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Effects of pulsed magnetic field on motor nerve conduction velocity in non-diabetic and diabetic rotenone-treated rats

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ABSTRACT: This study evaluated changes in motor nerve conduction velocity (MNCV) over a four-week follow-up period in non-diabetic and diabetic rats exposed to systemic rotenone and investigated the potential modulatory effect of pulsed magnetic field (PMF) application. Eight experimental groups were included: control (C), control+PMF (C+PMF), control+rotenone (C+ROT), control+rotenone+PMF (C+ROT+PMF), diabetes mellitus (DM), diabetes mellitus+PMF (DM+PMF), diabetes mellitus+rotenone (DM+ROT), and diabetes mellitus+rotenone+PMF (DM+ROT+PMF). The final analysis included 80 rats, with 10 animals in each group. MNCV was measured at study baseline and at the end of weeks 1, 2, 3, and 4. Rotenone-treated animals were also monitored qualitatively for Parkinsonian-like motor signs. Rotenone exposure was associated with a progressive reduction in MNCV. Compared with the C group, MNCV was significantly lower in the C+ROT group from week 1 through week 4. Similarly, MNCV was significantly lower in the DM+ROT group than in the DM group during weeks 2–4. MNCV was significantly higher in the C+PMF group than in the C group at week 4 and in the DM+PMF group than in the DM group during weeks 2–4. No significant differences were detected between the C+ROT and C+ROT+PMF groups or between the DM+ROT and DM+ROT+PMF groups at any measurement time. Overall, systemic rotenone exposure was associated with impaired peripheral motor nerve conduction in both non-diabetic and diabetic rats. The applied PMF protocol was associated with time-dependent changes in MNCV under non-rotenone conditions but did not significantly attenuate rotenone-associated impairment in peripheral motor nerve conduction.
KEYWORDS: Diabetes mellitus; motor nerve conduction velocity; peripheral neuropathy; pulsed magnetic field; rotenone.

1. INTRODUCTION

Parkinson's disease is a progressive neurodegenerative disorder characterized primarily by the loss of dopaminergic neurons in the substantia nigra and reduced striatal dopamine levels. Clinically, it is associated with motor manifestations including bradykinesia, rigidity, tremor, and postural instability [1,2]. However, current evidence indicates that Parkinson's disease is not limited to the dopaminergic system. α-Synuclein accumulation, mitochondrial dysfunction, oxidative stress, neuroinflammation, and peripheral nervous system involvement may also contribute to the disease process [2,3]. Therefore, evaluating peripheral nerve function in Parkinson's disease and related experimental conditions may help clarify the effects of these pathological processes beyond the central nervous system.

Rotenone is a lipophilic pesticide widely used to establish experimental models that reproduce selected pathological and motor features associated with Parkinson's disease. It inhibits mitochondrial complex I, thereby impairing cellular energy metabolism, increasing oxidative stress, and potentially damaging dopaminergic neurons [4]. Chronic rotenone administration in rats has been shown to produce Parkinsonian-like motor signs, nigrostriatal dopaminergic degeneration, and α-synuclein accumulation [4–6]. However, rotenone may also exert direct peripheral and systemic neurotoxic effects. Binienda et al. reported that chronic rotenone exposure caused peripheral neuropathy associated with reduced motor nerve conduction velocity and sciatic nerve damage [7]. Similarly, Sarkar et al. demonstrated electrophysiological and histopathological abnormalities in peripheral nerves following rotenone exposure, together with dopaminergic toxicity [8]. Therefore, reductions in peripheral nerve conduction following rotenone administration should not be interpreted solely as a consequence of central Parkinsonian pathology.

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Diabetes mellitus (DM) is a chronic metabolic disorder that frequently affects the peripheral nervous system. Diabetic peripheral neuropathy is among its most common long-term complications. Hyperglycemia, oxidative stress, mitochondrial dysfunction, neuroinflammation, microvascular damage, and abnormalities in axonal metabolism contribute to its development [9–11]. Reduced nerve conduction velocity is a characteristic electrophysiological finding in diabetic neuropathy, and decreased motor nerve conduction velocity has been demonstrated in streptozotocin-induced experimental diabetes [12]. Motor nerve conduction velocity is therefore a suitable functional parameter for evaluating peripheral nerve impairment under diabetic conditions.

Diabetes and Parkinson's disease share several pathophysiological features, including oxidative stress, mitochondrial dysfunction, inflammation, and metabolic abnormalities. Recent meta-analyses have reported associations between diabetes and both the risk and progression of Parkinson's disease [13,14]. Salem et al. also used a combined diabetes and rotenone model to investigate the neuroprotective effects of semaglutide and metformin in diabetic rats exposed to rotenone [15]. Thus, combined diabetes and rotenone models have been investigated previously. However, changes in peripheral motor nerve conduction over time under these combined experimental conditions, particularly in relation to pulsed magnetic field application, remain insufficiently characterized.

