Journal of Integrative Neuroscience

J. Integr. Neurosci. **2026**; 25(7): 52589
https://doi.org/10.31083/JIN52589

Original Research

Evaluation of the Behavioral and Histopathological Outcomes of Two Cuprizone Administration Routes in a Mouse Model of Multiple Sclerosis

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Academic Editor: Ulises Gomez-Pinedo
Submitted: 4 April 2026 Revised: 28 May 2026 Accepted: 8 June 2026 Published: 27 July 2026

Abstract

Background: The cuprizone (CPZ) model has been used to study de- and re-myelination in mice; Previous studies have used different routes for CPZ intoxication, but the results were highly variable. **Methods:** Here, we compared demyelination induced by the administration of 0.3% CPZ mixed with ground rodent chow and a 400 mg/kg CPZ suspension in carboxymethyl cellulose (CMC) via oral gavage in SWR/J mice. The mice were assigned into a control group, a CPZ diet group, and an oral CPZ groups (n = 6 mice/group). Rotarod, hanging wire, and open field tests were performed to evaluate locomotor activity. Histological analyses were conducted on the corpus callosum (CC) of the brain to determine myelin loss and on the liver to assess the toxicity of CPZ. **Results:** The administration of CPZ with ground rodent chow significantly reduced body weight gains, grip strength, and motor coordination performance during the 5-week demyelination period. In contrast, all analyzed parameters were less different in mice receiving CPZ via oral gavage. However, both CPZ routes significantly reduced myelin content in the CC. Additionally, the oral administration of CPZ had strong effects on liver toxicity compared to that of CPZ diet feeding. This study showed that both routes of CPZ intoxication could induce reproducible and consistent demyelination changes within the CC of the mouse brain, with the CPZ diet being more effective based on all analytical parameters and having fewer toxic effects on the liver compared with oral CPZ. **Conclusions:** In conclusion, the resultsassist in the selection of a suitable multiple sclerosis (MS) model for investigating disease mechanisms and therapies.

Keywords: multiple sclerosis; animal models; cuprizone; demyelinating diseases; motor activity

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory, neurodegenerative disease of the central nervous system (CNS), and between 2.1 and 2.5 million people worldwide suffer from MS. This makes it the most common cause of non-traumatic neurological impairment in young adults [1,2]. Common markers include demyelination, the loss of oligodendrocytes, and neuronal and axonal degeneration, which are also accompanied by very different clinical complications and histopathological manifestations depending on the affected region and stage of disease [3,4]. Demyelination is the main pathological feature of MS that distinguishes it from other inflammatory and neurodegenerative diseases [5]. Demyelination is often followed by remyelination, but this is usually not complete [6,7]. Understanding how remyelination fails and finding ways to restore myelination is a major problem in MS research. To develop new therapeutic techniques that target demyelination and remyelination, a thorough understanding of the complex interactions that occur during these processes is required [8]. Animal models are important tools to study the underlying mechanisms of demyelination and remyelination, as well as the associated cellular responses and interactions, and to identify potential therapeutic targets [8]. Some common animal models of MS can help to elucidate the important signaling pathways involved in the pathophysiology of the disease. These include viral models, such as those utilizing Theiler's murine encephalomyelitis virus, autoimmune models, such as experimental autoimmune encephalomyelitis, and toxin-induced demyelination models, such as those induced using lysolecithin and cuprizone (CPZ) [9]. However, each model has its own limitations, representing only part of the pathogenic spectrum and resulting in the simulation of only a subset of the disease signs and pathological features of MS [10]. A suitable model for MS research is therefore primarily determined by the specific research question.

CPZ (bis-cyclohexanone oxaldihydrazone) is a copper chelator that causes highly consistent demyelination in many parts of the brain, including the corpus callosum (CC), cerebral cortex, and superior cerebellar peduncle, and is associated with behavioral abnormalities similar to those seen in humans. CPZ is frequently used in MS research to study the de- and re-myelination phase of MS [11]. Many different routes of CPZ administration have been reported, including the traditional method of mixing it into powdered rodent chow, which requires daily preparation [12]. CPZ-

containing commercial pellets are also used; however, temperature control is essential during pellet preparation because the primary disadvantage of this method is that CPZ is a heat-sensitive chemical [13]. In contrast to CPZ pellet-mediated intoxication, Hochstrasser T et al. [14] found that intoxication caused by CPZ mixed with ground rodent chow is the typical way to induce reproducible and consistent demyelination. However, the main challenge with this method is the variation in consumption among different animals and the deterioration of CPZ due to environmental exposure. CPZ intoxication can also be achieved by mixing this chemical with the drinking water. The major limitations of this method are the poor solubility of CPZ in water and the need for a long duration of administration to induce complete demyelination [15]. Alternatively, a CPZ-containing liquid can also be administered orally [8]. In this case, each animal receives a standard daily dose of CPZ in suspension via oral gavage.

Numerous studies have shown that intoxication caused by CPZ, when mixed with ground rodent chow or administered via oral gavage, is the gold standard method for inducing consistent demyelination [8,14,16]. Notably, although both administration methods can induce demyelination, they can differ in efficiency, consistency, and associated systemic effects, such as liver damage. The lack of comparative data makes it unclear which administration route provides a more reliable balance between effective demyelination and minimal hepatic histopathological changes. Therefore, understanding the limitations of *in vivo* models and making informed decisions about the appropriate route of CPZ administration can greatly enhance the sensitivity and reproducibility of the results, as well as their application in evaluating new treatments. To date, there have been no studies comparing the differences between routes of CPZ administration (diet and oral) in SWR/J mice regarding behavioral and histological changes in the brain and liver. The purpose of this study was to compare whether intoxication in SWR/J mice using 0.3% CPZ mixed into ground rodent chow induces demyelination to the same extent as that with a CPZ suspension in carboxymethyl cellulose. To achieve this, CPZ intoxication was induced with different formulations to evaluate the behavioral changes associated with CPZ treatment and to quantify the degree of demyelination in the CC area of the brain. Finally, the toxicity of CPZ to the liver was also investigated. This study offers new insight into the advantages and limitations of each administration method. These findings enhance methodological rigor and may inform the selection of the most appropriate cuprizone-administration strategy in future MS research.

2. Materials and Methods

2.1 Animals

In total, 18 male SWR/J mice weighing approximately 19–21 g were sourced by the Animal House Unit of the King Fahd Medical Research Center, in Jeddah, Saudi Arabia. The mice were kept under standard humidity and temperature conditions with a 12-hour light/dark cycle and had unrestricted access to food and water. The study was conducted in compliance with the ARRIVE 2.0 guidelines (see **Supplementary Material**).

