BMC Pharmacology and Toxicology

RESEARCH Open Access

# Investigation of the protective effects of dichloroacetic acid in a rat model of diabetic neuropathy

Murat Arı[1*], Mumin Alper Erdogan[2] and Oytun Erbaş[3]

## Abstract

**Background** Diabetic neuropathy (DN) is a heterogeneous condition characterized by complex pathophysiological changes affecting both autonomic and somatic components of the nervous system. Inflammation and oxidative stress are recognized contributors to the pathogenesis of DN. This study aims to evaluate the therapeutic potential of dichloroacetic acid (DCA) in alleviating DN symptoms, focusing on its anti-inflammatory and antioxidant properties.

**Methods** Thirty-two adult male Sprague Dawley rats were divided into four groups: Control, Diabetic, and two DCA-treated groups receiving 5 mg/kg and 10 mg/kg of DCA, respectively. Diabetes was induced with streptozotocin (STZ) injections. Assessments included lipid peroxidation levels, plasma fibroblast growth factor-21 (FGF-21) and transforming growth factor-beta (TGF-β) levels, electrophysiological measurements, histological examination of the sciatic nerve, and motor function tests.

**Results** Treatment with DCA significantly reduced malondialdehyde (MDA) levels, indicating decreased lipid peroxidation. Plasma TGF-β levels were also lower in the DCA-treated groups, suggesting diminished inflammation. Conversely, plasma FGF-21 levels were elevated. Electrophysiological assessments revealed enhanced compound muscle action potential (CMAP) amplitudes and reduced distal latencies in DCA-treated rats, indicative of improved nerve conduction. Histopathological examinations showed reduced perineural thickness in the sciatic nerves of DCA-treated rats, pointing to decreased fibrosis. Enhanced performance in motor function tests was observed in these rats, implying improved muscle strength and motor capacity.

**Conclusions** The study demonstrates that DCA therapy significantly reduces oxidative stress and inflammation in a rat model of DN, thereby ameliorating neuropathic symptoms. These results support the potential of DCA as a promising therapeutic agent for DN treatment. Further research is warranted to explore its clinical applications and to provide more detailed insights.

**Keywords** Diabetes mellitus, Diabetic neuropathy, Dichloroacetic acid, Oxidative stress, Inflammation

*Correspondence:
Murat Arı
muratari60@gmail.com
[1]Söke Health Services Vocational School, Aydin Adnan Menderes University, Aydin, Turkey
[2]Faculty of Medicine, Department of Physiology, Izmir Katip Celebi University, Izmir, Turkey
[3]Faculty of Medicine, Department of Physiology, Istanbul Demiroglu Bilim University, Istanbul, Turkey

## Introduction

Diabetes Mellitus (DM) is a multifaceted syndrome characterized by impaired carbohydrate metabolism, with a significant global prevalence that correlates with elevated rates of mortality and morbidity [1]. The International Diabetes Federation reports that approximately 425 million people worldwide currently suffer from diabetes, a number projected to escalate to 643 million by 2030 and 783 million by 2045 [2, 3]. Among the myriad complications of diabetes mellitus, neuropathy stands out as a prevalent chronic manifestation. According to a recent study, approximately 12% of patients with diabetes mellitus had diabetic neuropathy. Diabetic neuropathy (DN) causes functional abnormalities, disability and peripheral nerve damage in the organ affected by long-term diabetes [4]. DN is broadly categorized as a disorder of peripheral nerves that can be hereditary, traumatic, compressive, metabolic, toxic, nutritional, infection-related, immune-mediated, or non-neoplastic in nature [5]. Epidemiological data suggest that neuropathy has a prevalence of 1–4%, with diabetes accounting for 40–55% of these cases [6, 7]. The incidence of neuropathy is notably higher in individuals with type 2 diabetes (42.2%) compared to those with type 1 diabetes (29.1%) [8]. Approximately 75% of DN cases are identified as distal symmetric polyneuropathy, a condition that progresses slowly and often leads to neuropathic pain, severely diminishing the quality of life and functional capacity of those affected [9]. The primary pathological mechanisms in diabetes-related neuropathy include inflammation and oxidative stress induced by chronic hyperglycemia. Effective blood pressure management has been shown to mitigate these adverse effects and reduce the incidence of DN. The relationship between effective blood pressure management and the reduction in the incidence of DN is well-documented in clinical studies. Research has demonstrated that maintaining blood pressure within the recommended range (typically < 140/90 mmHg, or even lower for diabetics with complications) reduces the risk of diabetic neuropathy. Trials such as the UK Prospective Diabetes Study (UKPDS) emphasise the importance of tight blood pressure control in reducing diabetes-related complications. Patients with controlled blood pressure exhibit a slower progression of neuropathic symptoms compared to those with uncontrolled hypertension [10, 11].

There is ample evidence that dichloroacetic acid (DCA) is a metabolic modifier which, by inhibiting pyruvate dehydrogenase kinase (PDK), restricts glycolysis and enhances mitochondrial oxidative metabolism. This fundamental mechanism of action has been repeatedly confirmed in a variety of tumours and circumstances, as shown in studies [12]. Additionally, it curtails the hepatic biosynthesis of cholesterol and triglycerides. While certain studies indicate DCA's neutral effects on hemodynamics. Others have documented its beneficial impact on cardiac function post-ischemia and its significant reduction of acute kidney injury [13, 14]. Further research has highlighted DCA's role in enhancing glucose oxidation in various mitochondrial disorders. However, the exploration of DCA's utility in cancer treatment remains limited to a few cell lines, with an incomplete understanding of the alternative apoptotic pathways it may regulate [15].

To our knowledge, to date, the potential effects of DCA on DN have not been thoroughly investigated. Considering the gaps in current research, this study posits that DCA could offer protective or therapeutic benefits in DN. We are of the opinion that the study is valuable in this respect. Accordingly, this investigation is designed to explore the efficacy of DCA as a preventive or therapeutic agent against the damage wrought by this condition.

