Original Article

Ferulic Acid Ameliorates Cell Injuries, Cognitive and Motor Impairments in Cuprizone-Induced Demyelination Model of Multiple Sclerosis

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Abstract

Objective: Ferulic acid (FA) is a phenolic compound that exhibits neuroprotective effects in the central nervous system (CNS). This study was conducted to evaluate the potential effects of FA on the cognitive and motor impairments in the cuprizone-induced demyelination model of multiple sclerosis (MS).

Materials and Methods: In this experimental study, demyelination was induced in mice by feeding them with chow containing cuprizone (CPZ) 0.2% for 6 weeks. Mice in the control group received normal chow. Mice in the CPZ+Veh, CPZ+FA10, and CPZ+FA100 groups received saline, and FA at a dose of 0, 10, or 100 mg/kg (intraperitoneal, I.P., daily) respectively. After cognitive and motor assessments, under anaesthesia, animal brains were removed for evaluating the histological, apoptosis, and molecular changes.

Results: The results showed that FA increased freezing behaviour in contextual ($P<0.05$) and cued freezing tests ($P<0.05$). FA also reduced the random arm entrance ($P<0.01$) and increased spontaneous alternations into the arms of Y-maze compared to the CPZ+Veh group ($P<0.05$). Time on the rotarod was improved in rats that received both doses of FA ($P<0.01$). Demyelination, apoptosis, and relative mRNA expression of p53 were lower in the FA-treated groups relative to the CPZ+Veh group ($P<0.01$). In addition, FA increased mRNA expression of brain-derived neurotrophic factor *(Bdnf), Olig2*, and *Mbp* ($P<0.05$) but decreased *GFAP* mRNA expression compared to the CPZ+Veh group ($P<0.01$).

Conclusion: The results of this study showed that FA plays a significant neuroprotective role in CPZ models of demyelination by reducing neuronal apoptosis and improving oligodendrocytes (OLs) growth and differentiation.

Keywords: Apoptosis, Cuprizone, Demyelination, Ferulic Acid, Oligodendrocyte

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Introduction

Multiple sclerosis (MS) is one of the frequent neurological diseases characterized by chronic inflammation and demyelination in the central nervous system (CNS) (1). Demyelination of neurons in the CNS that is caused by the destruction of oligodendrocytes (OLs) leads to an abnormal interplay of glial and neural cells. This abnormality has been considered the main pathological substrate for the structural and abnormal functional consequences of MS (2). OLs are necessary for the myelination of neurons in the CNS and the normal transmission of neural signals. Also, they have a crucial role in axonal maintenance and regeneration (3). In addition to severe axonal dysfunction, apoptosis of neuronal and glial cells occurs in the CNS of patients with MS (4). Obvious demyelination and apoptotic atrophy are also observed in the corpus callosum (CC), the prefrontal cortex (PFC), the external capsule, and the hippocampus of the CNS (5). It has been demonstrated that the extensive demyelination in the CNS following MS leads to cognitive and motor dysfunctions of the brain (6). While MS leads to several neurological disorders, optimal treatment for the disorder is yet to be discovered. Therefore, it is important to explore the potential novel neuroprotective compounds for MS therapy.

Ferulic acid (FA) is a phenolic compound widely found in vegetables, fruits, and some beverages such as coffee and beer (7). Previous studies have shown that FA has neuroprotective effects via antioxidant and anti-apoptotic mechanisms *in vitro* and *in vivo* (8), neural progenitor cell proliferation (9), differentiation of human bone marrow stromal cells into neural-like cells (10), prevention of neuronal apoptosis, acceleration of peripheral nerve regeneration, and neurogenesis (11).

There are experimental methods widely used for evaluating demyelination disorders. Cuprizone (CPZ) is a copper-chelating agent which is able to induce demyelination through the destruction of OLs. CPZ has been widely used for the induction of demyelination, and

CPZ-induced demyelination has been used as a model for the study of demyelination diseases such as MS (12). Administration of CPZ to C57BL/6 mice can induce apoptosis of mature OLs and subsequent demyelination accompanied by cognitive and motor disorders in the CC of the mice (13).

New complementary and alternative therapies that can increase OLs survival and repair the myelin sheaths are required to treat MS. Based on the present evidence for the beneficial effects of FA, we designed the study to evaluate the effect of FA on the OLs death and demyelination rate using the mouse model of CPZ-induced demyelination. The molecular and cellular mechanisms of the histological and cognitive effects of FA were targeted.

Materials and Methods

Chemicals

Cuprizone, Bis (cyclohexanone) oxaldihydrazone, FA (4-hydroxy-3-methoxy-cinnamic acid), Neutral Red, and Luxol- Fast blue powder were purchased from Sigma-Aldrich.co, Austria. RNA extraction kits were provided by Cinna Gen, Iran. RevertAid™ First Strand cDNA Synthesis kit was from TAKARA- BIO, Japan. Master Mix containing SYBR green DNA dye was purchased from Amplicon, Denmark, and the in-situ cell death detection kit POD (Cat no. 11 684 817 910) was obtained from Roche, Mannheim, Germany.

Animals

All experiments were performed according to the agreement of the Animal Ethics Committee of the Yazd University of Medical Sciences (IR.SSU.MEDICINE.REC.1393.111). Male C57BL/6 mice (6-7 weeks old, 20-25 g weight) were obtained from the animal facility of Shiraz University (Shiraz, Iran). The animals were maintained in Plexiglas cages on a 12 hours light/dark cycle, controlled temperature (20-22 °C) and had free access to water and food. Every effort was taken to minimize the suffering and damage to the animals and tried to use as few animals as possible.

