

MMP9 Gene Expression Variation by Ingesting Tart Cherry and P-Coumaric Acid During Remyelination in the Cuprizone Mouse Model

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Abstract- Matrix metalloproteinase-9 (GELB) as a member of gelatinases plays key role in the destruction of blood-brain barrier (BBB), T cells migration into the CNS, and demyelination induction. Considering remyelination induction in response to tart cherry extract and pure p-coumaric acid ingestion via tracking MMP9 gene expression in the cuprizone mouse model. Firstly, predicting the chemical interaction between p-coumaric acid and MMP9 protein was conducted through PASS and Swiss dock web services. Next, the content of p-coumaric acid in the tart cherry extract was analyzed by HPLC. Later, mice (male, female) were categorized into two groups: standard, cuprizone. After the demyelination period, mice classified into four groups: standard, natural chow, tart cherry extract, p-coumaric acid. Finally, brains were extracted from the skull, and MMP9 gene expression was evaluated by real time RT-PCR. Bioinformatics analysis displayed p-coumaric acid has potent inhibitory effect on MMP9 gene expression ($P=0.818$) with estimated ΔG (kcal/mol) -8.10. In addition, during the demyelination period, MMP9 expression was increased significantly in the male group that is related to myelin destruction. However, MMP9 was declined throughout remyelination in both male and female. It's remarkable that pure p-coumaric acid and tart cherry extract ingestion could decrease the gene expression ratio more than natural chow. According to the results, it's deduced the male mouse is more appropriate gender for demyelination induction via cuprizone. In addition, tart cherry extract and pure p-coumaric acid ingestion could decrease MMP9 gene expression level considerably during remyelination.

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Introduction

Multiple sclerosis (MS) is a chronic inflammatory immune mediated disease that demyelination is one of the most prominent properties of MS pathogenesis. MS pathological process contributes to blood brain barrier (BBB) disruption, migration of immune cells and targeting oligodendrocytes, and myelin protein in white matter (1,2). Matrix metalloproteinase (MMPs) known as matrixines are a family of zinc dependent endopeptidases that play crucial role in the homeostasis of the extracellular matrix (ECM) and are categorized into five subfamilies, including Collagenases (MMP-1, -8, -13, and -18), Stromelysins (MMP-3, -10 and -11), Gelatinases (MMP-2 and -9), Matrilysins (MMP-7 and -26), and Membrane bound MMPs (MMP-14, -15, -16, -17, and -25) (3,4). According to previous research, MMPs

have role in MS pathogenesis by applying two mechanisms: (a) MMPs are released from activated white blood cells and recruit them into the central nervous system. (b) MMPs also stimulate an elevation in the release of (TNF)- α and by cooperation with other factors such as cytokines, free radicals result in myelin destruction (5,6). MMPs are regulated by specific endogenous tissue inhibitors of metalloproteinases (TIMPs) include four protease inhibitors (TIMP1, TIMP2, TIMP3, and TIMP4).

Matrix metalloproteinase-9 (GELB) as a member of gelatinases group has Zn²⁺ and Ca²⁺ binding sites; it's involved in varied biological activities, for instance, collagen catabolic process (7), extracellular matrix organization, leukocyte migration (8). Matrix metalloproteinase 9 (MMP-9) is secreted by activated lymphocytes, endothelial cells, and leads to blood-brain

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barrier (BBB) disruption (5). Therefore, MMP9 is a neutral protease that via targeting extracellular matrix (ECM) in basal lamina surrounding BBB (e.g., fibronectin, vitronectin, entactin/nidogen, elastin, and collagen IV) results in BBB permeability (9,10). The MMP9 gene promoter possesses AP-1, nuclear factor- κ B (NF- κ B) sites that NF- κ B is inducible during the inflammatory response to secondary factors, such as TNF- α , IL-1 β , and Lipopolysaccharide (LPS). MMP9 is primarily produced as an inactivated form (pro-MMP9) and can be activated by removing pro-peptide region by MMP-3 (11).

Prior studies indicated MMP9 plays key role in the destruction of blood brain barrier (BBB), T cells migration into the CNS, and demyelination induction (12,13). MMP9/TIMP-1 gene expression ratio is considerably higher in active MS blood samples than the control group (14). In addition, high concentration of MMP9 is also apparent in MS CFS samples (15). As a result, compounds like IFN β by decreasing the MMP9/TIMP-1 ratio in active MS patients can exert a therapeutic effect and prevent from MS progression (16).

