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RESEARCH ARTICLE

Promising Effects of Curcumin on Axon Morphology in A Streptozotocin-Induced Diabetic Female Rat Model: Responses to Early, Simultaneous, And Late Treatments

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ABSTRACT

A common complication of diabetes mellitus (DM), peripheral neuropathy, is frequently discussed. This research examined the role of curcumin-induced treatment models against the devastating effects of diabetes. Wistar albino female rats (n=56) were randomly divided into seven groups. The control group did not receive any special procedure. Five mL/kg of corn oil by intragastric gavage was given to the Sham group. Streptozotocin was administered intraperitoneally at 50 mg/kg in the DM group. Curcumin (30 mg/kg) was given to the treatment groups for 14 days. These were then divided into three further groups: the early treatment group (DC1) was applied curcumin seven days after DM induction, the late treatment group (DC2) after 21 days, and the simultaneous treatment group (DC3) received curcumin concurrently with induction. The curcumin group received only 30 mg/kg of curcumin for 14 days. 30 mg/kg of curcumin was given to the DM + curcumin group (DC1) via intragastric gavage once daily for 14 days, starting seven days after diabetes induction. The DM + curcumin group (DC2) received 30 mg/kg curcumin intragastric gavage once daily for 14 days, starting 21 days after the onset of diabetes. 30 mg/kg of curcumin was applied to the DM + curcumin (Cur) group (DC3) via intragastric gavage once daily for 14 days, simultaneously with the onset of diabetes. 30 mg/kg of curcumin was given to the Cur group via intragastric gavage once daily for 14 days. Stereological methods were used for morphoquantitative analysis of myelin thickness, myelinated axon areas, and axon numbers. The axon area decreased in the DM group compared with the control group. Large-diameter axons were shown in the DC1 group. Decreased axon areas and myelin sheath thicknesses were observed in the DC2 and DC3 groups. Curcumin encourages axonal regeneration in terms of axon area and myelin sheath thickness when administered in the early stage of diabetes induction but not when given simultaneously or late.

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INTRODUCTION

Diabetes mellitus (DM) is created by both inherited and environment-related factors, a condition characterized by hyperglycemia (Boulton *et al.,* 2005, Hossain *et al*., 2024) that produces numerous symptoms, such as cardiovascular diseases, neuropathy, retinopathy, and nephropathy (Herman, 2007; Forbes and Cooper, 2013). Diabetes can occur in many pathological or physiological conditions, such as toxicity, pregnancy, pancreatic exocrine disease, and genetic disorders (Tremblay and Hamet, 2019). The literature mentions complex mechanisms in which genetic and environmental factors lead to various autoimmune responses in Type 1 DM. Exogenous insulin administration is essential since pancreatic beta cells are destroyed (Keenan *et al.,* 2010). In developed countries, the widespread Western-style diet, which occurs because of lifestyle changes brought about by urbanization and leads to obesity, may lead to a rise in the incidence of Type 2 DM (Forbes and Cooper, 2013). The fact that the number of patients with type 2 DM has

been increasing and will continue to increase is alarming in terms of its prevalence worldwide (Cho *et al.,* 2018).

Streptozotocin, an antibiotic, is frequently used in the diabetes model created in rats. It has been proven by stereological methods that streptozotocin causes destruction in the pancreatic β-cells and has a destructive effect on the liver of rats (Yıldız *et al.,* 2024). Additionally, streptozotocin-induced diabetic rat models are frequently used to determine the influence of oxidative stress in the etiology of diabetes-related neurological diseases (Al-hafidh and Abdulwahid, 2024; Saputri *et al.,* 2024). Similarly, alloxan-induced diabetes activation, a decrease in serum levels of glutathione, catalase, and superoxide dismutase enzymes, which inhibit a rise in lipid peroxidation in adult male rats and play an important part in defense against reactive oxygen species, was found. The decrease in antioxidant enzyme levels also leads to deterioration in cellular organelle and enzyme structure (Mukhlif *et al.,* 2020). In addition, Bakır (2023) reported that in a streptozotocin-induced diabetic rat model, it has been observed that there is an increase in TNF-α, IL-1β expression, and inflammation, apoptotic processes are triggered, and there is a reduced bcl-2 release and an enhanced bax release (Bakır, 2023). Al-hafidh and Abdulwahid (2024) suggested that cellular functions were impaired due to the increase in free radicals in the brains of rats. Salgıntas *et al.* (2022) also suggested the effects of oxidative stress on the etiology of DM. Curcumin had an active role in regulating oxidative stress resulting from diabetes in rats (Salgıntas *et al.*, 2022).