Experimental and clinical studies have suggested that pulsed magnetic field (PMF) application may have beneficial or modulatory effects on peripheral nerve regeneration, neuropathic pain, microcirculation, and nerve conduction parameters [16–19]. Nevertheless, whether PMF modifies changes in motor nerve conduction velocity in non-diabetic and diabetic rats exposed to rotenone remains unclear.

This study aimed to evaluate changes in motor nerve conduction velocity over a four-week follow-up period in non-diabetic and diabetic rats with or without rotenone treatment and to investigate the potential modulatory effect of PMF application. We hypothesized that rotenone treatment would be associated with a progressive reduction in motor nerve conduction velocity and that PMF might modify the pattern of these changes. Motor nerve conduction velocity was defined as the primary outcome of the study.

2. RESULTS

During the model-induction period, 13 animals died: three in the DM group, three in the C+ROT group, two in the C+ROT+PMF group, three in the DM+ROT group, and two in the DM+ROT+PMF group. No mortality occurred in the C, C+PMF, or DM+PMF groups. Following model induction, 85 animals survived. As the prespecified analytical sample size was 10 animals per group, five surviving reserve animals were not selected for the final analytical cohort. Selection was performed by simple lottery independently of physiological measurements, motor observations, treatment responses, and MNCV findings. Thus, the final analysis included 80 animals, with 10 animals in each experimental group. No additional mortality or missing MNCV measurements occurred during the four-week follow-up period.

During rotenone administration, the rotenone-treated animals exhibited qualitative motor abnormalities, including slowing of movement, difficulty initiating movement, postural impairment, and increased resistance to passive movement. These observations were considered supportive phenotypic findings and were not evaluated using a formal quantitative scoring system.

Motor nerve conduction velocity values at study baseline and during the four-week follow-up period are presented in Table 1. The pairwise comparisons relevant to the study objectives are shown in Figure 1.

Separate one-way ANOVAs demonstrated no significant difference among the non-diabetic groups at study baseline, $F(3,36)=0.093$, $p=0.9634$. Significant between-group differences were observed at week 1, $F(3,36)=16.379$, $p<0.0001$; week 2, $F(3,36)=98.712$, $p<0.0001$; week 3, $F(3,36)=287.082$, $p<0.0001$; and week 4, $F(3,36)=808.788$, $p<0.0001$.

Tukey-adjusted pairwise comparisons showed that MNCV was significantly higher in the C+PMF group than in the C group at week 4 ($p<0.01$), whereas no significant differences were detected at the other measurement times (Figure 1A). Compared with the C group, the C+ROT group had significantly lower MNCV at week 1 ($p<0.01$) and at weeks 2, 3, and 4 (all $p<0.001$; Figure 1B). No significant differences were detected between the C+ROT and C+ROT+PMF groups at any measurement time (Figure 1C).

Among the diabetic groups, no significant difference was detected at study baseline, $F(3,36)=0.603$, $p=0.6171$. Significant between-group differences were observed at week 1, $F(3,36)=3.266$, $p=0.0323$; week 2, $F(3,36)=96.202$, $p<0.0001$; week 3, $F(3,36)=284.763$, $p<0.0001$; and week 4, $F(3,36)=435.633$, $p<0.0001$.

Tukey-adjusted pairwise comparisons showed that MNCV was significantly higher in the DM+PMF group than in the DM group at week 2 ($p<0.01$), week 3 ($p<0.05$), and week 4 ($p<0.001$), whereas no significant

differences were detected at study baseline or week 1 (Figure 1D). Compared with the DM group, the DM+ROT group had significantly lower MNCV at weeks 2, 3, and 4 (all $p<0.001$), whereas no significant differences were detected at study baseline or week 1 (Figure 1E). No significant differences were detected between the DM+ROT and DM+ROT+PMF groups at any measurement time (Figure 1F).

Table 1. Motor nerve conduction velocity values in the experimental groups during the four-week follow-up period

	Groups participating in experiments							
Time	C (m/s)	C+PMF (m/s)	C+ROT (m/s)	C+ROT+ PMF (m/s)	DM (m/s)	DM+PM F (m/s)	DM+RO T (m/s)	DM+RO T+PMF (m/s)
Study baseline	49.13 ± 1.35	49.90 ± 1.47	48.70 ± 1.77	49.20 ± 1.87	44.79 ± 1.49	46.10 ± 1.02	43.70 ± 1.04	44.60 ± 1.52
Week 1	50.57 ± 1.11	49.57 ± 0.75	44.66 ± 1.97	45.77 ± 1.68	37.66 ± 0.75	37.83 ± 0.74	34.99 ± 0.61	36.93 ± 0.77
Week 2	50.34 ± 0.94	50.65 ± 0.87	33.75 ± 1.15	32.50 ± 1.06	35.68 ± 0.60	37.36 ± 0.52	24.87 ± 0.69	27.68 ± 0.65
Week 3	49.59 ± 1.02	50.30 ± 1.21	23.12 ± 0.74	23.77 ± 0.46	34.75 ± 0.70	36.07 ± 0.56	17.21 ± 0.62	18.13 ± 0.54
Week 4	48.64 ± 0.87	50.77 ± 0.67	19.02 ± 0.38	20.11 ± 0.39	33.27 ± 0.74	36.60 ± 0.65	13.47 ± 0.49	15.66 ± 0.29

