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Effects of Semelil (ANGIPARS™) on diabetic peripheral neuropathy: A randomized, double-blind Placebo-controlled clinical trial

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ABSTRACT

Background and the purpose of the study: Diabetic neuropathy is the most common diabetic complication that often is accompanied by significant morbidity, mortality and economic burden. The purpose of this study was evaluation of effect of Semelil (ANGIPARS™), a new herbal drug for treatment of diabetic foot ulcers or diabetic peripheral neuropathy.

Methods: In this double blind clinical trial, 49 type 2 diabetes patients with different degrees of neuropathy were evaluated in two groups (ANGIPARS™ and placebo groups). All patients were assessed at the start and 12 weeks after treatment, with laboratory tests, United Kingdom screening test, Michigan neuropathy screening score, Michigan diabetic neuropathy score, vibration perception thresholds, nerve conduction study, monofilament test and visual analog scale.

Results: Michigan diabetic neuropathy score was decreased notably in ANGIPARS™ group. In the nerve conduction study, appropriate meaningful changes were observed in the distal latency and amplitude in the motor Ulnar nerve in ANGIPARS™ group.

Conclusion: The results showed limited evidence of efficacy of ANGIPARS™ in diabetic neuropathy treatment and more studies with a larger sample size and longer duration are required.

Keywords: Diabetes Mellitus, Peripheral Neuropathy.

INTRODUCTION

Diabetic neuropathy is the most common diabetic complication associated with a 25-50% lifetime risk among diabetic patients. Diabetic peripheral neuropathy may be asymptomatic in up to 50% of cases, and in 15% symptomatic patients, symptoms of neuropathy are severe and require treatment (1, 2). It is often accompanied by significant morbidity, mortality and economic burden. The most troublesome and important problem due to neuropathy is foot ulcers and subsequent gangrene and foot amputation (3) which is responsible for 50-75% of non traumatic lower limb amputations (1,3). Amitriptyline, imipramine, gabapentin, duloxetine. have been used for symptomatic therapy of peripheral neuropathy but these drugs have adverse effects which limit their uses for a long time or at high doses (3,4). Therefore more effective and appropriate treatment based on the etiological and pathogenical factors are being investigated. Diabetic neuropathy has a multifactorial pathogenesis with different biochemical mechanisms such as increase in oxidative stress, neuro inflammation, decrease in neuronal perfusion and consequently interneuron hypoxia (1, 5, 6).

Semelil (ANGIPARS™) is a new herbal drug, an extract of *Melilotus officinalis*, for diabetic foot ulcers management that has been formulated by Iranian scientists in recent years and according to the results of pre-clinical and clinical trial (phase I-IV) studies, this drug is effective and safe (7-11). ANGIPARSTM may have anti-inflammatory effects as extract of *Melilotus officinalis* (12). Also this drug contains compounds such as 7-Hydroxycoumarin and flavonoids which have potent antioxidant effects and have been shown to be neuroprotective (13). Since a possible mechanism for this drug is angiogenesis and an increase in tissue blood flow and oxygenation (11) it is expected that ANGIPARS™ improve diabetic neuropathy and this study was designed to evaluate the beneficial effects of ANGIPARS™ on the treatment of diabetic peripheral neuropathy.

METHODS

Study design

This study was a 12-weeks randomized double-

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Table 1. United Kingdom screening test.

1. Abnormal sensation felt:	
Burning, numbness, or tingling	2 points
Fatigue, cramping, or pain	1 point
2. Location of symptoms:	
At the Feet	2 points
At the Calves	1 point
Elsewhere	0
3. Had the symptoms ever woken the patient fromsleep?	
Yes	1 point
4. The symptoms:	
Are nocturnal	2 points
Are both nocturnal and diurnal	1 point
Are only diurnal	0
5. The symptoms reduce with:	
Walking	2 points
Standing	1 point
Sitting or lying or no relief	0

Table 2. Michigan neuropathy screening instrument.