2.2 Treatment Preparation

In the current study, acute demyelination in mice was induced either through the diet or orally.

2.2.1 Diet CPZ Preparation

The 0.3% (w/w) CPZ diet was formulated by mixing 100 g of ground rodent chow and 0.3 g of CPZ powder (C9012-25G, Sigma-Aldrich, St. Louis, MO, USA) for 1 minute and then forming it into a pellet form [17]. To ensure the effectiveness of the CPZ, the pellet was freshly prepared, stored for less than 3 days, and changed daily in the cage.

2.2.2 Oral CPZ Preparation

To prepare a homogeneous CPZ–carboxymethyl cellulose (CMC) emulsion, the CPZ powder was mixed with 1% CMC [18]. The 1% CMC was prepared weekly by dissolving 1 g of CMC (21902-100G, Sigma-Aldrich) in 100 mL of water at a constant temperature of 60 °C, and the mixture was then kept at 4 °C [19]. The CPZ–CMC suspension was prepared fresh every day and administered once daily via oral gavage (0.2 mL/mouse) at a dose of 400 mg/kg [8,20]; the dosage was changed weekly depending on the weight of the animals.

2.3 Experimental Design

The mice were randomly allocated to one of three groups: (I) control group (received 0.2 mL of 1% CMC by oral gavage and normal chow daily), (II) CPZ-diet group, and (III) CPZ-oral group (n = 6 mice/group). The total duration of the study was 5 weeks. Behavioral tests were conducted under blinded conditions in an isolated behavioral laboratory at room temperature (21–23 °C) and under standard lighting conditions on different days from the first to the fifth week. Before testing, the mice were habituated to their environment. Fig. 1 illustrates the experimental design of the study.

2.4 Behavioral Tests

2.4.1 Open Field Test (OFT)

Locomotor activity was evaluated weekly by performing an OFT. Individual mice were positioned in the center of the open-field arena (45 cm × 45 cm square) and permitted to move freely and explore the environment for 3 minutes during the test. The movements of the mice were tracked and recorded by a video camera mounted above the arena. The total distance moved (TDM; cm) and velocity (cm/s) were measured using the EthoVision XT 8 track-

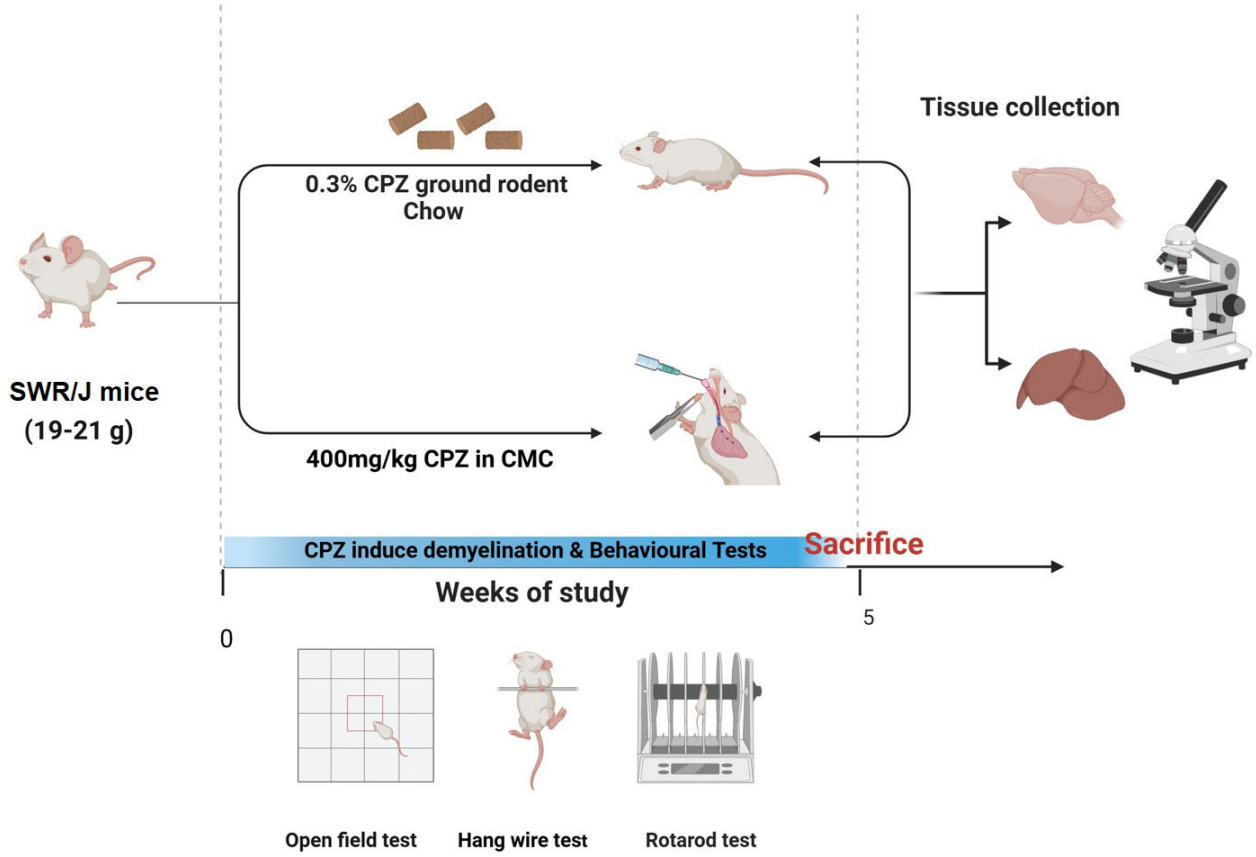

Fig. 1. Overview of the time course of the study and the behavioral tests. CMC, carboxymethyl cellulose; CPZ, cuprizone. Created with https://BioRender.com.

ing system (Noldus Information Technology, Wageningen, Netherlands) [21,22].

2.4.2 Wire Hang Test

The wire-hang test was used to assess the grip strength of mice. Briefly, each mouse was carefully placed individually in the center of a horizontal and narrow steel wire measuring 40 cm long, 40 cm high, and 5 mm in diameter. The latency to fall (the time each mouse remained on the wire before falling) was measured, with the cut-off time set at 180 seconds. Each mouse underwent three trials, and the average was calculated [18,23].

