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Chrysin suppress immune responses and protects from experimental autoimmune encephalomyelitis in mice

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ABSTRACT

We investigated the effects of chrysin in the experimental autoimmune encephomyelitis (EAE), a multiple sclerosis (MS) animal model. EAE was induced using myelin oligodendrocyte glycoprotein (MOG) 35–55 peptide in C57BL/6 mice. Chrysin reduced weight loss, attenuated clinical signs and blunted the EAE-induced increase in histone deacetylase (HDCA) activity, glycogen synthase kinase-3β (GSK-3β) levels and pro-inflammatory cytokine levels as well as in the EAE-induced decrease in histone acetyltransferases 3 and 4 (HAT3, HAT4). Altogether, results demonstrate beneficial effects and potential targets of chrysin in EAE.

1. Introduction

Multiple sclerosis (MS) is the most common chronic inflammatory demyelinating neurodegenerative disease of the central nervous system (CNS) (Zhang et al., 2015a,b). MS causes varying degrees of relapsing or progressive neurological impairments (Zhang et al., 2015a,b). The clinical course and disease progression of MS are highly variable and likely depend on complex heritable (genetic and epigenetic) and environmental factors (Kucukali et al., 2015). Experimental autoimmune encephalomyelitis (EAE) is an animal model of MS, which is often used for studies of the pathogenesis and therapeutic interventions for this disease (Zhang et al., 2015a,b).

Several immunomodulatory drugs have been introduced to treat MS, but current drugs are unsatisfactory because their limited efficacy and occurrence of severe toxicity in some patients (Ge et al., 2013). In recent years, there has been increasing interest in seeking alternative and complementary means to improve the outcomes of conventional therapies in MS patients (Wang et al., 2018). Regarding this point, the use of flavonoids may be considered an interesting strategy, since preclinical data indicate that flavonoids can reduce neuroinflammation through regulation of microglial cells (Spagnuolo et al., 2018). Moreover, flavonoids have received considerable attention as a dietary supplement since they modulate the immune and inflammatory responses (Kanwal et al., 2016; Wang et al., 2018). In this context, chrysin (5,7-dihydroxyflavone) is a natural flavonoid present in many plant extracts, flowers, honey and propolis (Borges et al., 2015).

Chrysin has several of pharmacological activities, such as anti-inflammatory, hipolipdemic, antioxidant, antidepressant (Borges et al., 2015; Souza et al., 2015; Zarzecki et al., 2014). For instance, Goes et al. (2018) reported that chrysin protected against behavioral alterations and neuroinflammation in a mouse model of the Parkinson's disease (PD) induced by 6-OHDA. Moreover, Zhang et al. (2015a,b) showed that chrysin inhibited human dendritic cell differentiation, maturation and function in experimental autoimmune encephalomyelitis (EAE), an animal model of MS. Importantly, chrysin modulates DNA and histone methylation (Kanwal et al., 2016), and an increasing number of studies have shown that histone deacetylase (HDAC) inhibitors have beneficial effects on autoimmune and inflammatory diseases (Ge et al., 2013). Therefore, the present study aimed to further explore the beneficial effects of chrysin on MS (EAE model), as well as potencial underlying

Abbreviations: MS, multiple sclerosis; HDAC, histone deacetylase; EAE, experimental autoimmune encephomyelitis; HAT3, HAT4, histones acetyltransferases 3 and 4; GSK-3β, glycogen synthase kinase-3β; CNS, central nervous system

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mechanisms such as GSK-3β, HDAC, HATs and interleukins on spleen, spinal cords, hippocampus and prefrontal cortex.

2. Material and methods

2.1. Animals and chemicals

Male C57BL/6 mice (20–25 g) in weight and adults (90 days old) were used. Animals were housed, divided in groups of 6 in Plexiglas cages (41 cm × 34 cm × 16 cm) with the floor covered with sawdust. They were kept in a room with light-dark cycle of 12 h with the lights on between 7:00 and 19:00 h and temperature controlled (20–25 °C) and received water and food ad libitum. The experimental protocol was approved by the Animal Research Ethics Committee of the Federal University of Pampa (# 034/2017) and conducted in accordance with national (guidelines of Brazilian Council of Animal Experimentation – CONCEA) and international (U.S. Public Health Service's Policy on Humane Care and Use of Laboratory Animals – PHS Policy) legislation.

