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RESEARCH ARTICLE

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Protective Effect of Celery (*Apium graveolens L.*) Essential Oil on the Experimental Model of Cuprizone-induced Multiple Sclerosis in Male C57BL/6 Mice

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ABSTRACT

Considering the beneficial effects of *Apium graveolens L.* (Celery) on the nervous system, this study elucidates the protective effect of CEO on the experimental model of cuprizone-induced MS in male C57BL/6 mice. Frothy mice were allocated into four experimental groups: control, cuprizone (chew pellet containing 0.2 %CPZ), CEO (800 mg/kg), and CPZ+CEO. Animals received treatments based on their groups for 5 weeks. Finally, reflexive motor behavior and serum antioxidant levels were determined. Based on the findings, ambulation score, hind-limb suspension, front limb suspension, and grip strength significantly decreed in the mice treated with CPZ ($p < 0.05$). Hind limb foot angle, surface rights, and negative geotaxis significantly increased in the animals treated with CPZ ($p < 0.05$). Co-administration of CPZ+CEO significantly reduced the adverse effects of CPZ on ambulation score, surface righting, hind limb suspension, grip strength, and negative geotaxis ($p < 0.05$). Co-administration of CPZ+CEO significantly diminished the adverse effects of CPZ on the number of crosses in the open field test and duration on the rotarod ($p < 0.05$). Serum MDA activity increased while GPx, SOD, and TAS decreased in the mice treated with CPZ ($p < 0.05$). Co-administration of CPZ+CEO significantly reduced the adverse effects of CPZ on serum antioxidant levels ($p < 0.05$). These results suggested the protective effect of CEO against CPZ-induced MS mediated by its antioxidant activity.

Keywords

Celery, Essential oil, Cuprizone, Multiple sclerosis, Mice

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Abbreviations

ANOVA: Analysis of variance
CEO: Celery essential oil
CPZ: Cuprizone
GPx: Glutathione peroxidase
MDA: Malondialdehyde
MS: Multiple sclerosis

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Introduction

MS is a complex neurodegenerative disease caused by the demyelination of neurons in the CNS [1]. This condition might emerge due to genetic risk factors and oxidative stress, representing fatigue, muscle weakness, ataxia, cognitive impairment, and depression [2]. As oxidative stress plays a primary role in the development of MS, an imbalance between antioxidant capacity and the production of ROS is responsible for the pathophysiology of MS [3]. Chemical and natural medications are widely used to decrease oxidative stress and cognitive deficits in MS patients. Even though these medications prevent immune cell-driven inflammation and reduce the relapse rate, they are ineffective at controlling the predominant neurodegeneration that happens later in the disease course processes [4].

Apium graveolens L. is a green-branched leaf stalk from the family *Apiaceae*. This plant is rich in phenolic compounds, flavonoids, L-3-n-butylphthalide, limonene, selinene, volatile oil, sedanolide, and linoleic acid [5]. It has several medical properties, mainly inflammatory, antimicrobial, antioxidant, and antiulcerogenic [6]. The beneficial effect of L-3-n-butylphthalide was demonstrated to improve cognitive impairment in Alzheimer's mouse models [7]. Battery test is routinely used to determine neurodevelopmental or neurodegenerative disorders. This method includes limb grasping and placing, cliff avoidance, righting, accelerated righting, gait, auditory startle, and posture [8]. Celery (300 and 600 mg/kg) ameliorates neurobehavioral and neurochemical disorders in perinatal lipopolysaccharides exposure in mice offspring [5].

Recent reports have been growing on the beneficial activity of *A. graveolens* on the nervous system. For instance, it has been reported that celery extract improved cognitive impairment in Alzheimer's [9] and Parkinson-like symptoms in an experimental mouse model [10]. It has been indicated that the oral administration of celery extract (125, 250, and 500 mg/kg) enhanced anxiety-like behavior using a battery of behavioral tests. In addition, celery extract decreased MDA production while increasing GPx levels in the cortex and striatum of the mice [11]. CPZ models were beneficial for the pathophysiology of MS [12]. Due to the lack of a straightforward way to treat this disorder, we investigated the protective effect of CEO on the experimental model of CPZ-induced MS in male C57BL/6 mice.

Results

Based on Figure 1, CPZ significantly decreased ambulation scores compared to the control mice (p = 0.043). Supplementation of CEO significantly amplified ambulation score (p = 0.022). Co-administration of CPZ+CEO significantly reduced the adverse effects of CPZ on ambulation scores compared to the CPZ group (p = 0.034). In this study, the hind limb foot angle significantly enlarged following CPZ treatment compared to the control group (p = 0.021). on the other hand, CEO significantly reduced hind limb foot angle rather than control mice (p = 0.044). Pretreatment with CPZ+CEO significantly minimized the influence of CPZ on the hind limb foot angle compared to the CPZ group (p = 0.036) (Figure 2).

It was observed that hind limb suspension significantly reduced in the CPZ-treated mice (p = 0.043). Hind limb suspension was not influenced by CEO compared to the control animals (p = 0.643). The combination of CPZ+CEO significantly decreased the adverse impact of CPZ (p=0.021) (Figure 3). According to Figure 4, surface righting was significantly raised by CPZ (p = 0.046), while CEO treatment significantly decreased surface righting compared to the control group (p=0.012). Pretreatment with CPZ+CEO significantly opposed the effect of CPZ (p = 0.023).

