RESEARCH PAPER

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# Response of miRNA to treatment with *Hypericum perforatum* L. oil in multiple sclerosis

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MicroRNA-regulated gene expression plays an important role in autoimmune diseases, such as multiple sclerosis (MS). This study investigated the expression patterns of microRNAs (miRNAs) in MS in brain tissues using an animal experimental autoimmune encephalomyelitis (EAE) model treated with *Hypericum perforatum* (HP) oil. C57BL/6 J mice were divided into two groups: MS and control. The MS group was subdivided into sham (MS) and MS+HP. After the EAE induction treatment protocol, the patterns of miRNA expression profiles were determined in brain samples of the groups. The array data identified eleven miRNAs and candidate miRNA validation was performed by RT-qPCR. A literature review of the validated miRNAs found that six of the eleven miRNAs (miR-200a-3p, miR-200b-3p, miR-200c-3p, miR-182-5p, miR-183-5p, and miR-1298-5p) were directly associated with MS. These miRNAs have been suggested as biomarkers of MS because they are highly correlated with the pathology of the disease. Furthermore, miRNA array analysis identified five candidate miRNAs (miR-299a-5p, miR-206-3p, miR-325-5p, miR-10b-5p, miR-429-3p) that are highly likely to be associated with MS pathogenesis, which could be helpful in the diagnosis and treatment of MS disease. This research offers vital insights that could be utilized in creating biomarkers and advancing treatments for MS.

**Key words:** multiple sclerosis, *Hypericum perforatum,* microRNA, experimental autoimmune encephalomyelitis

## INTRODUCTION

Multiple sclerosis (MS) is a progressive autoimmune disorder affecting the central nervous system (CNS), where the body's immune system attacks the white matter, leading to inflammation and demyelination. It is a major cause of neurological symptoms in young adults and currently has no cure. New treatments focus on managing symptoms and modulating immune function more effectively. While various environmental factors can raise the likelihood of developing MS, a significant portion of the risk is genetic, influenced by numerous common genetic variations that impact immune system regulation in specific ways (Yazdanpanah et al., 2021). Lately, there has been growing interest in the role of microRNAs (miRNAs), which are small, non-coding RNA molecules, in autoimmune conditions such as MS.

miRNAs have emerged as important regulators of many factors and have been proposed as disease bio-

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markers and therapeutic targets. They are endogenous non-coding RNAs with regulatory functions. miRNA consists of 21–25 nucleotides and mainly regulates mRNA expression at the post-transcriptional level (Lim et al., 2023). miRNA dysfunction is related to the pathology of MS dysfunction in CNS diseases and is closely associated with imbalances in miRNA expression (Doghish et al., 2023).

While mechanisms exist in the CNS to repair MS damage, they are disrupted in MS, and currently, there are no treatments to address this deficit. In recent decades, there has been a noticeable increase in the use of complementary and alternative medicine among MS patients, particularly herbal remedies (Alam, 2023).

Medicinal plants have various therapeutic effects on diseases (Agyare et al., 2018). Studies have shown that certain herbal compounds can improve myelin repair and inhibit inflammation (Mohajeri et al., 2015; Piao & Liang, 2012). *Hypericum perforatum* L. (HP), commonly called St. John's Wort, is a flowering plant belonging to the family *Hypericaceae*. While native to Europe and Asia, it has now spread across the globe (Beaubrun & Gray, 2000). Currently, HP is utilized for treating inflammatory conditions, cancers, and neurodegenerative diseases (Lu et al., 2004). Due to its antidepressant, antioxidant, and anti-inflammatory properties, HP may be considered a potential recommendation for MS patients (Naziroglu et al., 2014).

Although the etiology of MS is not well understood, animal models, especially the experimental autoimmune encephalomyelitis (EAE) model, can mimic many clinical, neuropathological, and immunological aspects of MS (Hohlfeld & Wekerle, 2001). The molecular mechanisms of MS have been determined by creating an EAE model in mice (Hohlfeld & Wekerle, 2001; Garcia-Alvarez et al., 2014; Steimle et al., 2024). In our previous study, we proved with biochemical and histological findings that feeding C57BL/6 J mice with HP oil helped to heal MS induced by the EAE method (Selek et al., 2019).

This research aimed to examine alterations in miRNA due to the therapeutic impact of HP oil, employing an animal model of MS induced through the EAE method.

## METHODS

### Animals

The experimental protocol was approved by the Animal Ethics Committee of Bezmialem University (approval number: 2015/53). C57BL/6 J mice (n=40) were purchased from Bogazici University Animal Laboratory (Istanbul, Turkey). Mice were housed at the laboratories of Bezmialem University under controlled lighting, with a 12 h light -12 h dark cycle and a temperature of 22°C. They had *ad libitum* access to food and water.