It has been reported that α1-antagonists and renin-angiotensin inhibitors, which increase neuronal blood flow, cause an enhancement in nerve conduction velocity. Neuronal and glial effects can lead to changes in neuronal blood vessels (Forbes and Cooper, 2013). In cases of diabetic neuropathy, distal damage can be mentioned, leading to severe pain and loss of sensation in the peripheral nerve (Boulton *et al.*, 2005; Forbes and Cooper, 2013). Natural alternative therapeutic agents such as herbs, flavonoids, and polyphenols are today frequently used in diabetic neuropathy for treatment and postoperative pain relief (Quintans *et al.,* 2014; Boadas-Vaello *et al.*, 2017; Forouzanfar and Hosseinzadeh, 2018; Basu and Basu, 2020; Basu *et al.,* 2021). Curcumin, which stands out with its anti-inflammatory and antioxidant properties, is considered a therapeutic option in peripheral neuropathy. In particular, it has an effective role in wound healing and postoperative pain relief (Lee *et al.,* 2013; Hewlings and Kalman, 2017). The role of curcumin treatment on the nervous system involves increasing neuron viability, inducing neuronal differentiation, and inhibiting apoptotic processes in neurons (Dai *et al.,* 2018; Sadeghi *et al.,* 2018; Ruan *et al.,* 2019; Chen *et al.,* 2022). A variety of studies performed in recent years have demonstrated that curcumin acts to repair damage to the sciatic nerve (Chen *et al.,* 2022; Wang *et al.,* 2022a). The current research examines the efficacy of curcumin administered at different times in the treatment of DM-related peripheral neuropathy.

MATERIALS AND METHODS

Animals and experimental groups: This experimental study was performed after the approval of the Ondokuz Mayıs University Experimental Animals Local Ethics Committee, Türkiye. In this research, cadaveric sciatic nerve tissues of the study with ethics committee approval number 2017/53 were analyzed (ethical committee decision dated 30.03.2018, document number: 68489742-604.01.03 E.8100). The sample size was determined using Minitab (version 18.0) power analysis software. Fifty-six 12-week-old adult female Wistar albino rats (250-300g) obtained from the university were used. During the experimental procedures, the rats were housed in separate cages at a room temperature of 22±2°C and a humidity of 45-50%, with a 12-hour dark/light cycle. In addition, rats were given *ad libitum* access to chow and tap water. Adult female rats (n=56) were divided into seven equal groups (n=8), and the groups were designed as follows:

Control group (Cont): No experimental procedure was performed in this group. This group was included in the study to obtain baseline values.

Sham group (Sham): Corn oil by intragastric gavage was given to the rats in this group received for 14 days, aiming to eliminate the effects caused by corn oil (1ml/kg).

Diabetes mellitus group (DM): The animals in this group intraperitoneally received a single dose of streptozotocin (STZ) (50 mg/kg) (Cayman Chemicals, USA).

DM + curcumin group (DC1): The rats in this group were given curcumin (30 mg/kg) by intragastric gavage once daily for 14 days after seven days of (STZ) (50 mg/kg) induction. 30 mg/kg of curcumin was dissolved in corn oil.

DM + curcumin group (DC2): Rats in this group were given curcumin (30 mg/kg) by intragastric gavage once a day for 21 days, seven days after (STZ) (50 mg/kg) induction. Rats in this group were given curcumin (30 mg/kg, dissolved in corn oil) by intragastric gavage once a day for 21 days, seven days after the onset of diabetes.

DM + curcumin group (DC3): Rats in this group were given curcumin by intragastric gavage once a day for 14 days, starting simultaneously with the development of diabetes. 30 mg/kg of curcumin was dissolved in corn oil.

Curcumin group (Cur): 30 mg/kg curcumin dissolved in corn oil by gavage once a day for 14 days was given to the rats in this group.

Experimental diabetes model: The experimental diabetes model was established by fasting the animals overnight before the administration of STZ (50 mg/kg, dissolved in 0.1 M citrate buffer) (Cayman Chemicals, USA) (Andrade Cetto *et al.,* 2000). In the first 12-24 hours after diabetes induction by STZ injection, 5% glucose was added to the drinking water to prevent hypoglycemia in the animals. *Ad libitum* access to chow was permitted after diabetes induction using STZ (Tufekci and Kaplan, 2023). The

fasting blood glucose of rats was measured 72 hours after diabetes induction with STZ, and those with blood glucose levels of 250 mg/dL or higher were included in the research as diabetics.

Perfusion: After the experimental process was completed, perfusion was performed for all rats under anesthesia, and the sciatic nerve samples were removed as previously done (Andrade Cetto *et al.,* 2000).

Light and electron microscopy: After fixation, tissue samples were washed in phosphate buffer (pH=7.4) and post-fixed with 1% osmium tetroxide. Electron microscopic tissue processing was performed using an Epoxy Resin (Araldite CY212) kit (Agar Scientific, UK). Images obtained from semi-thin sections taken by ultramicrotome (Thermo Scientific Shandon, USA) were captured using a program (cellSens Entry) with a computer-aided microscope (Olympus, BX43, Center Valley, PA, USA) with a camera attachment. Sections (70 nm) being treated with 0.5% uranyl acetate and 3% lead citrate placed on copper grids were examined under the electron microscope (JEOL JSM-7001F, JEOL Ltd., Tokyo, Japan) (Kaplan *et al.*, 2023; Delibaş and Kaplan, 2024).