In the current study, grip strength significantly decreased in mice that received CPZ (p = 0.013). CEO treatment significantly increased grip strength compared to the control mice (p = 0.043). Grip strength significantly improved in the CPZ+CEO group compared to the CPZ group (p = 0.05) (Figure 5).

Based on our findings, CPZ significantly diminished front limb suspension (p = 0.035). The CEO supplementation significantly improved front limb suspension compared to the control animals (p = 0.032). The effect of CPZ on front limb suspension was not suppressed in the group pretreated with CEO (p = 0.67) (Figure 6). As shown in Figure 7, the negative geotaxis significantly rose in the CPZ-treated mice (p = 0.023). Supplementation with CEO significantly decreased negative geotaxis in comparison with the control mice (p = 0.041). Co-administration of CPZ+CEO improved negative geotaxis compared to CPZ only (p = 0.14).

As presented in Figure 8, the number of crosses in the OFT significantly decreased in CPZ group following CPZ administration (p = 0.015). Supplementation with CEO increased the number of crosses in OFT compared to the control animals (p = 0.043). Co-administration of CPZ+CEO decreased the adverse effects of CPZ on OFT compared to the CPZ group (p

Abbreviations-Cont'd

OFT: Open Field Test
ROS: Reactive oxygen species
SOD: Superoxide dismutase
TAS: Total antioxidant status

= 0.024). As mentioned in Figure 9, the duration of stay on the rotarod decreased following CPZ administration (p = 0.021). Supplementation with CEO did not affect rotarod time in comparison with the control group (p = 0.23). Pretreatment with CEO diminished the impact of CPZ on rotarod time (p = 0.042).

According to Figure 10, CPZ administration significantly elevated serum MDA compared to the control mice (p = 0.031). Supplementation with CEO significantly decreased serum MDA (p = 0.027). Co-administration of CPZ+CEO significantly reduced CPZ-induced elevation in the MDA production rather than CPZ (p = 0.034). In this research, SOD activity decreased following CPZ administration in comparison with the control mice (p = 0.023), while SOD activity was enhanced by CEO supplementation (p = 0.014). Co-administration of CPZ+CEO improved serum SOD compared to the CPZ-only group (p = 0.043) (Figure 11).

Regarding the adverse effects of CPZ, the serum GPx activity significantly decreased (p = 0.033), whereas enhanced in CEO-treated mice (p = 0.014). Co-administration of CPZ+CEO reduced the adverse effects of CPZ on serum GPx in comparison with the CPZ-treated mice (p = 0.047) (Figure 12). Finally, serum TAS significantly declined in the CPZ group (p = 0.015) and supplementation with CEO increased its levels (p = 0.023). Co-administration of CPZ+CEO decreased CPZ-induced elevation in TAS compared to the CPZ-only group (p = 0.041) (Figure 13).

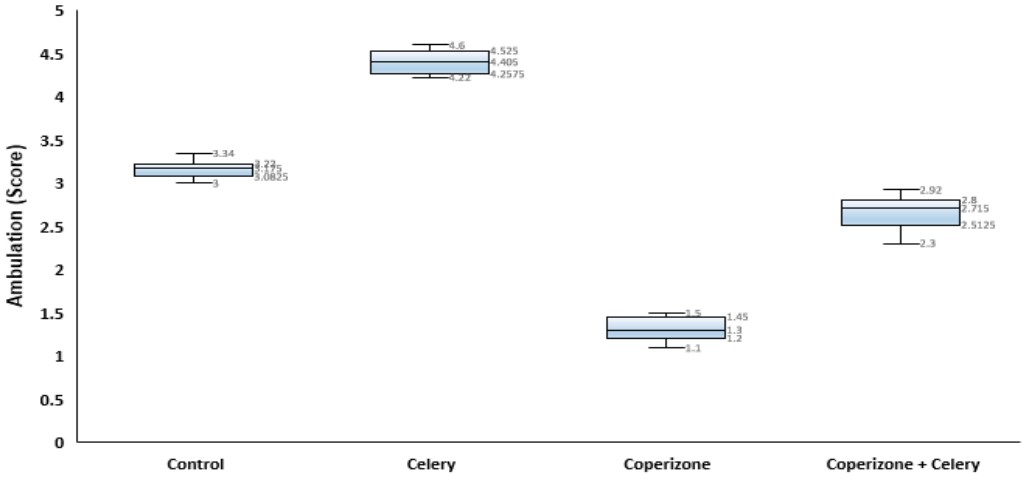

Figure 1.
Effects of cuprizone, celery extract and their combination on ambulation score in Cuprizone-induced model of multiple sclerosis mice (n = 10) (p < 0.05).