### EAE induction and treatment protocol

C57BL/6 J mice (n=40) were divided into two main groups: MS (n=30) and control (n=10). The MS group was subdivided into two groups: the sham group (MS, n=15), and the MS+HP group (MS+HP, n=15). An MS model was used for MS induction, as described previously (Selek et al., 2019). Briefly, the MS model was induced by subcutaneous immunization with 200 µg MOG35–55 peptides (Hooke Kit™ MOG35-55/CFA Emulsion PTX; Hooke Lab. Inc., Lawrence MA) in a 200 µl emulsion composed of equal volumes of MOG and Complete Freund's Adjuvant (CFA) supplemented with 10 mg/ml heat-killed *Mycobacterium tuberculosis* (H37Ra; Difco Adjuvant H37Ra, Fisher Scientific Ltd., Loughborough, UK). Mice were injected intraperitoneally with 500 ng pertussis toxin (Hooke Kit™ MOG35-55/CFA Emulsion PTX; Hooke Lab. Inc.) on day one. Clinical signs of disease were scored (Bebo et al., 1998) daily after two weeks, as follows: 0, no sign; 1, tail weakness or slightly clumsy gait; 2, tail paralysis and/or moderately clumsy gait and/or mild hind limb weakness; 3, moderate to severe hind limb paralysis or mild forelimb weakness or both; 4, complete hind limb paralysis or moderate to severe forelimb weakness or both; 5, quadriplegia or moribund state; 6, death during the six-week experimental period. After two weeks of immunization, plant oil treatments were performed. The food for the treatment groups was produced using HP extracts (Premium Organics Ltd., Turkey). The MS+HP group mice were fed food containing 18–21 g/kg HP extract. Mice in the control and MS groups were fed standard feed for six weeks (Mojaverrostami et al., 2018) (Fig. 1). At the end of the study, brain samples were collected from all mice under general anesthesia using 35 mg/kg ketamine and 80 mg/kg xylazine. Tissue samples were stored at –80°C in a freezer (Haier Co., Louisville, KY) until analysis.

### Tissue homogenization and total RNA isolation

All brain samples were homogenized at 30 m/s for 5 min in a homogenization device (MP Biomedical Fast Prep 24, South Korea), using lysis buffer and total RNA samples isolated with the RNA easy mini kit (Qiagen, Germany), according to the manufacturer's instructions. RNA quality and quantity were measured with a NanoDrop2000c (Thermo Scientific, USA). RNA integrity was analyzed by gel electrophoresis and the Spot Check Nucleic Acid Quantitation Kit (Sigma-Aldrich, St. Louis, MO).