Data are presented as mean ± standard error of the mean (SEM), expressed in m/s; n=10 animals per group. Study baseline refers to the MNCV measurement performed after confirmation of diabetes and before the initiation of PMF exposure. Rotenone administration had already begun in the ROT-treated groups at the time of the study-baseline measurement. C: control; DM: diabetes mellitus; MNCV: motor nerve conduction velocity; PMF: pulsed magnetic field; ROT: rotenone.

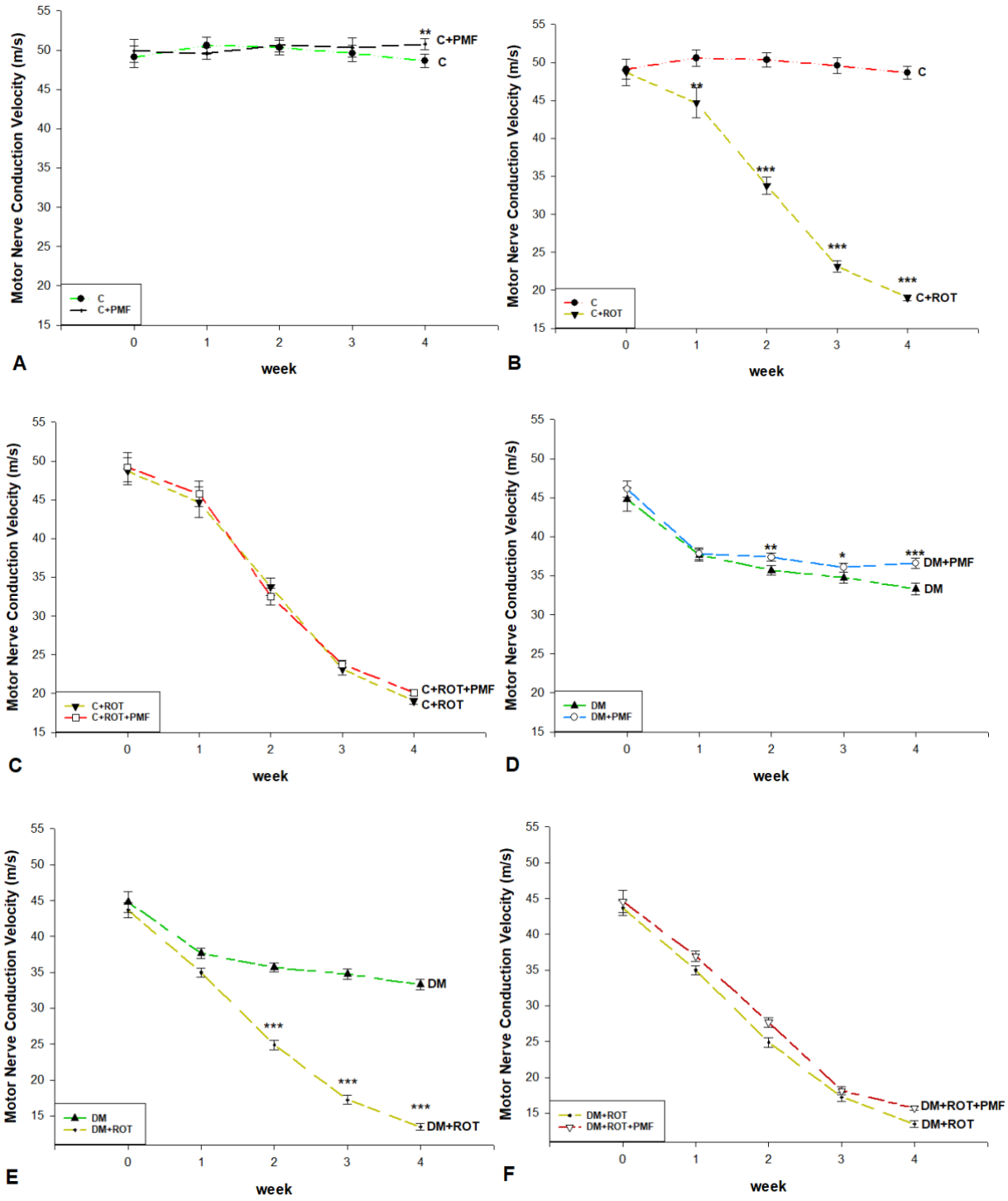


Figure 1. Pairwise comparisons of motor nerve conduction velocity between the experimental groups during the four-week follow-up period. (A) C and C+PMF; (B) C and C+ROT; (C) C+ROT and C+ROT+PMF; (D) DM and DM+PMF; (E) DM and DM+ROT; and (F) DM+ROT and DM+ROT+PMF. Data are presented as mean ± standard error of the mean (SEM), with 10 animals in each group. Between-group differences at each measurement time were evaluated using separate one-way ANOVAs followed by Tukey's honestly significant difference post hoc test. C: control; DM: diabetes mellitus; MNCV: motor nerve conduction velocity; PMF: pulsed magnetic field; ROT: rotenone. *p<0.05, **p<0.01, and ***p<0.001.

3. DISCUSSION

This study evaluated changes in motor nerve conduction velocity over a four-week follow-up period in non-diabetic and diabetic rats exposed to rotenone and investigated the potential modulatory effect of PMF application. Rotenone administration was associated with lower MNCV values over the follow-up period. In non-diabetic animals, MNCV was significantly lower in the C+ROT group than in the C group from week 1 through week 4. Similarly, in diabetic animals, MNCV was significantly lower in the DM+ROT group than in the DM group during weeks 2-4. MNCV was significantly higher in the C+PMF group than in the C group at week 4 and in the DM+PMF group than in the DM group during weeks 2-4. However, no significant differences were detected between the C+ROT and C+ROT+PMF groups or between the DM+ROT and DM+ROT+PMF groups at any measurement time. Overall, the response to PMF varied according to the experimental condition and measurement time and did not represent a uniform or sustained electrophysiological benefit.

Although Parkinson's disease is primarily characterized by degeneration of the nigrostriatal dopaminergic system, increasing evidence indicates that its pathological manifestations may extend beyond the central nervous system. Mitochondrial dysfunction, oxidative stress, neuroinflammation, and α-synuclein-related pathology are among the major processes implicated in the disease [1-3]. Peripheral neuropathy has also been reported in patients with Parkinson's disease and may contribute to gait and balance impairment [20,21]. These observations support the clinical relevance of evaluating peripheral nerve function in Parkinsonian conditions. However, peripheral electrophysiological alterations observed in rotenone-treated animals should be interpreted with caution, as they may reflect both Parkinsonian-associated peripheral involvement and the direct systemic neurotoxicity of rotenone.