Experimental protocols

In this experimental study, the animals were divided into 4 groups of 10 mice. Group 1 (control) received standard rodent chow; the other three CPZ-treated groups received standard rodent chow mixed freshly with 0.2% CPZ for 6 weeks. Group 2 (CPZ+Veh) was treated with cuprizone and saline; group 3 (CPZ+FA10) was treated with FA at a dose of 10 mg/kg. Group 4 (CPZ+FA100) was treated with FA at a dose of 100 mg/kg. Saline or FA was administered orally once a day for 6 weeks (Table 1).

For evaluation of memory impairment and locomotor incoordination, we used the fear conditioning, Y-maze, and rotarod tests. At the end of the experiment, under anaesthesia, the brains were removed and fixed for evaluating the histological changes and apoptosis rate. Also, fresh brain tissues were used to quantify relative mRNA expressions of *Bdnf*, *Olig2*, *Gfap*, *Mbp*, and *p53* genes.

Neurobehavioral assessments

Assessment of cognitive and memory impairments

Fear conditioning test

To determine the FA role in CPZ-induced memory impairment, we used a contextual fear conditioning test as previously described (14). On Day 1 (day of training), animals were placed into a fear conditioning chamber (Iran, Chamber: 25 × 24 × 21 cm) containing grey walls, a metal grid floor to deliver a shock, and equipped with a sound generator. The animal was first allowed to explore the chamber for 3 minutes, thereafter, presented the clicker sound for 30 seconds. At the end of the clicker sound, each animal received a foot shock (0.7 mA) at the last 2 seconds. Memory for the context (contextual memory) for each animal was determined by counting the freezing behavior, defined as the total lack of movement except for respiration. For the cued memory test, mice were placed into a novel context for 3 minutes, and after an initial 30 seconds of novel context evaluation, the same training tone was played for 3 minutes. Freezing behavior was scored over the 3 minutes testing period and data were presented as a percentage of time in the freezing over the total sampling period. Mice were habituated to the testing room for 30 minutes at the beginning of training and the test day. All tests were conducted by the same experimenter.

Table 1: The experimental groups and procedures

Group/Treatment	6 weeks administration	Behavioral assessments	Histological and molecular assessments
Group 1 (control)	Standard rodent chow	Motor and cognitive tests	LFB – TUNEL staining, real-time PCR
Group 2 (CPZ+Veh)	CPZ in food+Vehicle	Motor and cognitive tests	LFB – TUNEL staining, real-time PCR
Group 3 (CPZ+FA10)	CPZ in food+FA10	Motor and cognitive tests	LFB – TUNEL staining, real-time PCR
Group 4 (CPZ+FA100)	CPZ in food+FA100	Motor and cognitive tests	LFB – TUNEL staining, real-time PCR

CPZ; Cuprizone, FA; Ferulic acid, LFB-TUNEL; Luxol fast blue and TUNEL staining, and PCR; Polymerase chain reaction.

Y-maze study

Working memory impairment was evaluated using 3 arms Y-maze apparatus. Each arm was 50 cm in length, 15 cm in height, and 10 cm in width, with equal angles (named A, B, and C). The behavioral evaluation was performed according to the previous study (15). Briefly, Mice were placed in the centre of the Y-maze and allowed to move their arms freely for 8 minutes. The total number of entrances and the number of alternations or the count of sequential entrances in the three different arms (e.g., ABC, BCA, CBA …) were visually recorded. The percentage of alternation was calculated using the below acquisition:

Alternation%= (number of alternations/total number of arms entrances-2)×100.

Assessment of motor coordination

Rotarod test

The rotarod device (Borj Sanat Azma, Iran) was used to assess motor coordination and balance in the animals. The setup consists of a horizontal step ladder (2.5 cm in diameter, 38 cm in height, and a constant speed of 25 rpm). Before starting the experiment, mice were trained on the rotarod in 3 training sessions, each lasting 5 minutes for 3 days. At the end of the experiment, the performance of the mice was assessed for 5 minutes, and a fall off the rotarod within this period was recorded (16). Additionally, the number of falls and flips was counted during 300 seconds.

Histological assessment

Myelin staining

Luxol fast blue (LFB) staining was performed to evaluate the demyelination process in the CC. Mice were deeply anesthetized (urethane, 1 g/kg of body weight) and intracardially perfused with 100 µl of saline followed by the same volume of formalin (10%). Brains were post-fixed in 4% paraformaldehyde for 3 days and embedded in paraffin for 24 hours. Coronal sections (6 µm thickness) were prepared through the CC using a microtome (Leica RM2135, Germany). After dehydration with graded alcohols and clearing with xylene, the paraffinized sections were incubated overnight with 0.1% Luxol-Fast Blue and counterstained with neutral red according to the previously reported method (17). After capturing images of slides (Zeiss light microscope, 4 × objective magnification), the CC area of each slide was scored in a blinded manner by two investigators.

For the quantification of demyelination, two independent researchers in a double-blind fashion scored each region of interest and scored them from 0 to 3. A score of "0" referred to normal myelination, a score of "1" indicated demyelination of only one-third of the myelin fibers, and a score of "2" was equivalent to demyelination of two-thirds and a score of "3" referred to complete demyelination. Scores from different sections were summed up to calculate the average score for each group.