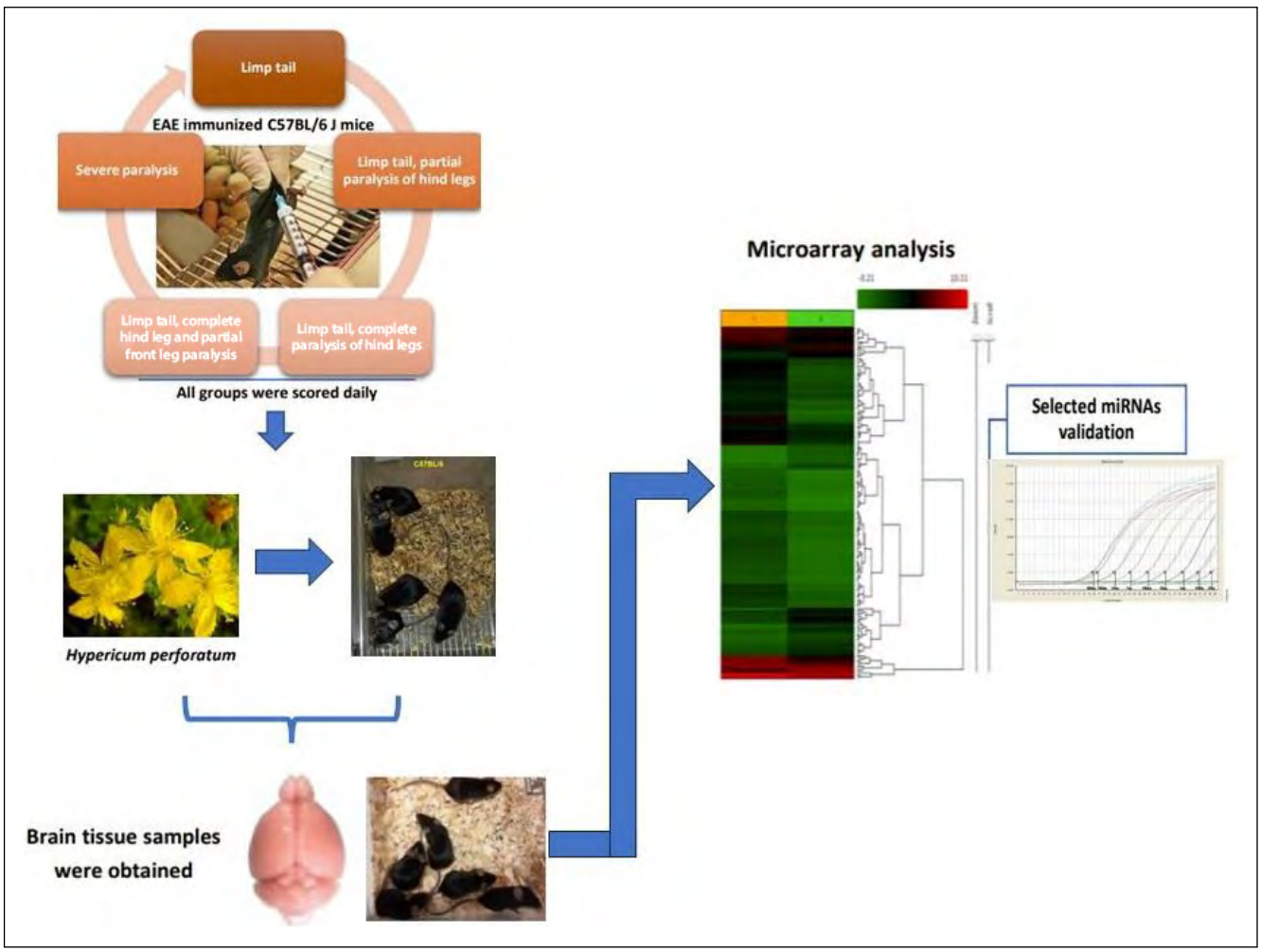


Fig. 1. Schematic of the experimental design.

## miRNA array and analysis of data

To determine the miRNA expression profiles, an Affymetrix GeneChip miRNA array v. 3.0- (902018) (Affymetrix, Santa Clara, CA, USA) was performed. The GeneChip miRNA 3.0 array contained 100% miRBase version v17 coverage and could provide 19.931 mature miRNA probe sets from all organisms. It also provides 2.999 probe sets specific to human, mouse, and rat pre-miRNA hairpin sequences. The three experimental groups (MS+HP, MS, control) were pooled, and each group contained 100 ng RNA. Total RNA was labeled with Biotin using a 3DNA Array Detection FlashTag™ Biotin HSR kit (Genisphere, Hatfield, PA, US), following the manufacturer's protocol, and was subsequently hybridized overnight. Using the Affymetrix GeneChip miRNA array, samples were loaded onto the hybridization array chip and left in an oven at 48°C for 16–18 h of hybridization (GeneChip® Hybridization Oven 645). Post-hybridization washing, staining (GeneChip® Fluidics Station 450), and scanning of chips were performed by following the manufacturer's technical manual (Affymetrix, USA). Hybridized chips were scanned with an Affymetrix GCS 3000 7G Scanner. All points were compared by t-test statistical analysis and scatter diagrams for miRNA expressions based on $p<0.05$ for repeatability and reliability. The raw data were processed using the Robust Multi-Array Mean (RMA) algorithm to perform background correction. The pre-processed data were then analyzed with the Expression Console (Affymetrix, Santa Clara, CA, USA) and other software (Affymetrix Expression Console Build 1.3.1.187 and Affymetrix Transcriptome Analysis Console v3.0).

## Candidate miRNA validation with RT-qPCR

Candidate miRNAs, determined from the expression profiles of microarray miRNAs, were validated by quantitative real-time reverse transcription polymerase

chain reaction (RT-qPCR). Briefly, a single-stage Poly-A insertion and reverse transcription reaction was performed with the Quanto qScript miRNA cDNA synthesis kit (QuantoBio Co., USA) to prepare cDNAs using miRNA samples derived from each sample in all groups. The miRNA expression profile from the Biorad iTaq™ Universal SYBR® Green Supermix (Bio-Rad Co., USA) and Quanta Perfecta universal PCR Primer (QuantoBio Co., USA) were determined using sequence-specific primers in an ABI PRISM 7900HT real-time PCR device (Applied Biosystems, Foster City, CA, USA). The reaction mixture of each sample was prepared in duplicate. The amplification profile was denaturation at 95°C for 30 s, followed by 40 cycles of 95°C for 15 s, 60°C for 30 s, and 70°C for 30 s. Specific miRNA expression levels were normalized with U6, a structurally expressed endogenous control miRNA, and calculated as the $2^{-}\Delta ct$ values. The fold change in miRNA expression levels was determined using the $2^{-}\Delta\Delta CT$ method as described by Livak and Schmittgen (2001).

### Statistical analysis

Statistical analyses were conducted using GraphPad Prism version 8.0.1 software (San Diego, CA). The Shapiro-Wilk test was conducted to assess the normality of the data. miR-1298-5p, miR-182-5p, miR-183-5p, miR-200a-3p, and miR-200c-3p were analyzed using Kruskal-Wallis, followed by a *post-hoc* Dunn test, while miR-10b-5p, miR-200b-3p, miR-206-3p, miR-299a-5p, miR-325-5p, and miR-429-3p were analyzed *via* a one-way ANOVA, followed by a *post-hoc* Bonferroni test. The Mann-Whitney U test was used in unpaired group comparisons to evaluate fold change ratios of miRNAs. $p<0.05$ was considered statistically significant, with mean ± SD.