Right foot	Left foot
Appearance of feet: deformities, dry skin, callus, infection, fissure	
Absence = 0	Absence = 0
Presence =1	Presence =1
Ulceration	
Absence = 0	Absence = 0
Presence =1	Presence =1
Achilles tendon reflex	
Presence = 0	Presence = 0
Present with reinforcement = 0.5	Present with reinforcement = 0.5
Absence = 1	Absence = 1
Vibration sense on the dorsum of the large toe	
Presence = 0	Presence = 0
Reduced = 0.5	Reduced = 0.5
Absence =1	Absence =1

blind placebo-controlled parallel-group clinical trial study which was carried out in 49 type 2 diabetic patients. The study protocol was approved by the ethics committee of Tehran University of Medical Sciences.

Selection of patients

The patients were selected from those with diabetic type 2 which were previously diagnosed according to the standard world health organization criteria and were referred to Shariati Hospital Diabetes Clinic over a period of 9 months (Aug 2008-May 2009). After the patient selection, all participants were fully received information about this study and they all provided a written informed consent.

Inclusion criteria were male and females aged between 20-60 years, confirmed type 2 diabetes and were treated with oral antidiabetic drugs.

Patients who had non diabetic neuropathy; foot ulcer; symptomatic peripheral vascular disease or pulselessness; other chronic diseases such as coronary artery disease, chronic liver disease, significant renal impairment (serum creatinine > 270 μmol/l), proliferative retinopathy or malignancy; history of allergic drug reactions and pregnancy were excluded from the study.

During the primary assessment a detailed patient's past medical history was obtained especially about diabetes mellitus duration and drug allergies. A comprehensive physical examination of the patients was carried out by a trained physician.

Baseline laboratory tests for all subjects were: complete blood count, fasting blood sugar, HbA1c, lipid profile, liver and renal function tests which were carried out in the hormone laboratory of Endocrine Metabolism Research Institute (EMRI), Shariati hospital (Tehran Medical Sciences University). Complete blood count was achieved by Sysmex (Japan). Biochemical tests were measured by enzymatic method using auto analyzer (Parsazmoon Co, Iran Kit). HbA1C was measured by Drew-DS5. The presence and severity of diabetic neuropathy was evaluated by following methods.

United Kingdom screening test (UKST): it is a simple questionnaire (score range, 0-9) which evaluates the neuropathy as shown in table 1 and based on this messurement patients were divided into two groups: 1;UK score 0-4 (normal or mild neuropathy) and 2; UK score 5-9 (moderate or severe neuropathy (14).

Michigan neuropathy screening instrument: Neuropathy was evaluated based on physical examination of the patient's feet in this 8 point screening method which is illustrated in table 2. Also in a similar manner patients can be divided into two groups: 1; score of 0-2; normal, 2; score of 2.5-8: naturopathic patient (14, 15).

Michigan diabetic neuropathy score: It has three components and score is given for each foot separately according to table 3. The maximum score of Michigan diabetic neuropathy score is 46 points (16).

The visual analog scale (VAS): It is a simple subjective method for assessment of the discomfort sensations and feelings intensity, such as foot pain due to diabetic neuropathy. It is a straight line which has been numbered 0 to10. One end (Number 0) means no distress and the other end (Number 10) means the worst imaginable discomfort. A Patient marks a point on the line based on the amount of discomfort that he or she feels (17).

Other neuropathic evaluations including nerve

Table 3. Michigan diabetic neuropathy score system.

