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# Effects of Alpha Lipoic Acid on Loss of Myelin Sheath of Sciatic Nerve in Experimentally Induced Diabetic Rats

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## ABSTRACT

**Objectives:** Diabetic neuropathy is the most frequent chronic complication of diabetes. It may attack to sensory, motor or autonomous fibers. Varied mechanisms account for the development of diabetic neuropathy such as metabolic disorders, microvascular damages, neurotrophic support deficit, alternation in neuro-immune interactions, neural and glial cell apoptosis, and inflammation. Alpha lipoic acid (ALA) is a potent lipophilic antioxidant in vitro and in vivo conditions, which plays a main role as cofactor in many mitochondrial reactions, easily absorbed from gastrointestinal tract and can easily cross the blood brain barrier (BBB). Apoptosis is an important mechanism of degenerative diseases, which is induced by some factors like hyperglycemia toxicity. In vivo and in vitro studies showed that hyperglycemia affected the cell survival and induced apoptotic changes in dorsal root ganglion neurons and Schwann cells. **Methods:** In this experiment we used a total of 28 rats. 14 rats were given 180mg/kg streptozotocin (STZ) dissolved by single intraperitoneally (i.p.) injection. Rats are divided into 4 groups; Control (group I), DM (group II), ALA (group III) and DM+ALA (group IV). Myelin sheaths of sciatic nerves were examined histologically for each group. **Results:** In the results of the histological examination, showed that loss of myelin sheath in sciatic nerves of rats while the group treated with ALA showed less myelin loss. **Conclusion:** This study might be suggested that ALA has a protective effect on peripheral neuronal cell damage generated with DM.

**Keywords:** alpha lipoic acid, diabetes mellitus, sciatic nerve, myelin sheath.

## 1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized by developing hyperglycemia related with the disruption in insulin secretion or activity. Biochemical, morphological and functional changes occur in tissues and organs of patients with DM. Disease course is also associated with especially lipid, carbohydrate and protein metabolisms disturbances and rapid atherosclerosis with microvascular and macrovascular complications (1). Considering the inhibitory effect of insulin on cellular apoptosis, lack of insulin in type I diabetes indicates for neuronal apoptosis (2). It is known that in hyperglycemic situations glucose can bind proteins randomly without assistance any enzymes and cause uncontrolled glycosylation reaction resulting in the production of glycosylation end products (AGEs) (3). Glycosylated protein causes the production of free oxygen radicals (4).

Diabetic neuropathy is the most frequent chronic complication of diabetes. It may attack to senso-

ry, motor or autonomous fibers (5). Varied mechanisms account for the development of diabetic neuropathy such as metabolic disorders, microvascular damages, neurotrophic support deficit, alternation in neuro-immune interactions, neural and glial cell apoptosis, and inflammation (6, 7). Free radical induced oxidative stress has been played an important role in the pathogenesis of diabetic neuropathy (6). Free radicals reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been implicated as a potential contributor to the pathogenesis of neurodegenerative diseases like diabetic, which seems appropriate for therapeutic interventions such as the use of free radical scavengers. neuropathy (6, 8).

Alpha lipoic acid is a cofactor for mitochondrial enzymes which have a role in energy production and metabolism and can be naturally synthesized in the body (9). A major role in mitochondrial  $\beta$ -oxidation reaction was identified in ALA metabolism (10). ALA and its reduced form, dihydrolipoic acid (DHLA) react with

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reactive oxygen species such as superoxide radicals, hydroxyl radicals, peroxy radicals and singlet oxygen (11). DHLA can regenerate ascorbate glutathione, coenzyme Q1 and vitamin E directly or indirectly (12).

The purpose of this study is the investigation of the protective effect of ALA on the myelin sheath of sciatic nerve in diabetic rat model produced using streptozotocin (STZ).

## 2. MATERIALS AND METODS

This study was conducted in Firat University Experimental Research Center (Firat Üniversitesi Deneyisel Araştırma Merkezi, FÜDAM) collaborating with Firat University Medical Faculty Department of Histology and Embryology after approval by Firat University Experimental Animal Ethical Committee (23.12.2011/143) and was in accordance with the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

### Animals

For this experiment, we used at least 8 weeks old Albino Wistar male rats which were provided from Firat University Experimental Research Center. Animals were maintained in FÜDAM Animal Laboratory at a fixed temperature between 22-23°C. Animals were followed in 12 light / 12 dark cycles and fed each day in special cages with a special aeration system and cages were cleaned every day. Foods were provided in a special steel container, water was provided through water dispenser with ball filled with normal tap water. Rats kept abovementioned conditions until the beginning of the experiment.