Chrysin and other reagents used in this experiment were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Treatment

Mice were fed diet AIN-93 M (Puro Trato, RS), ad libitum. After one week of acclimation, mice were randomly divided into 4 groups: Naïve/vehicle (group 1), Naïve/Chrysin (group 2), EAE/vehicle (group 3) and EAE/Chrysin (group 4).

Chrysin (20 mg/kg) was dissolved in a saline/propyleneglycol solution (80:20), and was administered intragastrically (i.g.) for 25 days (Borges et al., 2015), right after immunized to induce EAE as described below.

2.3. Induction, and clinical assessment of EAE

To induce EAE (Fig. 1), mice were immunized subcutaneously in the both flanks with 100 µg myelin oligodendrocyte glycoprotein (MOG) 35–55 peptide, and purity > 95% (Sigma, St. Louis, USA), dissolved in 200 µl of PBS and emulsified in 100 µl of Complete Freund's Adjuvant (CFA) (Sigma, St. Louis, USA) containing 200 µg of *Mycobacterium tuberculosis* H37RA (Difco, Detroit, USA). Immediately after immunization and again 48 h later, the mice were received a tail vein injection of 200 ng of pertussis toxin (List Biological Laboratories, CA, USA) in 100 µl of PBS (Zhang et al., 2017). The naive group received PBS instead of MOG35–55 peptide. The experimental protocol was repeated two times to ensure the reproducibility of the results.

2.4. Mean clinical score

The mean clinical scores (MCSs) were assessed daily according to a standard scoring system. The disease was graded on a scale of 0–5 of increasing severity: 0, no clinical signs; 1, tail paralysis; 2, one hindlimb paralysis; 3, complete hindlimb paralysis; 4, hindlimb and forelimb paralysis; 5, moribund/death. (Zhang et al., 2015a,b). Results are shown as average daily scores and area under curve (AUC).

2.5. Tissue preparation

After the mean clinical score evaluation, animals were euthanized, the spleen, spinal cords (SC), hippocampus (HIP), prefrontal cortex (PFC) were dissected, removed, weighed and homogenized in 50 mM Tris–Cl, pH 7.4. The homogenate was centrifuged at 2400 ×*g* for 15 min at 4 °C, and a low-speed, supernatant fraction (S1) was used for assays.

2.6. Glycogen synthase kinase-3β (GSK-3β)

The measurement of GSK-3β level was performed using a commercially available ELISA kit (YH Biosearch Laboratory, Shanghai, China), according to the manufacturer's instructions. All samples had their total protein levels adjusted to 1 mg/ml before starting ELISA procedures.

2.7. TNF, IL-17 and IFNγ

Levels of TNF, IL-17 and IFNγ in the spleen, SC, PFC, HP were determined using commercially available ELISA assays, following the instructions supplied by the manufacturer (DuoSet Kits, R&D Systems; Minneapolis). Results are shown as pg/mg tissue.

2.8. Histone deacetylase (HDAC) and histone acetyltransferases 3 and 4 (HATs)

The level of global HDAC and HATs 3 and 4 was estimated by using EpiQuik™ Global HDAC and HAT3 and HAT4 Assay Kit (as per manufacturer's instruction).