TUNEL assay

TUNEL staining was performed to determine DNA fragmentation as an index of cell apoptosis rate. Apoptotic cells were detected using an in-situ cell death detection kit (Roche, Mannheim, Germany) as per the manufacturer's instructions. The mice brains were removed and fixed in phosphate-buffered saline (PBS, 0.1 M Sodium phosphate, 0.14 M NaCl, pH=7.4) containing 3.7% paraformaldehyde. The fixed brains were histologically processed and embedded in the paraffine. Three sections (thickness: 5 µ, interval: 30 µ) were obtained through the CC. The sections were deparaffinised and hydrated. Thereafter, the sections were incubated with proteinase K (100 µg/mL), washed with PBS, and treated with 3% H_2O_2 for 10 minutes to inactivate endogenous peroxidase. Again, the sections were washed twice with PBS and incubated in the TUNEL reaction mixture for 1 hour. The tissues were rinsed and visualized using Converter-POD with 0.03% 3, 3′-diaminobenzidine (DAB). For counter-staining, the washed sections were stained with haematoxylin (DAKO, Denmark). The apoptotic cells were counted (3 windows per section/animal; 4 animals/group) and the mean value for each group was used for analysis. The percentage of apoptotic cells was calculated as TUNEL-positive cells/total numbers of cells (18).

Quantitative real-time polymerase chain reaction

mRNA levels of *Olig2* as an OLs precursor marker, *p53* as an apoptotic regulator marker, *GFAP* as an indicative of astrogliosis, *BDNF*, and *MBP* as the markers for the survival of neurons, glial cells, and remyelination were determined using semi-quantitative real-time polymerase chain reaction (qRT-PCR). RNA of each tissue sample of CC was extracted according to the kit instruction. The efficiency and quality of RNA extraction were determined using the 260/280 ratio of optical density from each sample (Microplate reader, Epoch, England). Then, Single-strand cDNA was synthesized from 1 µg of the extracted mRNA and finally, qRT-PCR was performed. The qPCR reaction contained 12.5 µl of Master Mix, 10.5 µl of purified water, 1 µl of 10 µM primer solution, and 1 µl of cDNA template. All values were normalized to the hypoxanthine-guanine phosphoribosyl transferase-encoding gene (HPRT), a housekeeping gene with minimal variability in different experimental conditions. Finally, the ΔΔCt method was applied to compare the relative gene expression. The primers sequences are indicated in Table 2.

Statistical analysis

Data were expressed as mean ± SEM and analysed by statistical software GraphPad Prism 7.0 (GraphPad, Inc., USA). One-way ANOVA was used to determine the significant differences between groups and post-hoc Bonferroni or Tukey's tests as required. The $P<0.05$ was considered statistical significance.

Table 2: The primer's sequences list

Gene	Primer sequence (5´-3´)
p53	F: TTATGTGCACGTACTCTCCTCC
	R: GCTGTGACTTCTTGTAGATGGC
Olig2	F: TTACAGACCGAGCCAACACC
	R: TCAACCTTCCGAATGTGAATTAG
Gfap	F: AACCGCATCACCATTCCTGT
	R: CAGGCTGGTTTCTCGGATCT
Bdnf	F: TACTTCGGTTGCATGAAGGC
	R: TACTGTCACACACGCTCAGCTCC
Mbp	F: GCACGCTTTCCAAAATCTTTA
	R: GCCATGGGACCAGAG
Hprt	F: GTGATTAGCGATGATGAACCAG
	R: AGCAAGTCTTTCAGTCCTGTCC

Results of cognitive evaluations

Fear conditioning

As expected, there was not a significant difference in freezing response between experimental groups on day 1. During 120 s of the test, all animals produced a brief transient freezing-like behavior. During the fear contextual test, mice in the CPZ+Veh showed a significantly lower freezing response compared to the control group (P<0.01). Post-hoc analysis revealed that FA at both doses increases freezing response in the fear contextual test (P<0.05). During the cue test, mice in the CPZ+Veh group showed a significantly lower percentage of the freezing response compared to the control group (P<0.05). Also, the post-hoc analysis revealed that FA at the higher dose increased freezing response in the fear contextual test (P<0.05). Data are shown in Figure 1.

Y-maze

As shown in Figure 1, the total number of random arm entries were significantly higher in CPZ+Veh group when compared to control mice (P<0.01). Treatment of mice with FA at both doses reduced the total number of random entries (P<0.01). The percentage of spontaneous alternations showed a significant decrease in the CPZ+Veh group compared to the control group (P<0.05), while FA treatment at both doses could significantly improve the percent of spontaneous arm alternation (P<0.05).

Results of motor coordination evaluations

Rotarod test

CPZ administration to mice in the CPZ+Veh group significantly decreased the time on the rotarod and increased the number of falls/flips from the rotarod compared to the control group (P<0.001). However, treatment of mice with FA at both doses increased time on the rotarod and decreased the number of falls/flips compared with the CPZ+Veh group (P<0.05). Data are shown in Figure 1.