2.4.3 Rotarod Test

The Rotarod device (BR1001, B.S Technolab Inc., Seoul, South Korea) was used to assess the motor coordination and balance of mice; the device consists of a variable-speed rotating rod driven by a motor. Each mouse was positioned on the rotating rod for 300 seconds with an acceleration of 4 to 40 rpm. Each mouse was subjected to three trials at 1-hour intervals on each test day, and the average latency to fall was calculated [23,24].

2.5 Tissue Preparation

The mice were anesthetized with isoflurane (Aerrane®, Baxter Healthcare Corporation, Deerfield, IL, USA) inhalation (induction at 5% and maintenance at 3% in 2 L/min oxygen in a sealed container) at the end of the study period. Adequate anesthesia depth was confirmed by the absence of reflex responses. The mice were then euthanized by decapitation, and the brains and livers were removed and fixed in 10% formaldehyde. After 48 hours of fixation, the tissues were processed and embedded in paraffin. Serial sagittal sections of the brains (5 µm) were prepared and stained with Luxol Fast Blue Stain Kit (LFB, Cat. No. RPSK345-500, Atom Scientific, Greater Manchester, UK), while 5 µm liver sections were prepared and stained with hematoxylin and eosin (H&E) kit (ab245880, Cambridge, MA, USA).

2.5.1 LFB Staining

Demyelination was detected histochemically through an examination of sagittal sections of the brain stained with the LFB Stain Kit (Cat. No. RPSK345-500, Atom Scientific) according to the Klüver–Barrera protocol (1953). In LFB staining, demyelinated areas appear white. A

light microscope (Olympus BX51, Olympus Corporation, Tokyo, Japan) was used at 10× magnification to examine the stained samples, and photographs were taken with a digital camera. Image J software (Version 1.53t, National Institutes of Health, Bethesda, MD, USA) was used to estimate the percentage of myelinated areas in the CC; three slices per group were analyzed, and the percentage of the myelination area was calculated as follows:

Myelination area% = LFB stained area / Total selected area × 100.

2.5.2 H&E Staining

H&E staining was performed to detect the histological morphological changes in the liver. The staining procedure was performed according to a previously reported protocol [25].

2.6 Statistical Analysis

GraphPad Prism 9.2.0 (GraphPad Software, La Jolla, CA, USA) was used for the statistical analysis, and all data were presented as the mean ± standard error of the mean (SEM). Statistical differences between groups were analyzed by performing a two-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons, except for the percent weight gain and myelin content, which were analyzed by performing an ordinary one-way ANOVA followed by Tukey's test for multiple comparisons. Significant differences were defined as $p < 0.05$.

3. Results

3.1 Effect of Different Routes of CPZ Administration on Body Weight

At the end of the 5-week CPZ-mediated intoxication period, body weights were significantly decreased in the CPZ-diet group when compared to those in the control group ($p = 0.0042$) and the CPZ-oral group ($p = 0.0364$), as shown (Fig. 2A). Weight gain results (in %) also showed a significant difference between the CPZ-diet group and the other groups, namely the control group ($p = 0.0031$) and the CPZ-oral group ($p = 0.0036$), with a non-significant difference between the CPZ-oral and control groups ($p = 0.9929$) (Fig. 2B).

3.2 Effect of Different Routes of CPZ Administration on Locomotor Activity (TDM and Velocity)

As shown in Fig. 3A, no significant differences in the TDM value were found between the control group and the other groups, namely the CPZ-diet ($p = 0.7844$) and CPZ-oral ($p = 0.4040$) groups, or between the CPZ-diet and CPZ-oral groups ($p = 0.8715$). Furthermore, no significant differences in velocity were found between the control group and the two CPZ groups, namely the CPZ-diet ($p = 0.8109$) and CPZ-oral ($p = 0.4908$) groups, or between the CPZ-diet and CPZ-oral groups ($p = 0.8720$) (Fig. 3B).

3.3 Effect of Different Routes of CPZ Administration on Grip Strength

The mice in the CPZ groups (diet and oral) exhibited shorter latency compared with control group, as shown in Fig. 4. Statistically significant differences were observed between the CPZ-diet and control groups at weeks 2, 3, and 4 ($p = 0.0131$, $p = 0.0028$, $p = 0.0262$, respectively). No significant differences were detected at weeks 1 and 5 ($p = 0.1986$ and $p = 0.2048$, respectively). In addition, there were no statistically significant differences between the CPZ-diet and CPZ-oral groups ($p = 0.9016$, $p = 0.7714$, $p = 0.1109$, $p = 0.5270$, and $p = 0.6825$, respectively) or between the CPZ-oral and control group at any week of the study.

3.4 Effect of Different Routes of CPZ Administration on Motor Coordination and Balance

In the last 3 weeks of the study, the motor performance in the CPZ groups decreased compared to that in the control group. A statistical analysis revealed significant differences between the control and CPZ-diet groups ($p = 0.0285$ and $p = 0.0149$) at weeks 4 and 5, respectively. In contrast, no significant differences were found between the control and CPZ-oral groups ($p = 0.2402$ and $p = 0.1186$) or between the CPZ-diet and CPZ-oral groups ($p = 0.5268$ and $p = 0.5854$). Of note, there were no significant differences in the first weeks among all groups (Fig. 5).

3.5 Effect of Different Routes of CPZ Administration on CC Demyelination

Demyelination was assessed based on a histological examination of LFB staining in sections of the CC, as this area is reproducibly affected by CPZ administration [26]. As shown (Fig. 6), complete myelination was observed in the CC of the control group, whereas almost complete demyelination was detected in all groups receiving CPZ (diet and oral). Using Image J software, myelin content was calculated by determining the percentage of the myelinated area. Quantitative analysis of the myelinated region in all areas of the CC showed that the percentage of the myelinated area decreased significantly ($p < 0.0001$) in both CPZ groups (CPZ-diet = 10.23% and CPZ-oral = 12.90%) compared to that in the control group (93.99%), with no statistically significant differences between the CPZ-diet and CPZ-oral groups (Fig. 6).