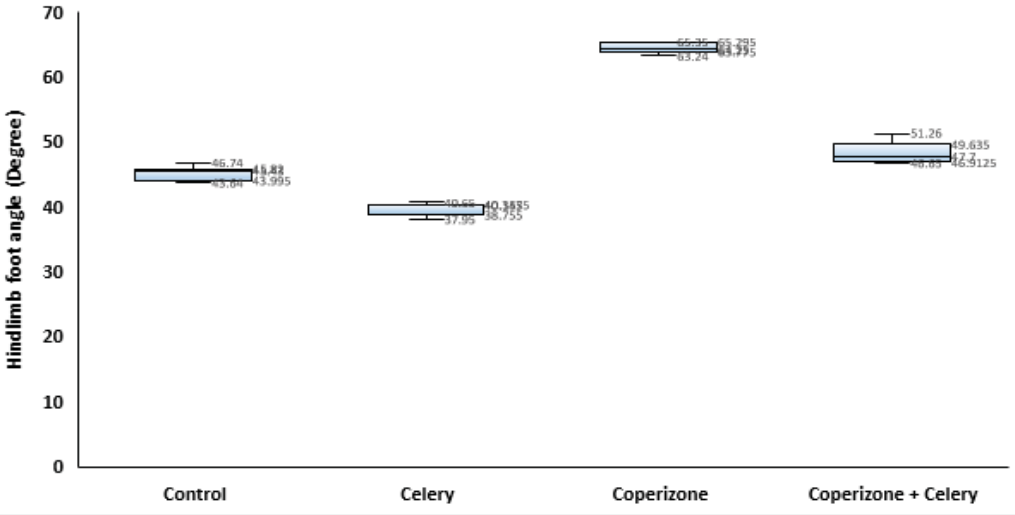

Figure 2.
Effects of cuprizone, celery extract and their combination on hindlimb foot angle in Cuprizone-induced model of multiple sclerosis mice (n = 10) (p < 0.05).

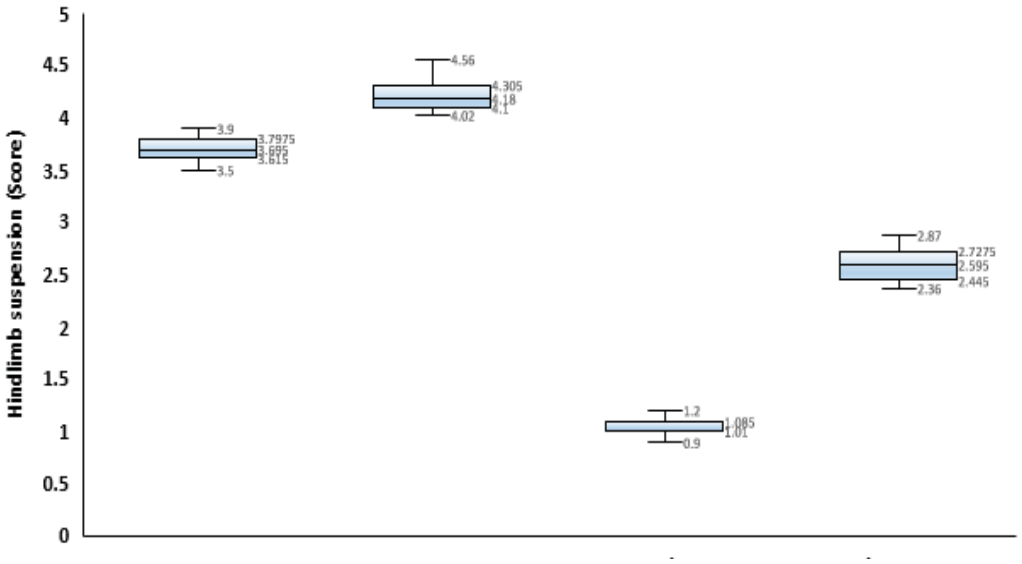

Figure 3.
Effects of cuprizone, celery extract and their combination on hindlimb suspension in Cuprizone-induced model of multiple sclerosis mice (n = 10) ($p < 0.05$).

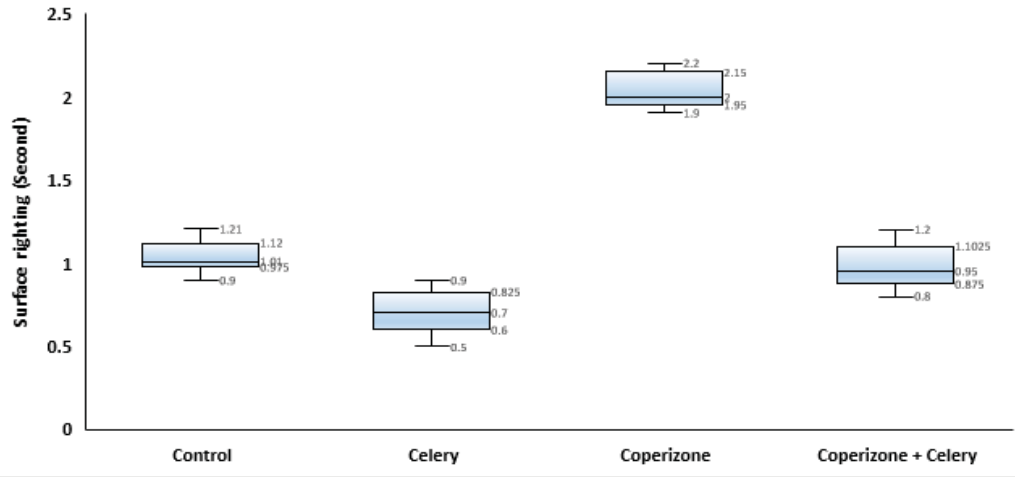

Figure 4.
Effects of cuprizone, celery extract and their combination on hindlimb foot angle in Cuprizone-induced model of multiple sclerosis mice (n = 10) ($p < 0.05$).