Tart cherries (*Prunus cerasus*) are a valuable source of vitamins (A, B1, B2, C, E, K, and Niacin), carotenoids, minerals (Ca²⁺, Fe²⁺, K⁺, Na⁺, Mn²⁺, and Phosphorous) (17), fiber, various sugar (e.g. fructose, glucose, and maltose), antioxidant agents (e.g., caffeic acid (PubChem CID: 689043), p-coumaric acid (PubChem CID: 637542), chlorogenic acid (PubChem CID: 1794427), gallic acid (PubChem CID: 370), quercetin (PubChem CID: 5280343), kaempferol (PubChem CID: 5280863), 1-(3',4'-dihydroxycinnamoyl)-cyclopenta-2,5-diol,1(3',4'-dihydroxycinnamoyl)-cyclopenta-2,3-diol, cyaniding-3-O glucosylrutinoside) (18-20).

Prunus cerasus has some health benefits, including decreased body weight and lowers blood cholesterol in diabetic patients, antioxidant and anti-inflammatory effects (21), control the sleep-wake-cycle (17), reduce muscle pain and prevent from symptoms of muscle damages (22,23). In addition, previous research demonstrated tart cherries because of their high content of hydroxycinnamic acids and anthocyanins have protective effect on neuronal cells and strong anti-neurodegenerative activity (24). It's remarkable that cherries could reduce oxidant stress and β -amyloid production; as a result, they have therapeutic effect on neurodegenerative diseases such as Alzheimer, Parkinson, Huntington (25).

In this paper, chemoinformatic analysis of MMP9 protein is conducted, and the role of pure p-coumaric acid

as a potent antioxidant agent and tart cherry extract (24) is examined on remyelination in cuprizone mouse model. Then, MMP9 gene expression is evaluated to consider remyelination stimulation in response to tart cherry extract and pure p-coumaric acid ingestion.

Materials and Methods

Chemo informatics analysis

Pass (Prediction of Activity Spectra for Substance) is a simple online computational tool that can predict over 4000 biological activities including, pharmacological effects, mechanisms of action, toxicity and adverse effects, interaction with metabolic, enzymes and transporters, and the effect on gene expression based on structural descriptors of compounds with over 85% accuracy. PASS prediction can be interpreted by Pa and Pi values. Pa and Pi values are as measures that determine activity and inactivity of compounds. Pa-the probabilities of being active and close to 1.000, Pi-the probabilities of being inactive close to 0.000; therefore, the Pa and Pi values are varied from 0.000 to 1.000 and in general Pa+Pi<1 (26). Firstly, the skeletal structure of p-coumaric acid was drawn by Chemschetch, Chemaxon version 5.4 software and saved in SDF format. Finally, biological activity prediction of p-coumaric acid was exploited by www.pharmaexpert.ru/passonline

Molecular docking using swiss dock

Swiss dock is a web service (<http://www.swissdock.ch/docking>) predicts the possible molecular interactions between a target protein and small molecule and is based on the docking software EADock DSS. CHARMM energies are estimated on a grid, and the most favorable energies are evaluated by FACTS, the predicted binding modes can be visualized by Chimera software. Hence, target and ligand selection can be searched via PDB code, protein name, amino acid sequences, ligand name, etc. Finally, the results are exhibited as full fitness (kcal/mol) and Estimated ΔG (kcal/mol).

Tart cherry analysis

Extraction method (shaking incubator)

Firstly, tart cherries were stored in -20° C, 5 g of fruit was measured by measurement with accuracy 0.001 and crushed with mortar. Then, 50 ml methanol 100% was added, the mixture was poured into the bottle, and put it into shaking incubator with speed 150 rpm at 40° C for 24 h. Lastly, the extract was filtered (27).

Total phenol analysis

Total phenol method was applied according to Folin-Ciocalteu procedure. Gallic acid was used as a standard; hence, 1 mg ml⁻¹ Gallic acid/distilled water was prepared. By raising the concentration of the standard from 10 to 60 µl, the color of the complex would be from yellow to green, and the rate of absorbance would be increased. For measuring TPC of fruit sample, five dilutions (50, 70, 90, 110, 130 µl) of fruit extract was analyzed. Therefore, for preparing aliquots (50 µl) fruit extract, (450 µl) distilled water and (500 µl) Folin-Ciocalteu were mixed, and after 3 min, 500 µl Sodium Carbonate (0.1 N) was added to the mixture, was placed 30 min in the dark at room temperature. Finally, the absorbance of the prepared mixture against the blank at 765 nm was read by UV-Vis spectrophotometer. The results were expressed by g Gallic acid (27).