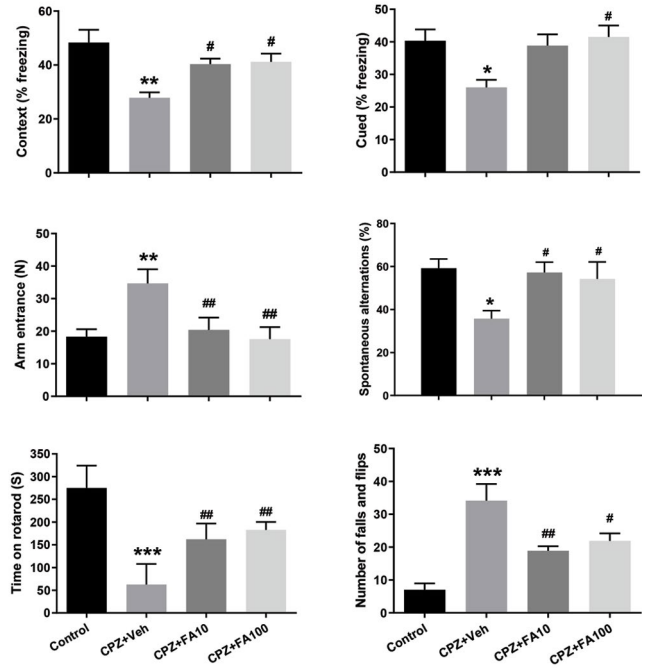

Fig.1: Effect of FA on cuprizone-induced impairments in cognitive function (freezing and cued behaviors in fear conditioning test, and the number of arm entries and spontaneous alternations in Y-maze) and motor coordination (time on road and the number of falling/flipping in rotarod). *; Significant difference when compared to control group, #; Significant difference when compared to CPZ+Veh group, *; P<0.05, **; P<0.01, ***; P<0.001, #; P<0.05, ##; P<0.01, FA; Ferulic acid, CPZ; Cuprizone, and Veh; Vehicle. Data are mean ± SEM.

Histological findings

The LFB is a sensitive tissue staining method used to detect the phospholipids of CC. Qualitatively, Chronic CPZ administration led to a prominent lesion in the myelin sheath in the CC compared to the control group (Fig.2A-D). In the presence of CPZ treatment, neuroprotection of CC occurred in the FA-treated mice (Fig.2E-H) but not in vehicle-treated mice (control group). The CC of mice that received FA at a dose of 10 mg/kg showed a lesser demyelination relative to the control group. Also, this protective effect was observed in the mice treated with 100 mg/kg of FA but to a lesser extent compared to the mice treated with 10 mg/kg. Semiquantitative analysis of LFB stained cells for scoring of demyelination revealed that CPZ significantly increased demyelination in CC (P<0.01). FA at both doses led to a significant decrease in the CPZ-induced demyelination (P<0.05).

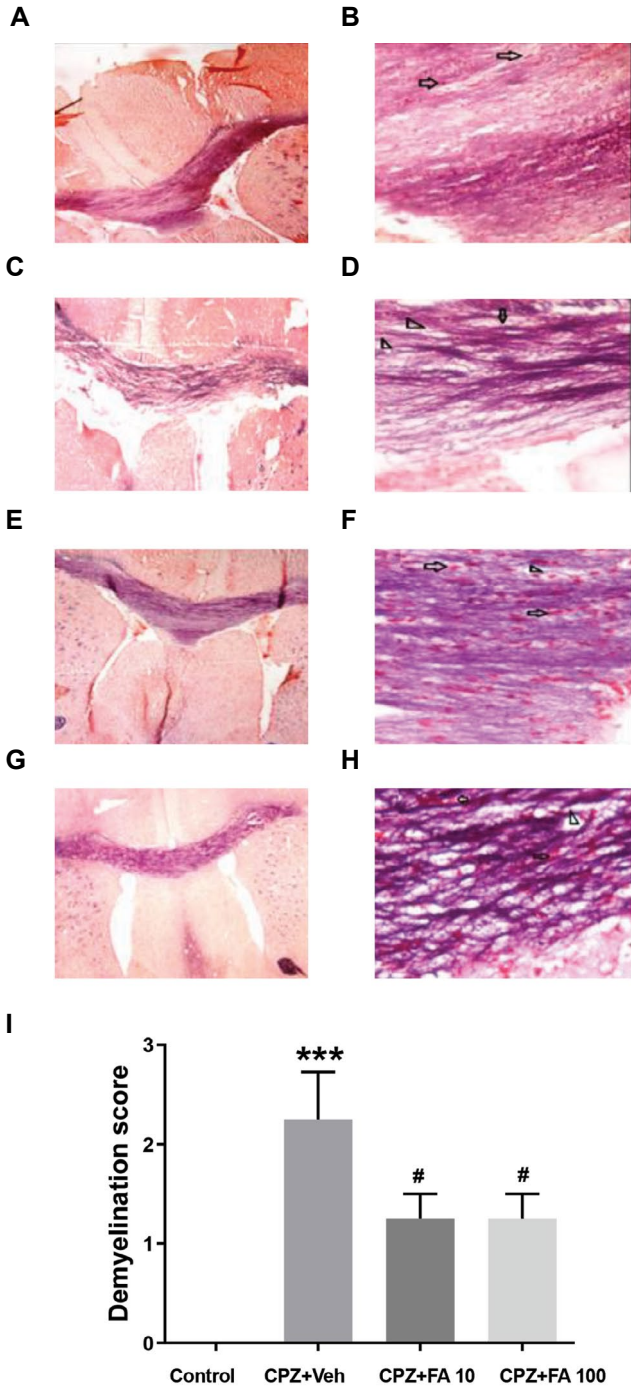

Fig.2: Light micrographs showing histological sections of brains from rats in CC area. Arrows and open triangles show OLs and demyelination loci respectively. **A, B.** Control group, **C, D.** CPZ+Veh group, **E, F.** CPZ+FA 10, **G, H.** CPZ+FA 100 (the right column, 40x and the left column 400×). **I.** The histogram represents demyelination scores of CC in different groups. Demyelination score data are expressed as the mean ± SEM and analysed using one-way ANOVA followed by post hoc Turkey's test. ***; P<0.001: significant difference when compared to the control group, #; P<0.05: significant difference when compared to CPZ+Veh group, OLs; Oligodendrocytes, FA; Ferulic acid, CPZ; Cuprizone, and Veh; Vehicle.

