Restorative Effect of Distillated Nerium Oleander Extract on Diabetic Neuropathy: Animal Model Study

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Abstract: The current study aims to investigate the possible role of *Nerium oleander* (NeOL) distillate in the treatment of diabetic neuropathy. The restorative affects of distillated NeOL on the diabetes-induced electrophysiological alterations were investigated. Induction of diabetes was done by the combination of single dose streptozotocin injection together with a high fat diet for four weeks. Experimental groups were designed as follows: control, diabetic, and NeOL treated diabetic. Nerve conduction velocity (CV) recordings were performed through a suction electrode from the tibia branch of the sciatic nerve trunk. Diabetes results an increase in rheobase, chronaxie values together with a decrease in maximum depolarization (MD), compound action potential (CAP) area and CV measurements. With its antioxidant nature, NeOL treatment produced nearly complete restorations of the diabetes-induced alterations. Current study has shown that distillated NeOL (375 µg /0.5 ml dH_2O /day) can be a highly potential therapeutic agent on the diabetes induced alterations.

Keywords: Type II Diabetes, Neuropathy, Nerium oleander, Rat, Sciatic Nerve.

INTRODUCTION

Diabetic neuropathies (DNPs) are a group of nerve disorders caused by diabetes mellitus (DM). Patients with DNPs can experience numbness, weakness and sometimes pain in the extremities due to the damage in nerves throughout the body [1]. Patients can develop nerve problems at any time during DM. However the one having longer DM has greater risk for neuropathtic problems. The highest rate of DNPs is seen among those who have had the disease for at least 25 years [2]. DNPs also seem to be more frequent in patients who have had problems in controlling their blood glucose levels, in those with high levels of blood fat and blood pressure, and in patients over the age of 40. As a result, DNPs are the most common cause of non-traumatic amputations and autonomic failure [3]. In their lifetime, patients with DNPs have 15% probability of undergoing one or more amputations [4].

For the majority of the cases, animal models of diabetes are the most extensively chosen for the examination of the DM induced secondary complications [5-10]. In these models, within a few days, hyperglycemia develops which is then followed by a reduction in nerve CV and a loss of sensitivity. Nearly one month after the onset of DM, a reduction in the size of the myelin sheath is also evidenced by histomorphometry in the sciatic nerves [11]. The severity of neuropathy in these experimental models, like in humans, depends on both the time and the level of exposure to high blood glucose levels [11]. Many of the research projects dealing with the DM related secondary complications ends up with the excessive free radical generation phenomenon. Increased production and/or attenuated clearance of reactive oxygen species (ROS), collectively named as oxidative stress, have been postulated as a possible mechanism for both defective nerve blood supply and endoneurial damage [12-15].

NeOL is an evergreen flowering shrub belongs to the dogbane family: Apocynaceae. The parts of NeOL that contain cardiac glycosides are collectively called as nerine and oleandrin [16]. Although NeOL had been used as a folklore medicine for the congestive heart failure diseases [17, 18]. It has been reported that the leaves of NeOL possess hypolipidemic [19], hypoglycemic [20, 21] and an anticonvulsant activity in some of the central nervous system activities [22].

Although the hypolipidemic and hypoglycemic potential of NeOL have been screened, there has been no report on the possible therapeutic and/or protective effect of NeOL on the DNP changes. Therefore current study aims to investigate the possible role of NEOL distillate (375 µg /0.5 ml dH_2O /day) on the DNP.

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METHODS AND MATERIALS

Experimental Animals

All animals were cared for in accordance with the National Institutes of Health's (NIH) Guide for the care and use of laboratory animals. These protocols were reviewed and approved by the animal use committee (Selçuk University Ethics Committee of the Faculty of Veterinary Medicine, Konya, Turkey, report no: 2010/058). To eliminate the sex dependent differences on CV measurements, male Sprague-Dawley rats of 12-14 weeks of age were used for the experiments. After birth, the animals were housed as three rats per cage at ambient (23±1 ^{o}C) temperature and a 12-h light/dark cycle. Unless otherwise stated throughout the experiments animals were fed with standard rat diet and water without restrictions.