### Induction of Diabetes

For this step of study 14 rats were given 180mg/kg streptozotocin dissolved in 0.4 mL (0.1M) sodium citrate buffer solution (pH: 4.5) by single intraperitoneally (i.p.) injection. After 72 hours, blood samples were collected from the tail vein and measured in glucometer device. According to measurement results, the ones with blood glucose >250 mg/dL are considered as diabetics. Measurement of blood glucose was done with Glucostix (Myles, Ekhart, IN). To determine fasting glucose levels of rats, blood samples were taken between 9-10 AM after 8-10 hours of fasting period. All groups were followed for eight weeks. ALA were given by oral gavage for eight weeks to drug-treated groups.

### Generation of experimental groups

In this experiment, we used a total of 28 rats. Rats' initial weights were recorded and they are divided into 4 groups. Control (group I), DM (group II), ALA (group III) and DM+ALA (group IV).

### Sample collection

Rats from all groups were decapitated at ninth week under anesthetic conditions by application of ketamine (75mg /kg, i.p.) and xylazine 10 mg/kg, i.p.). After decapitation, sciatic nerves were removed. These tissues

were kept at -80°C fixed in 10 % formaldehyde solution for histological studies.

### Blood Glucose levels

During this study, all blood glucose level measurement was done by glucometer (Glucostix, Myles, Ekhart, IN).

### Statistical analysis

SPSS (Statistical Package for Social Sciences) for Windows 21.0 software was used for statistical analysis. Data were expressed as the mean ± standard deviation. For assessment of the differences between groups, one-way ANNOVA and subsequently post-hoc Tukey test were used. P<0.05 was considered as significant.

### Histological examination of sciatic nerves

We took 2µm thick sections from two different levels of rat sciatic nerves. Tissue samples were fixed by exposing microwave radiation. From these tissue samples, two paraffin sections were obtained. One of these was stained with hematoxylin-eosin to determine fixation quality. These two sections were stained as stated in the literature by Weil in conventional settings with the help of microwave radiation (13).

## 3. RESULTS

### Clinical findings

In control and ALA groups, we observed a significant increase in weight at 4th and 8th week of the experiment compared to the initial weight of rats (p<0.05) while DM and DM+ALA groups showed a significant decrease compared to control (p<0.05) (Table 1).

### Biochemical Findings

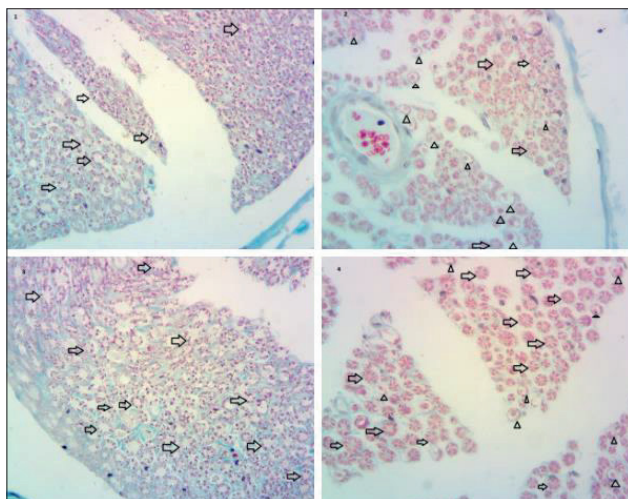
Rats with blood glucose level > 250 mg/dL were considered as diabetes according to blood glucose level measured during classification. We confirmed that high blood glucose level was maintained in DM groups by measurement at 4<sup>th</sup> week and we continued to experi-

	Group I (Control) (n=7)	Group II (DM) (n=7)	Group III (ALA) (n=7)	Group IV (DM+ALA) (n=7)
Initial body weight (gr)	273.8±4.4	277.7±4.31	295.2±4.35	306.2±1.25
Body weight at 4 <sup>th</sup> week (gr)	341.6±6.12 <sup>a</sup>	281.1 ±5.34 <sup>b</sup>	336.4±5.82 <sup>a</sup>	307.7±4.3 <sup>b</sup>
Final body weight (gr)	364.4±5.58 <sup>a</sup>	275.8 ±4.76 <sup>b</sup>	364.6±5.12 <sup>a</sup>	302.3±5.55 <sup>b</sup>

Table 1. Weight changes of rats during the experiment. Values are given as mean ± standart error. a There is a significant difference between the first and last mesurements in the same group, (p<0.05). b There is a significant differences when compared to the control group (group 1), (p<0.05).