3.6 Effect of Different Routes of CPZ Administration on Liver Toxicity

3.6.1 Control Groups

A microscopic examination of H&E-stained liver sections from the control-group mice revealed classic polyhedral lobules with portal vein spaces at the periphery of each lobule, indicating a normal architecture of the liver (Fig. 7A). Hepatocyte cords extending from the central vein to the periphery formed the hepatic lobular parenchyma. Nar-

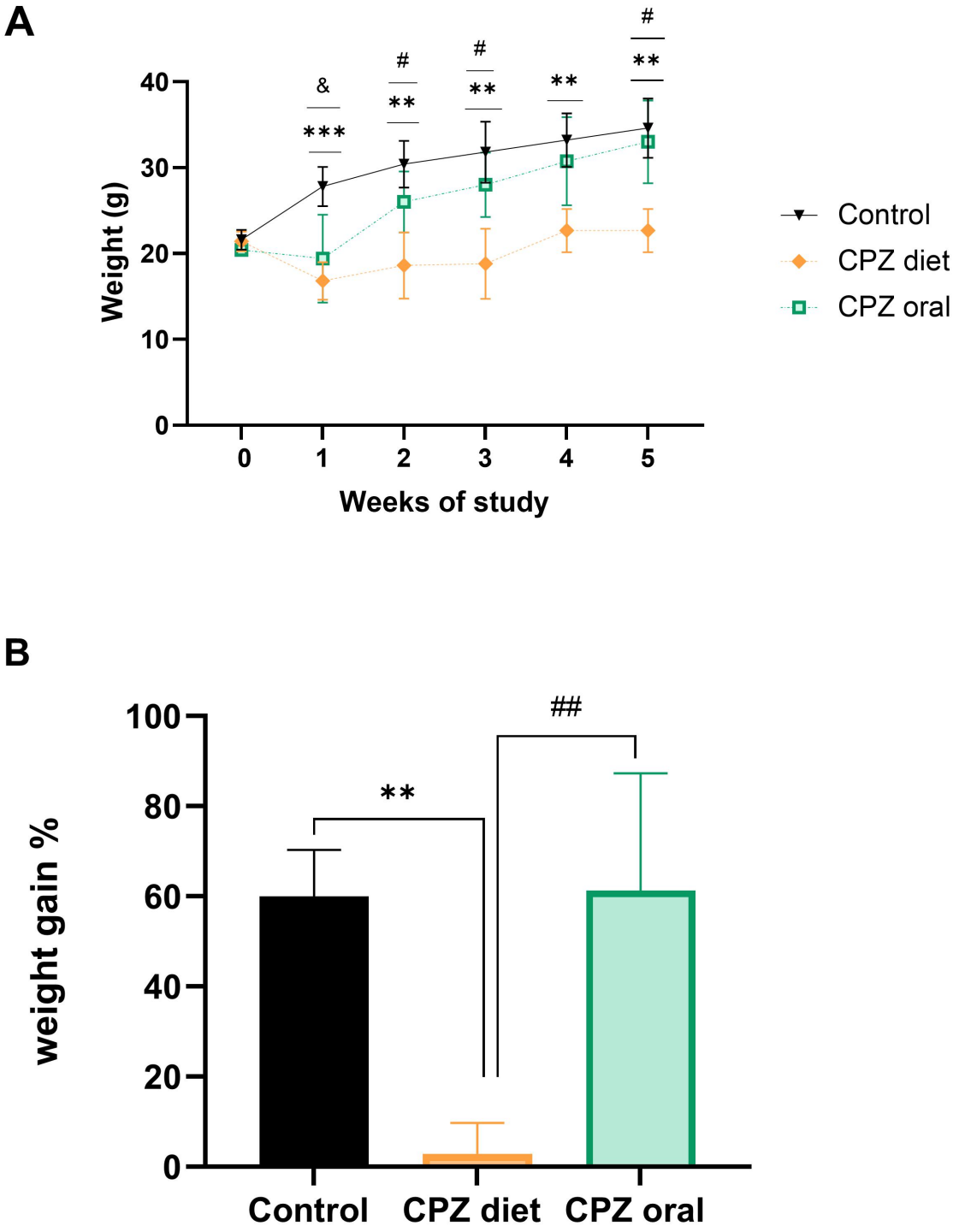

Fig. 2. Effect of different routes of CPZ administration on (A) body weight (g), and (B) percent weight gain. Data were analyzed via two-way ANOVA in (A) and ordinary one-way ANOVA in (B), followed by Tukey's multiple comparisons test. All data are presented as the mean ± SEM. g, gram; SEM, standard error of the mean; ANOVA, analysis of variance; 6 mice per group = 18 mice. (*) Indicates a significant difference between control and CPZ-diet groups, (&) indicates a significant difference between control and CPZ-oral groups, and (#) indicates a significant difference between CPZ-diet and CPZ-oral groups. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\&}p < 0.05$.