Rotenone is widely used to reproduce selected pathological and motor features associated with Parkinson's disease. Because of its lipophilic properties, rotenone crosses biological membranes and systemically inhibits mitochondrial complex I, thereby impairing cellular energy metabolism and increasing oxidative stress [4-6]. Betarbet et al. demonstrated that chronic systemic rotenone exposure reproduces several neuropathological features associated with Parkinson's disease, whereas Cannon et al. established a reproducible protocol associated with dopaminergic degeneration and progressive motor impairment [4,5]. However, systemic rotenone exposure may also affect neural structures beyond the nigrostriatal system [22,23]. Therefore, although rotenone-treated animals may exhibit selected Parkinsonian-like features, peripheral electrophysiological abnormalities in this model may also arise from rotenone's direct toxic effects on peripheral nerves.

The significantly lower MNCV observed in the C+ROT group than in the C group from week 1 onwards indicates that systemic rotenone exposure was associated with impaired peripheral motor nerve function. This finding is consistent with previous experimental studies reporting peripheral electrophysiological abnormalities and sciatic nerve injury following rotenone exposure [9,10]. Inhibition of mitochondrial complex I, reduced ATP production, increased reactive oxygen species generation, and subsequent disturbances in axonal or myelin function may contribute to this reduction [4-6,22,23]. However, axonal morphology, myelin integrity, mitochondrial function, and oxidative stress were not directly assessed in the present study. Therefore, the observed MNCV reduction should be interpreted as a peripheral electrophysiological alteration associated with systemic rotenone exposure rather than as definitive evidence of axonal degeneration, demyelination, or Parkinson's disease-related peripheral neuropathy.

Diabetic peripheral neuropathy is a common chronic complication of diabetes and is associated with hyperglycemia, oxidative stress, mitochondrial dysfunction, neuroinflammation, microvascular impairment, and abnormalities in axonal metabolism [9-11]. Reduced nerve conduction velocity is a characteristic electrophysiological manifestation of diabetic neuropathy. Coppey et al. reported that impaired arteriolar vasodilation supplying the sciatic nerve may contribute to slowed motor nerve conduction in streptozotocin-induced diabetic rats [12]. In the present study, the DM group showed a gradual decline in MNCV over the four-week follow-up period. This pattern is consistent with impaired peripheral nerve function in streptozotocin-induced diabetes; however, the metabolic, vascular, and structural mechanisms underlying this change were not directly assessed.