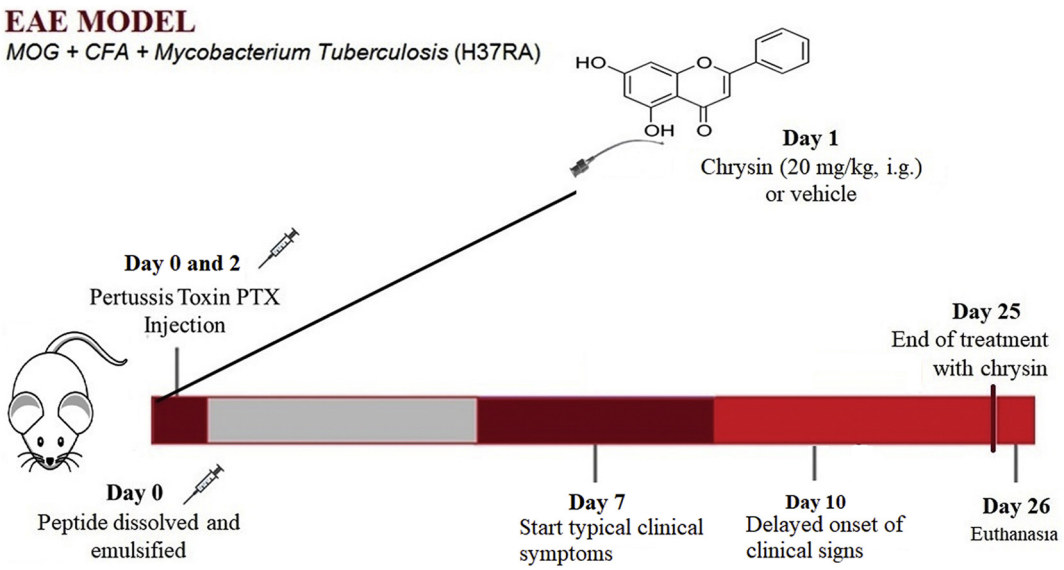

Fig. 1. Overview of study design.

2.9. Protein determination

Protein concentration was measured by the Bradford (Bradford, 1976), using bovine serum albumin (1 mg/ml) as the standard (Sigma). The Bradford assay is a protein determination method that involves the binding of Coomassie Brilliant Blue G-250 to proteins. It is this blue protein form that is detected at 595 nm in the assay using a microplate reader.

2.10. Statistical analysis

Results are presented as mean and S.E.M. Comparisons between the experimental and control group were performed by two-way analysis of variance (ANOVA), followed by Bonferroni test when appropriate. A value of $p < 0.05$ was considered to be significant. All tests were carried out using the GraphPad software (San Diego, CA, USA).

3. Results

3.1. Chrysin treatment attenuates the clinical signs and body weight loss caused by EAE

Typical clinical signs, such as tail atony and clumsy gait, appeared on day 7 post-MOG-injections and continue to rise until day 25 on untreated EAE group (Fig. 2A). Statistical analyses showed that control group differed from EAE in day 10 until day 26. In days 10, 11,13, 15–26 EAE differed from chrysin and from chrysin-treated EAE (Fig. 2A and B). Chrysin-treated EAE afforded a 55% improvement in clinical scores, as measured by the area under the curve (AUC) (Fig. 2B). Moreover, a progressive body weight loss paralleled disease severity in a mice model of EAE (Fig. 2C). In addition, chrysin treatment protected against marked loss of body weight induced by EAE (Fig. 2C).

3.2. Chrysin treatment modulates levels of GSK3β

EAE increased GSK3β levels in spleen, SC HIP and PFC and chrysin treatment (20 mg/kg) partially protected against this increase (Fig. 3A–D).

3.3. The EAE model induced desbalance of HAT/HDAC in the spleen, HIP, PFC and SC of mice

Activation and/or overexpression of specific HDACs have been associated with neurodegenerative diseases, so in this study we investigate the role of HDACs in a model of MS. In response to EAE model, a significant increase in HDAC activity was found in the spleen, HIP, PFC and SC when compared to naïve/vehicle group (Table 1). In contrast, HDAC activity in naive/chrysin group significantly decreased compared to control mice (naïve/vehicle), supporting a chrysin-induced inhibition of HDAC activity. Post hoc comparison showed that chrysin treatment normalized the increased HDAC activity observed in the spleen, HIP, PFC and SC of EAE/vehicle mice (Table 1).

EAE/Vehicle demonstrated a significant inhibition of HAT H3 and H4 activities in the spleen, HIP, PFC and SC when compared to control group (naïve/vehicle) (Table 1). However, chrsyin treatment increased the HAT H3 and H4 activities in the spleen, HIP, PFC and SC compared to naïve/vehicle group, demonstrating a modulatory effect. Post hoc comparisons indicated that the inhibition of HAT H3 and H4 activities in the spleen, HIP, PFC and SC of EAE/Vehicle mice was mitigated by chrysin treatment (Table 1).