Stereology: A stereo microscope (Labomed Luxeo 4Z, USA) was used to dissection the sciatic nerve tissue from the surrounding connective and muscle tissue. Stereological evaluations were performed on images obtained from toluidine blue-stained sections using Image J (http://rsb.info.nih.gov/ij/) software. The 2D disector method was performed to estimate the myelinated axon number. Axon area and myelin sheath thickness were estimated using a planimetric approach. The area of the axon and thickness of the myelin sheath were measured in myelinated axons that superimposed at the upper right corner of an unbiased counting frame (Fig. 1). The unbiased counting frame area was 2500 μm^2, and the step size area was 9606 μm^2. The myelinated axon number was estimated using the formula (Kaplan *et al.,* 2010). All stereological analysis was done blindly.

Statistics: GraphPad Prism version (8.3.1) was used for statistical evaluation. The one-way ANOVA (post hoc: Tukey test) test was performed in groups showing normal distribution, while the Kruskal-Wallis test was used in groups not showing normal distribution.

RESULTS

Myelinated axon numbers: In terms of overall myelinated axon numbers belonging to all groups, no difference was determined (P>0.05) (Fig. 2). All the stereological data sets are shown in Table 1.

Axon areas: The mean axon area was significantly smaller in the Sham group compared with the Cont group (P=0.0001). The axon area in the DM group was significantly smaller than the Cont group's (P=0.0066). Mean axon area values in the DC2 and DC3 groups were significantly reduced compared to the Cont group's (P=0.0001) (P=0.001). The mean axon area of the DC1 group was significantly increased compared to the Sham group (P=0.0341), and it was significantly decreased in the DC3 group compared to the Sham group (0.0009). Also, axon areas in the DC2 and DC3 groups decreased more than in the DM group (P=0.003 and P=0.001, respectively). The mean axon area significantly decreased in the DC2 and DC3 groups than in the DC1 group (P=0.001). Axon area values significantly decreased in the Cur group compared to the Cont group (P=0.001). Axon area in the DC3 group decreased than the curcumin-only group (P=0.009) (Fig. 3).

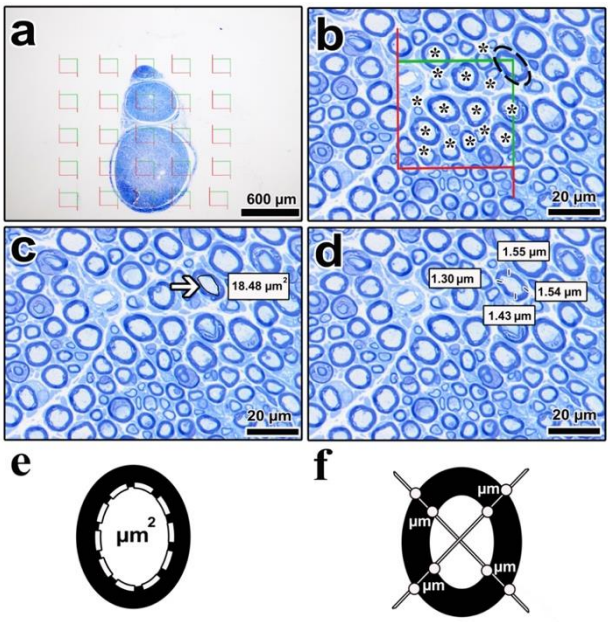

Fig. 1: (a) This representative image shows the field sampling according to the systematic random sampling rule in the stereological evaluation of the sciatic nerve. (b) The myelinated axon number was calculated using an unbiased counting frame. Sixteen axons were included in the count in a frame (including * and the axon circled in dashed lines). Myelin thickness and axon area were measured in the axon corresponding to the upper right corner of each counting frame (the axon in the dashed circle). (c, e) The area of an axon surrounded by its interior was measured with Image J analysis software. (d, f) The myelin sheath thickness was measured from four points and was averaged by arranging two orthogonal lines intersecting at the axon's center. (c, d) The numerical values shown are taken from our study data.

