846777.
- [28] Hao CZ, Wu F, Lu L, Wang J, Guo Y, Liu AJ, et al. Chinese herbal medicine for diabetic peripheral neuropathy: an updated meta-analysis of 10 high-quality randomized controlled studies. *PLoS One* 2013 Oct 16;8(10):e76113. <https://doi.org/10.1371/journal.pone.0076113>. PMID: 24146822; PMCID: PMC3797714.