Diabetes and Parkinson's disease share several pathophysiological features, including oxidative stress, mitochondrial dysfunction, inflammation, and metabolic abnormalities [13,14]. These common mechanisms provide a biological rationale for investigating the effects of systemic rotenone exposure under diabetic conditions. In the present study, MNCV was significantly lower in the DM+ROT group than in the DM group at weeks 2, 3, and 4, indicating that rotenone exposure was associated with additional impairment of

peripheral motor nerve conduction in diabetic animals. Rotenone-induced mitochondrial toxicity may act alongside the metabolic, oxidative, and microvascular abnormalities associated with diabetes; however, these mechanisms were not directly assessed. Importantly, the present pairwise comparisons do not establish a diabetes × rotenone interaction or demonstrate that diabetes specifically exacerbated the effect of rotenone.

The effects of PMF on peripheral nerve function have been investigated in experimental studies. De Pedro et al. reported that pulsed electromagnetic field exposure may promote peripheral nerve regeneration and induce changes at motor end plates [16]. Lei et al. demonstrated that a 15-Hz protocol alleviated neuropathic manifestations in a streptozotocin-induced model of diabetic peripheral neuropathy [24], while Mert et al. reported improvements in mechanical threshold and thermal latency following PMF application in rats with streptozotocin-induced diabetic neuropathy [25]. In the present study, no significant difference in MNCV was detected between the DM and DM+PMF groups at study baseline or week 1, whereas MNCV was significantly higher in the DM+PMF group at weeks 2, 3, and 4. These findings suggest that the response to PMF emerged after the first week of exposure and persisted during the remaining follow-up measurements. Previous findings suggest that the biological effects of PMF may vary according to stimulation frequency, exposure duration, and other application parameters [25,26]. However, because only one PMF protocol was evaluated and the underlying mechanisms were not directly assessed, these findings should be interpreted as a condition- and measurement-time-dependent electrophysiological association rather than evidence of uniform neuroprotection.

Clinical studies evaluating PMF in diabetic neuropathy have reported heterogeneous results. Graak et al. observed reductions in neuropathic pain and improvements in nerve-conduction parameters following low-frequency PMF application, while Musaev et al. reported beneficial effects on peripheral nerve function, particularly in patients with earlier-stage diabetic polyneuropathy [27,28]. Battecha similarly reported improvements in pain and peroneal nerve-conduction velocity when PMF was added to a conventional physical therapy program [18]. In contrast, Weintraub et al. found no significant improvement in the primary pain-intensity or sleep-disruption outcomes in a multicenter, randomized, double-blind, sham-controlled trial, although exploratory findings suggested possible neurobiological effects requiring further investigation [17]. Differences among these studies may be related to stimulation parameters, exposure duration, disease severity, study design, and the selected clinical or electrophysiological outcomes [29]. Therefore, although PMF may have potential as an adjunctive approach in diabetic neuropathy, the consistency and optimal conditions of its clinical effects remain uncertain. This heterogeneity is compatible with the present findings, in which differences between the DM and DM+PMF groups were observed during weeks 2–4 but were not present at study baseline or week 1.

No significant difference in MNCV was detected between the C and C+PMF groups at study baseline or during weeks 1–3. However, MNCV was significantly higher in the C+PMF group at week 4. Together with the differences observed between the DM and DM+PMF groups during weeks 2–4, this finding suggests that the electrophysiological response to PMF may depend on the underlying experimental condition and duration of exposure. Because microcirculatory, metabolic, inflammatory, and regenerative mechanisms were not directly assessed, these findings should be interpreted as electrophysiological associations rather than evidence of a specific neuroprotective mechanism.

In the rotenone-treated groups, PMF exposure was not associated with a statistically significant difference in MNCV. No significant differences were detected between the C+ROT and C+ROT+PMF groups or between the DM+ROT and DM+ROT+PMF groups at any measurement time. These findings indicate that the applied PMF protocol did not significantly attenuate the MNCV impairment associated with rotenone exposure. Rotenone-induced mitochondrial complex I inhibition, impaired cellular energy production, and oxidative injury may have limited the response to PMF [4–6]. Nevertheless, because mitochondrial function, oxidative stress, inflammation, and peripheral nerve structure were not directly assessed, these mechanisms should be considered possible explanations rather than demonstrated causes.