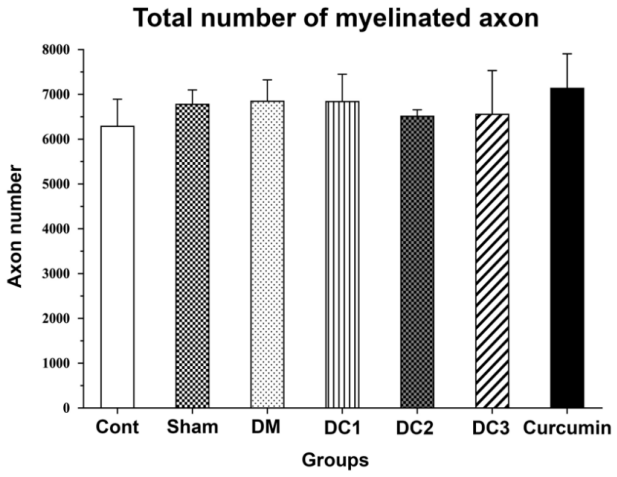

Fig. 2: The myelinated axon number of sciatic nerves in different studied groups can be seen. No significant difference between the groups was found.

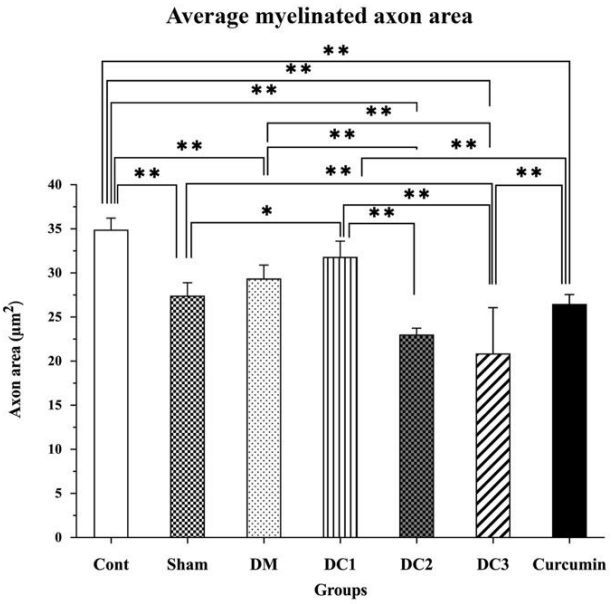

Fig. 3: The groups' average axon areas (µm²) in female rat sciatic nerves can be seen. Significant differences between groups are detailed in the graph. Statistical differences between groups are indicated with "*" and "**". *: P<0.05, **: P<0.01.

Table 1: The coefficient variation values of the total numbers of myelinated axons, axons area, and myelin sheath thicknesses estimation that obtained by stereological analysis in all the study groups

Groups	The total number of myelinated axons	Axon area	Myelin sheath thickness
Cont	0.04	0.01	0.03
Sham	0.04	0.05	0.08
DM	0.06	0.04	0.03
DC1	0.08	0.05	0.06
DC2	0.02	0.03	0.06
DC3	0.13	0.08	0.08
Curcumin	0.10	0.04	0.04

Myelin sheath thickness: Reduced thickness values in the Sham groups were observed compared to the Cont group's (P=0.001). Myelin sheath thickness in the DM group was lower than in the Sham group (P=0.04). The thickness of the DC1, DC2, and DC3 groups was also significantly lower than in the Cont group (P=0.02, P=0.0007, and P=0.0001, respectively). The myelin sheath of the DC2 and DC3 groups was thinner than in the DM group (P=0.02 and P=0.003, respectively) (Fig. 4).

Histopathology: The tissues of the Cont group exhibited a healthy morphology, and the integrity of the surrounding epineurium layer was well preserved. The fact that healthy axons in this group have a cylindrical structure, most axon diameters and myelin sheath thickness were large, structural integrity in the connective tissue adjacent to the axon was preserved, and the vessels in the tissue were the main histological criteria taken into consideration in evaluating the sciatic nerve structure of this group as healthy. In addition, another histological criterion indicating normal morphology is that the nuclei and nuclear borders of the Schwann cells around the myelinated axons were prominent and had euchromatic staining. The axon morphology was also healthy, with most samples containing large-diameter axons. The Schwann cell nuclei were lightly stained in the Cont group samples (Fig. 5a), while degenerated axons were found in sciatic nerve sections from the Sham group (Fig. 5b). It is noted that the axons in this group were stripped of their sheaths and surrounded by phagocytic cytoplasm and debris; this clearly indicates macrophage-mediated demyelination. When histological images of DM group were evaluated, irregularly structured, dark-stained, stained circular compact myelin profile was observed in degenerated axons. Intense vacuolization and deterioration were observed in the myelin sheaths of nerve fibers belonging to the DM group. Unmyelinated axon clusters were more common in the DM group. Swelling and myelin ovoid structures seen in axons from DM and Sham groups indicate Wallerian degeneration (Fig. 5c). Schwann cells in the sciatic nerves from the DC2 and DC3 groups were stained darker than in the Cont and DC1 groups (Fig. 5a, Fig. 5c, Fig. 5d, Fig. 5e, Fig. 5f). Small-diameter axons were more common in the DC2, DC3, and DM groups (Fig. 5c, Fig. 5d, Fig. 5e). The myelin sheaths from the DC2 group were almost free of vacuolization, and axonal integrity was better preserved compared to the DC1 and DC3 groups (Fig. 5d, Fig. 5e, Fig. 5f). Healthy axons in the DC2, DC3, and Cur groups were mainly small in diameter (Fig. 5e, Fig. 5f, Fig. 5g). Schmidt-Lanterman clefts were found in all groups and were particularly intense in the Sham, DC1, DC3, and Cur groups (Fig. 5d, Fig. 5f, Fig. 5g). Healthy axons were observed in the Cont and Sham groups (Fig. 6a, Fig. 6b). However, deterioration in the myelin sheath was observed in the DM group (Fig. 6c). Thin axonal myelin sheaths were observed in the DC1 group (Fig. 6d). The axon diameters of the DC3 group were smaller than those of the Cont group (Fig. 6a and Fig. 6e). Impairment and vacuolization in the axonal myelin sheath were also shown in some nerve regions in the Cur group (Fig.6f).