## Materials and methods

### Animal preparation

The study utilized 32 adult male Sprague Dawley rats, each weighing between 200 and 210 g, were obtained from the Demiroglu Science University Experimental Animal Research Center for the study. These animals were accommodated under controlled conditions with a 12-hour light/dark cycle at a stable room temperature of 22 ± 2 °C. Throughout the study, the rats had ad libitum access to a standard pellet diet and tap water. All experimental procedures adhered to the ethical guidelines approved by the Institutional Animal Care and Ethical Committee of the University of Science (Ethical Approval Number: 28210104, Date:13.02.2023). Moreover, all procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals endorsed by the National Institutes of Health (USA).

### Drugs and chemicals

Streptozotocin (STZ)-Sigma-Aldrich Inc, Saint Louis, Missouri.

Glucose oxidase reagent strip-Boehringer Mannheim, Indianapolis.

DCA- Sigma-Aldrich Inc.

Biopac bipolar subcutaneous needle stimulation electrode (BIOPAC Systems, Inc., Santa Barbara, CA, USA).

Transforming Growth Factor Beta (TGF-β) and Fibroblast Growth Factor-21 (FGF-21)-immunosorbent assay (ELISA) kits from Biosciences.

### Experimental design

In the study, diabetic mellitus (DM) was induced by streptozocin in 24 of 32 Sprague Dawley rats. A single dosage of STZ from Sigma-Aldrich, Inc., Saint Louis, Missouri, USA, was injected intraperitoneally at a concentration of 60 mg kg in 0.9% NaCl, which was then

adjusted to a pH of 4.0 using 0.2 M sodium citrate. The remaining eight rats served as the control group, receiving no chemical treatment and maintaining normal blood glucose levels below 120 mg/dl. DM confirmation occurred 24 h post-injection, utilizing glucose oxidase reagent strips for blood glucose evaluation with readings exceeding 250 mg/dL indicating diabetes. Subsequently, the diabetic rats were randomly assigned into three groups: Group 1 ($n = 8$; diabetic and saline-treated) received 1 ml/kg saline, Group 2 ($n = 8$; diabetic and low-dose DCA-treated) received DCA at 5 mg/kg/day, and Group 3 ($n = 8$; diabetic and high-dose DCA-treated) received DCA at 10 mg/kg/day, all via intraperitoneal administration for four weeks. Doses 5 and 10 mg/kg/day were determined based on previous studies. Higher doses may lead to liver toxicity and neurological side effects [16].

At the conclusion of the treatment period, electromyography (EMG) and inclined plane testing were conducted. Following these assessments, the animals were euthanized, and both blood and sciatic nerve samples were collected for biochemical and histopathological examination, respectively.

### Electromyography (EMG) assessment

EMG recordings were taken three times from the right sciatic nerve using a Biopac bipolar subcutaneous needle stimulation electrode (BIOPAC Systems, Inc., Santa Barbara, CA, USA) positioned at the Achilles tendon. Stimulations were supra-maximal (intensity 10 V, duration 0.05 ms, frequency 1 Hz, within a range of 0.5–5000 Hz, at a 40 kHz/sec sampling rate). Compound muscle action potentials (CMAPs) and changes in motor nerve conduction velocity (NCV) were captured using unipolar needle electrodes implanted in the 2–3 interosseous muscle. Data were analyzed using Biopac Student Lab Pro version 3.6.7 software (BIOPAC Systems, Inc.). During the EMG measurements, the rectal temperatures of the rats were monitored using a rectal probe (HP Viridia 24-C; Hewlett-Packard Company, Palo Alto, CA, USA) and maintained between 36 °C and 37 °C using a heating pad.

### Inclined plane test

Motor function of the rats was assessed using the inclined plane test, as described by Rivlin and Tator, one month following STZ administration [17]. The apparatus consisted of a 50 cm x 30 cm stainless steel plane. The maximum angle at which a rat could maintain its body position without limb slippage was determined. This test was conducted three times per animal for each head position, with trials averaged and interspersed with 1-minute intervals between attempts.

### Histopathological examination of the sciatic nerve

Sciatic nerve samples were fixed in formalin, sectioned at 4 μm thickness, and stained with hematoxylin and eosin. The perineural thickness of the sciatic nerve was quantitatively assessed using an Olympus C-5050 digital camera attached to an Olympus BX51 microscope.

### Measurement of lipid peroxidation

Lipid peroxidation in the plasma was quantified by measuring the levels of malondialdehyde (MDA) as thiobarbituric acid reactive substances (TBARS) [18]. The procedure involved adding trichloroacetic acid and TBARS reagent to the plasma samples, followed by mixing and heating at 100 °C for 60 min. After cooling on ice, the samples were centrifuged at 3000 rpm for 20 min, and the absorbance of the supernatant was measured at 535 nm. Calibration was performed using tetraethoxypropane, and MDA levels were reported in nM.

### Measurement of plasma TGF-β and FGF-21

The concentrations of plasma TGF-β and FGF-21 were determined using enzyme-linked immunosorbent assay (ELISA) kits from Biosciences. Levels of these biomarkers were expressed in pg/ml.

### Statistical analysis

Data were analyzed using SPSS software version 15.0 for Windows. The groups of parametric variables were compared using Student's t-test and analysis of variance (ANOVA), while the groups of nonparametric variables were analyzed with the Mann-Whitney U test and Kruskal Wallis test. Results were expressed as mean ± standard error of the mean (SEM). A $p$-value of $< 0.05$ was considered statistically significant.

## Results

### Evaluation of electrophysiological records

Electrophysiological assessments focused on measurements of compound muscle action potential (CMAP) and distal latency. In the control group, CMAP values were recorded at 10.7 ± 0.9 mV (Fig. 1a; Table 1). For the diabetic group receiving saline, there was a significant reduction in CMAP, with a recorded value of 7.8 ± 0.6 mV in comparison with control group ($p = 0.034$) (Fig. 1b; Table 1). Diabetic rats administered 5 mg/kg of DCA displayed an improvement in CMAP, with values reaching 8.3 ± 0.5 mV in comparison with diabetes + saline group ($p = 0.041$) (Fig. 1c; Table 1). Those treated with a higher dose of 10 mg/kg DCA showed further improvement, registering a CMAP of 9.1 ± 0.3 mV in comparison with diabetes + saline group ($p = 0.012$) (Fig. 1d; Table 1).