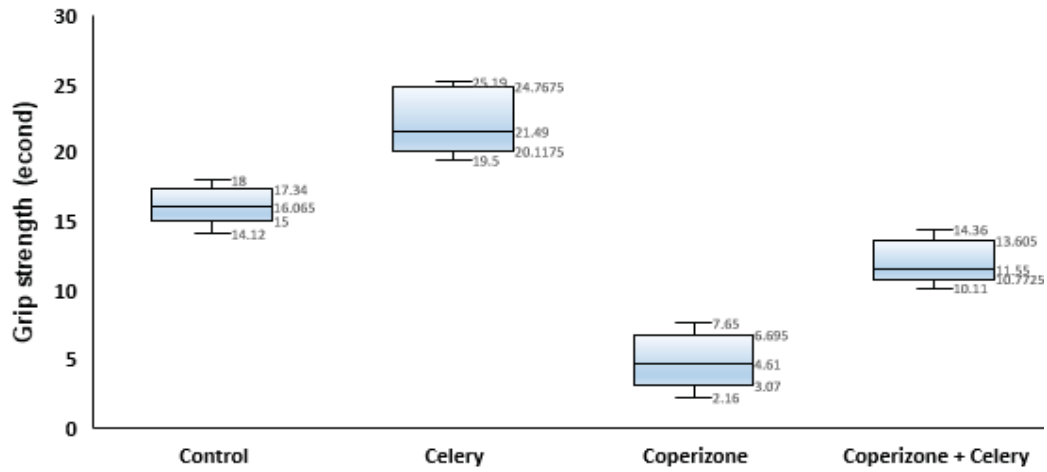

Figure 5.
Effects of cuprizone, celery extract and their combination on grip strength in Cuprizone-induced model of multiple sclerosis mice (n = 10) ($p < 0.05$).

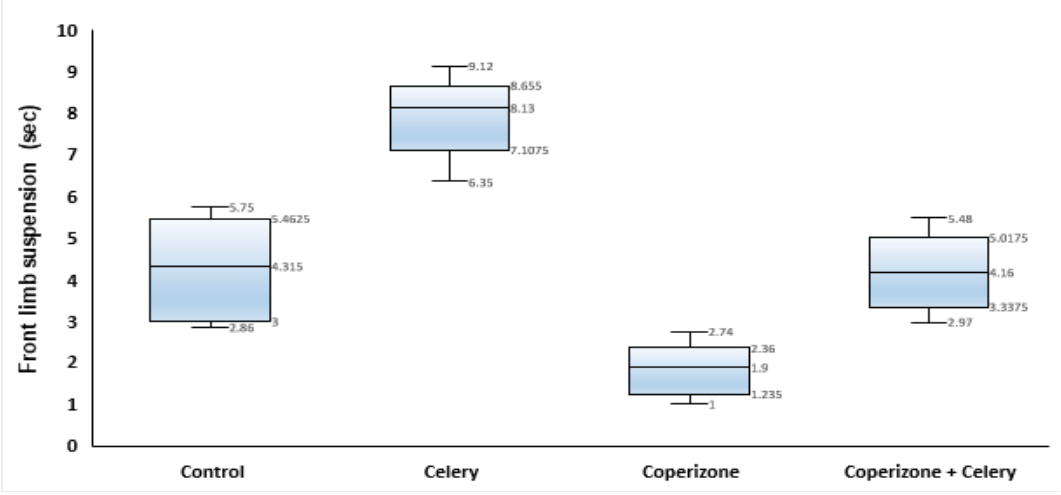

Figure 6.
Effects of cuprizone, celery extract and their combination on front limb suspension in Cuprizone-induced model of multiple sclerosis mice (n = 10) ($p < 0.05$).

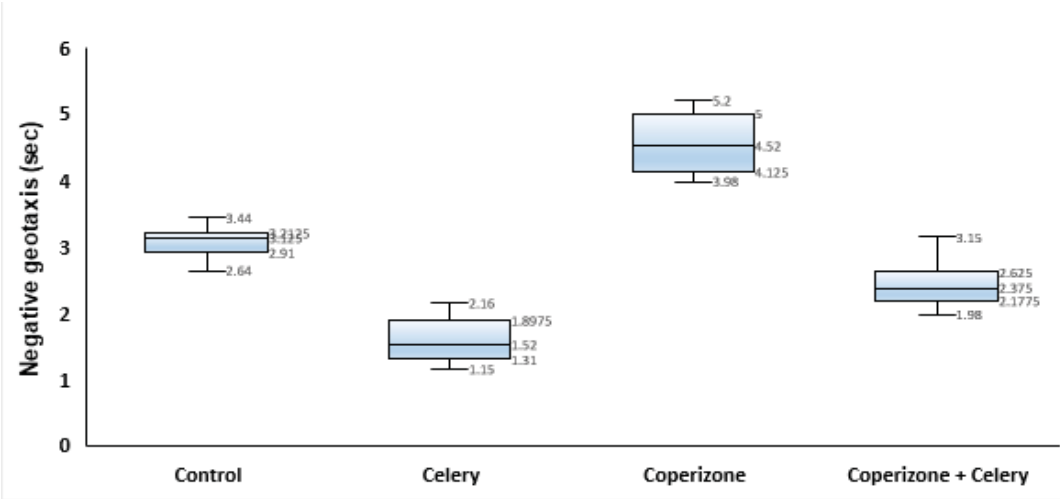

Figure 7.
Effects of cuprizone, celery extract and their combination on negative geotaxis in Cuprizone-induced model of multiple sclerosis mice (n = 10) ($p < 0.05$).