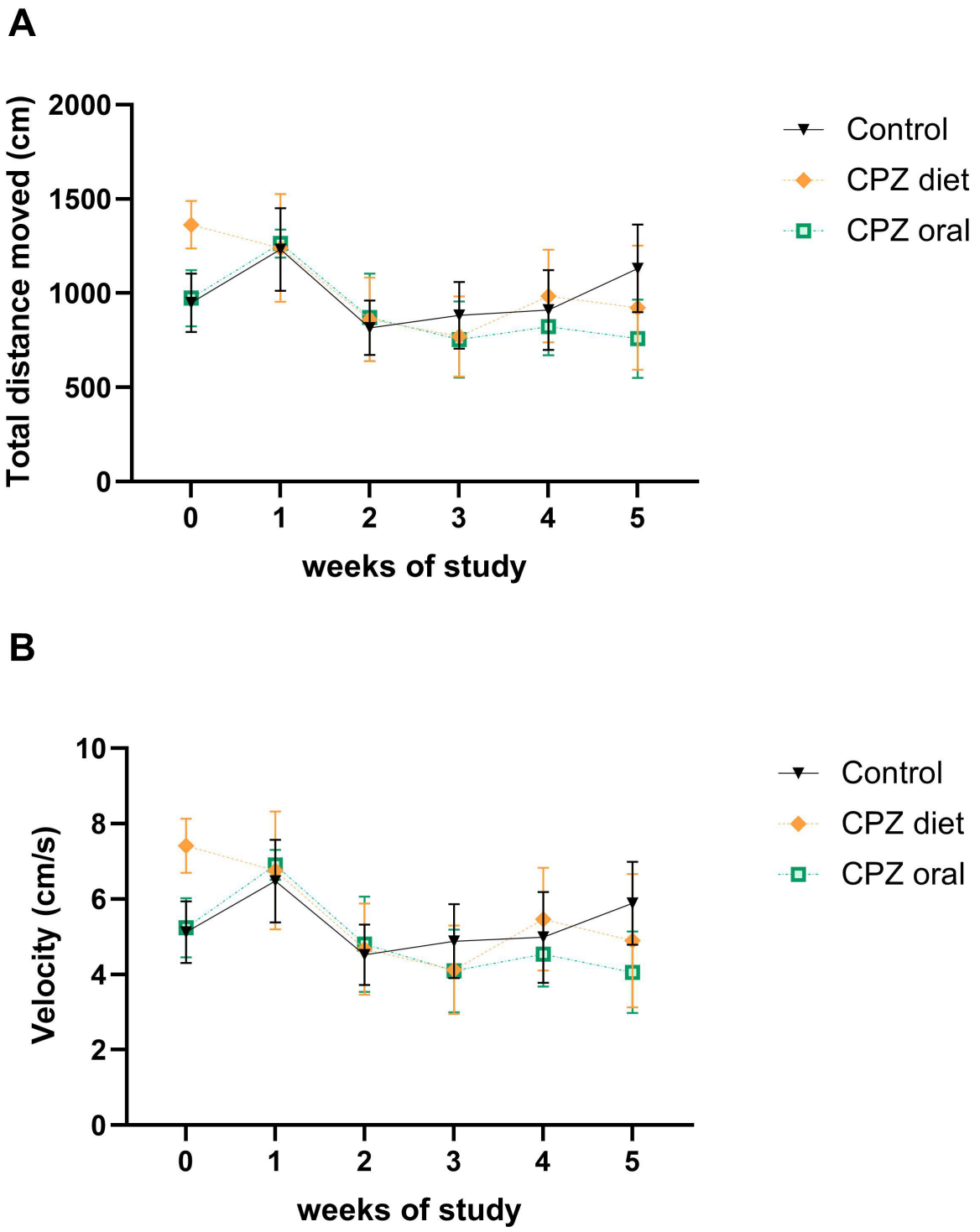

Fig. 3. Effect of different routes of CPZ administration on locomotor activity in OFT: (A) TDM, and (B) velocity. Data are displayed as the mean ± SEM and were analyzed via two-way ANOVA followed by Tukey's multiple comparisons test in (A,B), 6 mice/group. TDM, total distance moved; OFT, open field test.

row blood sinusoids, bordered by Kupffer cells and flat endothelial cells, filled the space between the cell cords and converged towards the central vein. The hepatocytes contained one or two spherical nuclei in the eosinophilic cytoplasm. The small branches of the bile duct, portal vein, and hepatic artery were visible in the portal vein spaces (Fig. 7B,C).

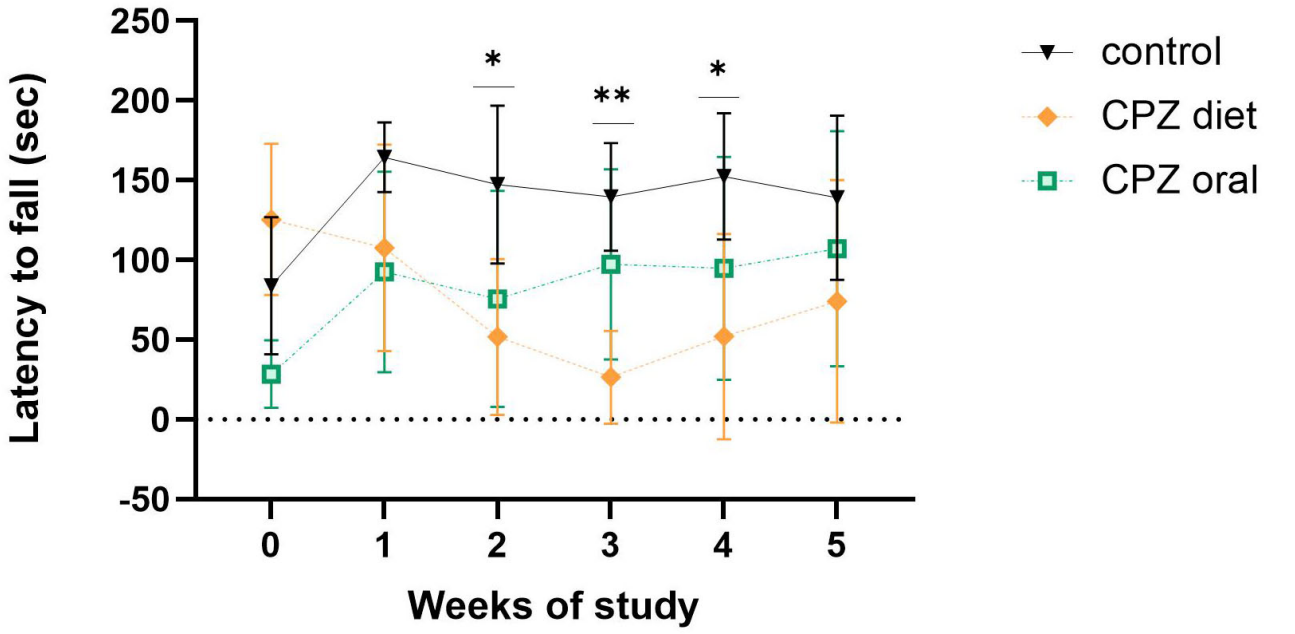

Fig. 4. Effect of different routes of CPZ administration on grip strength. Data are shown as the mean ± SEM; a two-way ANOVA followed by Tukey's multiple comparison test was performed; 6 mice/group. (*) Indicates a significant difference between the control and CPZ-diet groups, $^{*}p < 0.05$, $^{**}p < 0.01$.