Concerning distal latency, the control group exhibited a latency of 1.4 ± 0.03 ms (Fig. 1a; Table 1). This metric was prolonged in the saline-treated diabetic group, marked

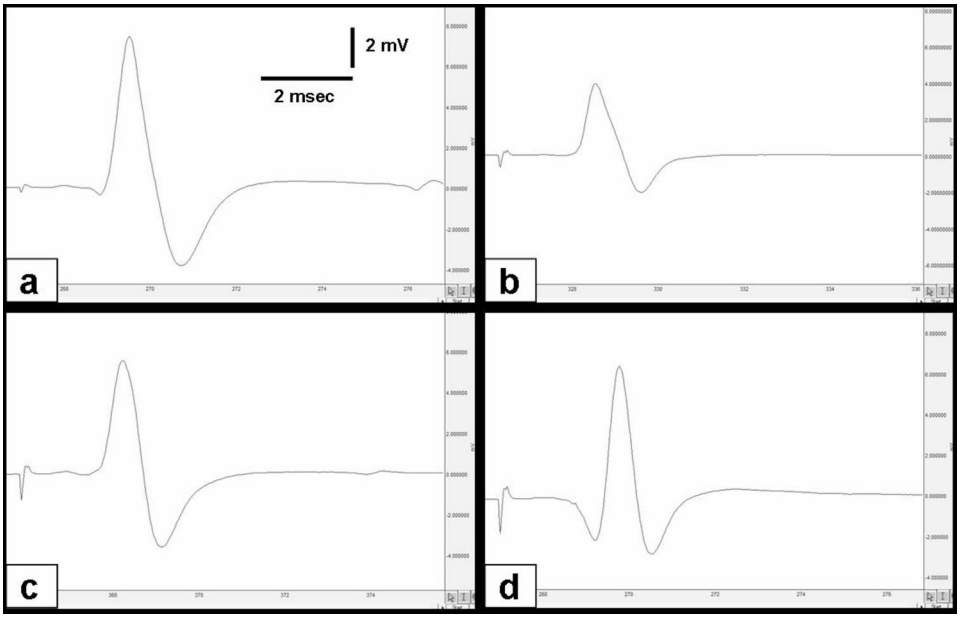


**Fig. 1** **a** Control group EMG, **b** Diabetic and saline treatment EMG, **c** Diabetic and DCA 5 mg/kg treatment EMG, **d** DCA 10 mg/kg treatment EMG

**Table 1** Impact of DCA treatment on CMAP amplitude and distal latency in diabetic rats

| | Control group | Diabetes and saline treatment | Diabetes and DCA 5 mg/kg treatment | Diabetes and DCA 10 mg/kg treatment |
|---|---|---|---|---|
| CMAP Amplitude (mV) | 10.7 ± 0.9 | 7.8 ± 0.6* | 8.3 ± 0.5# | 9.1 ± 0.3## |
| Distal Latency (ms) | 1.4 ± 0.03 | 1.7 ± 0.08* | 1.5 ± 0.1# | 1.5 ± 0.06# |

DCA: Dichloroacetic acid; CMAP Amplitude: Compound muscle action potential amplitude

Data are expressed as mean ± SEM

CMAP Amplitude:

Diabetes and saline treatment group vs. control group: *$p = 0.034$

Diabetes and 5 mg/kg DCA treatment group vs. Diabetes + saline group: #$p = 0.041$

Diabetes and 10 mg/kg DCA treatment group vs. Diabetes + saline group: ##$p = 0.012$

Distal Latency:

Diabetes and saline treatment group vs. control group: *$p = 0.022$

Diabetes and 5 mg/kg DCA treatment group vs. Diabetes + saline group: #$p = 0.038$

Diabetes and 10 mg/kg DCA treatment group vs. Diabetes + saline group: ##$p = 0.017$

by an increased latency of 1.7 ± 0.08 ms in comparison with control group ($p = 0.022$) (Fig. 1b; Table 1). Diabetic groups treated with 5 mg/kg ($p = 0.038$) and 10 mg/kg ($p = 0.017$) DCA showed notable reductions in distal latency, measuring 1.58 ± 0.1 ms (Fig. 1c; Table 1) and 1.5 ± 0.06 ms in comparison with diabetes + saline group (Fig. 1d; Table 1), respectively.

A comparison between the control group and the untreated diabetic group revealed significant differences in sensory nerve conduction studies ($p = 0.022$). Specifically, diabetic rats not treated with DCA exhibited considerably lower CMAP amplitudes and prolonged distal latencies in the sciatic nerve. In contrast, diabetic rats treated with DCA demonstrated a significant increase in CMAP amplitudes, with the most pronounced improvements observed in the group receiving 10 mg/kg DCA ($p = 0.017$). Furthermore, DCA treatment was associated with shorter distal latencies in diabetic rats compared to those not receiving DCA treatment (Fig. 1; Table 1).

### Histological assessment of the sciatic nerve

Histological analyses were conducted to measure the perineural thickness of the sciatic nerve. In the control group, the perineural thickness was measured at 3.5 ± 0.8 µm (Fig. 2a; Table 3). Diabetic rats that received saline showed a significant increase in perineural thickness, recording 24.5 ± 4.2 µm in comparison with control group ($p = 0.0087$) (Fig. 2b; Table 3). Treatment with 5 mg/kg of DCA in diabetic rats resulted in a decrease in perineural thickness to 14.8 ± 5.5 µm in comparison with diabetes + saline group ($p = 0.046$) (Fig. 2c; Table 3). More pronounced reductions were observed in diabetic rats treated with 10 mg/kg of DCA, where perineural thickness was further reduced to 10.1 ± 2.1 µm in comparison with diabetes + saline group ($p = 0.0013$) (Fig. 2d; Table 3).

The comparative analysis indicated that the perineural thickness in the sciatic nerves of diabetic rats receiving saline was significantly greater than that of the control group. Importantly, the reduction in perineural thickness was more significant in rats administered 10 mg/kg DCA compared to those given 5 mg/kg DCA (Fig. 2; Table 3).