Under light anesthesia (30 mg/kg i.p. sodium pentobarbital), sciatic nerves were dissected from the back foot of the Spraque Dawley immediately after the rats were sacrificed by cervical dislocation and exsanguinations. Nerves were then rapidly transferred to the recording chamber, which was super fused with a fresh modified Locke's solution (mmol/l): 140 NaCl, 5.6 KCl, 2.2 $CaCl_2$, 1.2 $MgCl_2$, 10 glucose and 10 Tris-(hydroxymethyl- aminomethane), pH 7.3 at a constant rate of 3 ml/min (which was controlled online by Biopack MP35 data acquisition system).

Obtaining a Lyophilized NeOL

NEOL plant was collected among new shoots in March-September period from Mediterranean region of Turkey, identified and authenticated at the department of biology. After washing the collected plant, fresh shoots were chopped, and distilled water (100 g NEOL plant/1000 ml water) were added. NeOL distillate was obtained by heating the mixture in heat resistant container. Then, NeOL distillate was lyophilized in small glass bottle (20 ml) by using lyophilizator (Patent No: WO/2010/082906 Use of Nerium Oleander for Diseases Manifested with Type II Diabetes, Obesity, High Cholesterol and Triglyceride). Lyophilized NeOL distillate was dissolved at the concentration of 750 µg/ml in distilled water.

Induction of Diabetes and Experimental Groups

Animals were randomly divided into three groups each of which having 10 rats. For the induction of type 2 diabetes (for two groups), streptozotocin (single STZ 35 mg/kg, which was dissolved in citrate buffer (pH 4.5) was administered intraperitoneally together with the high fat diet for four weeks. One week after STZ injection, rats with ≥300 mg/dl non-fasting blood glucose level were considered to be type 2 diabetic. The treatment and the diet content of experimental animal groups were as follows; Control group animals (Con): Healthy control rats fed with normal pellet diet and did not have the NeOL distillate; DM group animals (DM): Type 2 DM rats fed with high fat diet and did not have NeOL distillate; NeOL treated DM animals (DM+NeOL): Type 2 DM rats fed with high fat diet. The NeOL distillate at a dose of 375 µg /0.5 ml of distilled water was administrated by gavage once in a day from seventh day of diabetes induction to the end of the experiment.

CV Recordings

Following the integration of the isolated nerves to recording chamber, stimulations were applied from the proximal end of the nerve trunk while the recordings were done from the distal end of the nerve by means of suction electrode. The rectangular in shape stimulus have a 100-ms duration having supramaximal amplitude with a frequency of 1 Hz in nature. CV recordings were performed through a suction electrode from the tibia branch of the nerve trunk. Pre-amplified signals were digitized at 100 kHz sampling rate with Biopack MP35 data acquisition system and stored in a hard disk for further analysis.

Statistics

The measured parameters were compared with paired t-test, $p < 0.05$ values were taken as significant. Data are presented as mean ± s.e.m throughout the text.

RESULTS

General and Biochemical Characteristics of the Experimental Animals

Mean body weights, the blood glucose levels of the experimental groups were summarized in Table **1**. All the experimental group animals gained weight during the experimental period. Mean blood glucose levels of both non-treated (DM) and NeOL treated DM groups were found to be significantly higher than the control group animals at the end of the experiments. However, NeOL treatment decreased the BG values compared to DM group

Table 1: General Characteristic of the Experimental Animals

	Body Weight (g)		Blood Glucose (mg/dL)	
	Start	**End**	**Start**	**End**
CON (N=10)	231.2±5.8	353.8±7.9*	99.3±3.6	100.1±4.3
DM (N=10)	229.3±5.6	331.0±6.7*	443.0±35.3α	435.0±23.8α
DM + NeOL (N=10)	232.8±7.2	346.8±9.5*	497.5±32.4α	333.6±26.5α, #

In the table Control (Con), type 2 diabetic (DM), oral nerium oleander supplemented type 2 diabetic (DM + NeOL) group of animal values. Body weights and blood glucose represented as BW and BG respectively. * represents the degree of significance (P<0.05) compared to mean start values. α represents the degree of significance (P<0.05) compared to control while # represents the degree of significance (P<0.05) compared to DM group animals. Values are presented as the mean ± sem.