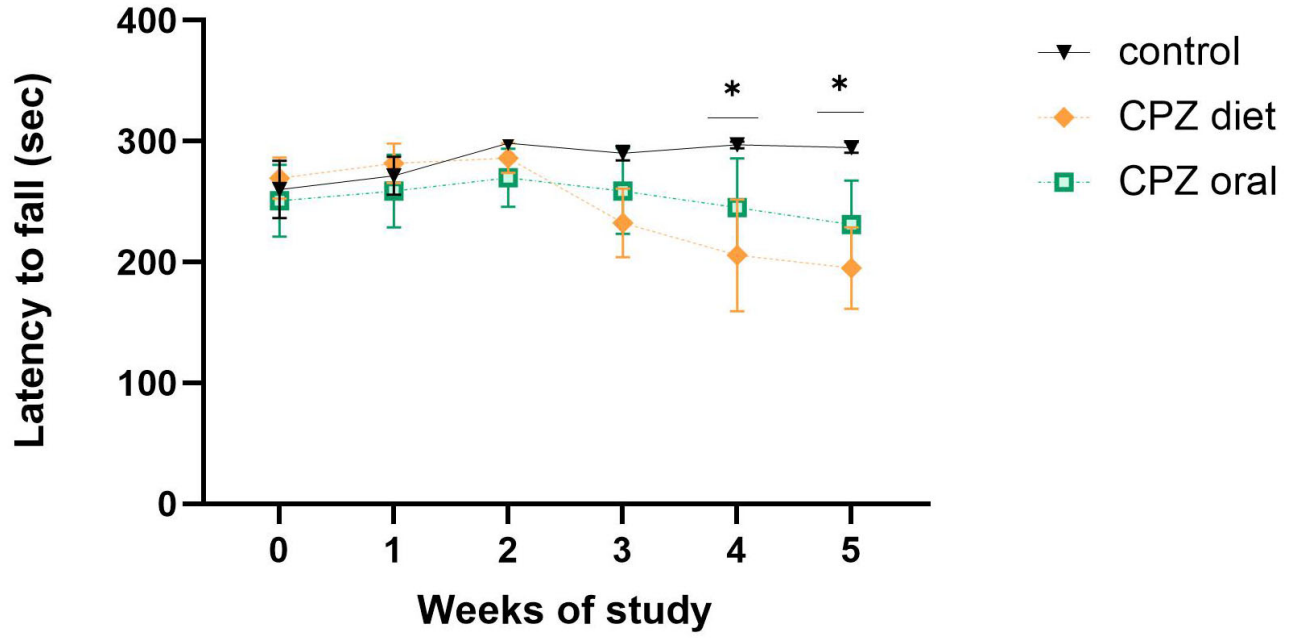

Fig. 5. Effect of different routes of CPZ administration on motor coordination and balance in the rotarod test. A two-way ANOVA was performed, followed by Tukey's multiple comparisons test, and the data are displayed as the mean ± SEM; 6 mice/group. (*) Indicates a significant difference between control and CPZ-diet groups, $^{*}p < 0.05$.

3.6.2 Diet Group

The microscopic examination of H&E-stained liver sections from the diet group revealed some disorganization of hepatic cords that radiate from the central vein with dilatation of the central vein, portal vein, and blood sinusoids (Fig. 7D). In addition, inflammatory cellular infiltration was detected around the central veins. The hepatocytes displayed a range of changes in the form of swelling, a vacuolated cytoplasm, and condensed nuclei, especially those around the central veins and portal spaces (Fig. 7E,F).

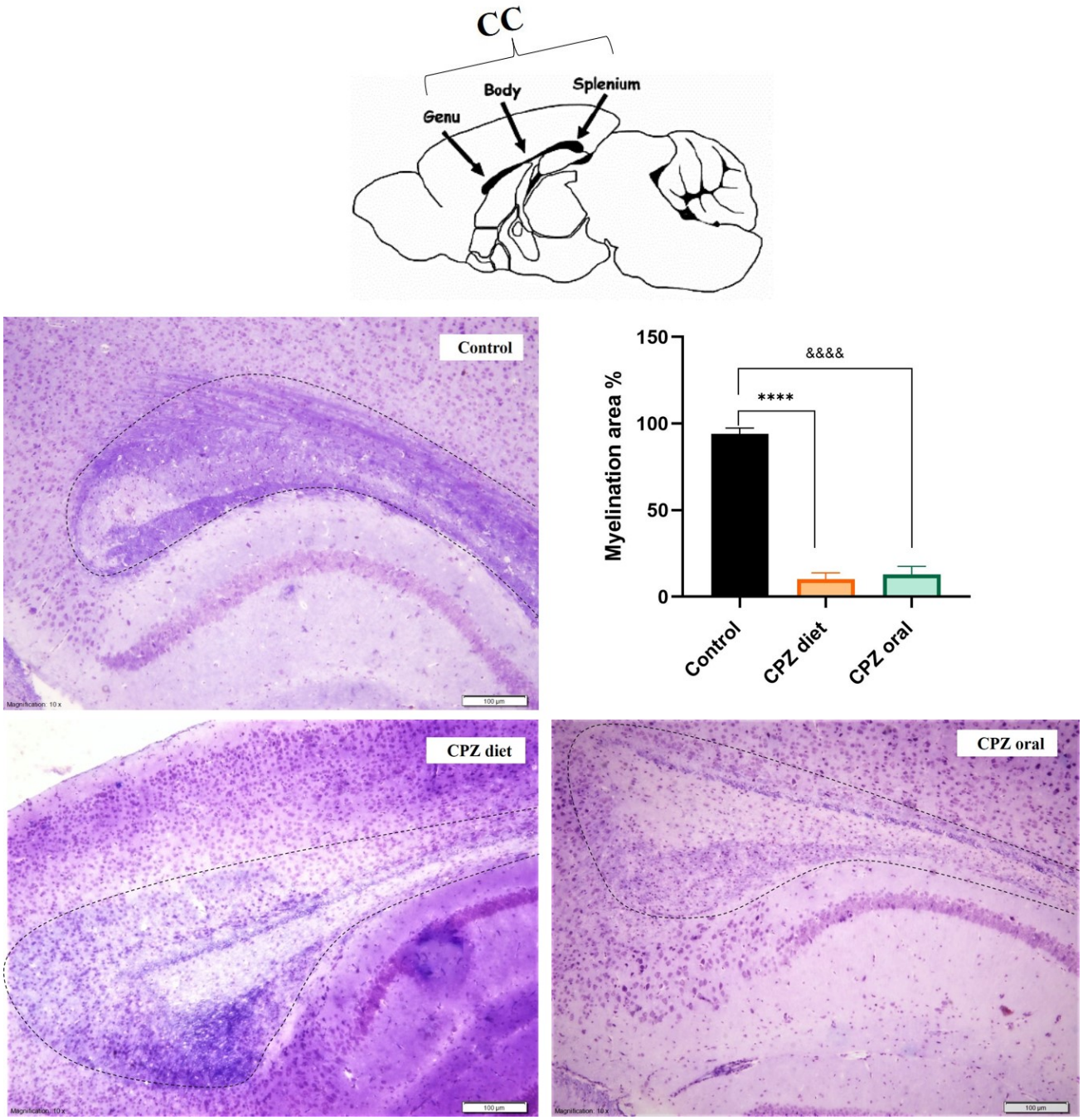

Fig. 6. Myelin content in the CC based on LFB staining and percentage of myelinated area in all groups at week 5. Quantification was performed using four mice per group, with multiple imaging fields analyzed from the corpus callosum region for each animal. Data are shown as the mean ± SEM; a one-way ANOVA was used followed by Tukey's multiple comparisons test. Scale bars: 100 µm. CC, corpus callosum; LFB, Luxol Fast Blue. $^{****}p < 0.0001$, $^{\&\&\&\&}p < 0.0001$; 10× magnification.