## RESULTS

Microarray analyses investigated the global miRNA profiles in the MS+HP, MS, and control groups. A heatmap representation of differentially expressed miRNAs was created, with the red color symbolizing high expression and the green color symbolizing low expression. Microarray profiling for the MS *vs.* control group revealed that 66 out of 327 miRNAs were downregulated (Fig. 2A), while in the MS+HP *vs.* MS group, 49 out of 215 miRNAs were up-regulated (Fig. 2B). The heatmap graph of 11 miRNAs that are detected in all three groups, and associated with MS and neurological diseases, is presented in Fig. 2C. The differentially expressed miRNAs are provided as a supplementary dataset (Supplementary File 1). The miRNAs that showed common expression patterns in all groups were determined comparatively. Eleven miRNAs were associated with MS and neurological diseases, and their expression was validated in brain samples by RT-qPCR (Table 1). Fold change ratios were calculated after validation results, and it was found that miRNA up- or down-regulated in MS, as compared to control, were generally downregulated after HP treatment (Table 2). The expression of miRNAs between

Table 1. miRNA array data validation of the groups.

| | MS Mean (SD) | Control Mean (SD) | MS+HP Mean (SD) |
|---|---|---|---|
| miR-1298-5p | 5.61±0.36* | 4.00±0.75 | 4.18±2.15$^{+}$ |
| miR-182-5p | 13.03±1.23* | 10.49±2.50 | 10.85±3.94$^{+}$ |
| miR-183-5p | 7.79±1.57 | 7.83±1.77 | 6.27±1.89*$^{+}$ |
| miR-200a-3p | 9.08±2.18* | 10.86±4.31 | 8.48±2.39*$^{+}$ |
| miR-200b-3p | 9.83±0.84* | 7.44±2.57 | 4.43±1.45***,$^{+++}$ |
| miR-200c-3p | 8.07±0.88* | 9.84±1.83 | 7.87±1.85*$^{+}$ |
| miR-10b-5p | 14.52±1.54* | 13.09±1.52 | 12.76±1.18$^{+}$ |
| miR-206-3p | 8.11±1.39** | 10.69±2.23 | 6.14±1.15**$^{++}$ |
| miR-299a-5p | 8.05±0.86** | 6.38±1.53 | 6.06±2.01$^{++}$ |
| miR-325-5p | 3.52±0.93** | 2.14±1.60 | 1.02±0.73*$^{++}$ |
| miR-429-3p | 14.36±0.81** | 11.59±2.46 | 12.03±1.85$^{++}$ |

*p<0.05, **p<0.01, ***p<0.001 statistically significance compared to the control group. $^{+}$p<0.05, $^{++}$p<0.01, $^{+++}$p<0.001 statistically significance compared to the MS group. Data is presented as mean ± standard deviation. MS: multiple sclerosis; HP: *Hypericum perforatum* oil treatment.