Antioxidant evaluation (DDPH)

DDPH (2, 2'-diphenyl-1-picrylhydrazyl) is a free radical scavenging assay that is on the basis of transferring electron to produce free radicals. Hence, free radicals are reduced in the presence of antioxidant molecules due to the fact that antioxidant agents act as H donor. Consequently, the rate of absorption in DPPH solution would be reduced by increasing the rate of antioxidant activity. 19.7 mg DPPH was measured and solute in methanol to reach 50 ml. Ascorbic acid was used as a standard; therefore, 17.5 mg ml⁻¹ Ascorbic acid/distilled water was prepared. Three dilutions of standard (2, 5, 10 µl) were mixed with 350 ml DPPH and reached at 2 ml with methanol, calibration curve was drawn to achieve the standard formulation. IC₅₀ values are attributed to the concentration of the test samples providing 50% radical scavenging were obtained from the graph-plotted scavenging percentage against extract concentration.

IC₅₀ Formula:

$$\%inhibition = \frac{A_{DPPH} - A_{Sample}}{A_{DPPH}} \times 100$$

For evaluating IC₅₀ of the sample, five dilutions of tart cherry extract (10, 20, 30, 40, 50 µl) was applied. Accordingly, 350 ml DPPH was added, then the whole mixture was reached to 2 ml of methanol, the mixture was placed in the dark at room temperature for 30 min to be observed by UV-Vis spectrophotometry. The absorbance of the prepared mixture against the blank at 517 nm was read by UV-Vis spectrophotometer. The results were expressed by g Ascorbic acid (27).

HPLC

In this experiment, 5 g of fruit sample was measured, crushed. 0.08 g Ascorbic acid (PubChem: 54670067) was dissolved in 5 ml water; then tart cherries are crushed and mixed with 25 ml methanol. Next, 3.5 ml HCL 37% was dissolved in 30 ml water to achieve HCl 1.2 mol. The solution was put in a water bath for 16 h at 35° C. After staying at room temperature, the solution was filtered and evaporated to dryness by evaporator rotary at 35° C. It's remarkable due to the existence of some oil in the extract; the residue will be one drop of oil after drying. The residue was dissolved in 2 ml methanol and filtered by Whatman® GD/X syringe filters with pore size 0.45 µm, diam 13 mm for HPLC injection. The HPLC that exploited for this research was equipped with Agilent 1200 series (Agilent technologies Walbronn, Germany). It consists of a G1312B binary pump, a G1376A capillary pump, G1330B FC/ALS, G1379B Degasser, and G1377A microwips, controlled by Chemstation software. Chromatographic separations were conducted on columns with 4.6×150 mm, 5 µm ZORBAX Eclipse XDB C18 column (Agilent Technology, Germany). Standard and extracts were run by two mobile phases: (A) 0.1% phosphoric acid, (B) (Methanol HPLC 100%). The elution conditions were as followed: 0-30 min from 5% B to 80% B; 30-33 min 80% B; 33-35 min from 80% B to 5% B; with flow rate=0.8 ml min⁻¹. Operating conditions were as follows: column temperature 20° C; injection volumes, 10 µl of the standards and samples. A 10 min re-equilibration period was applied between individual runs. 10 µl standard (1 ppm) and extract should be injected into the HPLC. The peak of p-coumaric acid was revealed at 320 nm UV-Vis spectra.

Demyelination and remyelination animal model

Female and male C57BL/6 mice at 6 weeks of age (n=50) were provided by the Pasteur Institute, and animals were housed according to the guidelines for the animal health care of the Pasteur Institute with ethical approval (code: IR.NIGEB.EC.1395.1.25.1) and direct supervision of animal sciences center at the Pasteur Institute, Production and Research Complex. Firstly, mice were categorized into two groups: standard (male, female), cuprizone (male, female), they were fed cuprizone (PubChem: 9723) during 6 weeks. It's worthy to note that the method of feeding cuprizone to mice was different from other experienced methods. In the previous method, cuprizone 0.2 % (w/w) were mixed with natural chow of rodent during 6 weeks; on the other hand, in our method, 0.2 gr cuprizone powder was mixed with 100 ml water and provided (0.2% w ml⁻¹) suspension.