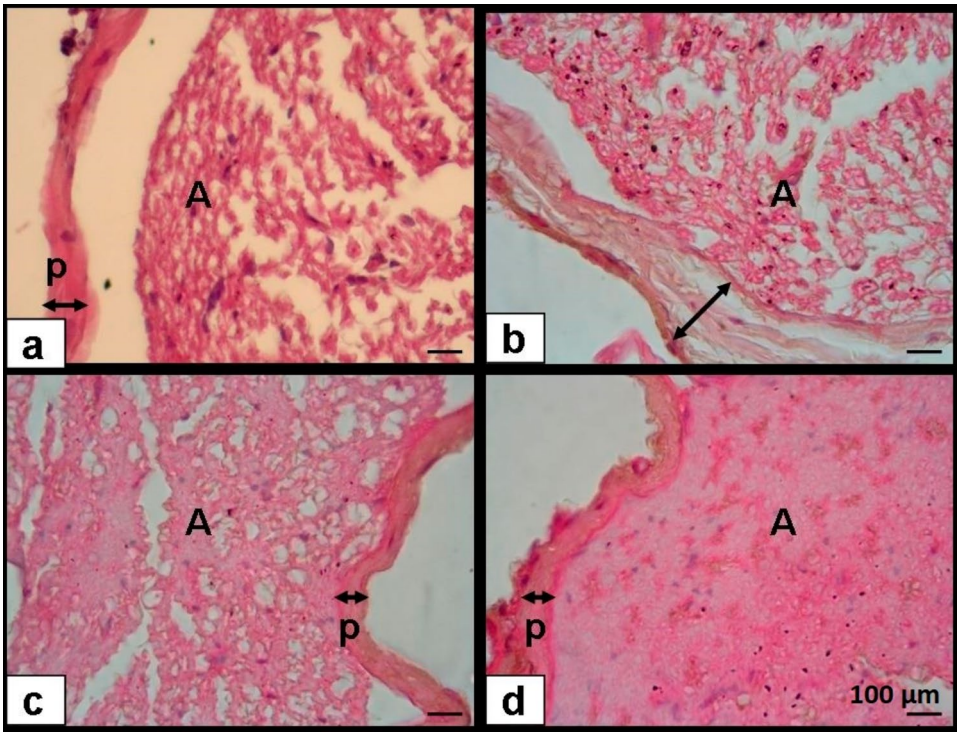


**Fig. 2** The histological sections of sciatic nerve. Hematoxylin and eosin stain (H&E) (x 40 magnification); (**a**) control group p: perineurium, two way arrow: perineural thickness, A: axon, (**b**) DM and saline treated group was shown increased perineural thickness (**c**) DM and 5 mg/kg DCA Group was shown decreased perineural thickness (**d**) DM and 10 mg/kg DCA group was shown decreased perineural thickness

**Table 2** Inclined plane performance and plasma glucose levels in diabetic rats under different treatments

| | Control group | Diabetes and saline treatment | Diabetes and 5 mg/kg DCA treatment | Diabetes and 10 mg/kg DCA treatment |
|---|---|---|---|---|
| Maximum angle of Inclined plane test (degree) | 81.5 ± 6.6 | 44.3 ± 3.2* | 67.9 ± 5.8# | 74.5 ± 7.3## |
| Plasma glucose (mg/dl) | 78.5 ± 4.7 | 421.9 ± 17.5* | 344.5 ± 12.1# | 318.4 ± 10.5## |

DCA: Dichloroacetic acid

Data are expressed as mean ± SEM

Inclined Plane Test (Maximum Angle):

Diabetes and saline treatment group vs. control group: *$p = 0.0004$

Diabetes and 5 mg/kg DCA treatment group vs. Diabetes + saline group: #$p = 0.0054$

Diabetes and 10 mg/kg DCA treatment group vs. Diabetes + saline group: ##$p = 0.0032$

Plasma Glucose Levels:

Diabetes and saline treatment group vs. control group: *$p = 0.0001$

Diabetes and 5 mg/kg DCA treatment group vs. Diabetes + saline group: #$p = 0.0073$

Diabetes and 10 mg/kg DCA treatment group vs. Diabetes + saline group: ##$p = 0.0045$

### Assessment of motor function via inclined plane test

The inclined plane test was employed to assess the motor function of the rats by measuring the maximum angle at which they could maintain their position without slipping. In the control group, the maximum angle achieved was 81.5 ± 6.6 (Table 2). Diabetic rats receiving saline exhibited a significantly lower maximum angle of 44.3 ± 3.2 ($p = 0.0004$) indicating impaired motor capabilities in comparison with control group. Conversely, diabetic rats treated with 5 mg/kg ($p = 0.0054$) DCA achieved a higher angle of 67.9 ± 5.8 and those treated with 10 mg/kg ($p = 0.0032$) DCA showed further improvement, achieving an angle of 74.5 ± 7.3 in comparison with diabetes + saline group (Table 2). These findings suggest that DCA treatment improves motor function in diabetic rats compared to those receiving saline.

### Evaluation of plasma glucose levels

Plasma glucose levels were analyzed across the various groups. In the control group, glucose levels averaged 78.5 ± 4.7 mg/dL. Diabetic rats receiving saline demonstrated significantly elevated glucose levels at 421.9 ± 17.5 mg/dL in comparison with control group ($p = 0.0001$). Treatment with 5 mg/kg ($p = 0.0073$) DCA reduced glucose levels of 344.5 ± 12.1 mg/dL, while diabetic rats treated with 10 mg/kg ($p = 0.0045$) DCA exhibited further reduced glucose levels of 318.4 ± 10.5 mg/

**Table 3** Comparative analysis of different treatment groups in a diabetic rat model

| | Control group | Diabetes and saline treatment | Diabetes and 5 mg/kg DCA treatment | Diabetes and 10 mg/kg DCA treatment |
|---|---|---|---|---|
| Perineural Thickness (µm) | 3.5 ± 0.8 | 24.5 ± 4.2* | 14.8 ± 5.5# | 10.1 ± 2.1## |
| Plasma FGF-21 (pg/ml) | 88.5 ± 9.4 | 285.1 ± 11.8* | 415.7 ± 24.2## | 448.9 ± 10.5## |
| Plasma TGF-Beta (pg/ml) | 4.2 ± 0.8 | 20.3 ± 4.8* | 13.2 ± 5.3# | 10.1 ± 2.2# |
| Plasma MDA (nM) | 58.4 ± 7.1 | 265.9 ± 12.4* | 124.8 ± 11.5# | 110.2 ± 18.6## |