The biological effects of PMF may vary considerably according to frequency, magnetic field intensity, waveform, session duration, total exposure period, and the characteristics of the target tissue [24–30]. Markov emphasized that biological responses to pulsed magnetic fields are determined by the interaction between the physical characteristics of the field, the exposure protocol, and the biological properties of the exposed tissue [30]. In the present study, frequencies of 1, 10, 20, and 40 Hz were applied sequentially at a magnetic field intensity of 1.5 mT. The differences observed in the C+PMF and DM+PMF groups, together with the absence of significant differences in the rotenone-treated comparisons, suggest that the effect of PMF may depend on both the underlying experimental condition and the selected stimulation protocol. However, because only one

PMF protocol was evaluated, the relative contributions of individual frequencies, field intensity, and exposure duration could not be determined. Further studies directly comparing different PMF parameters are required to identify protocols that may be more effective for diabetic- and rotenone-associated peripheral nerve dysfunction.

A strength of the present study is the repeated assessment of MNCV at weekly intervals over a four-week period in non-diabetic and diabetic rats exposed to rotenone, together with the evaluation of PMF under the same experimental conditions. Previous studies have separately investigated peripheral neuropathy in Parkinson's disease, rotenone-based experimental models, diabetic neuropathy, and PMF applications [20-26]. However, studies evaluating the temporal pattern of peripheral motor nerve conduction across combined diabetes, systemic rotenone exposure, and PMF conditions remain limited. Therefore, the contribution of the present study lies in characterizing changes in MNCV over time across these experimental conditions rather than in proposing a completely novel disease model or treatment approach.

The present study has several limitations. First, the rotenone-associated phenotype was supported by qualitative observations of Parkinsonian-like motor signs but was not validated using formal behavioral scoring, dopaminergic neurochemical measurements, or histopathological markers. Therefore, the findings should be interpreted in the context of systemic rotenone exposure rather than as definitive evidence obtained from a fully validated Parkinson's disease model. Second, MNCV was the principal electrophysiological outcome, and the structural, biochemical, and molecular mechanisms underlying the observed changes were not directly evaluated. Third, only one PMF protocol was investigated; therefore, the effects of different frequencies, magnetic field intensities, waveforms, and exposure durations could not be determined. Finally, separate citrate-buffer and rotenone-vehicle control groups were not included, and possible nonspecific effects associated with the vehicles cannot be completely excluded. Future studies that combine electrophysiological measurements with formal behavioral, histopathological, neurochemical, and mechanistic assessments and directly compare different PMF protocols may further clarify these findings.

4. CONCLUSION

Systemic rotenone administration was associated with lower motor nerve conduction velocity in both non-diabetic and diabetic rats during the four-week follow-up period. MNCV was significantly higher in the C+PMF group than in the C group at week 4 and in the DM+PMF group than in the DM group during weeks 2–4. However, no significant differences were detected between the C+ROT and C+ROT+PMF groups or between the DM+ROT and DM+ROT+PMF groups at any measurement time. These findings indicate that the applied PMF protocol was associated with time- and condition-dependent electrophysiological changes in animals not exposed to rotenone, but did not significantly attenuate rotenone-associated impairment in peripheral motor nerve conduction. Further studies incorporating formal behavioral assessment, histopathological and neurochemical validation, and comparisons of different PMF protocols are required to clarify the underlying mechanisms and potential translational relevance of these findings.

5. MATERIALS AND METHODS

5.1. Experimental animals and ethical approval

Male Wistar albino rats weighing 250-280 g were obtained from the Cukurova University Health Sciences Experimental Application and Research Center. Before the initiation of the experimental procedures, the animals were acclimatized to laboratory conditions for one week. During the acclimatization and experimental periods, the rats were housed under controlled environmental conditions at 22-23 °C and 40-50% relative humidity, with a 12 h light/12 h dark cycle. Standard laboratory chow and water were provided ad libitum.

All experimental procedures were approved by the Cukurova University Animal Experiments Local Ethics Committee (approval date: 26 September 2024; decision no. 4) and were conducted in accordance with accepted ethical principles for laboratory animal research. The ethics committee approval covered all animals entering the experimental procedures, including the prospectively allocated reserve animals.

5.2. Drugs and chemicals

Streptozotocin (STZ; catalog no. S0130; CAS no. 18883-66-4) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Rotenone (catalog no. T2970; CAS no. 83-79-4) was obtained from TargetMol (Boston, MA, USA). Reagent-grade dimethyl sulfoxide (DMSO; catalog no. DMS555; product code DMS555.250; CAS no. 67-

68-5) was obtained from BioShop Canada Inc. (Burlington, Ontario, Canada). Sunflower oil (Helianthus annuus seed oil; catalog no. S5007; CAS no. 8001-21-6) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Sevoflurane inhalation solution (Sevorane, 100%; AbbVie Tıbbi İlaçlar Sanayi ve Ticaret Ltd. Şti., Istanbul, Türkiye) was used for anesthesia.