	Sensory impairment		
	Normal	Decreased	Absence
Vibration perception at big toe	0	1	2
10 g filament	0	1	2
Pinprick on dorsum of great toe		painful	not painful
		0	2
	Muscle strength testing		
	Normal	Moderate	Severe	Absence
Finger spread	0	1	2	3
Great toe extension	0	1	2	3
Ankle dorsiflexion	0	1	2	3
	Reflexes		
	Normal	With reinforcement	Absence
Biceps brachii	0	1	2
Triceps brachii	0	1	2
Quadriceps femoris	0	1	2
Achilles	0	1	2

conduction study**,** monofilament test (with10g Semmes-Weinstein monofilament the 10 area on the plantar surface of the feet) and vibration perception thresholds (VPTs) (by a neurothesiometer at the dorsum of each large toe on the interphalangeal joint) were determined using standard methods.

Treatment protocol

The patients were randomized into two groups: A (n=25) and B (n=24). In group A, patients received oral ANGIPARS™ 100mg capsules and in group B, patients received placebo capsules (an inert polymer) twice a day for 12 weeks. Both physician and patient were blind to treatment.

Follow up assessments

The patients were visited monthly to evaluate the possible adverse drug reactions. After 12 weeks of therapy, all laboratory tests and neurological examination were repeated and patients were evaluated for possible side effects and drug compliance rate (ratio of observed/expected capsule consumption).

Statistical analyses

Results were analysed by SPSS version 14 software (SPSS .Inc) and data are presented as means±standard deviations (SD). Paired t-test was used for comparison between results at the start and the end of study in any groups and P values less than 0.05 were considered statistically significant.

RESULTS

This study as a double blind study was performed with 25 (51%) and 24 (49%) patients in the case and control groups respectively. There were not significant differences between two groups at the baseline in demographic profile with exception of cholesterol value (Table 4).

After completion of the study statistically significant alterations were observed in some parameters in some groups as it is shown in table 5 by highlighted P value.

Results of neurological examination are shown in table 6. These factors were not different at baseline in two groups. At the end of study factors didn't change statistically from baseline with the single exception of Michigan neuropathy score in the ANGIPARS™ group (p= 0.002).

Following study in the sub-analysis phase, patients were subdivided according to UK score and Michigan neuropathy screening instrument, then changes in other variables were assessed in these groups. Table 7 includes only those variables that had significant changes in these group.

In this study significant adverse effect was not observed by the laboratory evaluations.

DISCUSSION

Previous studies have shown benefits of ANGIPARS™ for diabetic foot ulcers treatment (9-11). Also this medication was expected to be effective in diabetic peripheral neuropathy through different mechanisms and thus help to diabetic foot management since it contains coumarins and flavonoides which have neuroprotective properties (13).

An appropriate approach for the assessment of the effects of a drug on neuropathy should focus on two factors: First, the effect of the drug on the patient's

Table 4. Patient Demographics and Baseline Characteristics.

Variable		Placebo	ANGIPARS™	P value
Age (year)*		51±5.5	52.9±5.6	NS
Sex (%)	male	20.8	20	NS
	female	79.2	80	NS
BMI (kg/m^2)*		29.9±4.8	28.3±3.9	NS
Duration of diabetes (year)*		10.1±10.3	8±4.6	NS
FBS (mg/dl)*		166.4±70.1	162.8±50.6	NS
HbA1C (%)*		7.6±1.8	8.4±1.6	NS
Triglyceride (mg/dl)*		148.3±72.6	209.4±134	0.055
Cholesterol (mg/dl)*		157.7±46.6	185.6±45.8	0.040

*: Mean±SD; BMI: Body Mass Index; FBS: fasting blood sugar; NS: not significant.

Table 5. Baseline and end point results of Nerve Conduction Study in two groups (Means±SD).