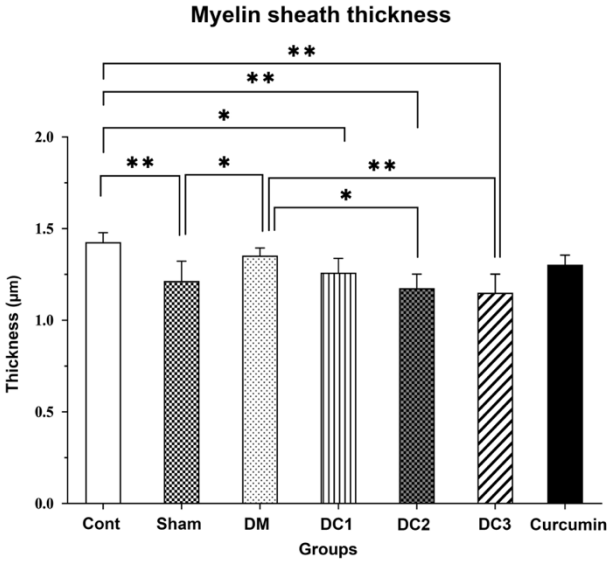

Fig. 4: The myelin sheath thicknesses (µm) of female rat sciatic nerves from all groups can be seen. Significant differences between groups are detailed in the graph. Myelin sheath thickness (µm) measurements of different groups of female rat sciatic nerves are given. Statistical differences between groups are indicated with "*" and "**". *: P<0.05, **: P<0.01.

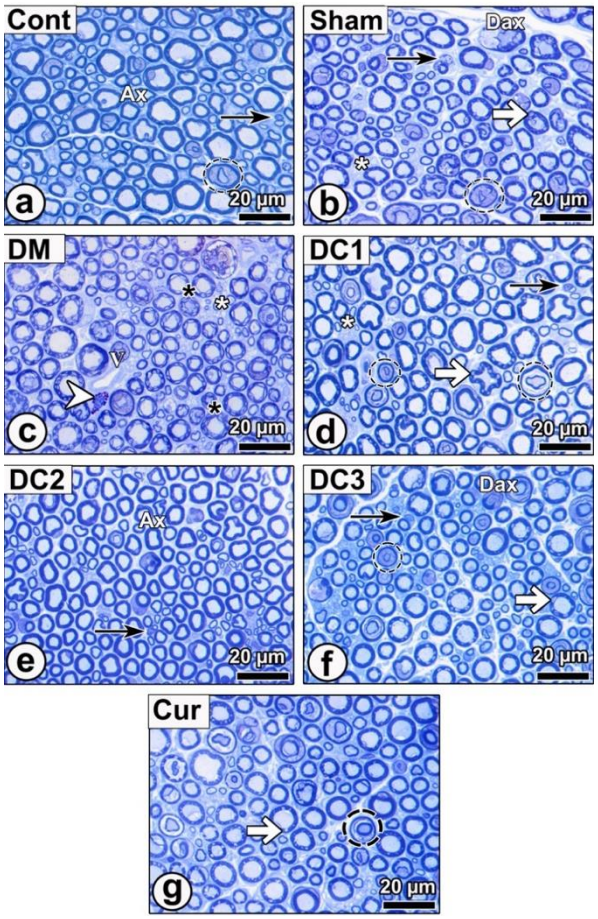

Fig. 5: (a-g) Light microscope images of the groups are seen in images taken from semi-thin sections. (a) The axons are seen in the Cont group, (b) Sham group, (c) DM group, (d) DC1 group, (e) DC2 group, (f) DC3 group, and (g) Cur group. Arrowhead: Mast cell, Ax: Healthy myelinated axon structure, Black arrow: Schwann cell nucleus around the myelin sheath, Black asterisk: Myelin sheath deteriorations, Dashed circle: Schmidt-Lanterman cleft, Dax: Degenerated axon, White arrow: Swelling in the myelin sheath, White star: unmyelinated axon region, V: Vessel. Section thickness is 0.5 µm—toluidine blue staining.