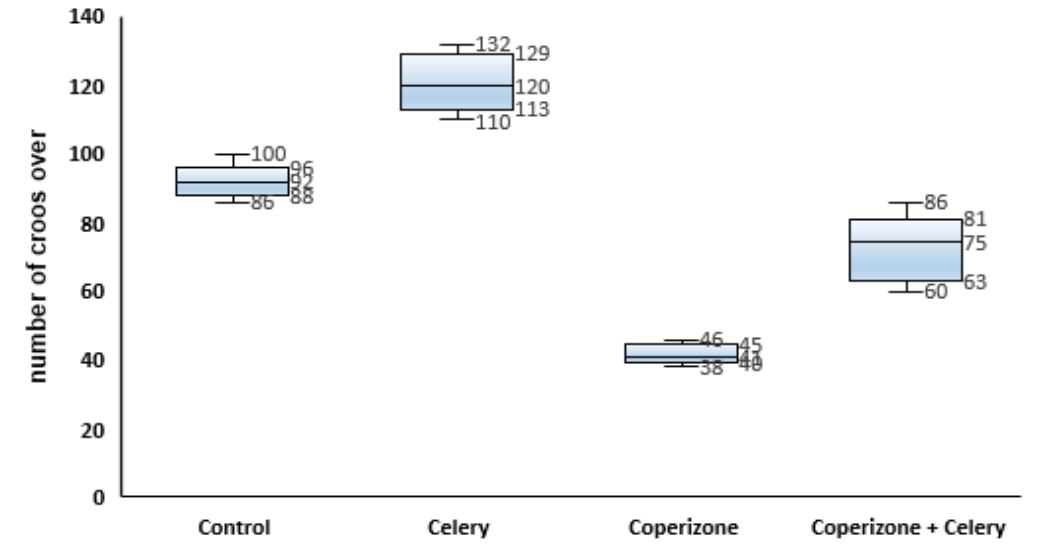

Figure 8.
Effects of cuprizone, celery extract and their combination on number of cross on open field test (OFT) in Cuprizone-induced model of multiple sclerosis mice (n = 10) ($p < 0.05$).

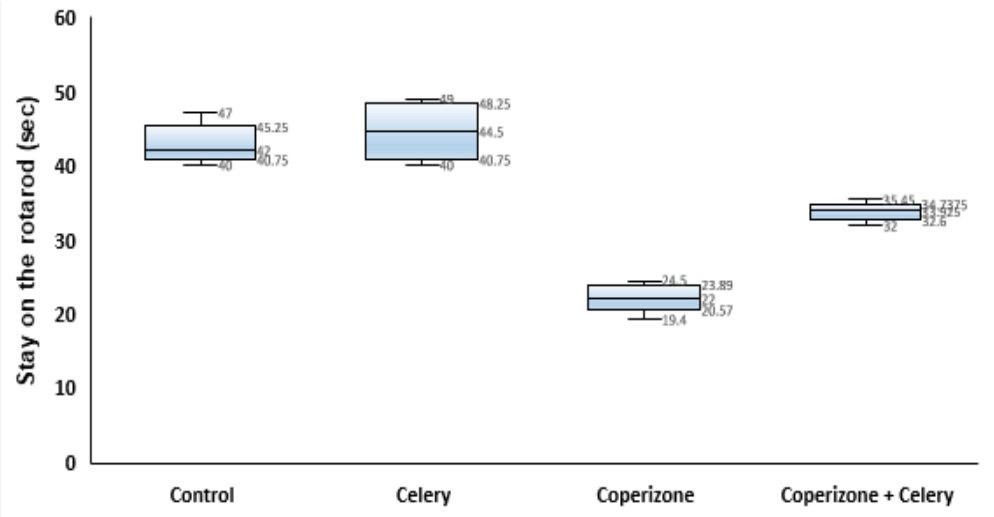

Figure 9.
Effects of cuprizone, celery extract and their combination on stay on the rotarod in Cuprizone-induced model of multiple sclerosis mice (n = 10) ($p < 0.05$)