Immediately before administration, STZ was freshly dissolved in cold 0.1 M citrate buffer adjusted to pH 4.6. The citrate buffer was prepared using citric acid monohydrate and trisodium citrate dihydrate and was maintained at approximately 4 °C until use. Rotenone was freshly prepared each day at a final concentration of 2 mg/mL in a vehicle consisting of 2% DMSO and 98% sunflower oil. The rotenone solution was protected from light and used on the day of preparation.

5.3. Sample size, randomization, experimental groups, and animal flow

The final sample size was determined in consultation with a faculty member from the Department of Biostatistics, Faculty of Medicine, Cukurova University, taking into account the experimental design and the planned statistical analyses. Based on this consultation, a minimum final sample of eight animals per group was considered sufficient. To improve the robustness of the findings, the prespecified analytical sample size was set at 10 animals per group.

Because mortality was anticipated during STZ- and/or rotenone-based model induction, three additional reserve animals were prospectively allocated to each group receiving STZ and/or rotenone. These reserve animals were included in the initial allocation before any experimental intervention and were not introduced after mortality occurred. Animals were assigned to the experimental groups using a simple lottery method.

Ten animals were initially allocated to each of the control (C) and control+pulsed magnetic field (C+PMF) groups. Thirteen animals were initially allocated to each of the C+ROT, C+ROT+PMF, DM, DM+ROT, DM+PMF, and DM+ROT+PMF groups. Thus, a total of 98 rats entered the experimental procedures.

The eight experimental groups were defined as follows:

1. Control group (C): No experimental treatment was administered.

2. Control + pulsed magnetic field group (C+PMF): PMF was applied without STZ or rotenone administration.

3. Control + rotenone group (C+ROT): Rotenone, prepared in 2% DMSO and 98% sunflower oil, was administered subcutaneously.

4. Control + rotenone + pulsed magnetic field group (C+ROT+PMF): Rotenone was administered subcutaneously, and PMF was applied.

5. Diabetes mellitus group (DM): STZ dissolved in citrate buffer was administered intravenously through the tail vein.

6. Diabetes mellitus + pulsed magnetic field group (DM+PMF): STZ was administered intravenously through the tail vein, and PMF was applied.

7. Diabetes mellitus + rotenone group (DM+ROT): STZ was administered intravenously through the tail vein, and rotenone was administered subcutaneously.

8. Diabetes mellitus + rotenone + pulsed magnetic field group (DM+ROT+PMF): STZ was administered intravenously through the tail vein, rotenone was administered subcutaneously, and PMF was applied.

The control group received no injection or PMF intervention. Separate citrate-buffer and DMSO–sunflower oil vehicle-control groups were not included in the experimental design.

During the model-induction period, 13 animals died: three in the DM group, three in the C+ROT group, two in the C+ROT+PMF group, three in the DM+ROT group, and two in the DM+ROT+PMF group.

No mortality occurred in the C, C+PMF, or DM+PMF groups. Following model induction, 85 animals survived: 10 each in the C, C+PMF, C+ROT, DM, and DM+ROT groups; 11 each in the C+ROT+PMF and DM+ROT+PMF groups; and 13 in the DM+PMF group.

When more than 10 eligible animals remained in a group, 10 animals were selected for the final analytical cohort using a simple lottery method. This selection was performed independently of physiological measurements, motor observations, treatment responses, and MNCV findings. Consequently, one surviving reserve animal from the C+ROT+PMF group, three from the DM+PMF group, and one from the DM+ROT+PMF group were not selected because the prespecified analytical sample size had already been reached.

The final analytical cohort comprised 80 rats, with 10 animals per experimental group. No additional mortality or exclusion occurred after formation of the final cohort, and complete longitudinal MNCV data from all 80 animals were included in the statistical analyses. The investigators performing the experimental interventions, electrophysiological measurements, and statistical analyses were aware of the group assignments; therefore, blinding was not applied.

5.4. Induction and confirmation of diabetes

Diabetes mellitus (DM) was induced by a single intravenous injection of STZ at a dose of 45 mg/kg through the tail vein. During STZ administration, the animals were anesthetized with sevoflurane delivered through a vaporizer. STZ was freshly dissolved in cold 0.1 M citrate buffer adjusted to pH 4.6 and was administered immediately after preparation [31].

Blood glucose concentrations were measured 72 h after STZ administration using blood obtained from the tail vein and a glucometer (Accuro PM1-300, Plusmed/Trimpeks İth. İhr. Tur. ve Tic. A.Ş., Istanbul, Türkiye). Animals with blood glucose concentrations greater than 250 mg/dL were considered diabetic [32].

5.5. Rotenone administration and assessment of motor signs

Rotenone administration was initiated on experimental day 1, which corresponded to 1 day after STZ administration in the diabetic groups. Rotenone was freshly prepared at a final concentration of 2 mg/mL in a vehicle consisting of 2% DMSO and 98% sunflower oil. Based on a previously described protocol, rotenone was administered subcutaneously in the nuchal region at a dose of 2 mg/kg and an injection volume of 1 mL/kg once daily for 10 consecutive days [33].