DISCUSSION

DM is a disease that reduces the functional and structural capacity of endocrine hormones that regulate blood glucose levels (AlMasoud *et al.,* 2024). It also has a dual effect on appetite-regulating hormones such as leptin and ghrelin (Bingöl *et al.,* 2024). In this context, new herbal treatment approaches are gaining momentum today despite pharmaceutical agents produced as anti-diabetics (AlMasoud *et al.,* 2024). Axon regeneration with impaired structural integrity is the prominent pathology of diabetic neuropathy. Nerve regeneration after crushing or transection in diabetic animal models is slow (Kennedy and Zochodne, 2005; Nishida *et al.,* 2013). Diabetes causes axonal degeneration and inhibits the regenerative pathways that appear to reverse existing damage. This phenomenon is known as "double hitting." Regenerative cluster densities decrease in advanced stages of neuropathy (Kennedy and Zochodne, 2005). The main pathological findings in diabetic peripheral neuropathy are demyelination, axonal atrophy, fewer nerve fibers, and decreased regeneration capacity (Yasuda *et al.,* 2003). It has recently been found that diabetic peripheral neuropathy results in edema and fiber deterioration in the tissue of the sciatic nerve (Gopalan, 2024). Similarly, when the diabetic Wistar Bonn Kobori rat model's tibial and sciatic nerve structures were examined, endoneurial fibrosis, axonal degeneration and microangiopathy were observed (Ozaki *et al.,* 2013). The decrease in the DM group axonal area may indicate axonal shrinkage and loss. In a previous morphological study, when the ultrastructural structure of the diabetic nerve was examined, morphological and morphometric changes in the myelin sheath and axon were accepted as the distinguishing feature of diabetic neuropathy (Evangelista *et al.,* 2018). The axonal area decreased and degenerated axons were encountered over the course of time. This may be evidence of nerve fibers undergoing apoptosis in the diabetes group.

In the current study, the Schwann cell nuclei of the DM group were darkly stained. These cells undergo apoptosis, and myelination is adversely affected. Detachments in the myelin sheath structure, a greatly distorted lamellar structure, and vacuolization were observed in some areas in the DM group. The absence of any change in the myelin sheath despite the shrinkage in the axon area may be an indicator of axon degeneration.

Diabetic sensorimotor polyneuropathy, another common form of complications resulting from type 2 diabetes, is characterized by decreased nerve conduction. It has been suggested that this neuropathy is similar in motor and sensory nerves (Numan *et al.,* 2021). In diabetic neuropathy, the reduced peripheral nerve conduction velocity may be associated with axonal atrophy rather than a decrease in myelin sheath thickness (Higashimori *et al.,* 2005). In the current research, degenerative axons and intense vacuolization in the DM group support the evidence of axonal shrinkage leading to cell death. Morphoquantitative changes in the Sham group may indicate the effect of stress on the tissue. However, the thin myelin sheaths in this group may indicate newly sprouting axons. Considering the pathological changes in diabetic peripheral neuropathy, therapeutic agents that prevent degeneration and enhance regeneration are essential. In an early experimental model of diabetes, the reduced diameter of myelinated fibers and impaired nerve conduction velocity is seen (Yang *et al.,* 2023). Histopathological images also support the decrease in the myelinated axon area of the DM group. In addition, the stereological results of myelin sheath thickness may have been high due to the presence of vacuolations and degenerations in the myelin sheath structure of the DM group. Curcumin has recently become prominent in treating diabetic peripheral neuropathy (Ma *et al.,* 2016; Zhang *et al.,* 2022). Curcumin's supportive impact on axonal regeneration in diabetic rats has been demonstrated previously in another research (Banafshe *et al.,* 2014; Zhao *et al.,* 2014; Daugherty *et al.,* 2018). The impacts of curcumin on axon damage have been found to support regeneration. Research showed that the effect of curcumin on recovery of motor capabilities after crush injury in non-diabetic rat nerves was dose-dependent (Ma *et al.,* 2013; Mohammadi and Mahmoodi, 2013). In addition, recently, the protective role of curcumin against axonal damage in the optic nerves of diabetic rats has been revealed with morphological and cellular level evidence (Şahin *et al.,* 2024).