3.4. Cytokines levels in the spleen, HIP, PFC and SC of mice

The levels of IFNγ, IL-17 and TNF levels were analyzed in the spleen, HIP, PFC and SC (Table 2). EAE/vehicle group increases the levels of IFNγ, IL-17 and TNF compared to the naïve/vehicle group. Chrysin treatment reduces the increased inflammatory cytokines observed in the spleen, HIP, PFC and SC of EAE/vehicle mice (Table 2).

4. Discussion

In this study, we explored the effects of flavonoid chrysin on EAE model of MS. Chrysin attenuated mean clinical score, mitigated EAE-induced increases in HDCA activity, GSK-3β, IFNγ, IL-17 and TNF levels as well as EAE-induced decrease in HAT3 and HAT4 activities.

One of the first hallmarks of disease in the MOG-induced EAE model is a reduction of body weight and onset of signs such as tail tonus and hind limb paralysis (Giacoppo et al., 2015). As expected, mice belonging to EAE/vehicle group significantly lost weight and showed the highest score of disease (about 5 points in the grading scale of disease), in accordance with previous studies by Giacoppo et al. (2015), Khezri et al. (2018) and Mahmoodi et al. (2019). Mice treatment with chrysin at the dose of 20 mg/kg afforded significant protection against disease induction, delayed onset of clinical signs and attenuated clinical scores compared with the vehicle-treated counterparts.

The neuroprotective and immunosuppressive effects of histone deacetylase inhibitors (HDACi) suggest that HDACi may potentially be useful for treatment of neuroinflammatory diseases including MS. Recent studies using HDACi trichostatin A (TSA) and valproic acid, as well as Vorinostat (which preferentially inhibits class I and HDAC6, although it is not highly selective), have been shown to ameliorate EAE (Camelo et al., 2005; Ge et al., 2013; Sun et al., 2018; Zhang et al., 2012). In the present study chrysin modulated HDAC activity by blocking increased activity in EAE-treated mice. Inhibition of HDACs by chrysin was previously reported in vitro by Pal-Bhadra et al. (2012) and Kanwal et al. (2016). Importantly, here we show for the first time that such inhibition of HDAC by chrysin also occurs in vivo, which is agreement with the fact that HDAC inhibitors have been shown to have good prophylactic effects in rodent EAE model (Camelo et al., 2005; Ge et al., 2013). HDAC inhibitors and their derivatives have attracted attention as potent neuroprotective drugs (Leng et al., 2010) that trigger beneficial effects in EAE and could be useful in the treatment of

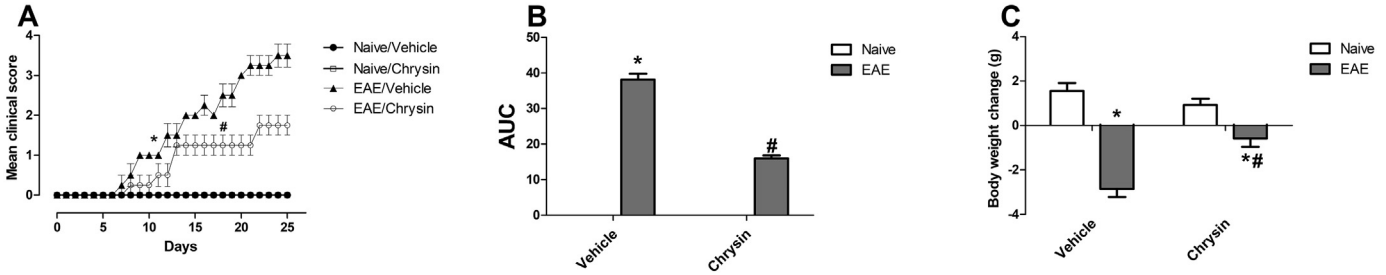

Fig. 2. Effect of treatment chrsyin and MOG_{35-55}/CFA on the clinical scores (A), area under curve (AUC) and body weight changes (C) in mice in a model of EAE. (Values are means and S.E.M. ($n = 6$). *$P < 0.05$ when compared with naïve/vehicle; #$P < 0.05$ when compared with EAE/vehicle (Two-way ANOVA, Bonferroni post hoc test).