Effect of NeOL Treatment on Compound Action Potential Parameters

Sciatic nerve CAPs were recorded by suction electrode, and parameters for general CAP appearance are shown in Figures (**1, 2, 3** and **4**). Figure **1** shows the representative original traces that were obtained from the experimental group of animals. In parallel to previous findings DM results in an increase in both the time required to reach the MD and the repolarization phases of the CAP recordings. Treatment of the NeOL for four weeks on the other hand results in a restoration to certain extend but this restoration was still found to be longer than the aged matched control group animal values (Figure **1**).

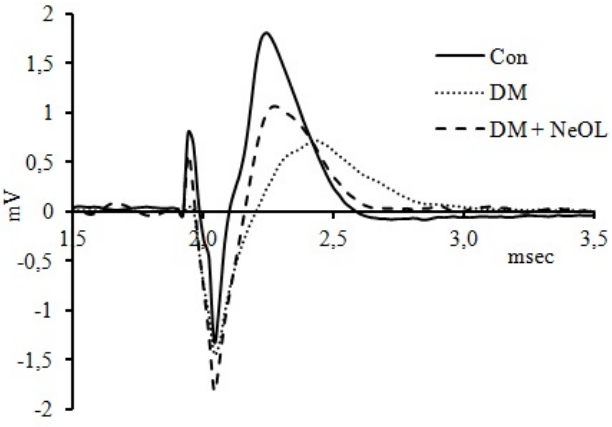

Figure 1: Representative sample sciatic nerve compound action potential traces from Control (Con), Type 2 diabetic (DM) and nerium oleander treated diabetic (DM + NeOL) animals were given.

The excitability parameters, summarized in Figure **2**, were significantly influenced by the period of diabetes mellitus. In Figure **2**, both rheobase (A) and chronaxie (B) values were found to be increased at the end of the DM period. Four weeks of NeOL treatment ends up with a complete normalization to age-matched control group animals (Figures **2A**, **B**).