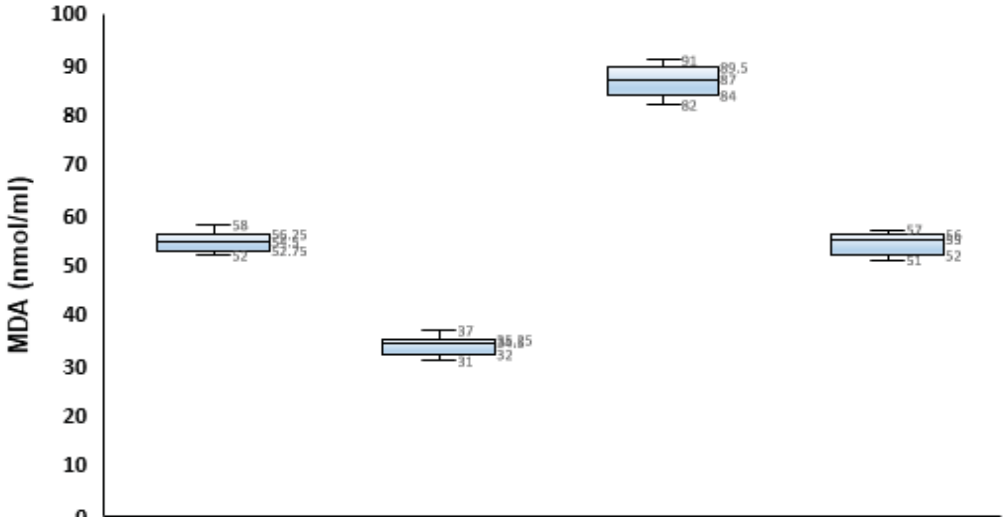

Figure 10.
Effects of cuprizone, celery extract and their combination on serum Malondialdehyde in Cuprizone-induced model of multiple sclerosis mice (n = 10) ($p < 0.05$)

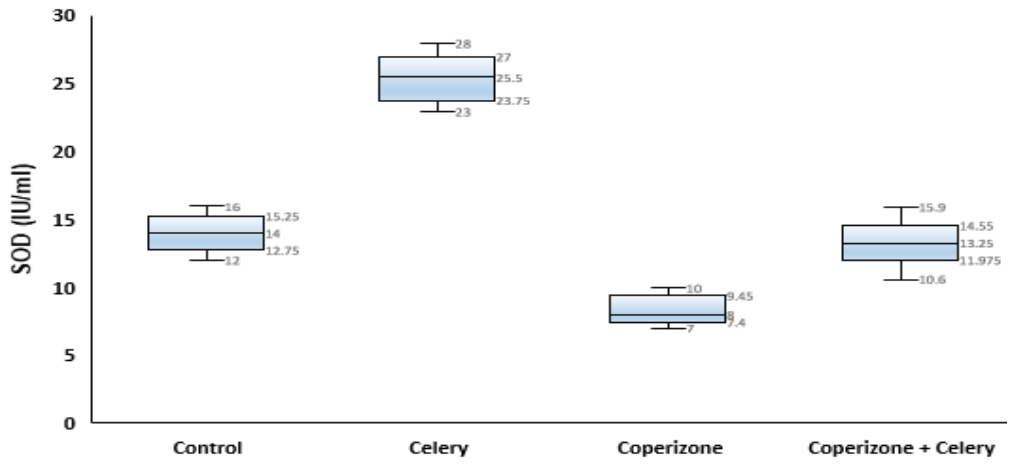

Figure 11.
Effects of cuprizone, celery extract and their combination on serum Superoxide dismutase in Cuprizone-induced model of multiple sclerosis mice (n = 10) ($p < 0.05$)

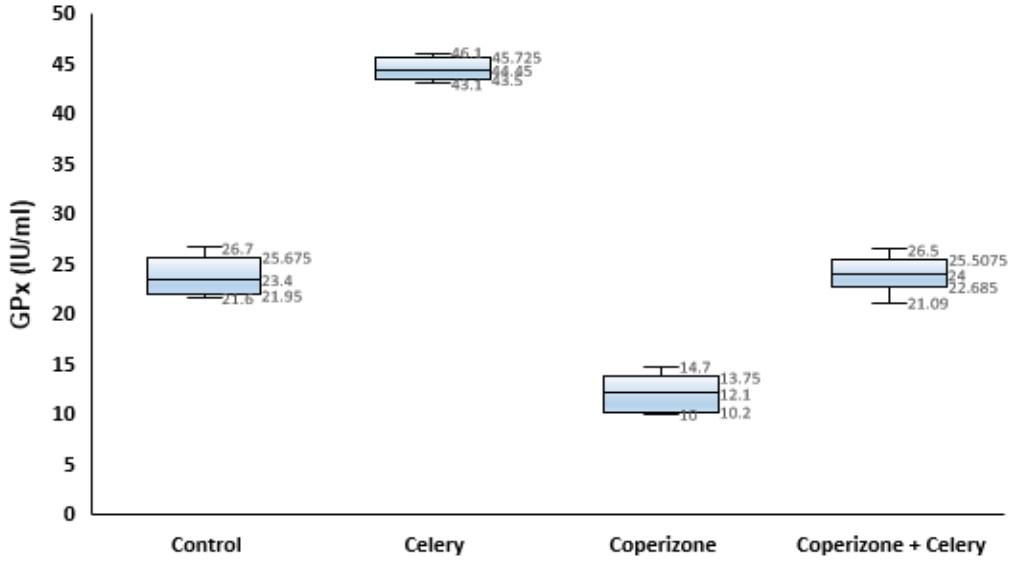

Figure 12.
Effects of cuprizone, celery extract and their combination on serum Glutathione peroxidase in Cuprizone-induced model of multiple sclerosis mice (n = 10) ($p < 0.05$).