DCA: Dichloroacetic acid; Plasma FGF-21: Plasma fibroblast growth factor 21; Plasma TGF-Beta: Plasma transforming growth factor beta; Plasma MDA: Plasma malondialdehyde

Data are expressed as mean ± SEM

Perineural Thickness:

Diabetes and saline treatment group vs. control group: *$p = 0.0087$

Diabetes and 5 mg/kg DCA treatment group vs. Diabetes + saline group: #$p = 0.046$

Diabetes and 10 mg/kg DCA treatment group vs. Diabetes + saline group: ##$p = 0.0013$

Plasma FGF-21 Levels:

Diabetes and saline treatment group vs. control group: *$p = 0.0058$

Diabetes and 5 mg/kg DCA treatment group vs. Diabetes + saline group: #$p = 0.0014$

Diabetes and 10 mg/kg DCA treatment group vs. Diabetes + saline group: ##$p = 0.0010$

Plasma TGF-Beta Levels:

Diabetes and saline treatment group vs. control group: *$p = 0.0046$

Diabetes and 5 mg/kg DCA treatment group vs. Diabetes + saline group: #$p = 0.033$

Diabetes and 10 mg/kg DCA treatment group vs. Diabetes + saline group: ##$p = 0.021$

Plasma MDA Levels:

Diabetes and saline treatment group vs. control group: *$p = 0.0079$

Diabetes and 5 mg/kg DCA treatment group vs. Diabetes + saline group: #$p = 0.042$

Diabetes and 10 mg/kg DCA treatment group vs. Diabetes + saline group: ##$p = 0.0028$

dL in comparison with diabetes + saline group (Table 2). Both 5 mg/kg and 10 mg/kg DCA treatment group showed significantly lower glucose levels, especially 10 mg/kg DCA doses were reduced more effectively. This highlights the potential of higher doses of DCA to more effectively manage hyperglycemia in diabetic rats.

### Evaluation of plasma FGF-21, TGF-Beta, and MDA levels

#### *MDA levels for assessing lipid peroxidation*

MDA levels were quantified to assess lipid peroxidation across different groups. The control group exhibited MDA levels of 58.4 ± 7.1 nM. In contrast, diabetic rats receiving saline showed significantly elevated MDA levels at 265.9 ± 12.4 nM in comparison with control group ($p = 0.0079$), Diabetic rats treated with 5 mg/kg of DCA had reduced MDA levels of 124.8 ± 11.5 nM ($p = 0.042$), and those treated with 10 mg/kg DCA demonstrated further reduction to 110.2 ± 18.6 nM in comparison with diabetes + saline group ($p = 0.0028$). These findings indicate that DCA treatment significantly lowers lipid peroxidation in diabetic rats compared to untreated ones (Table 3).

#### *Plasma levels of FGF-21 and TGF-Beta*

In the control group, plasma levels of FGF-21 and TGF-Beta were measured at 88.5 ± 9.4 pg/mL and 4.2 ± 0.8 pg/mL, respectively. Diabetic rats receiving saline exhibited increased levels of FGF-21 and TGF-Beta at 285.1 ± 11.8 pg/mL and 20.3 ± 4.8 pg/mL, respectively in comparison with control group ($p = 0.0058$; $p = 0.0046$). Treatment with 5 mg/kg DCA resulted in elevated FGF-21 levels of 415.7 ± 24.2 pg/mL ($p = 0.0014$), and decreased TGF-Beta levels to 13.2 ± 5.3 pg/mL in comparison with diabetes + saline group ($p = 0.033$). Rats treated with 10 mg/kg DCA showed FGF-21 levels of 448.9 ± 10.5 pg/mL ($p = 0.0010$), and further reduced TGF-Beta levels of 10.1 ± 2.2 pg/mL in comparison with diabetes + saline group ($p = 0.021$). These results suggest that while FGF-21 levels were elevated in all diabetic groups, TGF-Beta levels decreased significantly in those receiving DCA treatment, indicating a potentially beneficial therapeutic effect of DCA on inflammatory markers in diabetic rats (Table 3).

## Discussion

Diabetic neuropathy is a complex and multifaceted disorder with a yet unclear mechanism and pathophysiology, affecting both the autonomic and somatic components of the nervous system [10]. It is a complication of diabetes mellitus that causes mellitus that affects the peripheral nerves, causing uncontrollable pain in the in the upper and lower extremities. The treatment of DN is difficult because no effective drugs are are available. In recent years, studies have been carried out to been conducted, focusing on the pathophysiology and various of DN [19]. It is primarily driven by chronic hyperglycemia, which increases free radical formation and overwhelms the body's endogenous antioxidants, leading to an accumulation of toxic reactive oxygen products. Neuropathic damage is often precipitated by increased glycosylation of myelin sheath proteins and other nerve cell biomolecules, resulting in oxidative damage [20]. In the context of hyperglycemia, glycosylated lipoproteins facilitate lipid oxidation, leading to elevated levels of oxidized low-density lipoprotein (LDL) in diabetics. This process contributes to endothelial damage, stimulates vasculitis development, and negatively impacts nerve blood flow, thereby exacerbating neuropathy [21]. Sasaki et al. reported a significant reduction in sciatic nerve blood

flow in diabetic rats, a condition that can precipitate neuronal ischemia and subsequent oxidative stress, potentially leading to sensory neuropathy [22].