Nerve	Test	Placebo			ANGIPARS™			P value ANGIPARS vs. placebo
		baseline	endpoint	P[a]	baseline	endpoint	P[b]	at baseline
Sural	DL	2.99±0.71	3.33±0.77	**0.001**	3.06±0.75	3.18±0.44	0.25	0.76
	AM	12.56±8.92	9.67±5.15	0.10	11.52±5.62	11.84±5.99	0.72	0.63
	NCV	39.83±11.82	36.5±12.5	0.08	38.44±11.65	38.96±5.36	0.80	0.68
Median (sensory)	DL	4.02±0.95	4.35±1.27	0.07	4.13±1.04	4.39±1.02	**0.016**	0.70
	AM	23.72±14.63	22.62±14.04	0.67	23±13.53	21.19±12.6	0.31	0.86
	NCV	37.71±12.72	34.62±12.13	**0.01**	34.96±9.83	32.72±9.51	**0.009**	0.40
Ulnar (sensory)	DL	3.44±1.04	3.56±1.03	0.11	3.36±.056	3.40±0.40	0.67	0.73
	AM	27.33±12.90	30.92±15.65	0.13	25.37±12.97	26.28±11.98	0.72	0.60
	NCV	43.33±8.52	42.42±10.78	0.43	43.56±6.26	43.76±5.18	0.84	0.92
Proneal	DL	5.49±2.10	5.46±1.87	0.83	4.90±0.77	4.91±0.78	0.96	0.21
	AM	1.55±1.13	1.24±1.01	**0.02**	1.40±0.86	1.28±0.78	0.33	0.60
	NCV	39.08±9.98	41.67±5.89	0.08	40.88±4.30	40.60±4.36	0.57	0.41
Median (motor)	DL	4.94±2.09	4.73±1.98	0.057	4.83±1.27	4.73±1.33	0.30	0.83
	AM	5.56±3.84	5.49±2.86	0.92	5.38±3.12	6.10±3.59	0.25	0.86
	NCV	58.12±9.65	54.17±7.99	**0.04**	53.76±8.82	53.08±6.57	0.73	0.10
Ulnar (motor)	DL	3.66±1.24	3.39±0.41	0.21	3.48±0.34	3.28±0.33	**0.002**	0.49
	AM	6.10±2.06	6.45±2.47	0.44	6.18±2.11	7.23±1.91	**0.004**	0.89
	NCV	52.83±6.67	56.12±7.84	**0.008**	53.56±7.25	57.36±6.43	0.059	0.72
Tibial	DL	5.72±1.59	5.62±1.67	0.55	5.71±0.81	5.56±1.21	0.61	0.99
	AM	2.3±1.68	2.47±1.69	0.82	3±2.06	2.89±1.59	0.77	0.20
	NCV	37.21±9.68	40.13±5.70	0.07	39.4±4.84	38.8±4.47	0.63	0.32

DL: Distal Latency (millisecond); AM: Amplitude (microvolt); NCV: Nerve Conduction Velocity (m/s); a: P value endpoint vs. baseline in the placebo group; b: P value endpoint vs. baseline in the ANGIPARS group.highlight P value is related to the item with significant alterations.

Table 6. Baseline and end point results of neurological examination in two groups (Means±SD).

Test	Placebo			ANGIPARS™			P value ANGIPARS vs. placebo at baseline
	baseline	endpoint	P[a]	baseline	endpoint	P[b]	
VAS	3.7±3.1	3.1±2.6	0.17	2.7±3.5	2.0±2.9	0.059	0.33
UK	4.1±2.9	3.7±3.1	0.52	3.6±3.5	2.5±3.2	0.08	0.58
Michigan neuropathy screening	2.3±1.8	2.0±1.7	0.17	1.9±1.5	1.3±1.3	0.08	0.37
Michigan neuropathy score	4.9±4.5	4.3±4.5	0.19	4.1±3	2.8±2.9	0.002	0.44
Monofilament test (R)	9.6±1.0	9.8±0.5	0.10	9.9 ±0.3	9.8±0.5	0.57	0.25
Monofilament test (L)	9.6±1.1	9.8±0.6	0.10	9.8±0.5	9.9±0.4	0.66	0.39
Vibration perception(R) (Hz)	8.3±4.4	8.5±5.9	0.92	7.6±2.8	7.0±2.9	0.29	0.53
Vibration perception(L) (Hz)	8.2±4.8	8.1±4.9	0.67	7.3±2.6	7.3±2.8	0.97	0.41

VAS: visual analog scale; UK: United Kingdom neuropathy screening score; R: Right side; L: Left side; a: P value endpoint vs. baseline in the placebo group; b: P value endpoint vs. baseline in the ANGIPARS group.