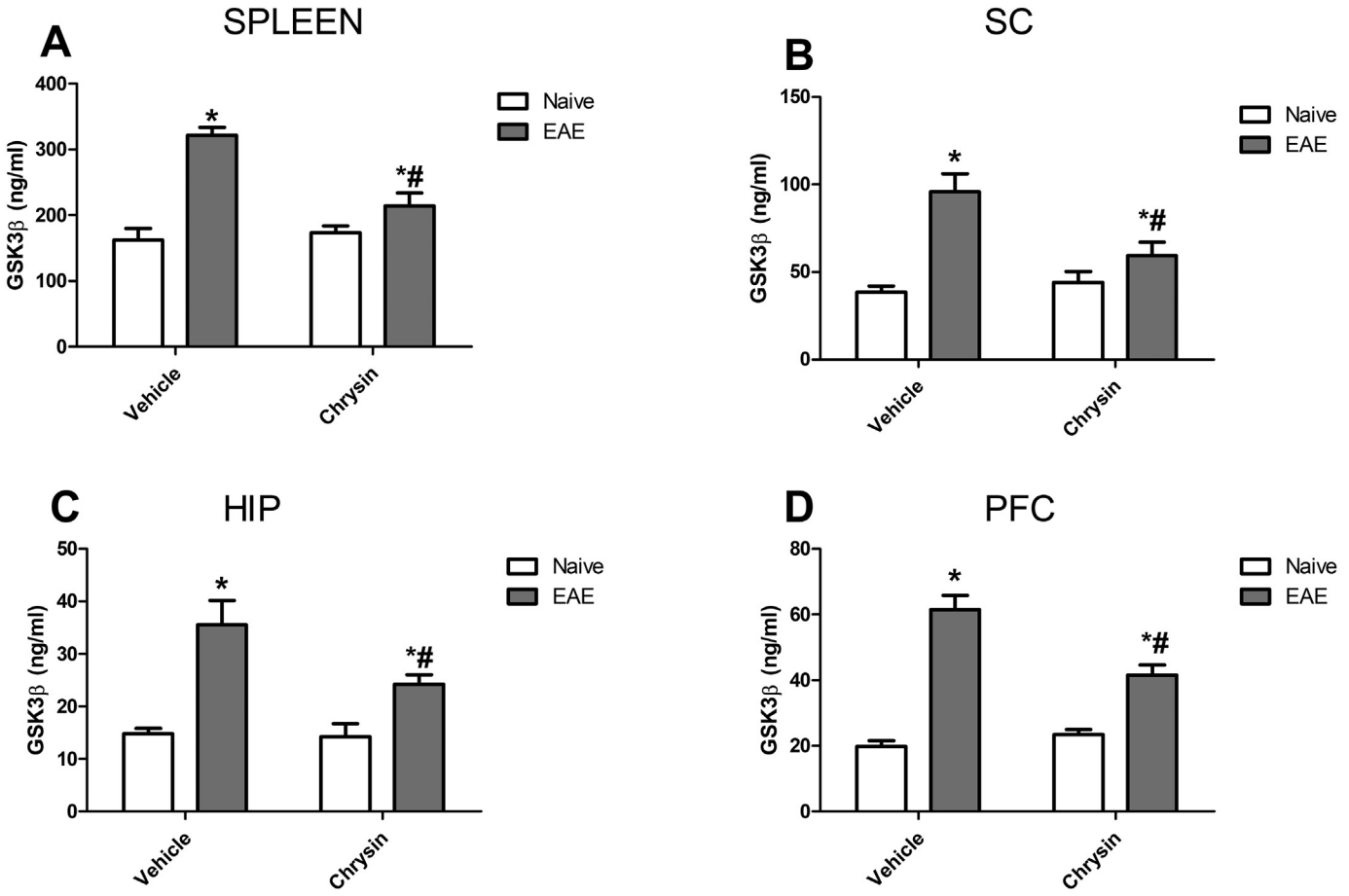

Fig. 3. Effect of treatment chrsyin and $MOG_{35–55}$/CFA on the GSK3β levels in the SPLEEN (A), SC (B), HIP (C) and PFC (D) (Values are means and S.E.M. (n = 6). *$P < 0.05$ when compared with Naïve/vehicle; # $P < 0.05$ when compared with EAE/vehhicle (Two-way ANOVA, Bonferroni post hoc test).