MMP9 gene expression variation

After six weeks of feeding cuprizone, mice were categorized into four groups: standard (female, male), natural diet (female, male), tart cherry (female, male), p-coumaric acid (female, male). Thus, in the tart cherry group, each mice consume natural juice of 1 g tart cherry every day, and the p-coumaric acid content of each g tart cherry was estimated at 21.6 ppm by HPLC. Hence, the same amount of p-coumaric acid was dissolved in water (21.6 ppm solution) and fed to the p-coumaric acid group. During the weeks of 0-6-9-12, mice from each group were euthanized by cervical dislocation method, brains were extracted from the skull and stored in a cryotube at -80° C.

Genetic molecular analysis

Total RNA was extracted from whole brain tissue, according to manufacturer's instruction of Tripure Isolation Reagent RNA extraction kit protocol

(Germany/Roche). cDNA synthesis was performed using Thermo Scientific Revert Aid RT Kit cDNA synthesis protocol (USA). Real time PCR primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and MMP9 were purchased from the Gene Fanavaran Company (Table 1). Relative quantitative real-time PCR on the basis of SYBR green was conducted according to AccuPower® 2X GreenStar Master Mix Solution Bioneer kit (Korea) protocol for producing cDNA from the reverse transcription of purified RNA. Real time PCR was applied according to program preamplification 95° C 10 min, the PCRs were amplified for 42 cycles (95° C 20 s, 60° C 15s, and 72° C 20s) on qPCR ROTTER GENE 6000 device. The expression of each mRNA was normalized against GAPDH mRNA expression. The data were evaluated by REST-RG © Version 2006 software according to formula.

Table 1. Primer sequence of GAPDH and MMP9

| Name of Gene | Primer sequence | Length of product |
|--------------|-------------------------------|-------------------|
| GAPDH | F: GGT GAA GGT CGG TGT GAA CG | 233 |
| | R: CTC GCT CCT GGA AGA TGG TG | |
| MMP9 | F: CTT CAC CGG CTA AAC CAC CT | 277 |
| | R: TGT CCC TAA CGC CCA GTA GA | |

Results

Bio and chemo informatics results

In analyzing the biological activity of p-coumaric acid by PASS software (Figure 1, 2), it was predicted that p-coumaric acid with Pa=0. 818, Pi=0.003 has potent

MMP9 inhibitory activity. In addition, evaluating the molecular interaction between MMP9 (PDB code: and p-coumaric acid by the Swiss dock (Figure 3) exhibited the clustering results during 251 runs, p-coumaric acid showed -1923.27kcal mol⁻¹ (Full Fitness) and -8.10 kcal mol⁻¹ (ΔG) (Table 2).

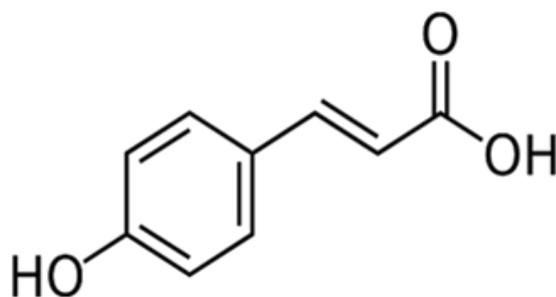


Figure 1. Skeletal structure of p-coumaric acid

All
 Pa>Pi
 Pa>0.3
 Pa>0.7

| Pa | Pi | Activity |
|-------|-------|---|
| 0.968 | 0.001 | Feruloyl esterase inhibitor |
| 0.954 | 0.003 | Membrane integrity agonist |
| 0.950 | 0.001 | Benzoate 4-monooxygenase inhibitor |
| 0.935 | 0.001 | 4-Hydroxybenzoate 3-monooxygenase inhibitor |
| 0.931 | 0.004 | Mucomembranous protector |
| 0.923 | 0.002 | Catechol oxidase inhibitor |
| 0.912 | 0.004 | Chlordecone reductase inhibitor |
| 0.897 | 0.002 | Benzoylformate decarboxylase inhibitor |
| 0.899 | 0.005 | CYP2J substrate |
| 0.896 | 0.002 | Pyruvate decarboxylase inhibitor |