	Group I (Control) (n=7)	Group II (DM) (n=7)	Group III (ALA) (n=7)	Group IV (DM+ALA) (n=7)
Initial blood glucose level (mg/dl)	94.40 ± 2.,90	93.51 ± 4.85	95.10 ± 3.25	95.6 ± 7.15
Blood glucose level at 4 <sup>th</sup> week (mg/dl)	98.20 ± 5.25	387.20 ± 15.15 <sup>ab</sup>	96.70± 6.00	390.45 ± 18.65 <sup>ab</sup>
Final blood glucose level (mg/dl)	96.20 ± 5.50	415.40 ± 12.85 <sup>ab</sup>	95.30 ± 5.55	410.95 ± 12.20 <sup>ab</sup>

Table 2. Blood glucose levels changes of rats during the experiment. Values are given as mean ± standart error a There is a significant difference between the first and last mesurements in the same group, (p<0.05). b There is a significant differences when compared to the control group (group 1), (p<0.05).



**Figure 1. Histological findings in the sciatic nerve 1: Sciatic nerve control group:** Tissue was stained with hematoxylin-eosin. Arrows show normal myelin sheath in histological imaging of sciatic horizontal section of control group. **2: Sciatic nerve DM group:** Tissue was stained with hematoxylin-eosin. Arrows show normal myelin sheath, arrowheads show myelin obliteration in histological imaging of sciatic horizontal section of DM group. **3: Sciatic nerve ALA group:** Tissue was stained with hematoxylin-eosin. Arrows show normal myelin sheath in histological imaging of sciatic horizontal section of ALA group. **4: Sciatic nerve DM+ALA group:** Tissue was stained with hematoxylin-eosin. Arrows show normal myelin sheath, arrowheads show myelin obliteration in histological imaging of sciatic horizontal section of DM+ALA group.

ment. DM + ALA rats showed a significant increase in blood glucose values at 4<sup>th</sup> and 8<sup>th</sup> week of the experiment compared to initial values ( $p < 0.05$ ). Compared to control group, DM, and DM+ALA rats have significant increase in blood glucose level in 4<sup>th</sup> and 8<sup>th</sup> week ( $p < 0.05$ ). There is no significant difference in blood glucose level of control and ALA groups throughout the experiment (Table 2).

#### Histological findings

In the histological observation of the tissue, we did not observe an apparent loss in myelin sheath in the control group (Figure 1.1). The loss of myelin is more in DM group than control, ALA and DM+ALA groups (Figure 1.2). There is no significant difference in ALA group compared to other groups in terms of myelin loss (Figure 1.3). The loss of myelin is more in DM+ALA group than control and ALA whereas we observe less intense loss of myelin in this group compared to DM (Figure 1.4).

## 4. DISCUSSION

Diabetic neuropathy is the most common complication of DM. Studies have shown that diabetic neuropathy is a progressive process, in spite of sufficient control of blood sugar, which might be owing to accumulation of reactive oxygen derivatives in neural tissue (14).

Alpha lipoic acid (ALA) is a potent lipophilic antioxidant in vitro and in vivo conditions, which plays a main role as cofactor in many mitochondrial reactions, easily absorbed from gastrointestinal tract and can easily cross the blood brain barrier (BBB) (15).

Application of ALA to rodents has been indicated to reduce the damage that occurs after ischemia-reperfu-

sion injuries in the cerebral cortex and peripheral nerve (15, 16).

A study by Senoglu et al have been showed that the neuroprotective effect of ALA after sciatic nerve crush injury by measuring of superoxide dismutase (SOD), catalase (CAT) activities, and malondialdehyde (MDA) levels (15).