autoimmune demyelination (Wang et al., 2015a,b; Azuchi et al., 2017). Understanding the involvement of particular HDACs may help to develop selectively acting medication and reduce unwanted side effects (Wang et al., 2018). Many genes are associated with the development of neurodegenerative diseases, and are susceptible to changes in expression by epigenetic mechanisms. (Neal and Richardson, 2018). The epigenetic regulation, through your components, may critically affect immune system activation, neurogenesis and myelin formation in MS (Castelo-Branco et al., 2014). HDAC2 is a histone deacetylase that has been shown to reduce the acetylation of learning and memory-related genes (Gräff et al., 2012), besides that selected human studies identified changes in specific histone marks or changes in the levels of the enzymes that are involved in these posttranslational modifications in specific regions of AD postmortem brains (Ding et al., 2008).

Table 1
Effect of treatment chrysin and EAE on HDAC, HAT 3 and HAT 4 activities in the in spleen, HIP, PFC and SC of mice.

	Naive		EAE	
	Vehicle	Chrysin	Vehicle	Chrysin
HDAC				
Spleen	5615 ± 321.1	5141 ± 295.5*	7281 ± 198.1*	4812.2 ± 177.9#
HIP	2532 ± 233.6	1887 ± 179.1*	3774 ± 312.5*	2150 ± 119.2#
PFC	2612 ± 221.1	1949 ± 244.3*	4208 ± 150.3*	2331 ± 224.1#
SC	5478 ± 410.2	4179 ± 314.1*	7171 ± 421.3*	4518 ± 229.0#
HAT 3				
Spleen	473.2 ± 52.7	606.5 ± 53.7*	279.2 ± 11.7*	504.2 ± 22.5*#
HIP	119.5 ± 10.1	168.2 ± 8.90*	84.5 ± 8.70*	125.1 ± 8.50*#
PFC	126.0 ± 6.33	172.1 ± 12.1*	82.5 ± 4.56*	136.1 ± 6.20*#
SC	244.2 ± 21.6	353.7 ± 22.6*	157.2 ± 15.3*	216.5 ± 13.9*#
HAT 4				
Spleen	294.2 ± 15.8	463.7 ± 20.6*	197.0 ± 7.10*	261.5 ± 24.3*#
HIP	89.2 ± 8.10	146.2 ± 8.90*	59.5 ± 4.90*	92.7 ± 9.60*#
PFC	90.2 ± 7.60	128.5 ± 4.50*	65.2 ± 5.30*	95.7 ± 7.60*#
SC	339.5 ± 22.5	489.7 ± 44.0*	212.7 ± 32.0*	354.5 ± 38.2*#

The values were analyzed by two-way ANOVA and Bonferroni multiple comparison test. Each value is expressed as the mean ± S.E.M. (n = 6).
* $P < 0.05$ when compared with Naïve/vehicle.
# $P < 0.05$ when compared with EAE/vehicle.

Table 2
Effect of treatment chrysin and EAE on pro-inflammatory cytokines levels in the spleen, HIP, PFC and SC of mice.