Table 7. Variables with significant changes in divided patients according to United Kingdom and Michigan screening.

Group	UK score				Michigan score			
	0-4		5-9		0-2		2.5-8	
	variable	df	variable	df	variable	df	variable	df
ANGIPARS™	DL of Median s.	0.33	DL of Ulnar m.	-0.30	DL of Median s.	0.36	DL of Ulnar s.	-0.27
	AM of Ulnar m.	0.97	AM of Ulnar m	1.11	NCV of Median s.	-2.83	DL of Ulnar m.[4]	-0.21
	DL of Tibial[1]	-0.76	AM of Median s.[3]	-2.40	DL of Ulnar m.	-0.19	Michigan screening	-1.71
			Michigan score	-1.31	AM of Ulnar m	0.93		
					NCV of Ulnar m.	4.28		
					Michigan score	-1.11		
Placebo	DL of Sural	0.31	DL of Sural	0.35	DL of Sural	0.38	AM of Sural	-6.27
	NCV of Tibial	5.14	NCV of Sural	-5.50	DL of Median s.	0.40	AM of Proneal	-0.50
	NCV of Ulnar m[2]	5.00	NCV of Median s.	-3.44	NCV of Median s.	-3.85	NCV of Ulnar m.	5.00
			AM of Proneal	-0.34	NCVof Median m	-5.46	Michigan screening	-0.91
			DL of Median m.	-0.27	NCV of Tibial	1.75		
			VAS	-1.19				

1-Pvalue=0.055; 2- P value=0.053; 3- P value=0.052; 4- P value=0.057; DL: Distal Latency (millisecond); AM: Amplitude (microvolt); NCV: Nerve Conduction Velocity (m/s); m: motor; s: sensory; df: difference between variable mean at the endpoint versus baseline; VAS: visual analog scale.

symptoms and complaints that were determined by UKST and VAS which did not show statistically significant changes. The second factor is the effect of the drug on the common neuropathic process prevention of neuropathic advancement and even for a therapeutic improvement in the function of peripheral nerves. For this purpose NCS and VPT and monofilament tests were performed and it was found that the VPT and monofilament test alterations were not significant.

Some meaningful results of improvement in NCS in the ANGIPARS™ group (Table 5) probably show a positive but small effect of ANGIPARS™ in this study. In the placebo group distal latency of the Sural nerve increased and amplitude of Proneal nerve and NCV of median nerve decreased meaningfully during the study and these changes are completely in accordant with usual diabetic neuropathy process. Although there were some alterations in treatment group but they were lower than placebo group and were not statistically significant. In addition, in the upper limbs in the ANGIPARS™ group but not in the placebo group, distal latency of the Ulnar nerve decreased and it's amplitude increased (these findings were also all confirmed by sub-group analysis) that these positive changes can be due to systemic effects of this drug.

In spite of reported neuroprotective power of coumarin and flavonoides via mechanisms of reduction in oxidative stress in the cellular-molecular studies (13), results of this study did not reveal convincing evidence to support positive effect of ANGIPARS™ on diabetic neuropathy and previous study also did not show anti-oxidative effect of this

drug during 12 weeks treatment (18).

There were some limitations in this study that may affect its outcome should be considered. These limitations were: Mild degree of neuropathy, good sensory nerve of the patients, short duration of the study and the lack of performance of biopsy to determine angiogenesis and tissue blood flow improvement.

The alterations mentioned above show limited evidence of efficacy of ANGIPARS™ in diabetic neuropathy treatment and suggest that more evaluations are required.

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