Animals receiving rotenone were monitored daily for the development of Parkinsonian-like motor signs. Qualitative observations included bradykinesia, reflected by slowing of spontaneous movement and difficulty initiating movement; postural abnormalities and impaired balance, reflected by difficulty maintaining normal body posture; and rigidity, reflected by increased resistance of the extremities to passive movement. These observations were considered supportive phenotypic findings, and no formal behavioral scoring system was applied. Because central dopaminergic, neurochemical, or histopathological validation was not performed, the animals are described as rotenone-treated rats displaying Parkinsonian-like motor signs rather than as constituting a definitively validated Parkinson's disease model.

5.6. Pulsed magnetic field exposure

The pulsed magnetic field was generated by two Helmholtz coils, each 60 cm in diameter (radius: 30 cm), connected to a programmable signal generator manufactured by ILFA Elektronik (Adana, Türkiye). The magnetic field intensity was verified using a gaussmeter equipped with a Hall-effect probe (F.W. Bell Model 6010, Sypris Company, Orlando, FL, USA).

Animals assigned to the PMF groups were exposed to a magnetic field intensity of 1.5 mT for 60 min per day over four consecutive weeks. Each daily exposure session consisted of four sequential 15-min periods at frequencies of 1, 10, 20, and 40 Hz, respectively. The exposure parameters were selected based on previous experimental studies investigating low-frequency and sequential pulsed magnetic field applications in experimental diabetic neuropathy [25,26].

5.7. Experimental timeline and animal monitoring

STZ was administered on experimental day 0. Rotenone administration was initiated on experimental day 1 and continued once daily for 10 consecutive days. Diabetes was confirmed in the STZ-treated groups 72 h after STZ administration. At the same time, the study baseline MNCV measurement was performed in all

groups before the initiation of PMF exposure. PMF exposure was initiated immediately after the baseline MNCV measurement and was continued daily for four consecutive weeks.

Because rotenone administration had begun before the study baseline measurement, the remaining rotenone injections overlapped with the PMF exposure period in both the C+ROT+PMF and DM+ROT+PMF groups. MNCV was measured at study baseline and at the end of weeks 1, 2, 3, and 4.

Body weight and blood glucose concentrations were monitored once weekly, on Fridays, as part of routine clinical follow-up. Blood glucose was measured using blood obtained from the tail vein and an Accuro PM1-300 glucometer. These monitoring variables were not included in the statistical analyses. Animals were also observed daily for mobility, food intake, paresis, general clinical condition, and mortality.

5.8. Motor nerve conduction velocity measurement

Motor nerve conduction velocity was measured using a BIOPAC MP35 data acquisition system (BIOPAC Systems, Inc., Goleta, CA, USA) with bipolar needle recording electrodes and a stimulating electrode. Measurements were performed repeatedly in the same animals at five time points: study baseline, obtained at the experimental time corresponding to diabetes confirmation in the STZ-treated groups and before the initiation of PMF exposure, and at the end of weeks 1, 2, 3, and 4.

During the measurements, the rats were maintained under inhalation anesthesia with 2–3% sevoflurane delivered in oxygen. The right hindlimb was shaved before electrode placement, and body temperature was maintained at approximately 37 °C throughout the recordings.

The recording electrodes were positioned over the biceps femoris muscle, and the ground electrode was placed on the tail. The sciatic nerve was stimulated percutaneously using supramaximal square-wave electrical pulses with a duration of 0.1 ms. Stimulation was first applied at the distal site near the muscle and then at a proximal site located 3 cm above the distal stimulation point.

Distal and proximal response latencies were recorded. MNCV was calculated by dividing the distance between the proximal and distal stimulation sites by the difference between the corresponding proximal and distal response latencies and was expressed in m/s.

5.9. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 20.0, and graphs were generated using SigmaPlot version 12.5. Data are presented as mean ± standard error of the mean (SEM). A p-value of <0.05 was considered statistically significant.

To evaluate between-group differences at individual measurement time points, separate one-way ANOVAs were performed at study baseline and at weeks 1-4. The non-diabetic groups (C, C+PMF, C+ROT, and C+ROT+PMF) and the diabetic groups (DM, DM+PMF, DM+ROT, and DM+ROT+PMF) were analyzed separately. When the omnibus ANOVA was significant, pairwise comparisons were performed using Tukey's honestly significant difference post hoc test.

Complete MNCV data were available for all 80 animals at all five measurement time points; therefore, no missing-data imputation was required. Statistical analyses were restricted to the longitudinal MNCV dataset. Body weight and blood glucose monitoring variables were not included in the statistical analyses.

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