3.6.3 Oral Group

The microscopic examination of H&E-stained liver sections from the oral group revealed more diverse forms of histopathological changes. This included the disorganization of hepatic cords around the central veins, which appeared dilated and congested (Fig. 7G). Dilatation of the portal veins and blood sinusoids was also observed, and focal areas of inflammatory cellular infiltrates were observed around the central veins. The hepatocytes displayed a range of changes in the form of swelling, a vacuolated cytoplasm, and pyknotic changes of the nuclei, in different parts of the hepatic lobules (Fig. 7H,I).

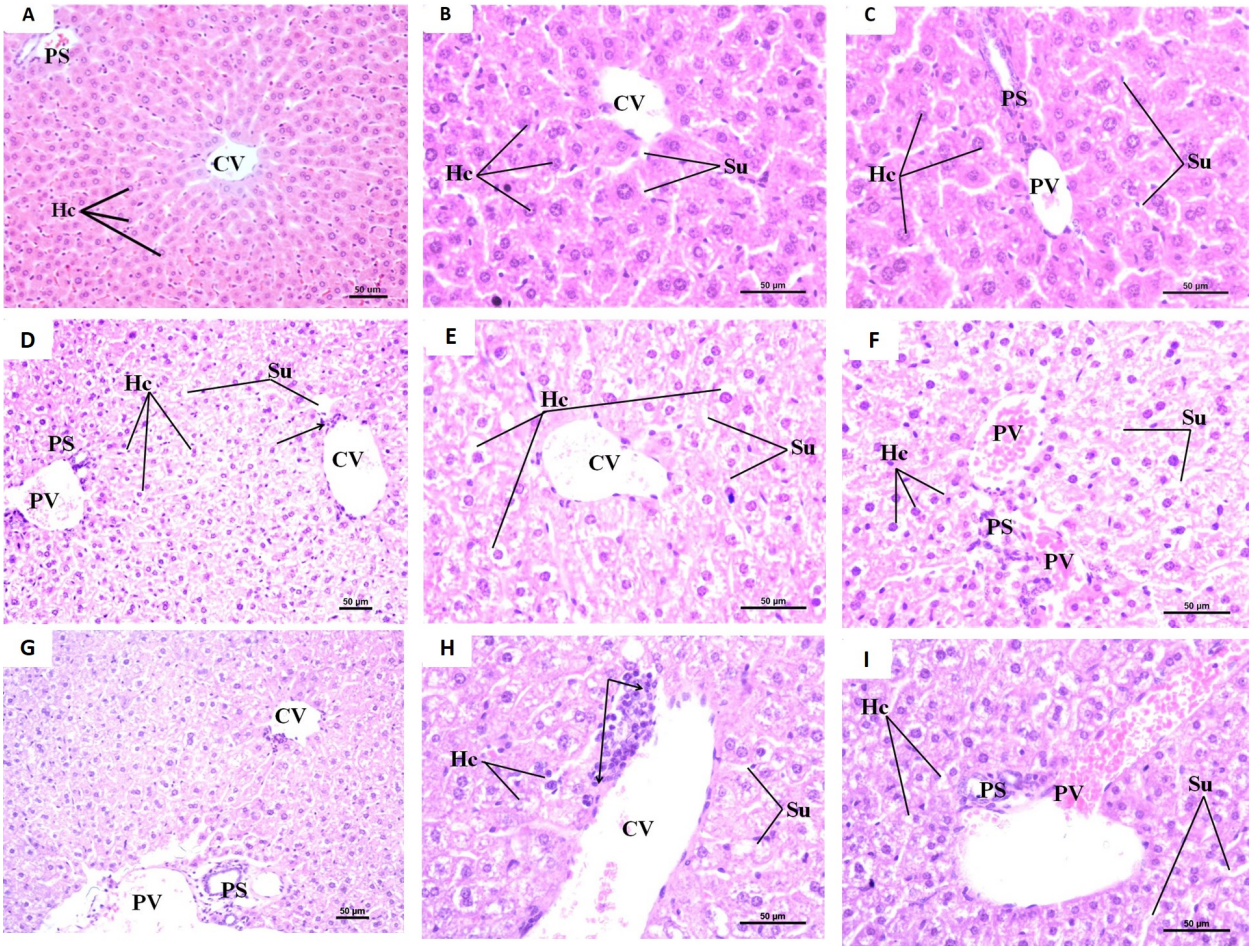

Fig. 7. Photomicrographs of liver sections from the control group showing the following. (A) normal histological structure of the liver in the form of cords of hepatocytes (Hcs) radiating from the central vein (CV) towards the portal spaces (PSs); H&E, ×200. (B) The hepatocytes had an eosinophilic cytoplasm and contain one or two rounded nuclei. Notice the normal hepatic sinusoids (Su); H&E, ×400. (C) Normal Hcs are arranged around the PS, which contains a portal venule (PV). Notice the normal hepatic sinusoids (Su); H&E, ×400. Photomicrographs of liver sections from the diet group showing the following: (D) disorganization of normal histological architecture of the liver with little dilation of the CV, PV, and Su. Many Hcs appeared to be vacuolated; H&E, ×200. (E) Little dilation of the CV and Su. The Hcs showed a vacuolated cytoplasm; H&E, ×400. (F) Little dilation of the CV and Su. The Hcs showed a vacuolated cytoplasm; H&E, ×400. Photomicrograph of liver sections from the oral group showing the following: (G) disorganization of normal histological architecture of the liver with dilation and congestion of the CV and PV; H&E, ×200. (H) Dilation of the CV and Su with areas of inflammatory cellular infiltrates (arrow) observed around the CV. The Hcs showed a vacuolated cytoplasm; H&E, ×400. (I) Dilation and congestion of the PV and Su; 4 mice/group. The Hcs showed a vacuolated cytoplasm; H&E, ×400. H&E, hematoxylin and eosin. Scale bar: 50 µm.