Figure 2. The results of p-coumaric acid prediction by PASS software



Figure 3. Exhibition of p-coumaric acid/MMP9 interaction profile by Swiss dock

Table 2. Classification of results obtained from the docking of p-coumaric acid into MMP9 by Swiss dock

| Receptor | No. of swiss dock clusters | Culster rank | Full fitness (kcal/mol) | Estimated ΔG (kcal/mol) |
|----------|----------------------------|--------------|-------------------------|---------------------------------|
| MMP9 | 41 (256runs) | 1 | -1923.27 | -8.10 |
| | | 2 | -1923.27 | -8.10 |
| | | 3 | -1923.24 | -8.10 |
| | | 4 | -1923.24 | -8.10 |
| | | 5 | -1923.24 | -8.10 |
| | | 6 | -1922.79 | -8.06 |
| | | 7 | -1922.79 | -8.06 |

Phyto analysis results

Phyto analysis was conducted on *Prenusis cerasus* extract to examine total phenolic compound (TPC) and antioxidant activity. Therefore, it's illustrated that total phenolic compound by Folin-Ciocalteu procedure is 1260 ± 310 mg kg⁻¹ and antioxidant evaluation via DPPH approach is 5.85 ± 1.18 mg ml⁻¹.

p-coumaric acid was distinguished by exploiting HPLC procedure to determine the quantity of this agent in tart cherry. p-coumaric acid was detected at ret time 17.985, 320 nm HPLC runs, and the graphs are shown in Figure 4, and the concentration of the agent was about 108 ppm in tart cherry.