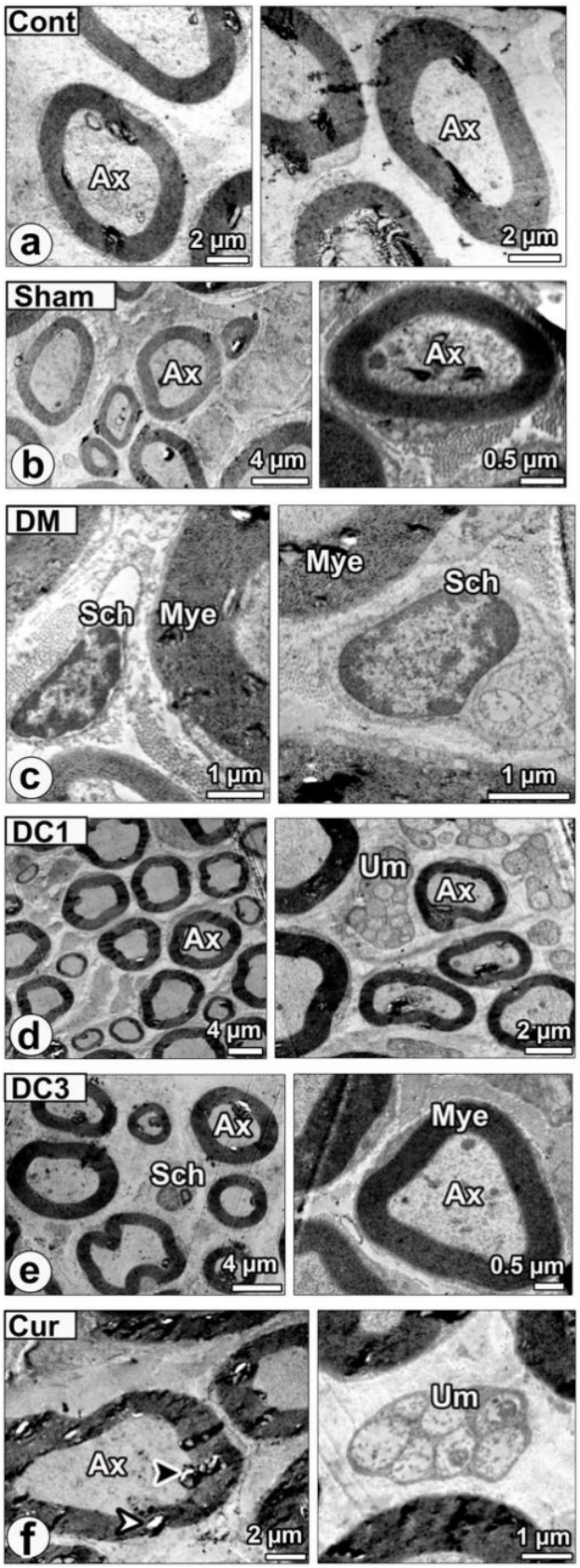

Fig. 6: Electron microscopic images of groups are seen. The myelin sheath thickness in the Cont and Sham groups was normal. Some myelin discontinuities and electron-dense areas were observed in the DM group's myelin sheath (Mye). The myelin sheath morphology was preserved in the DC1 and DC3 groups. In the Cur group, some myelin dissociation thought to be due to deficiencies in fixation in the myelin sheath (arrowhead) was observed. Unmyelinated axons (Um) in the DC1 and Cur groups were also healthy at the organelle level. Ax, Axon; Sch, Schwann cell.

High-dose curcumin was more effective in inducing axonal regeneration. Due to curcumin's role in triggering axonal regeneration, it can be regarded as a therapeutic option in diabetic neuropathy (Ma *et al.,* 2013). Female rats were used in this study because gender differences may affect the therapeutic effects of diabetes (Tramunt *et al.,* 2020). Female rats have been reported to exhibit less improvement in HbA1c than males (Kautzky-Willer *et al.,* 2015; Tramunt *et al.,* 2020). They have also been found to exhibit lower endogenous glucose production and hormonal response than males (Kautzky-Willer *et al.,* 2015). The effects of diabetes on health and treatment options have usually been studied using male animals (Ma *et al.,* 2013, Ma *et al.,* 2016; Kaplan *et al.,* 2023). We, therefore, decided to use female adult rats in this study. Gender differences are important in defining treatment options (Tramunt *et al.,* 2020).

The axonal area decreased in the groups that received curcumin 21 days after diabetes induction and concurrently with diabetes. More rapid myelination was observed in the DC1 group. Significant decreases can occur in nitric oxide synthases such as endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthases (iNOS) in the early phase of diabetes. Neuronal NOS (nNOS) is needed to produce peroxynitrite for the continuity of peripheral nerve functions. In diabetes, nNOS insufficiency exhibits signs such as axonal degeneration and nerve ischemia (Suresh and Reddy, 2021). It has been previously suggested that under hyperglycemic conditions in diabetic rats, oxidative stress caused by excessive production of reactive oxygen species and nitric oxide is reduced by the role of curcumin in regulating intracellular enzyme activity (Salgıntaş *et al.*, 2022). It was demonstrated that hyperglycemia in diabetic rats causes a higher level in pro-inflammatory cytokines, that in turn activate nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and elevate free oxygen radicals. In this case, oxidative damage occurs, and pancreatic β-cell devastation is seen (Susanti *et al.,* 2023). Diabetic neuropathy is significantly influenced by oxidative stress. Furthermore, there is an important connection between an anti-inflammatory reaction and neuropathic pain in rats with diabetes. Likewise, it was found that the sciatic nerves of diabetic rats had elevated levels of IL-6 and TNF-α (Wang *et al.,* 2022b). In this case, in diabetic rats, therapeutic approaches targeting the reduction of increased TNF-α levels are important (Susanti *et al.,* 2023). Despite the fact that there are numerous pharmaceuticals with anti-diabetic properties for the treatment of diabetes, interest in plant-based therapeutics is growing because of their minimal adverse effects (AlMasoud *et al.,* 2024). Curcumin is known as an alternative treatment for diabetes because of its anti-inflammatory and antioxidant benefits (Zhang *et al.,* 2022; Şahin *et al*., 2024).