Free radicals accumulate in the endothelium of peripheral nerves and cause inhibition of nitrous oxide and peripheral neuronal loss mediating apoptosis in Schwann cells (17).

A Mayo foundation study found normalization of blood circulation after 1 months of treatment with ALA while it improves the nervous system in patients (18).

Apoptosis is an important mechanism of degenerative diseases, which is induced by some factors like hyperglycemia toxicity. In vivo and in vitro studies showed that hyperglycemia affected the cell survival and induced apoptotic changes in dorsal root ganglion neurons and Schwann cells (19). Immunohistochemical results of a study by Nasiry et al. showed that administration of STZ considerably increased the expression of caspase-3, which plays a critical role in apoptosis (19).

In their study Sun *et al.* demonstrated discontinuous and continuous exposure to high glucose cause development of apoptosis in Schwann cells and ALA treatment cause decrease in Bax expression, cytochrome c, and apoptosis inducer factor (AIF) translocation while it cause an increase in Bcl-2 expression. In ALA-treated group, reduced Caspase 3 and 9 activity and a decrease in the division of PARP (poly ADP ribose polymerase) were observed. These results showed that ALA decreases apoptosis in Schwann cells via reducing oxidative damage and inhibition mitochondrial pathway (20).

In a study by Frøkjær et al. demonstrated that thinning in brain cortex in patient with long term diagnosis for type I DM which might be the underlying cause of peripheral neuropathy in patients with diabetes (21).

The electron microscopy results of a study of effects of ALA on experimental sciatic nerve crush injury in rats by Demir et al proved that the axon diameter, the myelin diameter, the area of regenerating axon and myelin were better in the treatment group than in the control group. These results suggest that ALA is a neuroprotective agent for peripheral nerve injury and supported peripheral nerve regeneration via its anti-inflammatory and antiapoptotic effects (22).

The effectiveness of ALA alone and in combined preparations in peripheral neuropathy was assessed in various clinical trials.

The SIDNEY 2 Trial was a multicenter study which used doses of ALA ranging from 600 to 1800 mg daily and also showed a recovery in total symptom score (TSS) (6). Neurological Assessment of Thioctic Acid in Diabetic Neuropathy (NATHAN) 1 Trial was a multicenter study which used 600 mg of ALA daily for four years with Neuropathy impairment score (NIS)-Lower Limb + seven neurophysiologic tests as primary outcome. In this study after a four-year treatment with ALA in mild-to-moderate Symptomatic distal symmetric dia-

betic polyneuropathy(DSPN) did not effect the primary composite end point but resulted in a significant clinical recovery and protection of progression of neuropathic impairments. As the primary composite end point did not get worse in placebo-treated subjects, secondary protection of its progression by ALA according to the trial design was not possible (23). All these latter studies concluded that the usual dose of 600 mg has efficacy and safety and adverse events, mainly in the gastrointestinal tract, that were dose dependent (24).

In a recent meta-analysis involving 1,258 diabetic patients treated with intravenously 600 mg of ALA for three weeks, induced that individualized total symptoms score such as pain, numbness and burning decreased significantly with ALA in comparison to placebo (25). In another a non-randomized, open-label and prospective study involving 50 patients with diabetes and symmetric sensorimotor polyneuropathy treated with a new oral formulation combining ALA 400 mg/daily and superoxide dismutase 140 IU/daily for four months has shown a recovery in pain and electroneurographic parameters, primarily in sensory nerve transmission (6).

In our study, we evaluated the effect of ALA in sciatic nerve of diabetes-induced rats. In the results of the histological examination, we observed loss of myelin sheath in sciatic nerve of rats while the group treated with ALA showed less myelin loss. Regarding this, we thought that ALA has a protective effect on peripheral neuronal cell damage generated with DM.

In this study rats were received 100 mg/kg ALA by oral gavage however this dosage was higher than dosage in previous studies and it was higher than the dosage which is used for the treatment of slides. Neuroprotective and antioxidant activity of ALA may increase with the increase in dosage. The weakness of our study comes from the lack of knowledge about the dosage, if it is tolerable in human or not, however, evaluation is not possible.

Consequently, it might be suggested that neuroprotective effect of ALA, which prevents oxidative damage thereby decreasing loss of myelin sheath in sciatic nerve of rats. However, further studies are necessary to support this idea.

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- **Conflict of interest:** none declared.

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