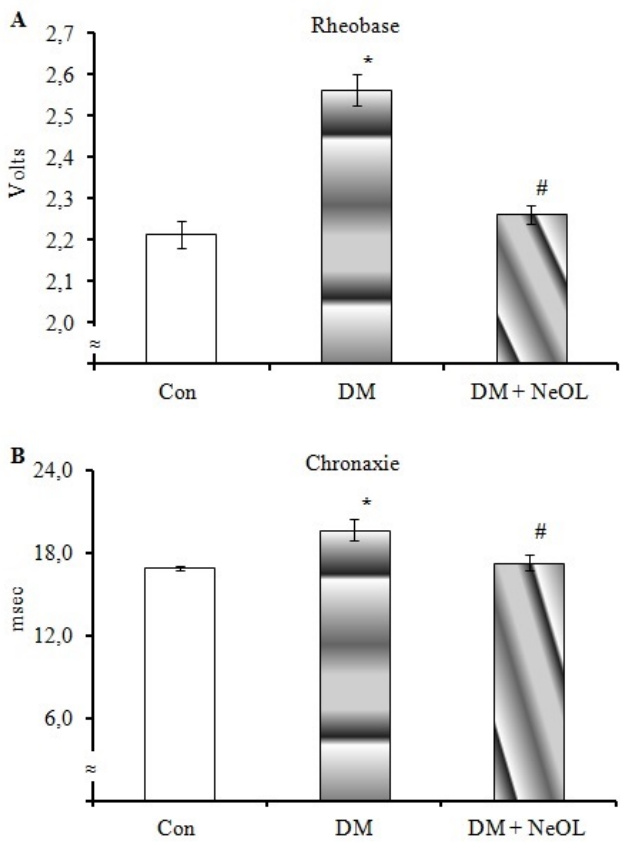

Figure 2: Mean measured rheobase (**A**) and chronaxie (**B**) values of experimental groups of animals. For details and number of animals used, see Materials and methods. Values are given as mean ± sem. CON, control; DM, Type 2 diabetic group; DM + NeOL, Nerium oleander treated diabetic group. *P < 0.05 vs. control; #P < 0.05 vs. diabetic group.

As expected, increased TP (time required to reach the MD) nature resulted in decreased maximum depolarization (MD) values of type 2 DM animal values. NeOL treatment to DM animals resulted in a nearly complete restoration (Figure **3A**). Mean area measurements, as a measure for the actively contributing nerve fibers, were found to be abolished in DM group and treatment here again results in a restoration for these parameters (Figure **3B**).

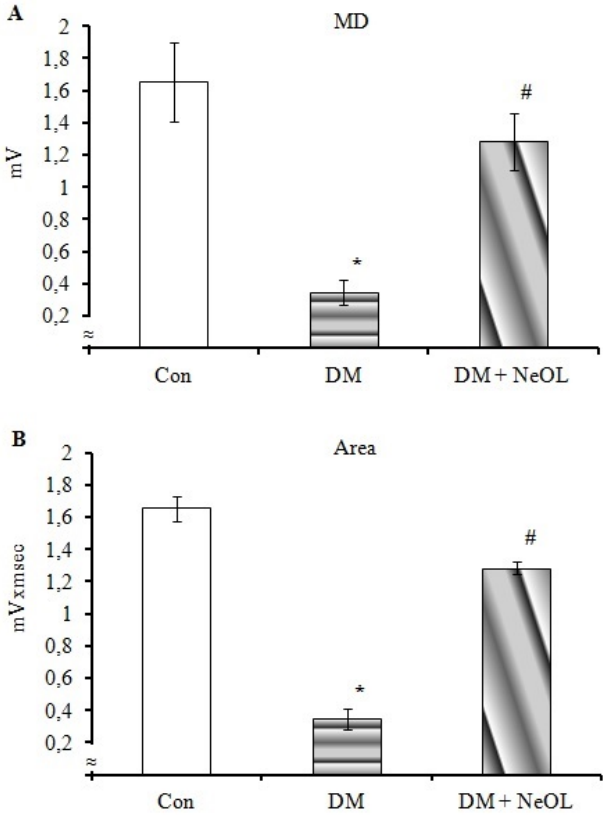

Figure 3: Mean measured maximum depolarization (MD) (**A**) and area under the CAP traces (Area) (**B**) values of experimental groups of animals. Values are given as mean ± sem. CON, control; DM, Type 2 diabetic group; DM + NeOL, Nerium oleander treated diabetic group. *P < 0.05 vs. control; #P < 0.05 vs. diabetic group.

Two-conduction velocity results were based on the two latency measurements shown in Figures **4A, B**. Latencies from stimulus artifact to start of action potential were considered the fastest conduction velocity (Figure **4A**), while latencies to peak of CAP attributed to intermediate conduction velocity (Figure **4B**). DM disturbed not only the intermediate conduction velocity (due to structural difference, it is the first to be affected), but also the fastest conduction velocity. DM-induced reduction in conduction velocity on the other hand was normalized by 4-weeks of NeOL treatment (Figures **4A, B**).