DCA was first considered a dangerous toxic industrial waste product, then a potential treatment for lactic acidosis. A recent studies found that DCA had anti-cancer effects in experimental animals. It is a compound that has been studied for its therapeutic effects in various areas such as cancer and metabolic diseases. However, the safety of its long-term use is controversial, especially due to its potential toxicity on the esophagus and kidney. Data on long-term use of DCA in studies are more limited. In addition, studies have found that low-dose use is effective in reducing toxicity risks. Our study is also a 4-week study. The doses we used are also in the low range. Studies have shown that DCA synergises with conventional therapies and other repurposed drugs. These findings have led to renewed interest in DCA [15]. Diabetes mellitus has been demonstrated to modify the toxicity of hazardous chemicals in a number of ways, as a result of its own pathophysiology. A study examining one of the high-risk nitrogenous disinfection byproducts, dichloroacetonitrile (DCAN), evaluated the neurotoxicity of DCAN (11, 44, and 88 mg/kg) orally in normoglycemic and streptozotocin (STZ)-induced diabetic rats for 28 days. The results of this study demonstrated that STZ-induced diabetes significantly prolonged the median survival time and total lethality after exposure to DCAN (88 mg/kg) compared to that observed in normoglycemic rats. Additionally, the study revealed that DCAN induced brain oxidative damage by increasing glutathione content and decreasing malondialdehyde levels; however, it was found that it did not cause oxidative damage in diabetic rats. In conclusion, the present study demonstrated that STZ diabetic rats exhibited resistance to DCAN-induced neurotoxicity with the dosage and dosage schedule employed in the 28-day subacute toxicity test [16]. To circumvent the potential for both toxicity and tolerance development, our study evaluated low-dose administration of 5 mg/kg and 10 mg/kg DCA. This study's findings, which were obtained using low doses, support the hypothesis that effective results can be achieved even at low doses.

Furthermore, MDA, a critical marker of oxidative stress, increases in diabetic conditions due to enhanced lipid peroxidation. A decrease in MDA levels is an indicator of reduced oxidative stress. In a study conducted by Gönüllü et al. in 2023, it was shown that MDA levels in diabetic rats decreased significantly after Aesculus hippocastanum (AH) treatment compared to those not receiving AH [23]. In our study, MDA levels were reduced in rats treated with DCA. This means an increase in antioxidants such as glutathione, superoxide dismutase and catalase [24]. DCA has also been shown to induce apoptosis, crucial for removing damaged cells, which underscores the importance of both antioxidant and apoptotic pathways in mitigating inflammation [25]. Our findings are consistent with these observations, as MDA levels were significantly higher in diabetic rats compared to controls but were substantially reduced in the DCA-treated groups.

Several oxidative stress-related molecular pathways are implicated in nerve dysfunction and pathological neuronal changes. These include activation of the polyol pathway, protein kinase C, and the formation of advanced glycation end products. These processes contribute to the degeneration seen in distal symmetrical depletion in the peripheral terminals of nociceptors and intraepidermal nerve fibers. More proximal nerve changes, such as demyelination of myelinated nerve fibers, axonal degeneration, necrosis, schwannopathy, and microangiopathy are also observed [26]. At the onset of type 1 and type 2 diabetes, nerve conduction abnormalities are evident in 29–70% and 45–60% of patients, respectively, with noted improvements in electromyographic readings following hyperglycemia management [27]. Reduced CMAP amplitude, a hallmark of axonal pathology in DN, along with prolonged distal latency and slowed nerve conduction velocity, are indicative of demyelination [28].

In our current study, comparisons between the control group and untreated diabetic rats revealed significantly lower CMAP amplitudes and prolonged distal latencies in untreated diabetic rats. Conversely, DCA treatment improved these conduction abnormalities. Notably, rats receiving 10 mg/kg DCA showed the most pronounced improvements in CMAP amplitude and the shortest distal latencies compared to untreated groups. These improvements suggest enhanced axonal regeneration and remyelination, indicative of an increase in the number of functional muscle units and axons. These observations reinforce the therapeutic potential of DCA in mitigating the impairments associated with diabetic neuropathy, highlighting its role in improving nerve function through mechanisms that likely involve both neuroprotection and enhancement of nerve repair processes.

The inclined plane test, a reliable method for evaluating muscle strength and motor performance, particularly measures the maximal angle a rat can maintain before slipping—a critical indicator in neuropathic disease [29]. Despite the predominance of sensory deficits in DN, motor impairments such as those affecting 1–6% of the population, can lead to significant clinical consequences including muscle atrophy, particularly of the extensor digitorum brevis (EDB), alterations in foot posture, and severe outcomes such as ulcers and amputation [30–32]. Our findings revealed that diabetic rats had a markedly lower inclination angle compared to the control group, indicating reduced motor capabilities. However, rats

treated with DCA exhibited significantly improved motor function, maintaining their position at higher inclination angles.

Fibroblast growth factor-21 (FGF-21) has been identified as a potent metabolic regulator, influencing lipid, glucose, and energy metabolism. Initially discovered for its role in enhancing glucose uptake in adipocytes, FGF-21 has also shown protective effects against diet-induced obesity in transgenic mice [33, 34]. Further studies have validated the beneficial impacts of FGF-21 on metabolic parameters without notable side effects, positioning it as a promising therapeutic for type 2 diabetes mellitus (T2DM) [33–36]. Interestingly, a 2019 study by Min et al. demonstrated that DCA administration could induce hepatic FGF-21 mRNA expression [37]. Consistent with these findings, our study observed elevated plasma levels of FGF-21 in diabetic rats, which were further increased with DCA treatment.

Transforming growth factor-beta (TGF-β), a key player in fibrosis, wound healing, and inflammation, typically sees increased levels following trauma [38]. Our study noted elevated serum TGF-β levels in diabetic rats, indicative of tissue damage and fibrosis. However, these levels significantly decreased following DCA treatment, suggesting a potential anti-fibrotic effect of DCA in diabetic neuropathy.

Hyperglycemia-induced pathways are a primary cause of DN, with literature highlighting the hypoglycemic effects of DCA [39]. It has been reported that DCA can enhance insulin secretion and improve glucose utilization, which aligns with findings from studies on certain saponin-containing plants [40]. In our research, diabetic rats exhibited significantly higher blood sugar levels compared to controls. Although the reduction in glucose levels was not significant in the group receiving 5 mg/kg DCA, a substantial decrease was observed in rats administered 10 mg/kg DCA.

Histopathological examinations have shown that DN often leads to axonal and myelin degeneration, alongside increased perineural thickness due to fibrosis [41]. Our study confirmed these findings, with untreated diabetic rats showing significantly increased perineural thickness compared to controls. However, DCA treatment was associated with reduced perineural thickness, with the most notable decrease observed in rats treated with 10 mg/kg DCA. This reduction likely results from DCA's anti-inflammatory properties, which mitigate fibrosis and thereby reduce perineural thickening.