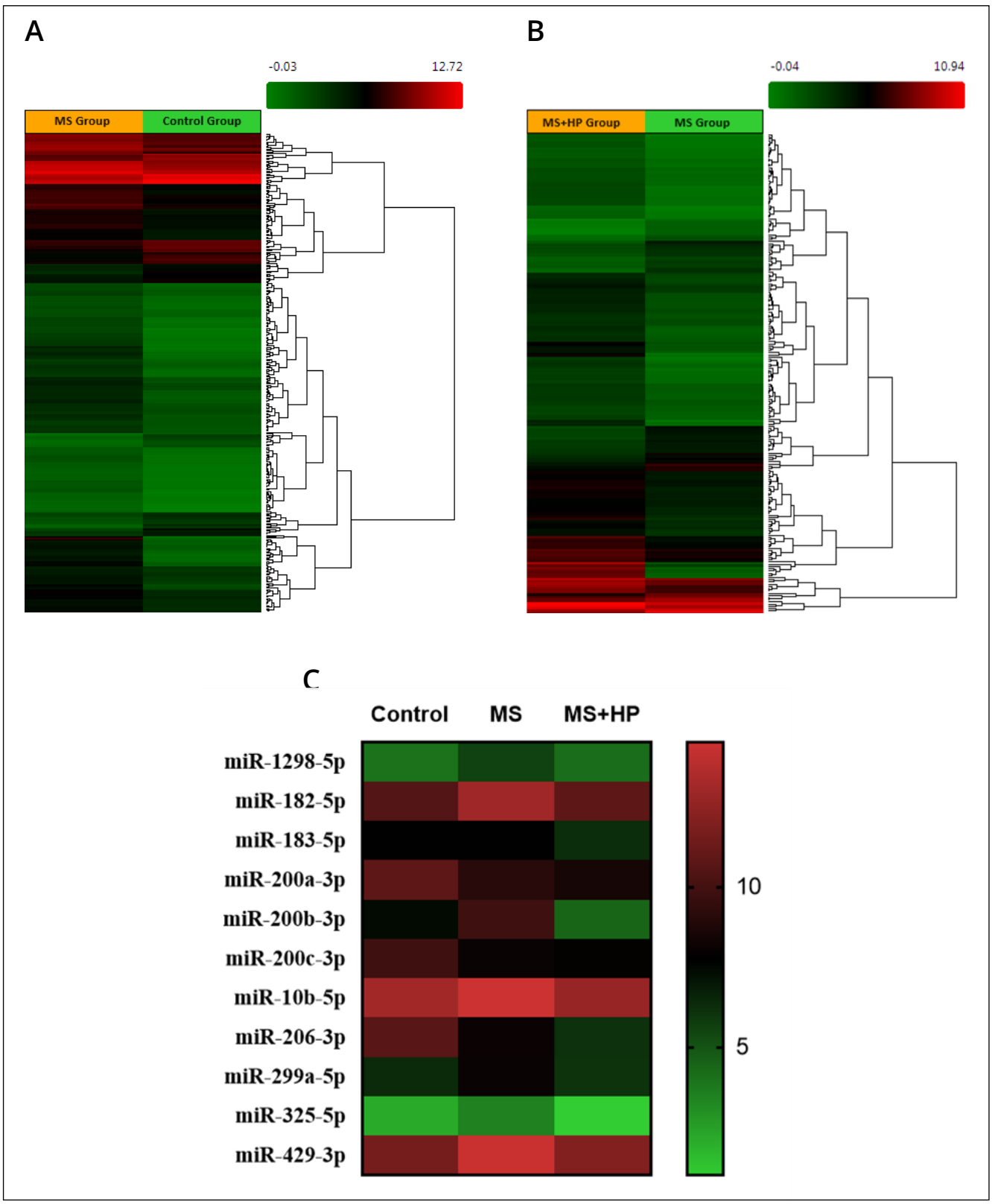


Fig. 2. Heatmap hierarchical clustering representation and scatterplots of the groups' differentially expressed microRNAs (miRNA). Microarray analysis comparing the MS group to the control group identified 66 downregulated miRNAs out of 327 (Fig. 2A), whereas in the MS+HP *vs.* MS group, 49 miRNAs out of 215 were found to be up-regulated (Fig. 2B). Corresponding scatterplots were also provided (Fig. 2C). MS: multiple sclerosis; HP: *Hypericum perforatum* oil treatment.

Table 2. Fold change ratios of validated eleven miRNAs.

| | MS *vs.* Control | | MS+HP *vs.* MS | |
|---|---|---|---|---|
| | Fold change | Regulation | Fold change | Regulation |
| miR-1298-5p | 3.1 | *Up-regulated* | 2.7 | *Downregulated* |
| miR-182-5p | 5.8 | *Up-regulated* | 4.5 | *Downregulated* |
| miR-183-5p | 1.0 | *Downregulated* | 2.9 | *Downregulated* |
| miR-200a-3p | 3.4 | *Downregulated* | 1.5 | *Downregulated* |
| miR-200b-3p | 5.2 | *Up-regulated* | 42.2 | *Downregulated* |
| miR-200c-3p | 3.4 | *Downregulated* | 1.1 | *Downregulated* |
| miR-10b-5p | 2.7 | *Up-regulated* | 3.4 | *Downregulated* |
| miR-206-3p | 6.0 | *Downregulated* | 3.9 | *Downregulated* |
| miR-299a-5p | 3.2 | *Up-regulated* | 4.0 | *Downregulated* |
| miR-325-5p | 2.6 | *Up-regulated* | 5.7 | *Downregulated* |
| miR-429-3p | 6.8 | *Up-regulated* | 5.0 | *Downregulated* |