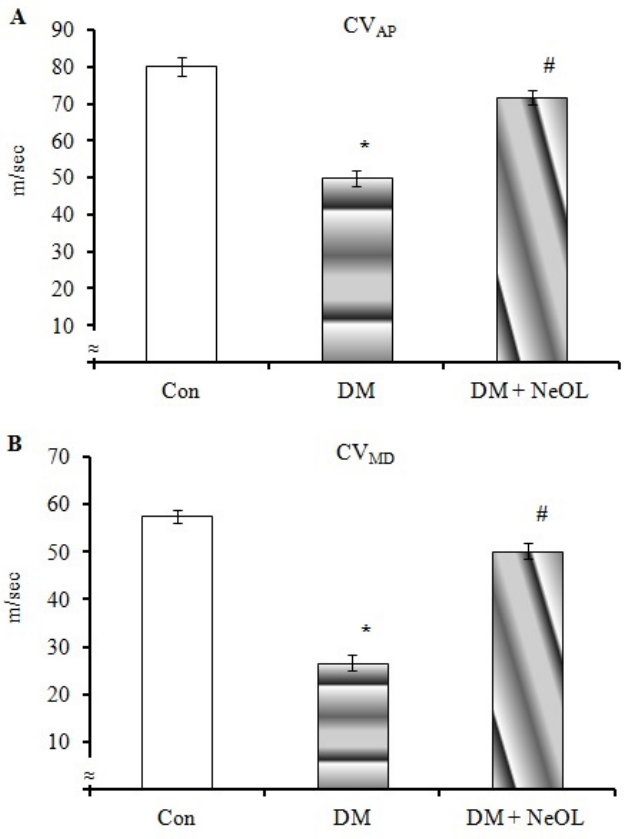

Figure 4: Results of two latency measurements on the experimental group of animals. Conduction velocities of A: the fastest group of nerve fibers (CV_{AP}) B: the intermediately conducting group (CV_{MD}). Values are given as mean ± SEM. CON, control; DM, Type 2 diabetic group; DM + NeOL, Nerium oleander treated diabetic group. *P < 0.05 vs. control; #P < 0.05 vs. diabetic group.

DISCUSSIONS

DNP, type independently, is one of the leading secondary complications seen in diabetes. Indeed, it increases the incidence of the DM patient's mortality and morbidity seen in longer periods of exposure to diabetes. Experimental models of diabetes are the most preferred method for studying diabetes induced secondary complications since they produce similar complications seen in humans within a shorter period of time.

In wide-ranging terms, oxidative stress can be described as an imbalance between the generation and removal of the reactive oxygen species. Although reactive species were originally considered to be entirely detrimental to cells, it is now recognized that the regulation of reduction-oxidation states of the molecules are the key points in modulation of the critical cellular functions [23]. This is particularly the case for neurons, astrocytes, and microglia in regard to

mitogen-activated protein kinase cascade activation, ion transport, calcium homeostasis, and apoptosis program activation. Furthermore, the role of oxidative stress is significant in neurological manifestation of mitochondrial diseases, as well as the new concept of "mitochondrial respiratory chain diseases" [24]. Antioxidant therapy in experimental DNP blocks the observed decrease in nerve growth factor expression and restores nerve function [5, 25]. In addition to many of the parameters, antioxidant therapy restores nerve conduction velocities in experimentally induced diabetes mellitus [13, 14, 26-29]. Therefore, to preserve the reduction-oxidation environment of cellular and mitochondrial function, it is helpful to increase antioxidant defenses with fruits and vegetables rich in antioxidants. Studies have shown that NeOL is a potential antioxidant that involve in the defense against reactive oxygen species toxicity [30]. It has also been shown that the antioxidant nature of NeOL includes free radical scavenging and reducing power in a concentration dependent manner [31, 32].

Current study for the first time showed that NeOL distilate supplementations (375 µg/0.5 ml dH_2O/day) can prevent DM induced CV dysfunctions. This restorative effect covers the excitability (rheobase and chronaxie), contributes to conduction velocities (intermediately and fastest nerve fibers). Although we believe that the positive effect seen in here is most probably due to NeOL's antioxidants nature and its anti-hyperglycemic effect, still it is obvious that molecular mechanism of the mode of action needs further investigations.

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