MMP9 gene expression variation

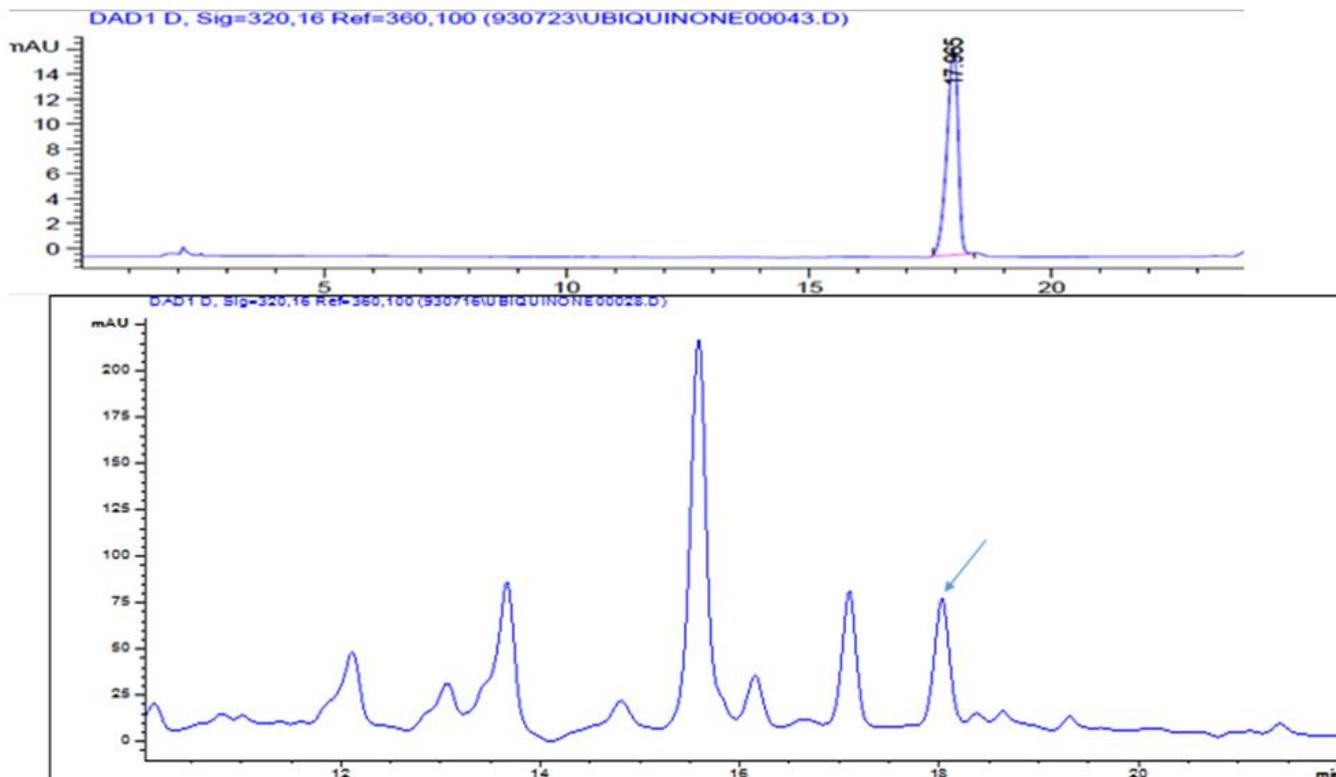


Figure 4. HPLC chromatogram of p-coumaric acid, *Prunus cerasus* that were detected at ret time 17.985 min, 320 nm and p-coumaric acid estimation was about 108 ppm

MMP9 gene expression in animal groups

Figures 5, 6 indicate to MMP9 ratio gene expression in sample groups in both genders. It can be seen from Figure 5, MMP9 gene expression was elevated considerably during demyelination (1-6 weeks) while it

decreased significantly during remyelination (6-12 weeks). By comparing MMP9 gene expression in natural diet, tart cherry, and p-coumaric acid groups, gene expression was reduced more sharply in the p-coumaric acid group (almost 10 times) than other ones.

MMP9 gene expression in the male group

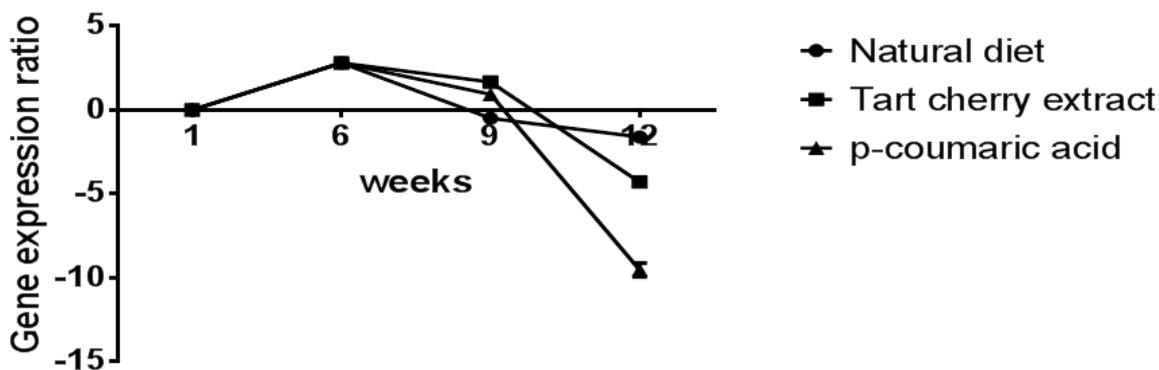


Figure 5. Natural diet, tart cherry extraction, and p-coumaric acid consumption in the male gender that has effect on MMP9 gene expression during remyelination. The MMP9 gene expression is increased during demyelination that implies to myelin destruction, although during remyelination, MMP9 experienced a downward trend (12th week) that is an indication of health of mice and inhibitory effect of antioxidant agents on MMP9

MMP9 gene expression in the female group

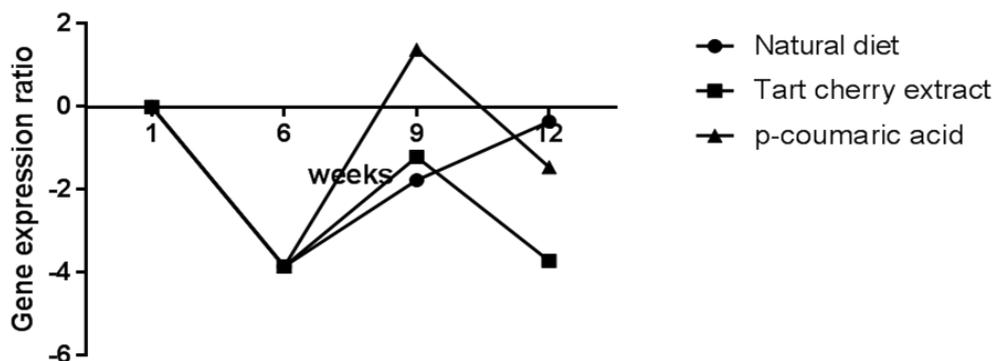


Figure 6. Natural diet, tart cherry extraction, and p-coumaric acid consumption in the female gender that has influence on MMP9 gene expression during remyelination. The expression ratio of MMP9 declined that it refers to intact myelin. In addition, natural diet during remyelination could reach the ratio of MM9 to the standard level; however, both tart cherry and p-coumaric acid could dwindle MMP9 gene expression that tart cherry was more potent in reduction than p-coumaric acid