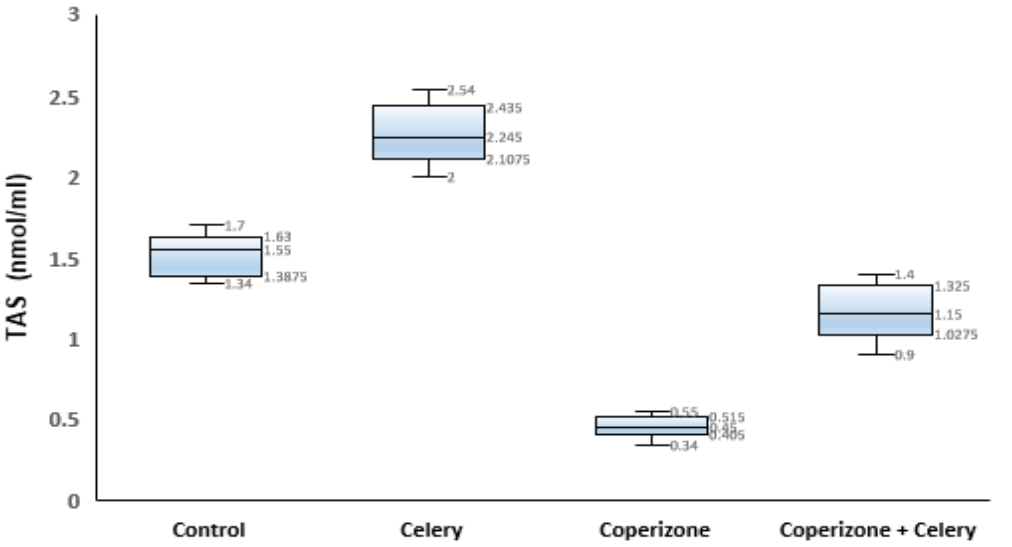

Figure 13.
Effects of cuprizone, celery extract and their combination on total antioxidant status in Cuprizone-induced model of multiple sclerosis mice (n = 10) ($p < 0.05$).

Discussion

Despite several types of research on the effectiveness of celery on brain and nervous system-related disorders, this study was performed for the first time on the effect of celery on CPZ-induced MS in mice. Based on the main findings, CPZ significantly decreased reflexive motor behavior in mice. Several methods were introduced for a model of demyelination in which a CPZ-containing diet for 4-6 weeks is preferred by many researchers [12]. This leads to oligodendrocytes damage, followed by microglia and astroglia activation, which disrupts energy metabolism in the mitochondria. Therefore, C57BL/6 mice are suggested [13], and here, we used a CPZ-containing diet for 5 weeks using C57BL/6 mice. According to our results, CPZ significantly decreased the crosses and rotarod time, and the administration of CPZ with CEO reduced the adverse effects of CPZ. In this regard, Xu et al. [14] reported that the mice exposed to 0.2% CPZ for 4-6 weeks had impaired sensorimotor gating and less social interaction, resulting in staying in the open arms of the maze and more memory impairment. Demyelination mainly occurs in white matter. However, there is evidence of grey matter [15].

As observed, CEO had a governing role in reflexive motor behavior in mice, and even the co-administration of CPZ with CEO significantly reduced the adverse impact of CPZ on reflexive motor behavior. In a similar report, different levels of A. graveolens extract (125-500 mg/kg) amplified activity and decreased anxiety-related behaviors (peak effect at 125 mg/kg) [11]. It has been well documented that antioxidants play a protective role against CPZ-induced demyelination [16]. Natural phenols have antioxidant properties in brain neurodegenerative diseases, such as MS [17]. It has been reported that A. graveolens methanolic extract (125-500 mg/kg) enhanced novel exploration and memories [18]. It seems that l-3-n-butylphthalide is responsible for augmented long-term spatial memory and reduced β-amyloid deposition in transgenic Alzheimer's disease mice [7]. A. graveolens methanolic extract (125 and 250 mg/kg) raises the number of living neurons in the cortex and hippocampus brain areas [18]. However, we could not conduct a histopathological investigation in this study due to limitations. It has been shown that L-3-n-butylphthalide, as the main bioactive component of A. graveolens, increased the transcription of neuroprotective factors, brain-derived neurotrophic factor, and klotho in mice with chronic epilepsy (19). The positive effects of celery extract on MS might be related to its main bioactive components, and we were not able to determine the effect of celery on these trophic factors because of some limitations.

Here, CPZ significantly elevated serum MDA while reducing GPx, SOD, and TAS levels. CEO decreased serum MDA while enhancing serum SOD, GPx, and TAS levels in the CPZ-treated mice. Based on the evidence, there is a close interrelation between the pathophysiology of MS and oxidative stress in humans and animals [4]. Because of high oxygen consumption and ROS production, the brain tissue is highly susceptible to oxidative damage due to polyunsaturated fatty acids constituting the neuronal membranes. Thus, the overproduction of MDA and reduced intracellular antioxidative protection (i.e., catalase, GPx, and SOD) leads to dysfunction and, ultimately, neuronal cell death. Tanasawet et al. [11] reported that A. graveolens (125, 250, and 500 mg/kg) decreased MDA production and enhanced GPx levels in the cortex and striatum of the mouse, and our findings were in agreement with this report. Celery contains several bioactive compounds, such as flavonoids and L-3-n-butylphthalide [5]. The anxiolytic activity of A. graveolens might result from its antioxidant properties [11].

These results suggested the protective effect of CEO against CPZ-induced MS mediated by its antioxidant activity. Further investigation is needed to determine its active constituents and precise mode of action.