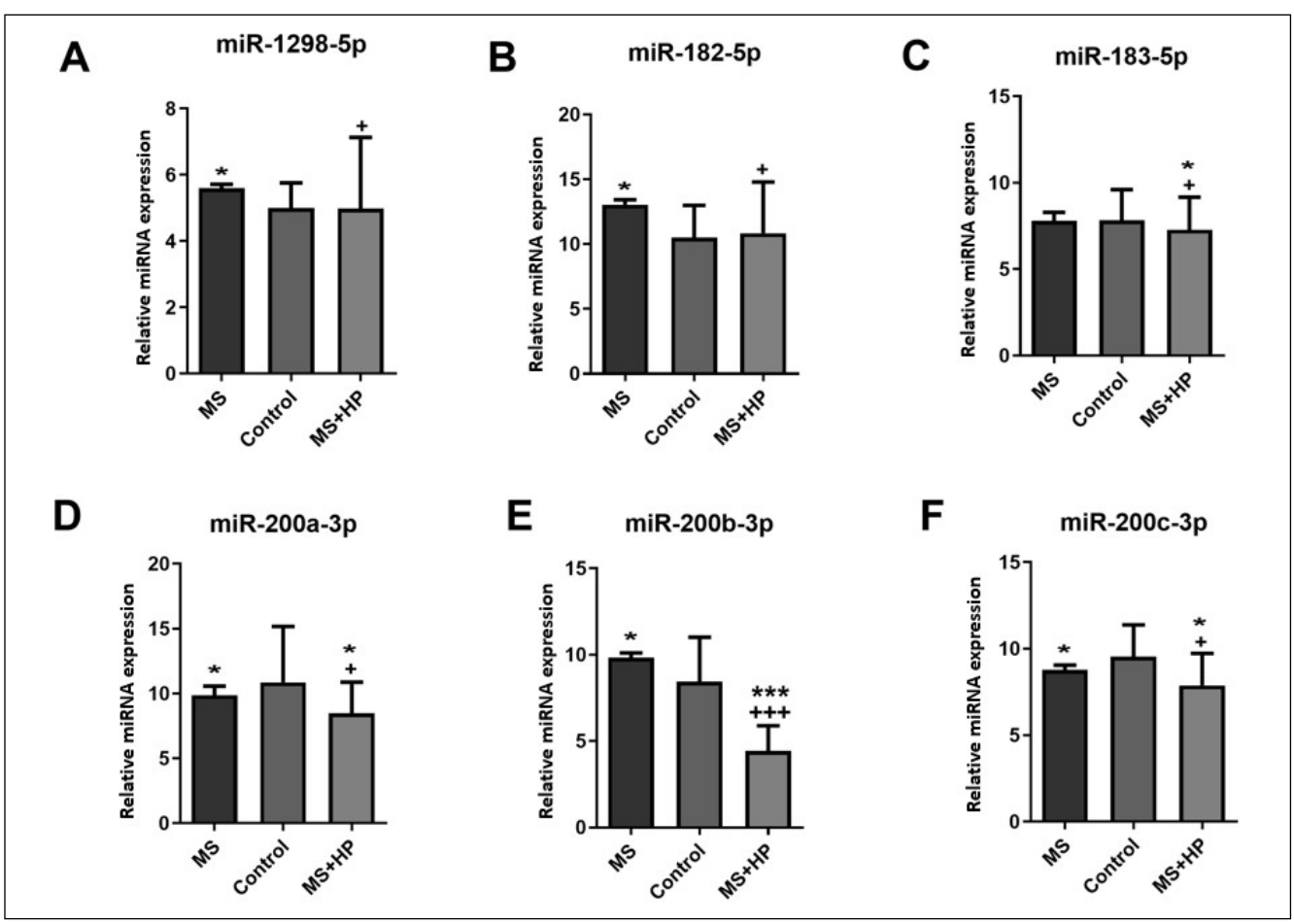


Fig. 3. microRNA (miRNA) array data validation by RT-qPCR and changes in miRNA expression in MS treated with HP extracts. (A) miR-1298-5p; (B) miR-182-5p; (C) miR-183-5p; (D) miR200a-3p; (E) miR-200b-3p; (F) miR-200c-3p, analyzed by Kruskal-Wallis followed by *post-hoc* Dunn test. Each bar is the mean ± SE from experiments performed. *p<0.05, ***p<0.001 statistically significant compared to the control group. +p<0.05, +++p<0.001 statistically significant compared to the MS group. MS: multiple sclerosis; HP: *Hypericum perforatum* oil treatment.

groups is shown in Fig. 3 and Fig. 4. The expressions of miR-1298-5p, miR-182-5p, miR-299a-5p, miR-429-3p, miR-10b-5p, miR-200b-3p and miR-325-5p were up-regulated in MS and down-regulated in the MS+HP group compared to the MS group (Table 2). Moreover, the expression miR-200b-3p, which was up-regulated in the MS group, was downregulated approximately 8-fold with HP treatment (Table 2). The expressions of miR-183-5p, miR-200a-3p, miR-200c-3p, and miR-206-3p were down-regulated both in the MS *vs.* control comparison and the MS+HP *vs.* MS comparisons (Table 2). The expression of miR-1298-5p was significantly higher (P<0.05) in the MS group than the control group, and in the MS+HP group, it was significantly lower (P<0.05) than the MS group, similar to the control group. (Fig. 3A). The expression of miR-182-5p was significantly higher (P<0.05) in the MS group compared to the control group, while it was significantly lower (P<0.05) in the MS+HP group than in the MS group (Fig. 3B). There were no significant differences in the expression of miR-183-5p in the MS group compared to the control; also, the MS+HP group was significantly lower (P<0.05) than the MS and control groups (Fig. 3C). The expressions of miR-200a-3p and miR-200c-3p in the MS group were lower than the control (P<0.05), and in the MS+HP group were significantly lower than the MS group (P<0.05, Fig. 3D and 3F). The expression of miR-200b-3p was significantly higher in the MS group than the control group (P<0.05), while significantly lower in the MS+HP group compared to the MS and control groups (P<0.001 and P<0.001, respectively, Fig. 3E). The expressions of miR-10b-5p and miR-325-5p in the MS group were significantly higher (P<0.05 and P<0.01, respective-