In the female groups, gene expression experienced variant trend than male. Consequently, the MMP9 gene expression was decreased sharply during demyelination phase to about 4 times. In the middle of remyelination (6-9 weeks), gene expression was elevated in three groups, although p-coumaric acid experienced a more significant increase than other groups. Then, during 9-12 weeks, natural diet could increase gene expression to its primer level; however, both tart cherry extract and pure p-coumaric acid ingestion could decrease the ratio of expression to a lower level.

Discussion

In this research, the effect of p-coumaric acid as a potent antioxidant agent and tart cherry extract was examined for MMP9 gene expression during remyelination in cuprizone mouse model to track their inhibitory effect on MMP9.

Cuprizone (bis (Cyclohexanone) oxaldihydrazone) is a white powder that is soluble slightly in water by forming complex with two copper ions, generate Cu-CPZ complex to reduce free Cu. Therefore, enzymes (e.g., superoxide dismutase-1) use copper as cofactor can't work properly due to the fact that cuprizone will reduce the amount of free Cu. Consequently, it results in reversible demyelination and oligodendrocyte apoptosis during cuprizone consumption that these changes are apparent in the corpus callosum (28,29). After withdrawing cuprizone and consuming natural diet, OPC (oligodendrocyte precursor cells) will be changed to their

mature form and remyelination is induced (30).

Cuprizone mouse model provides an appropriate pattern of demyelination and remyelination, although there are some obstacles in working with this animal model. One of the most important problems is related to the hazardous effect of cuprizone powder when is spread in the cage of mice. As a result, it will be in the direct contact of the researcher and current paper introduces a new method of exerting cuprizone to resolve this problem. Therefore, by diluting 0.2 g powder with 100 ml drinking water 25° C, a suspension %0.2 g ml⁻¹ was achieved and each mouse drank drinking water included cuprizone. Hence, cuprizone powder didn't distribute, and it was ensured that mouse consumes cuprizone every day.

It is remarkable that in the most of the cuprizone research, male gender was applied for demyelination induction due to the fact that sex female hormones (e.g., prolactin, oxytocin. etc.) have protective and therapeutic influence on demyelination (31,32). On the other hand, female gender has been applied in some experiments (33), and it's believed that there is no sex difference in the outcome of demyelination and remyelination (34). In the current study, both genders were applied to compare demyelination, remyelination, and their responding to tart cherry extract and pure p-coumaric acid ingestion.

Weight variation is one of the hallmark symptoms of demyelination and remyelination. Thus, it's revealed that the rate of gaining weight was considerably slower in the cuprizone group in comparison with the natural chow group (35) that is compatible with our results. Current

MMP9 gene expression variation

paper concentrated on weight variation in both genders during demyelination and remyelination; thus, we observed the rate of weight was declined about 2 g than standard groups during demyelination (first-6th weeks) in both genders. Then, by inducing remyelination with a natural diet, tart extract, and pure p-coumaric acid ingestion, the weight was increased slightly throughout 6th to 12th weeks. Consequently, pure p-coumaric acid consumption (17.6 ppm) could increase the weight to more than the standard group in the male group; in addition, natural diet and tart cherry extract consumption also balanced the weight to the standard level. In the female group, tart cherry extract could upsurge the weight to the standard level; however, natural diet and pure p-coumaric acid ingestion increased the weight less than the standard level.

MMP9 is involved in varied neurological health problems, including cerebral ischemia, bacterial and viral meningitis, stroke, HIV dementia, Guillain Baarré, brain tumors, and Multiple sclerosis (5). According to the role of MMP9 in stimulating BBB destruction, it was proposed that MMP9 normally isn't expressed in brain and after stroke or inducible factors, its gene expression is augmented and is responsible for primary tissue destruction (36). MMP9 overexpression is not limited to the lesions and spreads on the normal appearing white matter (4). As can be seen from Figure 5, it's visible throughout demyelination period (1-6 weeks), the MMP9 gene expression is elevated that indicates to demyelination occurrence in the male gender. However, due to the fact that demyelination is prevented through the protective effects of feminine hormones in the female gender, the MMP9 gene expression is declining significantly (Figure 6) that doesn't attribute to demyelination. It's noteworthy the induction of MMP9 gene expression contributes to intracerebral hemorrhage in neurological health problems (37) that in our experiment, the brain of cuprizone mice was bloody and regards to hemorrhage manifestation.