This comprehensive analysis underscores the multidimensional benefits of DCA in managing DN. By significantly improving nerve conduction properties, enhancing motor functions, and moderating molecular markers of oxidative stress and inflammation, DCA has demonstrated a capacity to address both the underlying metabolic dysfunctions and their neurological consequences. The correlation between improved metabolic regulation, as evidenced by elevated levels of FGF-21, and the reduction in TGF-β levels highlights DCA's role not only in mitigating hyperglycemia but also in ameliorating the associated cellular and tissue damage. This holistic approach to treatment, focusing on both metabolic control and neuroprotection, aligns with the emergent needs for comprehensive therapeutic strategies in DN, suggesting a promising avenue for further research and clinical application. This study not only reinforces the therapeutic potential of DCA but also sets a foundation for future investigations to explore its full spectrum of benefits in chronic diabetic conditions.

This study demonstrates that DCA significantly mitigates symptoms of DN in rats. Treatment with DCA notably enhanced nerve conduction, as evidenced by increased compound muscle action potential (CMAP) amplitudes and reduced distal latencies. Histological assessments revealed that DCA decreased perineural thickness, suggesting a reduction in nerve fibrosis. Biochemically, DCA was associated with lower levels of oxidative stress markers, particularly MDA, highlighting its antioxidant properties. Furthermore, the observed increases in FGF-21 and decreases in TGF-β underscore DCA's anti-inflammatory effects. These findings underscore the potential of DCA as a therapeutic agent for DN and support the need for further research into its clinical applications.

The study, however, is not without limitations. Firstly, it is based on a rat model, and the results may not be directly applicable to humans without comprehensive preclinical testing. Secondly, the number of rats used was constrained due to ethical considerations and limited funding. It is anticipated that future studies, potentially involving larger human cohorts and exploring different DCA dosages, modes of administration, and durations, will provide deeper insights into the protective and therapeutic effects of DCA against DN.

**Abbreviations**

| | |
|---|---|
| DN | Diabetic neuropathy |
| DCA | Dichloroacetic acid |
| STZ | Streptozotocin |
| FGF-21 | Plasma fibroblast growth factor-21 |
| TGF-beta | Transforming growth factor-beta |
| MDA | Malondialdehyde |
| CMAP | Compound muscle action potential |
| LDL | Low-density lipoprotein |
| AH | Aesculus hippocastanum |

**Acknowledgements**
Not applicable.

**Author contributions**
MA, MAE and OE concept and design the manuscript. MA, MAE and OE searched a literature. MAE and OE collected the data. MAE and OE performed all the experiments. MA, MAE and OE analyzed and interpreted the data.

MA was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

**Funding**
None.

**Data availability**
No datasets were generated or analysed during the current study.

## Declarations

**Ethics approval and consent to participate**
All experimental procedures adhered to the ethical guidelines approved by the Institutional Animal Care and Ethical Committee of the University of Science (Ethical Approval Number: 28210104, Date:13.02.2023).

**Clinical trial number**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

Received: 17 November 2024 / Accepted: 20 January 2025
Published online: 22 January 2025