In the late phase of diabetes, the nNOS content is completely depleted from the neurons. This stimulates the apoptotic process (Suresh and Reddy, 2021). Seven days after induction, large-diameter axons were observed in a treatment-initiated group in another study, which may indicate axonal maturation. It appears that nNOS does not contribute to the neuroprotective role of curcumin (Gilhotra and Dhingra, 2010). No studies have described the nNOS-related mechanism of action of curcumin.

However, the small diameter and thinly myelinated axons in the simultaneous treatment group may have resulted from curcumin's regenerative and protective effects. In the Cur group, curcumin was given earlier and exhibited a neuroprotective effect on nerve fibers. Recent research has shown that 100 mg/kg of curcumin supports regeneration in the damaged nerve fiber (Moattari *et al.,* 2018). It has been reported that 150 mg/kg of curcumin is more sensitive to the apoptosis of Schwann cells, but 50 mg/kg of curcumin triggers neural growth factors more effectively (Zhang *et al.,* 2022). This is evidence of controversies about curcumin's dose-dependent effects in diabetic neuropathy. The findings for the DC1 group are consistent with Kaplan *et al*. (2023) regarding curcumin treatment.

In one of our previous studies, curcumin did not affect the axon area or axon number during axonal regeneration but increased the myelin sheath thickness (Kaplan *et al.,* 2023). In the current study, when curcumin treatment was evaluated in diabetic animals, it was revealed that curcumin administered simultaneously with the induction of diabetes or in the late period did not show a protective effect on axon area or myelin thickness. Wallerian degeneration commences within hours after injury and is completed in 6-8 weeks. Functional recovery usually takes place between 14 and 90 days. After peripheral nerve damage, the myelin sheath becomes a regular, dense structure after 4-12 weeks (Moattari *et al.,* 2018). Curcumin administration after nerve injuries can be effective in high doses and for extended periods (Moattari *et al.,* 2018). Curcumin administered seven days after diabetes induction increased the axon area but had a minimal effect on myelin sheath thickness in this study. The treatment that started after seven days was more effective in terms of myelination than the treatment that started after 21 days and simultaneous treatment.

The structure of the myelin sheath in the Cur group at electron microscopic evaluation was mainly intact. On the other hand, vacuolization was found in the myelin sheaths of nerve fibers in the Sham group. This research investigated the effects of 14-day 30 mg/kg curcumin administration on diabetic animals. In this study, one of the main limitations is that the function of the sciatic nerve was not evaluated. This might have yielded a clearer idea about the efficiency of curcumin treatment and optimal exposure time. Another limitation is that electron microscopic micrographs of the DC2 group could not be provided. Electron microscopic evaluation was, therefore, not possible in the DC2 group.

Conclusions: Due to its ability to repair the myelin sheath structure, curcumin given seven days after diabetes induction may be a potential therapy option for diabetic neuropathy. New studies should be designed to compare the gender-related effects of curcumin forms applied at different times and doses. Although curcumin is a widely used and neuroprotective agent, further molecular-level studies are required regarding its effects.

Diabetes causes degenerative effects on the sciatic nerve. The axon has been shown to shrink and degenerate over time in the case of diabetes, and the structure of the myelin sheath deteriorates. Curcumin given seven days after the diabetic induction and exhibited a healing effect, especially on the structure of the myelin sheath. Curcumin is a promising substance for diabetic peripheral neuropathy. Further investigation of gender-dependent curcumin treatment options is needed.

Conflict of interest: The authors declare no conflict of interest.

Authors contribution: The researchers listed as authors in the study have made significant contributions regarding their areas of expertise and their methods. This study was designed by SK, GA, and KKT. Under the supervision of SK, GA, JA, KKT, and MBT conducted the experiments, and obtained the study data by performing tissue analyses. SK, who served as the project manager, managed the processes from the beginning to the end of the project, from the provision of the project budget to the biological interpretation of the data and the preparation of the draft article and the finalization of the article.

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