The significant role of MMP9 in myelin destruction is related to its the digestive effect on Myelin Basic Protein (MBP) is located in the myelin sheath and preserves the precise structure of myelin (38). Hence, in vitro injection of MMP9 exerted two cleavages in conserved region of MBP and the distribution of MBP debris might stimulate a severe autoimmune response that contributes to myelin destruction (39,40). Thus, an MMP9 increase in CFS sample was suggested as a marker of disease progression in MS (6). Meanwhile, MMP9 expression stimulates oligodendrocytes maturation and remove NG2 debris as an inhibitory proteoglycan have a preventive effect on

remyelination, play crucial role in reforming myelination (41-43). Furthermore, it was suggested that sildenafil as a drug with the protective impact on myelination/remyelination augmented MMP9 gene expression during remyelination that is attributed to the role of MMP9 in tissue remodeling and cleaning debris (44).

Decreasing the MMP9 gene expression as one of the key factors can reduce inflammatory reactions that Saja *et al.*, introduced curcumin as one of the anti-inflammatory agents could exert its effect via downregulating MMP9 in blood mononuclear cells (45). p-coumaric acid as a member of the hydroxycinnamic acid family is frequent in diverse natural resources (e.g., honey, peanuts, red fruits, garlic, grapes, spinach, red fruits, etc.) (46,47). Hydroxycinnamic acids because of their chemical structure possess the potent antioxidant and anti-inflammatory capabilities that are a precious candidate for cancer and neurological problems treatments (48,49).

NF- κ B involved in triggering inflammatory response transcriptional factor is composed of two subunits (p65, p50) and is bound to I- κ B α as an inhibitory factor in the cytosol. Therefore, in response to the inflammatory cytokines (e.g., IL-1, TNF- α), I- κ B α will be degraded and NF- κ B will migrate to the nucleus in order to transcript I- κ B α , pro-inflammatory mediators (e.g., IL-1, TNF- α , iNOS, COX-2, MMP9, etc.), chemokines, and adhesion molecules (50,51).

Phenolic compounds because of their anti-inflammatory effect have been used for therapeutic purposes, and they exert their influence by preventing from NF- κ B: DNA complex in the nucleus; indeed, the production of inflammatory factors will be blocked at the transcriptional level (52). In the research conducted by S.Pragasam *et al.*, was suggested p-coumaric acid inhibits from NF- κ B gene expression and consequently TNF- α expression will be declined (46). In addition, another study revealed that p-coumaric acid through phosphorylation of p65 component, impede NF- κ B nuclear translocation. p-coumaric acid also obstructs expression of COX-2 and iNOS significantly that is related to NF- κ B inhibition pathway (53). It's remarkable that blueberry extract comprised of various phenolic compounds and anthocyanins suppressed COX-2 and iNOS expression via inhibiting NF- κ B nuclear translocation (54). Moreover, the application of predictive binding modes could expect that hydroxycinnamic structure might generate possible interaction among MMPs and phenolic compounds that our docking results confirm this hypothesis (55).

According to our data, by changing the diet to natural chow, tart cherry extract, and pure p-coumaric acid, MMP9 gene expression dwindled significantly in both genders. In the male gender, p-coumaric acid could reduce it to about 10 times and tart cherry could reduce the MMP9 gene expression to about 5 times. Consequently, this reduction in gene expression can be related to direct binding of antioxidant agents to MMP9, preventing from NF- κ B migration to nucleus to inhibit MMP9 gene expression.

Matrix metalloproteinase (MMPs) through destructing blood brain barrier and myelin destruction results in multiple sclerosis pathogenesis. MMP9 is grouped as gelatinases plays key role in blood brain barrier (BBB) destruction, T cell migration into the CNS, and demyelination induction. Therefore, agents can decrease the ratio expression of MMP9 have a therapeutic effect on MS progression. Consequently, according to our results tart cherry extract and pure p-coumaric acid ingestion could decline MMP9 gene expression significantly, and it's deduced that antioxidant agents exert their influence via reducing MMP9 gene expression.

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