**References**

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalance of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004;27:1047–53.
2. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Result from the international diabetes federation diabetes atlas, 9th edition. Diabetes Res Clin Pract. 2019:157.
3. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res Clin Pract 2022; Vol. 183.
4. Bana S, Kumar N, Sartaj A, et al. *Rubia cordifolia* L. Attenuates Diabetic Neuropathy by Inhibiting Apoptosis and Oxidative Stress in Rats. Pharmaceuticals (Basel). 2023;16(11):1586. Published 2023 Nov 9. https://doi.org/10.3390/ph16111586.
5. Sing R, Kishore I, Kaur N. Diabetic Peripheral Neuropathy: current perspective and future directions. Pharmacolog Res. 2014;80:21–35.
6. Feldman EL, Callaghan BC, Pop-Busui R, Zochodne DW, Wright DE, Bennett DL, et al. Diabetic neuropathy. Nat Rev Dis Primers. 2019;5(1):42.
7. Callaghan BC, Kerber KA, Lisabeth LL, Morgenstern LB, Longoria R, Rodgers A, et al. Role of neurologists and diagnostic tests on the management of distal symmetric polyneuropathy. JAMA Neurol. 2014;71:1143–9.
8. Pfannkuche A, Alhajjar A, Ming A, Walter I, Piehler C, Mertens PR. Prevalence and risk factors of diabetic peripheral neuropathy in a diabetics cohort: Register initiative diabetes and nerves. Endocr Metab Sci 2020;1.
9. Weng YC, Tsai SS, Lyu RK, Chu CC, Ro LS, Liao MF, et al. Diabetic distal symmetrical polyneuropathy: Correlation of clinical, laboratory, and electrophysiologic studies in patients with type 2 diabetes mellitus. J Diabetes Res 2020:2020.
10. Gonullu E, Dagistan G, Özsezer Y, Erdogan MA, Erbas O. Aesculus hippocastanum alleviates diabetic neuropathy by reducing MMP-9 and MMP-10 levels. Int J Pharmacol. 2023;19:862–71.
11. Huang L, Zhang Y, Wang Y, Shen X, Yan S. Diabetic Peripheral Neuropathy is Associated with higher systolic blood pressure in adults with type 2 diabetes with and without hypertension in the Chinese Han Population. Can J Diabetes. 2020;44(7):615–23. https://doi.org/10.1016/j.jcjd.2019.11.005.
12. Schoonjans C, Joudiou N, Brusa D, Corbet C, Feron O, Gallez B. Acidosis-induced metabolic reprogramming in tumor cells enhances the anti-proliferative activity of the PDK inhibitor dichloroacetate. Cancer Lett. 2020;470:18–28.
13. Gang BP, Rooke M, Sun RC, Banskota S, Mishra S, Dahlstrom JE, Blackburn AC. Pyruvate dehydrogenase kinase expression profile is a biomarker for cancer sensitivity to dichloroacetate-mediated growth inhibition. bioRxiv 2023;12.
14. Oh CJ, Ha CM, Choi YK, et al. Pyruvate dehydrogenase kinase 4 deficiency attenuates cisplatin-induced acute kidney injury. Kidney Int. 2017;91(4):880–95. https://doi.org/10.1016/j.kint.2016.10.011.
15. Koltai T, Fliegel L. Dichloroacetate for Cancer Treatment: some facts and many doubts. Pharmaceuticals (Basel). 2024;17(6):744. https://doi.org/10.3390/ph17060744. Published 2024 Jun 6.
16. Li F, Dong Y, Shen H, et al. Tolerance to dichloroacetonitrile-induced neurotoxicity in streptozotocin-induced diabetic rats. Environ Toxicol Pharmacol. 2017;56:61–7. https://doi.org/10.1016/j.etap.2017.08.037.
17. Rivlin AS, Tator CH. Objective clinical assessment of motor function after experimental spinal cord injury in the rat. J Neurosurg. 1977;47(4):577–81.
18. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by Thiobarbituric Acid reaction. Anal Biochem. 1979;95:351–8.
19. Bana S, Gaur P, Kumar N, Goel R. Lett Appl Nanobiosci. 2020;9(1):853–60.
20. Babizhayev MA, Strokov IA, Nosikov VV, Savel'yeva EL, Sitnikov VF, Yegorov YE, et al. The role of oxidative stress in diabetic neuropathy: generation of free radical species in the glycation reaction and gene polymorphisms encoding antioxidant enzymes to genetic susceptibility to diabetic neuropathy in population of type I diabetic patients. Cell Biochem Biophys. 2015;71:1425–43.
21. Sasaki H, Schmelzer JD, Zollman PJ, Low PA. Neuropathology and blood flow of nerve, spinal roots and dorsal root ganglia in longstanding diabetic rats. Acta Neuropathol. 2002;93:118–28.
22. Ezberci F, Ünal E. Aesculus hippocastanum (aescin, horse chestnut) in the management of hemorrhoidal disease: review. Turk J Colorectal Dis. 2018;28:54–7.
23. Gonullu E, Dagistan G, Ozsezer G, Erdogan Y, Erbas MA. Aesculus hippocastanum alleviates diabetic neuropathy by reducing MMP-9 and MMP-10 levels. Int J Pharmacol. 2023;19:862–71.
24. Valauri-Orton A, Bschorer F, Bernd KK. Dichloroacetate Decreases Cell Health and Activates Oxidative Stress Defense Pathways in Rat Alveolar Type II Pneumocytes. Biomed Res Int. 2015;2015:129031.
25. Wong JY, Huggins GS, Debidda M, Munshi NC, De Vivo I. Dichloroacetate induces apoptosis in endometrial cancer cells. Gynecol Oncol. 2008;109(3):394–402.
26. Young RJ, Ewing DJ, Clarke BF. A controlled trial of sorbinil, an aldose reductase inhibitor, in chronic painful diabetic neuropathy. Diabetes. 1983;32:938–42.
27. Akbar DH, Mira SA, Zawawi TH. Subclinical diabetic neuropathy. A common complication in Saudi diabetics. Saudi Med J. 2000;21:433–7.
28. Partanen J, Niskanen L, Lehtinen J, Mervaala E, Siitonen O, Uusitupa M. Natural history of peripheral neuropathy in patients with non-insulin-dependent diabetes mellitus. New Engl J Med. 1995;333:89–94.
29. Dyck PJ, Kratz KM, Karnes JL, Litchy WJ, Klein R, Pach JM, et al. The prevalence by staged severity of various types of diabetic neuropathy, retinopathy, and nephropathy in a population-based cohort: the rochester diabetic neuropathy study. Neurology. 1993;43. https://doi.org/10.1212/WNL.43.4.817.
30. Happich M, Breitscheidel L, Meisinger C, Ulbig M, Falkenstein P, Benter U, et al. Cross-sectional analysis of adult diabetes type 1 and type 2 patients with diabetic microvascular complications from a German retrospective observational study. Curr Med Res Opin. 2007;23(6):1367–74.
31. Andersen H. Motor dysfunction in diabetes. Diabetes Metab Res Rev. 2012;28:89–92.
32. Zochodne DW. Diabetes mellitus and the peripheral nervous system: manifestations and mechanisms. Muscle Nerve. 2007;36:144–66.
33. Kharitonenkov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ, et al. FGF-21 as a novel metabolic regulator. J Clin Invest. 2005;115:1627–35.
34. Kharitonenkov A, Wroblewski VJ, Koester A, Chen YF, Clutinger CK, Tigno XT, et al. The metabolic state of diabetic monkeys is regulated by fibroblast growth factor-21. Endocrinology. 2007;148:774–81.
35. Wente W, Efanov AM, Brenner M, Kharitonenkov A, Köster A, Sandusky GE, et al. Fibroblast growth factor-21 improves pancreatic β-cellfunction and survival by activation of extracellular signal–regulated kinase 1/2 and akt signalingp athways. Diabetes. 2006;55:2470–8.
36. Min BK, Oh CJ, Park S, Lee JM, Go Y, Park BY, et al. Therapeutic effect of dichloroacetate against atherosclerosis via hepatic FGF21 induction mediated by acute AMPK activation. Exp Mol Med. 2019;30(10):1–12.

37. Pscherer S, Freude T, Forst T, Nussler AK, Braun KF, Ehnert S. Anti-diabetic treatment regulates pro-fibrotic TGF-β serum levels in type 2 diabetics. Diabetol Metab Syndr. 2013;31(1):48.
38. Nall AV, Brownlee RE, Colvin CP, Schultz G, Fein D, Cassisi NJ, et al. Transforming growth factor beta 1 improves wound healing and random flap survival in normal and irradiated rats. Arch Otolaryngol Head Neck Surg. 1996;122:171–7.
39. Avci G, Küçükkurt I, Akkol EK, Yeşilada E. Effects of escin mixture from the seeds of Aesculus hippocastanum on obesity in mice fed a high fat diet. Pharm Biol. 2010;48:247–52.
40. Choudhary N, Khatik GL, Suttee A. The possible role of saponin in type-II diabetes-A review. Curr Diabetes Rev. 2021;17:107–21.
41. Cavusoglu T, Karadeniz T, Cagiltay E, Karadeniz M, Yigitturk G, Acikgoz E, et al. The protective effect of losartan on diabetic neuropathy in a diabetic rat model. Exp Clin Endocrinol Diabetes. 2015;123:479–84.

## Publisher's note