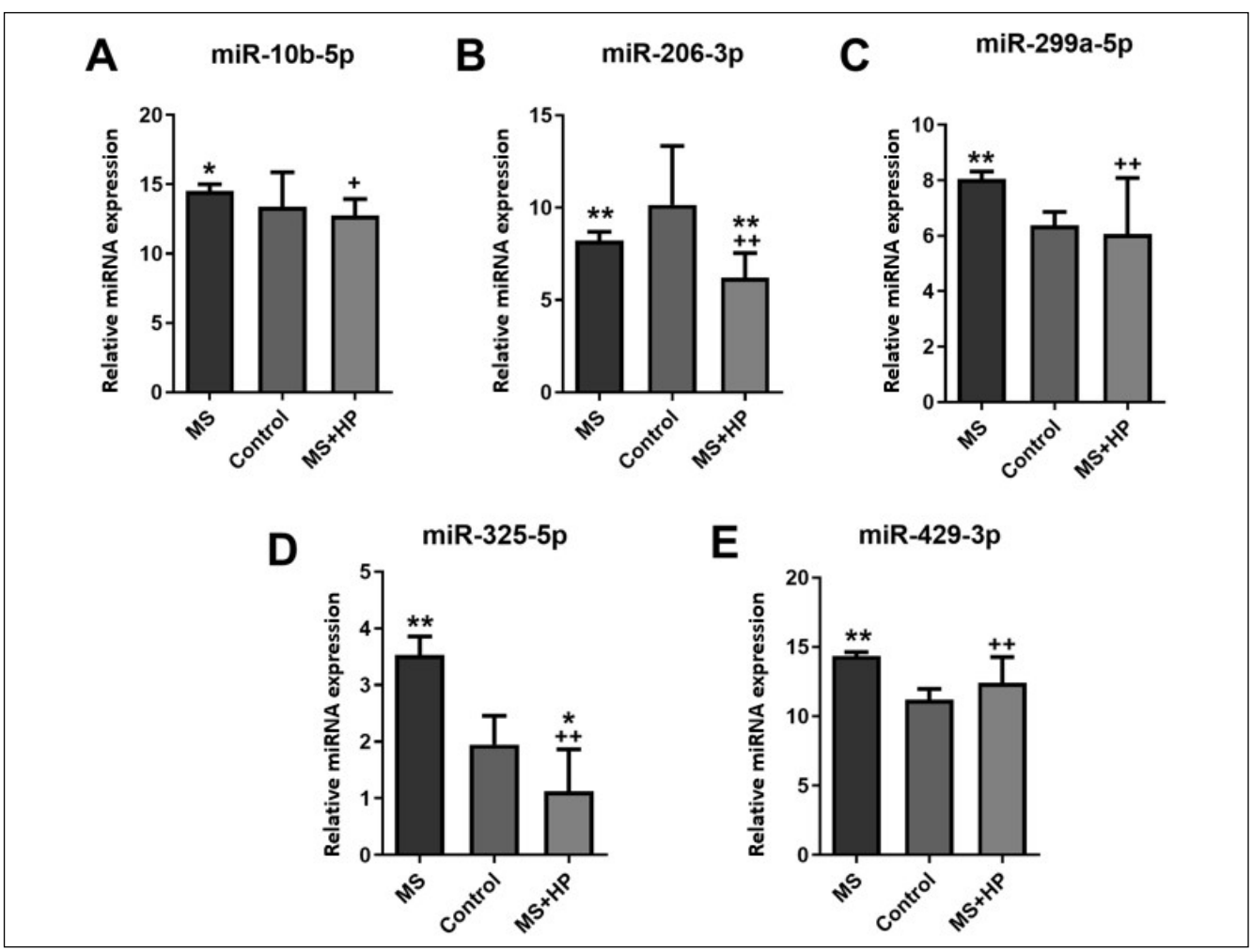


Fig. 4. microRNA (miRNA) array data validation by RT-qPCR and changes in miRNA expression in MS treated with HP extracts. (A) miR-10b-5p; (B) miR-206-3p; (C) miR-299a-3p; (D) miR-325-5p; (E) miR-429-3p analyzed using a one-way ANOVA followed by a *post-hoc* Bonferroni test. *p<0.05, **p<0.01 statistically significant compared to the control group. +p<0.05, ++p<0.01 statistically significant compared to the MS group. MS: multiple sclerosis; HP: *Hypericum perforatum* oil treatment.

ly) than the control, and significantly lower in the MS+HP group compared to the MS group (P<0.05 and P<0.01, respectively) (Fig. 4A and 4D). In addition, the expression of miR-206-3p was significantly lower in the MS group compared to the control group (P<0.01), and was significantly lower in the MS+HP group than in the MS group (P<0.01, Fig. 4B). The expression of miR-299a-5p was significantly higher in the MS group compared to the control group and MS+HP groups (P<0.01, Fig. 4C). Additionally, the expression of miR-429-3p was significantly higher in the MS group than in the control or MS+HP groups (P<0.01, P<0.01, respectively, Fig. 4E).

## DISCUSSION

In recent years, there has been growing interest in the impact of small, non-coding RNA molecules in autoimmune diseases, including MS (Krützfeldt et al., 2006). This study examined miRNAs present in MS brain samples by employing an animal model of MS treated with HP oil.

Eleven candidate miRNAs thought to be associated with MS obtained from microarray data were validated by RT-qPCR. A literature search of the validated miRNAs identified six of the eleven (miR-200a-3p, miR-200b-3p, miR-200c-3p, miR-182-5p, miR-183-5p, and miR-1298-5p) as being directly associated with MS disease (Naghavian et al 2015; Singh et al., 2016; Bencurova et al., 2017; Baulina et al., 2018; Liguori et al., 2018; Fu et al., 2019; Groen et al., 2020; Martinez & Peplow, 2020). These miRNAs have also been suggested as candidate biomarkers for MS (Kye et al., 2014; Naghavian et al, 2015; Singh et al., 2016; Bencurova et al., 2017; Regev et al., 2017; Juźwik et al., 2018; Baulina et al., 2018; Groen et al., 2020; Liguori et al., 2018; Wu & Chen, 2019; Martinez & Peplow, 2020).

The study further suggests that the remaining five out of eleven candidate miRNAs found (miR-206-3p, miR-10b-5p, miR-299a-5p, miR-325-5p, miR-429a-3p) may also play a role in several pathways and processes related to MS since all eleven miRNAs showed similar behavior and expression among the different groups.