Materials & Methods

Animals

Forty male C57BL/6 mice (aged 4-6 weeks and weighting 19 ± 2 g) were kept under laboratory conditions (temperature of 22°C ± 2°C and 12/h light/dark cycle) with adequate food and water in Razi laboratory complex (Islamic Azad University, Science and Research Branch, Tehran, Iran). One week after acclimatization, the mice were randomly allocated into four experimental groups (n=10). The research committee of Islamic Azad University, Science and Research Branch approved all study protocols (IR.IAU.SRB.REC.1401.112).

Preparation of celery crude essential oil

Apium graveolens L. was identified at the Faculty of Agriculture, Science and Research Branch, Islamic Azad University, Tehran, Iran. The Apium graveolens L. leaves (100 g) shade dried at room temperature (25°C ± 2°C). The samples were hydro-distilled by a Clevenger-type apparatus for 3.5 hours until the complete recovery of essential oil. The essential oil on top of the distillate was collected, dried, and stored in a dark glass bottle covered with aluminum foil at 4°C±1°C [20].

Study protocol

The control group received a regular diet. In group 2, acute demyelination was induced by feeding mice with 0.2% (w/w) CPZ (Sigma Aldrich, St. Louis, MO, USA) mixed with ground chow for 5 weeks [15]. In group 3, a regular diet was provided, and mice were administered daily p.o. with CEO (800 mg/kg) for 5 weeks [21]. In group 4, mice received a diet containing CPZ (0.2% w/w) for 5 weeks and were administered p.o. with CEO (800 mg/kg). Finally, reflexive motor behavior and serum antioxidant levels were determined. MS corresponding animal model, experimental autoimmune encephalomyelitis, is widely used to understand disease pathogenesis and test novel therapeutic agents. These defects are quantified using a standard experimental autoimmune encephalomyelitis scoring system on a 0-5 disease severity scale as 0: no disease; 1: loss of tail tone; 2: hind limb weakness; 3: hind limb paralysis; 4: hind limb and forelimb paralysis or weakness; and 5: moribund/death [22].

Ambulation

Ambulation test as crawling behavior is used to determine the ability to walk following MS [23]. In this test, mice are motivated to walk, then a scoring system is used for the quality of walk in which no movement is scored as zero, asymmetric walking is scored as 1, symmetric slow movement is scored as 2, and finally, fast walking mice are given score 3. This test was completed in triplicate at 3 minutes and the average score was recorded [24].

Hind limb foot angle

Following the signs of MS, hind limb positions change wherein walking [24]. Consequently, the movements of mice in an open field box were recorded by a camera. Images obtained from the hind limb positions and pictures in which mice had full stride in a straight line were used. A line was drawn from the end of the heel to the tip of the toe and the angle between them was measured [24].

Front limb suspension

This test was used to assess the ability of animals to hang with front limbs. Briefly, mice were allowed to grasp a wire which was tied to two ends between a wall. After grasping the wire, the time until the

mice released the wire was recorded. This test was repeated in triplicate at 3 minutes and the average time in seconds was recorded [24].

Hind limb suspension

This test described mice's ability to hang over their hind legs [24]. Following the signs of MS, hind limb strength decreases in mice. The hind legs of mice were hung over the wire which was tied at two ends between a wall and a scale system, and hind limb posture was determined. After mice hung over the wire, the hind limb posture score was recorded as not able to grasp the wire (score 0); the hind limb easily released from the grasped wire in a clasped position (score 1); by rising the tail, the hind limb was easily released from the grasped wire and stayed close to each other (score 2); by rising the tail, the hind limb of the mice was easily released from the grasped wire but stayed normal (score 3); by rising the tail, hind limb separation was normal with force [24].

Surface righting

This test assesses the ability of animals to return to their normal position [25]. Briefly, mice were kept in the pine position for 5 seconds. Next, they were released and the time needed for flipping the mice to the normal position onto the feet was recorded [24].

Grip strength

Mice were placed on a 16×18 fiberglass screen and slowly rotated from a horizontal to a vertical location. In this test, mice try to grasp the screen to not release from the surface [26]. The latency to fall was recorded [27].

Negative geotaxis

Mice were placed face down on a 45° surface. Then, they were released and the time needed to face the hill upward was recorded [24].
Open field test
This test describes the locomotor and exploratory activities of animals. Mice were allocated into an OFT apparatus (45×45×30 cm3 with nine divided squares wooden box). Mice were allocated at the center of the box and the number of squares passed was recorded in 6 minutes [28].

Rotarod test

This test evaluates animals' motor coordination and ability to stay running on accelerated rods. Mice were laced on rotarod apparatus and the test was performed with an acceleration of 0-20 rpm in 10 minutes. The time until mice fell off the rod was recorded [29].

Antioxidant activity

After determining the behavioral tests, blood samples were taken from each mouse from cardiac and serum MDA levels, and SOD, GPx, and TAS activities were obtained using Zell Bio GmbH (Germany) assay kits [30-32].

Statistical analysis

Data were analyzed using one-way ANOVA and were presented as mean±SE (standard error) using SPSS version 22.0. For treatments showing significant differences by ANOVA, between-group evaluations were performed using the Tukey posthoc test ($p < 0.05$).

Authors' Contributions

Tahoura Mohammadi-Ghohaki : collect data, draft of paper, Shahin Hassanpour: thesis supervisor, revise paper, study protocol, Morteza Zendehdel : thesis advisor.

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Competing Interests

There is no conflict of interest.

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