	Naive		EAE	
	Vehicle	Chrysin	Vehicle	Chrysin
IFNγ				
Spleen	1569 ± 152.9	1539 ± 205.7	4961 ± 412.1*	2778 ± 189.1*#
HIP	578.1 ± 41.2	548.5 ± 46.8	957.8 ± 75.8*	729.5 ± 34.6*#
PFC	601.4 ± 38.2	494.8 ± 27.9*	1045 ± 53.3*	753.0 ± 70.6*#
SC	377.8 ± 36.2	371.8 ± 28.4	813.5 ± 48.4*	425.0 ± 61.0#
IL-17				
Spleen	1025 ± 82.7	861.5 ± 77.5*	1764 ± 139.9*	1299 ± 55.1*#
HIP	242.3 ± 23.5	186.3 ± 18.9	424.8 ± 17.8*	341.3 ± 22.1*#
PFC	178.8 ± 13.6	169.9 ± 10.3	409.8 ± 32.9*	224.1 ± 19.7#
SC	336.3 ± 33.2	325.1 ± 30.7	849.5 ± 29.8*	527.5 ± 25.3*#
TNF				
Spleen	2774 ± 162.3	2192 ± 87.9*	3753 ± 156.6*	2947 ± 138.6*#
HIP	171.3 ± 12.4	155.32 ± 11.2	427.0 ± 32.7*	237.0 ± 26.3*#
PFC	172.0 ± 20.4	179.3 ± 10.4	512.0 ± 59.1*	353.0 ± 19.2*#
SC	270.1 ± 25.7	241.3 ± 14.8	769.3 ± 70.1*	398.6 ± 52.2*#

The values were analyzed by two-way ANOVA and Bonferroni multiple comparison test. Each value is expressed as the mean ± S.E.M. (n = 6).
* $P < 0.05$ when compared with Naïve/vehicle.
# $P < 0.05$ when compared with EAE/vehicle.

Recent studies suggest the potential advantages of combined treatment of GSK-3β and HDAC inhibition in various in vitro and in vivo models of neurological diseases (Sharma and Taliyan, 2015). GSK-3β is a multifunctional protein kinase that phosphorylates several brain proteins, including neurofilaments (Nicolia et al., 2017). It is an important proinflammatory molecule in autoimune CNS diseases (Beurel, 2011), being involved in differentiation of Th17 cells, which is important for the induction of EAE in mice and plays an important role in neurodegeneration (Beurel et al., 2013). Thus, the blockade of GSK-3 signaling may be a therapeutic strategy for neuroprotection (Ahn et al., 2017). HDACs are the enzymes which remove acetyl groups from lysine residues in proteins and play pivotal roles in epigenetic regulation of gene transcription by remodeling chromatin structure. Activation and/or overexpression of specific HDACs have been associated with neurodegenerative diseases (Bardai and D'Mello, 2015). In the present study chrysin treatment reduced GSK-3β levels EAE/Chrysin group and inhibited the activity of HDAC, agreing with the scenario described by Bardai and D'Mello (2015), who proposed a possible link between GSK-3β and histone deacetylases (HDACs). Moreover, Ryves et al. (2005) reported that lithium chloride (LiCl), a well-known mood stabilizer, possibly act via inhibition of GSK-3β and HDAC. In addition, Valproic acid (VPA) is an HDAC and GSK-3β inhibitor and has been widely clinically used as a pharmacological agent in the treatment of epilepsy, bipolar disorder, spinal muscular atrophy and migraine headaches for nearly 50 years (Cho et al., 2012; Wang et al., 2015a,b. These lines of evidence clearly point toward a possible link between GSK-3β and HDAC in neurodegenerative processes involved in neuroinflamation. Regarding this point, simultaneous attenuation of HDAC and GSK-3β may also explain the presently-reported beneficial effects of chrysin in the EAE model.

Proper histone acetylation homeostasis is maintained by the antagonistic activity of histone acetlytransferases (HATs) and HDACs that activate and repress gene expression, respectively (Panikker et al., 2018). In the present study chrysin treatment up-regulated HAT3 and HAT4, enzymes with opposite function to HDAC and that are decreased during neurodegeneration (Schmalbach and Petri, 2010). Once the balance is disturbed and the HAT/HDAC ratio shifts in favor of HDAC in terms of availability and enzymatic functionality, an altered transcription profile is observed, typically represented by the repression of pro-survival molecules and the decrease of several pro-apoptotic gene products. Thus, in the use of HDAC inhibitors has been considered a potential and attractive therapeutic approach (Garbes et al., 2013). Beyond chrysin, others bioactive compounds could also regulate HATs and HDACs. Priyadarsini et al. (2011) reported that quercetin inhibits HDAC and Ruiz et al. (2007) related that quercetin inhibits the expression of TNF, IFN-γ and macrophage inflammatory protein 2 (MIP-2) and post-translational modifications (acetylation and phosphorylation) of HAT3. In line of this view, other flavonoids are also investigated in EAE model. Ciftci et al. (2015) demonstrated hesperidin treatment prevents the oxidative stress caused by EAE in mice via a decrease in lipid peroxidation and increase in elements of the antioxidant defense systems in brain tissue. Wang et al. (2016) investigated the beneficial effects of resveratrol in protecting the integrity of the BBB in EAE mice and observed improved clinical outcome after resveratrol treatment. Additionally, Haghmorad et al. (2017) demonstrated that hesperidin inhibited development of EAE in mice. Xie et al. (2018) demonstrated kurarinone significantly inhibits the clinical progression of EAE through the inhibition of Th1 and Th17 cell differentiation and proliferation.