The effects of FA on apoptosis and expression of the *p53* gene in CC

As shown in Figure 3, the number of TUNEL-positive cells significantly elevated in cuprizone-treated mice compared to control rats. FA treatment at both doses decreased the number of TUNEL-positive cells in the medial region of the CC compared to the CPZ+Veh group. The quantitative analysis revealed that the number of TUNEL-positive cells in the CPZ+Veh group was significantly more than in the control group (P<0.001), while FA treatment significantly reduced the number of TUNEL-positive cells (P<0.01, Fig.2E). The mRNA level of the *p53* gene was significantly higher in the CPZ+Veh mice while it was decreased by treatment of rats with FA 10 and 100 mg/kg (P<0.01, Fig.3F).

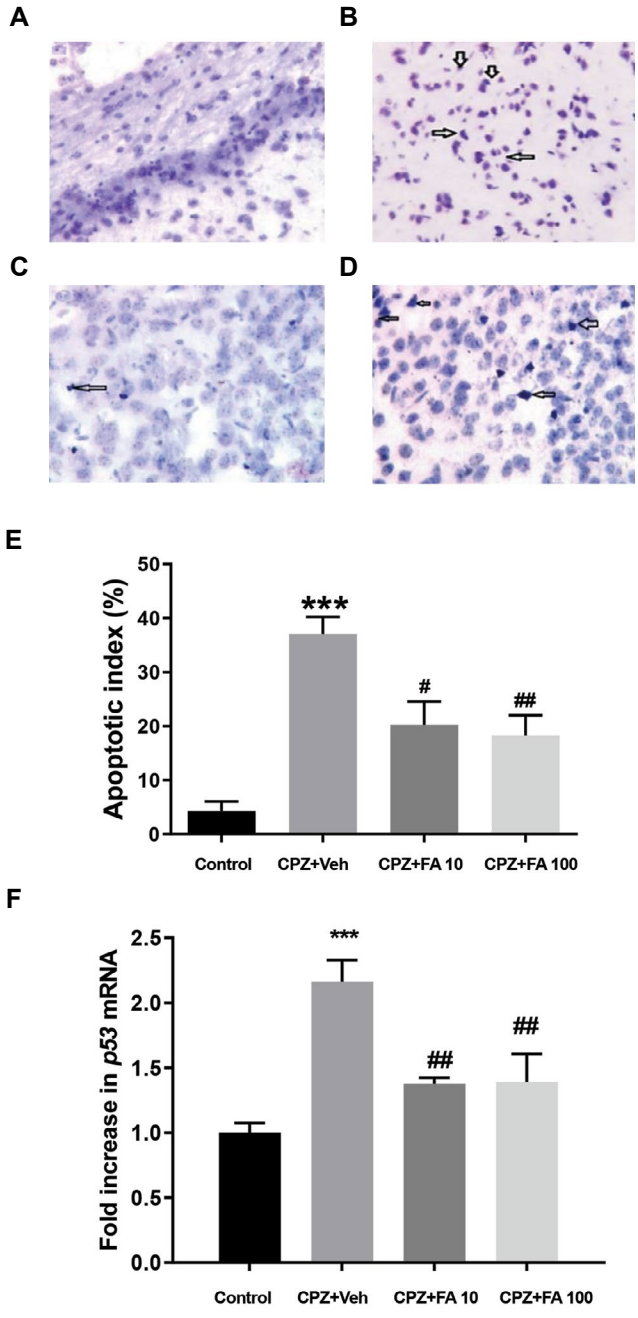

Fig.3: The apoptotic rate in different experimental groups light micrograph pictures (400×) of CC in experimental groups. **A.** Control group, **B.** CPZ+Veh treated group, **C.** CPZ+FA10 treated group, **D.** CPZ+FA100 treated group. The arrow indicates apoptotic cells. **E.** Quantitative analysis for calculating the ratio of TUNEL-positive cells to total cell number. **F.** The mRNA levels of *P53* relative to HPRT in the corpus callosum as determined by RT-PCR, data are expressed as the mean ± SEM and analysed using one-way ANOVA followed by post hoc Turkey's test. ***; P<0.001: significant difference when compared to the control group, #; P<0.05, and ##; P<0.01: significant difference when compared to CPZ+Veh group. CC; Corpus collosum, CPZ; Cuprizon, Veh; Vehicle, FA; Ferulic acid, HPRT; Hypoxanthine phosphoribosyltransferase 1 and R.

The effects of FA on gene expression of glial markers in corpus collosum

Evaluation of mRNA gene expression in CC is shown in Figure 4. The mRNA expression level of *BDNF* in CPZ+Veh groups was significantly decreased (P<0.01) when compared to the control group but FA at a dose of 10 mg/kg (P<0.01) and at a dose of 100 mg/kg (P<0.05) increased expression of *BDNF* mRNA relative to CPZ+Veh group. Relative mRNA expression of olig2 indicated a significant decrease in CPZ+Veh group when compared to the control. However, FA at a dose of 100 mg/kg could significantly increase the mRNA expression of Olig2 (P<0.01). The relative mRNA expression level of GFAP was significantly increased in the CPZ+Veh treated mice compared to the control mice (P<0.001) while both doses of FA could significantly decrease mRNA expression of GFAP compared to the CPZ+Veh group (P<0.01). The mRNA expression level of MBP in the CPZ+Veh group didn't show a significant difference compared to the control group but FA at a dose of 10 mg/kg causes a significant increase in the mRNA expression level of MBP compared to CPZ+Veh group (P<0.01).