4. Discussion

Several animal models have been established for studying the pathophysiology of MS. The use of CPZ comprises a valuable model to assess the effect of potential treatments, which has been used over several decades [27,28], as both de- and re-myelination occur simultaneously, as in MS [29,30]. Currently, most researchers generally use CPZ models, in which animals are fed either ground chow mixed with CPZ or pellets containing CPZ [16]. In addition, other routes of CPZ administration are also used, such as mixing CPZ into drinking water [15] or the oral administration of a suspension of CPZ in CMC [8]. Therefore, in accordance with previously reported CPZ-administration protocols, the aim of this study was to compare two standard routes of CPZ administration (diet and oral) using SWR/J mice, focusing on their effects on behavioral changes, CC demyelination, and liver toxicity.

Several studies have reported that CPZ intoxication can lead to weight loss, which provides evidence of the desired demyelinating effect of CPZ *in vivo* [8,31,32,33]. Our results showed that mice fed 0.3% CPZ in ground rodent chow exhibited a significant reduction in weight gain. In

contrast, the effect was less pronounced in mice receiving CPZ in a suspension formulation via oral gavage. This result is in agreement with previous studies showing that CPZ administration causes a decrease in body weight [8,34]. There could be several reasons for the difference in weight gain between the two groups of mice treated with CPZ, including differences in the route of administration and the resulting differences in pharmacokinetics and pharmacodynamics. Chronic exposure to lower dietary levels of CPZ may result in cumulative toxicity, gastrointestinal irritation, decreased nutrient absorption, or other systemic effects that may reduce weight gain more than that with acute, high-dose exposure via gavage.

Behavioral assessments using animal models are crucial to understanding the pathophysiology of a disease and finding possible treatments [35]. In the current study, various parameters of locomotion were investigated, including the TDM, velocity, grip strength, motor coordination, and balance. Regarding the OFT, our results showed no difference in locomotor activity between the CPZ groups (diet and oral) and the control group, as indicated by the similar TDM and velocity between the two groups. This result aligns with previous reports showing that motor performance and locomotor activity are unaffected by CPZ administration [36,37]. However, contradictory findings have also been reported, with some studies showing reduced locomotion and motor impairment [38]. These differences may be due to several experimental variables, such as the dose of cuprizone, length of exposure, route of administration, mouse strain, age, and the specific behavioral paradigms used. The wire-hang test was used to investigate the effects of CPZ on neuromuscular function and to evaluate the grip strength of mice. Our results showed that the administration of CPZ (diet and oral) induced muscle weakness in mice, compared to results from the control group, as indicated by a reduced latency to fall. These results are consistent with those of previous studies that demonstrated the effect of CPZ intoxication on muscle weakness using a wire-hanging test [18,39]. Interestingly, we found that the group of mice that received CPZ mixed with ground rodent chow had a significant decrease in grip strength compared to the group that received 400 mg/kg CPZ via oral gavage. Regarding the rotarod test, our results showed a decrease in motor coordination in the CPZ groups (diet and oral) compared to that in the control group, as evidenced by a decreased latency to the fall time. This finding is in agreement with previous reports that CPZ administration reduces the latency to fall from the rotarod [39,40,41]. However, the CPZ-diet group showed a more significant reduction than the CPZ-oral group. These results suggest that exposure to CPZ at low doses over a prolonged period, through the diet, produces long-lasting systemic effects. The observed behavioral abnormalities in the CPZ-diet group could be due to chronic metabolic disturbances caused by this continuous exposure. In contrast, acute, high-dose administration via gavage could lead to the rapid absorption and metabolism of CPZ, resulting in higher peak concentrations but a shorter duration of action. This could explain the less severe effects on the behavioral abnormality parameters compared to those with dietary administration. To the best of our knowledge, no previous study has compared routes of CPZ administration (dietary and oral) using SWR/J mice regarding behavioral deficits.

Demyelination in the CNS is a prominent feature of MS [42,43]. In the current study, we used LFB staining to observe myelin loss in the CC of CPZ groups. Our results showed severe demyelination in the genu part of the CC and a significant reduction in the percentage of myelin content in all parts of the CC of the mouse brain in both CPZ groups compared to that in the control group, which is consistent with the results of previous studies [8,14,31]. Our findings confirmed the previous results showing that behavioral impairment in mice is associated with demyelination in the brain [44,45]. In addition, an examination of the histologic effects of CPZ on liver tissue showed the presence of various forms of histopathologic changes in all CPZ groups. However, the group that was administered CPZ orally showed a more pronounced change than the group that was administered CPZ via the diet. In line with our data, the Hiremath MM et al. [32] study showed that administering dietary CPZ at 0.2%, to C57BL/6 mice, induces reliable demyelination without harmful systemic toxic effects. However, no previous study has reported the effect of oral gavage administration on histopathological liver alterations alone or compared it with the dietary route.

5. Conclusions

The current results provide new insights into the routes of CPZ administration for animal models, which could significantly result in a better understanding of the pathophysiology of MS and thus facilitate the identification of biomarkers and drug development. These results suggest that the CPZ-diet model is more appropriate for modeling MS because it comprises consistent and sustained effects on demyelination and behavior while minimizing acute liver toxicity. This model better mimics the chronic progression and multifaceted nature of MS, making it a more reliable and representative model for studying the disease. However, the current study has some limitations, including a small sample size and short duration of CPZ administration. In the future, biochemical and molecular analyses should be performed, including an assessment of antioxidant markers and myelin protein quantification in the CC. Future studies should also evaluate liver toxicity using biochemical assays to support the histological findings. These studies will offer greater insights into how various CPZ routes affect demyelination dynamics, behavioral results, and systemic toxicity, thereby improving model selection and increasing the translational value of experimental demyelination investigations.

Availability of Data and Materials

The original contributions presented in this study are included in the article; all data reported in this paper will be shared by the lead contact upon request.

Author Contributions

KMA-O conceived the study, conducted the experiments, analyzed and interpreted the data, and wrote the manuscript. The author read and approved the final manuscript. The author has participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was approved by the Biomedical Ethics Research Committee of King Abdulaziz University (Approval No. 551-20) and was in accordance with the guidelines of the Animal Care and Use Committee (ACUC) of King Fahd Medical Research Centre and the "System of Ethics of Research on Living Creatures" of King Abdulaziz City for Science and Technology, approved by Royal Decree No. M/59 dated 24 August 2010.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflicts of Interest

The author declares no conflicts of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/JIN52589.

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