Dysregulation of the immune system in EAE/MS is a fundamental aspect of both initiation and progression of disease. $CD4^+$ T cell-mediated autoimmunity is widely regarded as one of the most important aspects of MS pathogenesis (Zhang et al., 2015a,b). The immune response in MS is generally considered to be shifted toward T helper type 1 (Th1) cytokine production, including TNF and IFNγ (Kleinewietfeld and Hafler, 2014; Zhang et al., 2014). However, recent studies have indicated that IL-17 producing Th17 cells are involved, and are as critical as Th1 cells in this (Kebir et al., 2009), Komiyama et al. (2006) reported mice deficient for IL-17 are protected from the development of EAE. In the present study, chrysin treatment reduces pro-inflammatory cytokines IFNγ, TNF and IL-17 corroborating with the data described by Meng et al. (2017). The Th1 lineage of cytokine can help Th17 cells invade the brain and spinal cord, thus trigger EAE (Reboldi et al., 2009). The high percentage of Th17 cells has an impact on the inflammation in the brain and the severity of disease (Stromnes et al., 2008).

Another feature of EAE is the presence of infiltrating inflammatory cells in SC (Rossato et al., 2017). These infiltrating cells consist primarily of T cells and macrophages. Between these inflammatory cells, the Th1 cells (IFN-γ $CD4^+$) have been considered to play an important role in EAE development, principally by producing IFN-γ (Renno et al., 1995). In this sense, infiltrating T cells secrete large amounts of IFN-γ which will activate resident glial cells during EAE (Juedes et al., 2000; Renno et al., 1995). IFN-γ can enhances pro-inflammatory cytokines (IL-1β, IL-12) via monocytes and macrophages activation. Activated macrophages destroy axonal myelin and secrete countless cytokines, including the TNF, which would perpetuate inflammatory reactions, non-specific and would contribute to tissue damage (Rodrigues et al., 2010).

It's known that cognitive impairment is a significant symptom of multiple sclerosis (MS) affecting up to ~70% of patients (Rao et al., 1991). The hippocampus plays an important role in episodic memory, one of the most frequently affected cognitive disorder in MS (DeLuca et al., 1998; Yanike et al., 2004). Moreover, it has been shown that hippocampal demyelination is common in postmortem of MS brain of patients and that demyelinated hippocampi show decreased expression of neuronal proteins involved in a number of biological processes. (Dutta et al., 2011; Koenig et al., 2014).

The prefrontal cortex is one of the first regions showing changes at the beginning of the disease, where focal lesions, demyelination, and functional alterations were associated to cognitive decline and fatigue (Jehna et al., 2013). Since functional changes and atrophy in the frontal cortex may play a crucial role in MS progression these results may have broad implications for understanding the mechanism of cortical neuronal dysfunction in EAE and MS.

5. Conclusion

Chrysin treatment afforded significant protection against multiple parameters in EAE model of MS. These include clinical scores, levels of pro-inflammatory cytokines, and HDAC, HAT3 and HAT4 activities. Altogether, these results demonstrate beneficial effects and potential targets of chrysin in this model of EAE. Pharmacological and nutraceutical effects of chrysin may be explored in other autoimmune and chronic inflammatory disorders.

Declaration of Competing Interests

The authors have no conflicts of interest to disclose.

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