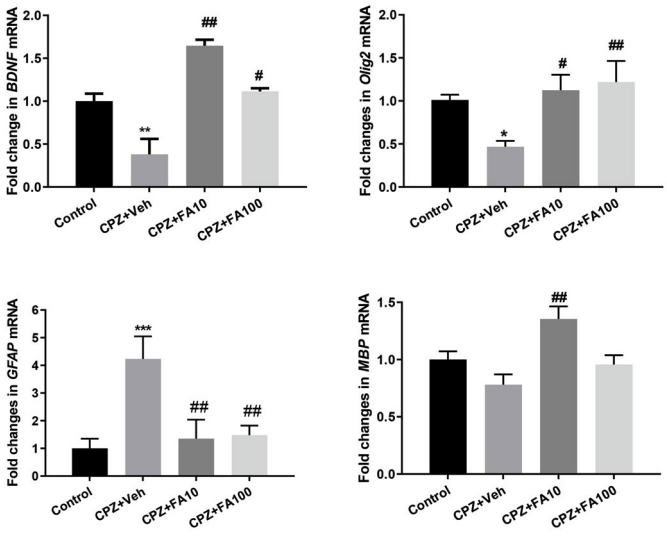

Fig.4: The effect of different doses of FA on mRNA expression of the genes involved in myelination. Data are expressed as the mean ± SEM and analysed using one-way ANOVA followed by post hoc Turkey's test. *; Significant difference when compared to the control group, #; Significant difference when compared to CPZ group, *; P<0.05, #; P<0.05, **; P<0.01, and ##; P<0.01.

Discussion

Our findings showed that oral administrations of FA could improve cognitive impairment and motor coordination in the CPZ model of demyelination and MS in mice. FA can also reduce CC demyelination, apoptosis, the expression of the genes involved in demyelination (GFAP), and increase the expression of genes involved in the myelination, neuronal, glial protection, and growth (e.g. *Olig2*, *MBP*, and *BDNF*).

The CPZ model has been widely used for experimental studies on demyelination disorders and MS, and this chemical is able to cause behavioral, histological, and molecular changes such as those seen in MS (19, 20). Moreover, studies have shown that CPZ causes demyelination, apoptosis, and activation of neuroglia in the CNS (21, 22).

The results of the present study on the effect of CPZ on cognitive function are consistent with the previous studies showing that the CPZ-treated mice had higher arm entrance (13) and a lower percentage of spontaneous alternation in the Y-maze apparatus (23). Also, cognitive impairment in the contextual/cued fear conditioning tests observed in this study was similar to the previous study that indicated the CPZ-treated mice had significantly lower levels of freezing behavior than vehicle-treated mice (24).

FA is an antioxidant, found in vegetables, fruits, cereal, coffee, and beer (25). It can protect cells from oxidative stress by increasing the activity of several antioxidant enzymes and by modulating several cellular functions (26). Our results indicated that FA decreased the number of arm entries and spontaneous alternation behavior compared to CPZ+Veh mice in Y-maze tasks. Some reports indicated that FA could improve impaired cognitive function through different mechanisms (27, 28). While other few studies have shown that FA does not affect the number of arm entries or spontaneous alternation behavior (29, 30). The controversial results can be attributed to the different doses or time courses of FA administration.

CPZ could induce apoptosis in OLs, the reaction that activates microglia/macrophages and leads to the destruction of myelin sheets (31). The *p53* mRNA level expression upregulated in CC after administration of CPZ treated diet in mice (32). The p53 as a proapoptotic protein has an essential role in OLs death so that in the transgenic animals, the *p53* gene has been knocked out, revealing no obvious apoptosis and OLs death followed by CPZ treatment (32, 33). Also, repeated treatments with pifithrin-α, as a p53 inhibitor, could reduce demyelination and microglial activation (32) . In this study, FA could attenuate the apoptotic rate in the CC of the mice treated with CPZ; the findings can be attributed to the p53 inhibition by FA. These reports are also in accordance with the previous studies that showed FA is an anti-apoptotic factor that can exert its effect by suppressing caspase3 activity (34) and reducing p53 level in the neuronal cells (35).

Olig2 is a transcription factor that regulates neuronal and glial specifications and promotes OLs differentiation from its precursor cells, especially after cortical injury (36). The increased level of Olig2 in the subventricular zone of CPZ-feeding mice indicates that Olig2 is a key regulator in the period of remyelination after myelin sheet destruction (37). It has been reported that

in vivo and *in vitro* administrations of FA accelerate the proliferation and differentiation of neural progenitor (9) and Schwann cells (38). Indeed, our study results are in agreement with the above-mentioned evidence that implies FA could increase Olig2 mRNA expression in CC.

MBP is a major constituent of myelin sheet and its expression significantly decreased in the CC of mice treated with CPZ (21). As observed in our study, FA could increase the expression of MBP mRNA in the Schwann cells (38). The increment in myelin sheet synthesis and development of OLs may be attributed to the effect of FA on the elevation in MBP expression. Previous studies reported that FA treatment could increase the population of myelinated axons after sciatic nerve injury (11).

BDNF is a neurotrophic factor demonstrating an essential role in accelerating of OLs precursor proliferation and development (39). Also, BDNF promotes the synthesis of the protein molecules such as MBP which is a necessary base material for synthesizing myelin and thickening it. It has been reported that the level of BDNF was reduced in the CC of the mice treated with CPZ (40). We found that FA treatment could potentiate mRNA expression of BDNF in CC. It may be concluded that the part of beneficial effects of FA exert through the increase in the OLs precursor proliferation and development mediated by BDNF and MBP.