Valsecchi et al. (2020) reported that miRNA-206 is up-regulated in the brainstem of spinal muscular atrophy mouse models in the early stage of the disease. Studies using an Alzheimer's model found that miRNA-206-3p expression increased in the brain (Sorenson et al., 2014; Wang et al., 2017), while another study determined that miR-206 has a retrograde regulatory role in the neuromuscular junction (Mu et al., 2015). In this study, miRNA-206-3p was downregulated 6.0-fold in the MS group, and interestingly, downregulation was detected in the MS+HP treatment group as well. These results are essential for investigating the role of this candidate miRNA in MS.

In many vertebrate species, miR-10 paralogs are expressed at the borderline level with Hox4 paralogs early in development (Mansfield & McGlinn, 2012). One study showed irregular miRNA expression in the SMA mouse model, and most miRNAs in motor neuron cultures were miR-10a and miR-10b. One study reported that miR-10b-5p was consistently downregulated in amyotrophic lateral sclerosis/motor neuron patients. However, since the role of miR-10 is not yet known, further investigation into the role of miR-10 families is required (Gonçalves et al., 2018). In this study, miR-10b-5p expression was up-regulated 2.7-fold in the MS group compared to the control group. In addition, expression was downregulated 3.4-fold in the MS+HP treatment group. This result may show us that the treatment with HP oil may contribute to recovery by lowering the miRNA expression, contrary to the control group. The expression of miR-10b-5p under hypoxic stress was investigated in C57BL/6 mice with a myocardial infarction (MI) or ischemia/reperfusion model, and overexpression of miR-10b-5p significantly reduced the size of MI, improved cardiac function, and inhibited apoptosis (Wu et al., 2019). Clinical studies also support the theory that hypoxia exists in MS patients (Yang & Dunn, 2019). Hence, examining miR-10b-5p in conjunction with targets from hypoxia-related pathways is crucial, as they may serve as potential biomarkers or therapeutic agents in MS.

miRNA-429 is a member of the micro-200 family, which is thought to be important in neurodegenerative diseases (Humphries & Yang, 2015). In diseases such as MS and prion, miR-200 family members are involved in various cellular processes, including regulation of DNA repair, cellular apoptosis, alpha-synuclein aggregation, FUS protein production, and differentiation of Th17 cells, and it has been reported that miR-141 and miR-200a are highly expressed in the relapsing stages of MS (Fu et al., 2019; Naghavian et al., 2015). In addition, miR-200 family members can also induce differentiation of Th17 cells, which may be an essential factor in the progression of MS (Naghavian et al., 2015). A study investigating the miRNA profile during ischemic preconditioning found that miR-429 may play a neuroprotective role in ischemia *in vitro* (Lee et al., 2010). In this study, miR-200a-3p and miR-200c-3p, belonging to the miR-200 family, were downregulated compared to the control group, while miR-200b-3p was up-regulated 5.2-fold compared to the control group, and downregulated by varying rates (1.1-42.2) with HP treatment. The dramatic downregulation of miRNAs associated with MS pathogenesis by treatment demonstrates the therapeutic powers of HP

oil. The fact that miR-429-3p was downregulated 5.0-fold in the treatment group in this study strengthens the possibility of it being a candidate miRNA.

While there is no data on the expression of miR-325-5p in MS models in the literature, a recent study showed that miR-325-3p targets receptor-interacting serine-threonine kinase 3 in neurons, which leads to the inactivation of the mitogen-activated protein kinase pathway and protects against injury caused by cerebral ischemia-reperfusion (Yi et al., 2020). Furthermore, this miRNA family plays a role in pain formation in the peripheral nervous system (Genda et al., 2013). In this study, alteration in the expression of miR-325-5p was determined with HP treatment. miR-325-5p expression was up-regulated 2.6-fold in the MS group compared to the control group, and a dramatic 5.7-fold downregulation was observed in the group treated with HP oil. These findings imply that miR-325-5p contributes actively to the development of MS. Additionally, HP may hold significance in treating MS.

Recent studies indicate that miR-299a-5p acts as an autophagy inhibitor by antagonizing caspase-dependent apoptosis (Zhang et al., 2016). Autophagy is crucial for maintaining neuronal balance and is associated with various neurodegenerative diseases. Autophagy deficiency was found to partially contribute to neuronal damage induced by EAE, suggesting that pharmacological intervention targeting autophagy could be a potential therapeutic approach for MS (Feng et al., 2017). Therefore, decreasing miR-299a-5p levels might be useful in controlling autophagy in MS and increasing neural survival; the repression of autophagy by miR-299a-5p helps membrane elongation (Zhang et al., 2016). In addition to its effect on autophagy, miR-299a-5p also increases endocytosis and controls levels of inflammation (Nardelli et al., 2017). Since autoimmunity is crucial to the pathology of MS, reducing the inflammation level may positively affect the disease course. In this study, the level of miR-299a-5p expression was found to be down-regulated compared to the MS group. The treatment effect may be explained by an alteration in the levels of autophagy and endocytosis controlled by miR-299a-5p.

## CONCLUSION

In this study, the effects of HP oil on the miRNA profile using an animal EAE model, which was shown to have a positive impact on the recovery of multiple sclerosis in our previous study, were investigated. Treatment with HP oil significantly altered the expression of several miRNAs associated with MS. This study provides crucial information that can be used to develop biomarkers and treat MS.

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