Astrogliosis, as a response to acute or chronic demyelination, was observed after different types of demyelination disorders or in animals that received a CPZ diet (39). In agreement with our findings, some studies show that FA could reduce the level of GFAP after CNS injury which indicates that it can attenuate the rate of gliosis (7).

Conclusion

The results of the present study showed that FA improved demyelination, motor incoordination, and cognitive dysfunction in the laboratory model of MS. FA could increase myelin density and decrease the apoptotic rate in the brain affected by a CPZ. These effects are partially mediated by reducing the expression of genes involved in apoptosis and an increase in the expression of the OLs precursor proliferation and development.

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Authors' Contributions

M.Gh.; Performed the induction of MS and evaluation of drug effects and data collection. B.A.; Prepared tissue samples and histological processing. M.Y.; Interpreted the histological cells for evaluation of necrotic and apoptotic cells. M.E.; Participated in drafting and statistical analysis. F.H.-M.; Conducted to design of the study and molecular experiments and RT-qPCR analysis. M.E.R.; Participated in design of study, performed editing, and approving the final version of this paper for submission. All authors read and approved the final manuscript.

References

1. Vercellino M, Masera S, Lorenzatti M, Condello C, Merola A, Mattioda A, et al. Demyelination, inflammation, and neurodegeneration in multiple sclerosis deep gray matter. J Neuropathol Exp Neurol. 2009; 68(5): 489-502.
2. Mi S, Hu B, Hahm K, Luo Y, Hui ES, Yuan Q, et al. LINGO-1 antagonist promotes spinal cord remyelination and axonal integrity in MOG-induced experimental autoimmune encephalomyelitis. Nat Med. 2007; 13(10): 1228-1233.
3. Moore S, Meschkat M, Ruhwedel T, Trevisiol A, Tzvetanova ID, Battefeld A, et al. A role of oligodendrocytes in information processing. Nat Commun. 2020; 11(1): 5497.
4. Mahad DH, Trapp BD, Lassmann H. Pathological mechanisms in progressive multiple sclerosis. Lancet Neurol. 2015; 14(2): 183-193.
5. Treaba CA, Herranz E, Barletta VT, Mehndiratta A, Ouellette R, Sloane JA, et al. The relevance of multiple sclerosis cortical lesions on cortical thinning and their clinical impact as assessed by 7.0-T MRI. J Neurol. 2021; 1: 2473-2481.
6. Llufriu S, Blanco Y, Martinez-Heras E, Casanova-Molla J, Gabilondo I, Sepulveda M, et al. Influence of corpus callosum damage on cognition and physical disability in multiple sclerosis: a multimodal study. PLoS One. 2012; 7(5): e37167.
7. Mancuso C, Santangelo R. Ferulic acid: pharmacological and toxicological aspects. Food Chem Toxicol. 2014; 65: 185-95.
8. Ren Z, Zhang R, Li Y, Li Y, Yang Z, Yang H. Ferulic acid exerts neuroprotective effects against cerebral ischemia/reperfusion-induced injury via antioxidant and anti-apoptotic mechanisms in vitro and in vivo. Int J Mol Med. 2017; 40(5):1444-1456.
9. Srinivasan M, Sudheer AR, Menon VP. Ferulic acid: therapeutic potential through its antioxidant property. J Clin Biochem Nutr. 2007; 40(2): 92-100.
10. Wang Y, Deng Z, Lai X, Tu W. Differentiation of human bone marrow stromal cells into neural-like cells induced by sodium ferulate in vitro. Cell Mol Immunol. 2005; 2(3): 225-229.
11. Lee SC, Tsai CC, Yao CH, Chen YS, Wu MC. Ferulic acid enhances peripheral nerve regeneration across long gaps. Evid Based Complement Alternat Med. 2013; 2013: 876327.
12. Praet J, Guglielmetti C, Berneman Z, Van der Linden A, Ponsaerts P. Cellular and molecular neuropathology of the cuprizone mouse model: clinical relevance for multiple sclerosis. Neurosci Biobehav Rev. 2014; 47: 485-505.
13. Makinodan M, Yamauchi T, Tatsumi K, Okuda H, Takeda T, Kiuchi K, et al. Demyelination in the juvenile period, but not in adulthood, leads to long-lasting cognitive impairment and deficient social interaction in mice. Prog Neuropsychopharmacol Biol Psychiatry. 2009; 33: 978-985.
14. Tomas-Roig J, Agbemenyah HY, Celarain N, Quintana E, Ramió-Torrentà L, Havemann-Reinecke U. Dose-dependent effect of cannabinoid WIN-55,212-2 on myelin repair following a demyelinating insult. Sci Rep. 2020; 10(1): 590.
15. Xiao L, Xu H, Zhang Y, Wei Z, He J, Jiang W, et al. Quetiapine facilitates oligodendrocyte development and prevents mice from myelin breakdown and behavioral changes. Mol Psychiatry. 2008; 13(7): 697-708.
16. Jones BJ, Roberts DJ. The quantitative measurement of motor inco-ordination in naive mice using an accelerating rotarod. J Pharm Pharmacol. 1968; 20(4): 302-304.
17. Carriel V, Campos A, Alaminos M, Raimondo S, Geuna S. Staining methods for normal and regenerative myelin in the nervous system. Methods Mol Biol. 2017; 1560: 207-218.
18. Faheem H, Mansour A, Elkordy A, Rashad S, Shebl M, Madi M, et al. Neuroprotective effects of minocycline and progesterone on white matter injury after focal cerebral ischemia. J Clin Neurosci. 2019; 64: 206-213.
19. Matsushima GK, Morell P. The neurotoxicant, cuprizone, as a model to study demyelination and remyelination in the central nervous system. Brain Pathol. 2001; 11(1): 107-116.

20. Stangel M. Neurodegeneration and neuroprotection in multiple sclerosis. Curr Pharm Des. 2012; 18(29): 4471-4474.
21. Babbs RK, Beierle JA, Yao EJ, Kelliher JC, Medeiros AR, Anandakumar J, et al. The effect of the demyelinating agent cuprizone on binge-like eating of sweetened palatable food in female and male C57BL/6 substrains. Appetite. 2020; 150: 104678.
22. Bénardais K, Kotsiari A, Škuljec J, Koutsoudaki PN, Gudi V, Singh V, et al. Cuprizone [bis (cyclohexylidenehydrazide)] is selectively toxic for mature oligodendrocytes. Neurotox res. 2013; 24(2): 244-250.
23. Yan JJ, Jung JS, Kim TK, Hasan MA, Hong CW, Nam JS, et al. Protective effects of ferulic acid in amyloid precursor protein plus presenilin-1 transgenic mouse model of Alzheimer disease. Biol Pharm Bull. 2013; 36(1): 140-143.
24. Kopanitsa MV, Lehtimäki KK, Forsman M, Suhonen A, Koponen J, Piiponniemi TO, et al. Cognitive disturbances in the cuprizone model of multiple sclerosis. Genes Brain Behav. 2021; 20(1): e12663.
25. Zheng YZ, Zhou Y, Guo R, Fu ZM, Chen DF. Structure-antioxidant activity relationship of ferulic acid derivatives: Effect of ester groups at the end of the carbon side chain. LWT. 2020; 120: 108932.
26. Wang H, Sun X, Zhang N, Ji Z, Ma Z, Fu Q, et al. Ferulic acid attenuates diabetes-induced cognitive impairment in rats via regulation of PTP1B and insulin signaling pathway. Physiol Behav. 2017; 182: 93-100.
27. Mhillaj E, Catino S, Miceli FM, Santangelo R, Trabace L, Cuomo V, et al. Ferulic acid improves cognitive skills through the activation of the heme oxygenase system in the rat. Mol Neurobiol. 2018; 55(2): 905-916.
28. Mori T, Koyama N, Guillot-Sestier MV, Tan J, Town T. Ferulic acid is a nutraceutical β-secretase modulator that improves behavioral impairment and alzheimer-like pathology in transgenic mice. PLoS One. 2013; 8(2): e55774.
29. Mamiya T, Kise M, Morikawa K. Ferulic acid attenuated cognitive deficits and increase in carbonyl proteins induced by buthionine-sulfoximine in mice. Neurosci Lett. 2008; 430(2): 115-118.
30. Yan G, Dai Z, Shen Z, Wu R. Behavioral and neurochemical alterations in C57BL/6 mice exposed to cuprizone: An in vivo 1H-MRS STUDY at 7.0 T. J Neurol Sci. 2013; 333: e629.
31. Mason JL, Jones JJ, Taniike M, Morell P, Suzuki K, Matsushima GK. Mature oligodendrocyte apoptosis precedes IGF-1 production and oligodendrocyte progenitor accumulation and differentiation during demyelination /remyelination. J Neurosci Res. 2000; 61(3): 251-262.
32. Li J, Ghiani CA, Kim JY, Liu A, Sandoval J, DeVellis J, et al. Inhibition of p53 transcriptional activity: a potential target for future development of therapeutic strategies for primary demyelination. J Neurosci. 2008; 28(24): 6118-6127.
33. Hesse A, Wagner M, Held J, Brück W, Salinas-Riester G, Hao Z, et al. In toxic demyelination oligodendroglial cell death occurs early and is FAS independent. Neurobiol Dis. 2010; 37(2): 362-369.
34. Picone P, Nuzzo D, Di Carlo M. Ferulic acid: a natural antioxidant against oxidative stress induced by oligomeric A-beta on sea urchin embryo. Biol Bull. 2013; 224(1): 18-28.
35. Zhang Z, Wei T, Hou J, Li G, Yu S, Xin W. Iron-induced oxidative damage and apoptosis in cerebellar granule cells: attenuation by tetramethylpyrazine and ferulic acid. Eur J Pharmacol. 2003; 467(1-3): 41-47.
36. Marshall CA, Novitch BG, Goldman JE. Olig2 directs astrocyte and oligodendrocyte formation in postnatal subventricular zone cells. J Neurosci. 2005; 25(32): 7289-7298.
37. Chen LP, Li ZF, Ping M, Li R, Liu J, Xie XH, et al. Regulation of Olig2 during astroglial differentiation in the subventricular zone of a cuprizone-induced demyelination mouse model. Neuroscience. 2012; 221: 96-107.
38. Zhu X, Li K, Guo X, Wang J, Xiang Y. Schwann cell proliferation and differentiation that is induced by ferulic acid through MEK1/ERK1/2 signalling promotes peripheral nerve remyelination following crush injury in rats. Exp Ther Med. 2016; 12(3): 1915-1921.
39. Langhnoja J, Buch L, Pillai P. Potential role of NGF, BDNF, and their receptors in oligodendrocytes differentiation from neural stem cell: an in vitro study. Cell Biol Int. 2021; 45(2): 432-446.
40. Nguyen HT, Wood RJ, Prawdiuk AR, Furness SG, Xiao J, Murray SS, et al. TrkB agonist LM22A-4 increases oligodendroglial populations during myelin repair in the corpus callosum. Front Mol Neurosci. 2019; 12: 205.