A comparative study on *Echinococcus granulosus* and curcumin therapeutic effect in mouse model of multiple sclerosis

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Abstract

Multiple sclerosis is a chronic autoimmune disease of the central nervous system, characterized by demyelination and neurodegeneration of the brain, spinal cord. Echinococcus granulosus the causative agent of Cystic echinococcosis induces host immunomodulation which protect it from the anti-parasitic immune response. Curcumin is a natural polyphenol component with proved anti-inflammatory and antioxidant properties. Current study was conducted to explore the immunomodulatory effects of Echinococcus granulosus protoscolices in experimental autoimmune encephalomyelitis as a model of multiple sclerosis in rats and to compare it with therapeutic effect of curcumin. 40 Sprague Dawley rats were randomly divided into four groups (ten animals per group), include: normal control, experimental autoimmune encephalomyelitis group, experimental autoimmune encephalomyelitisrats treated with Echinococcus granulosus protoscolices and experimental autoimmune encephalomyelitisrats treated with curcumin. Proinflammatory cytokines, brain nitric oxide concentration, serum myeloperoxidase activity and serum malondialdehyde level was evaluated. Based on our results, experimental autoimmune encephalomyelitiscaused significant increase in proinflammatory cytokines, myeloperoxidase activity, malondialdehyde levels and nitric oxide concentration as compared with the control group. Treatment with curcumin and Echinococcus granulosus protoscolices reduced the release of inflammatory cytokines, myeloperoxidase activity, malondialdehyde levels and nitric oxide concentration compared to experimental autoimmune encephalomyelitis group. No difference was observed between curcumin and Echinococcus granulosus protoscolices treated groups. The results of current study showed that Echinococcus granulosus protoscolices confer a therapeutic effect on experimental autoimmune encephalomyelitis as a model of Multiple sclerosis by decreasing the

production of pro-inflammatory cytokines and inhibiting oxidative stress. The similar role was observed for curcumin. The efficacy of Echinococcus granulosus protoscolices was equal to curcumin.

Abbreviations

- MS: Multiple sclerosis
  - EAE: Experimental autoimmune encephalomyelitis
  - PSCs: Protoscolices
  - MPO: Myeloperoxidase
  - NO: Nitric oxide
  - MDA: Malondialdehyde
  - SOD: Superoxide dismutase
  - TNF-α: Tumor necrosis factor-alpha
  - IL: Interleukin
  - iNOS: Inducible nitric oxide synthase
  - ROS: Reactive oxygen species
  - Th1: T-helper 1
  - Th2: T-helper 2
  - Th17: T-helper 17
  - FCA: Freund's complete adjuvant
  - PBS: Phosphate-buffered saline
  - ELISA: Enzyme-linked immunosorbent assay
  - CAT: Catalase
  - GPx: Glutathione peroxidase
  - Nrf2: Nuclear factor erythroid 2–related factor 2
  - AREs: Antioxidant response elements
  - NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells
  - JAK/STAT: Janus kinase/signal transducer and activator of transcription

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Highlights

1- Proinflammatory cytokine and oxidative stress markers increased in EAE rats

2- Treatment with *E. granulosus* alive protoscolices markedly reduced proinflammatory cytokine production and oxidative stress markers

3- Treatment with curcumin markedly reduced proinflammatory cytokine production and oxidative stress markers

4- *E. granulosus* protoscolices and curcumin showed similar therapeutic efficacy in EAE rats

1. Introduction

Multiple sclerosis (MS) is a chronic autoimmune neurodegenerative disease affecting around 2.8 million people worldwide. It is characterized by myelin loss, axonal pathology, presence of lesions in the brain and spinal cord and progressive neurological dysfunction. The disease is found the main cause of neurobiological disability in young adults with wide psychological, financial, social, and health consequences. Certain regions around the world have experienced a notable rise in the prevalence of multiple sclerosis (MS). In 2016, the global number of MS cases was estimated at 2,221,188, corresponding to 30.1 cases per 100,000 people [95% uncertainty interval (UI): 27.5–33.0]. Between 1970 and 2015, the Middle East and North Africa (MENA) region reported a high prevalence of MS. Among the countries in the Eastern Mediterranean Region (EMR), Iran had the highest rate, with 72.11 cases per 100,000 population. Tehran, the capital and most populous city of Iran, recorded a prevalence of 79.3 cases per 100,000 people in 2006, which increased to 162.38 cases per 100,000 [95% confidence interval (CI): 160.27–164.52] by 2019 [1].

Despite advances in the management of multiple sclerosis, current treatment options remain limited in their ability to halt disease progression or reverse neurological damage. Most available therapies focus on modulating the immune system to reduce the frequency and severity of relapses; however, they often fail to prevent long-term disability, particularly in progressive forms of the disease. Moreover, these treatments are frequently associated with significant adverse effects, including increased risk of infections, liver toxicity, cardiovascular complications, and diminished quality of life due to the burden of continuous administration.The heterogeneity in patients' responses to disease-modifying therapies (DMTs) further complicates treatment strategies, underscoring the urgent need for more personalized and targeted approaches. As the underlying mechanisms of MS continue to be elucidated, there is a growing demand for innovative therapies [2].

Although several environmental and genetic factors are introduced as a risk factor, the exact etiology of MS remains unknown. In experimental studies, the experimental autoimmune encephalomyelitis (EAE) in animals is used as a model of MS for pharmacological studies and drug discovery. Accumulating evidences in animals with EAE and MS patients suggest that

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inflammation plays a fundamental role in MS pathogenesis. The disease progression is mediated by T cells, B cells, macrophages and activated microglial cells [3]. Cytokines produced by T-helper 1 (Th1) and T-helper 17 (Th17) cells, such as interferon-γ (IFN-γ) and tumor necrosis factor-alpha (TNF-α) were considered primarily responsible for the induction of the inflammatory process during the development of MS. Proinflammatory molecules secreted during MS development cause myelin and oligodendrocyte destruction [4].

In addition, there is a close correlation between oxidative stress and progression of neuroinflammation. Previous studies found that the central nerve system (CNS) damage was induced by the reactive oxygen species (ROS) such as nitric oxide (NO) and hydrogen peroxide (H2O2) in MS patients [5,6].

Pharmacological treatments for MS are based on immunomodulatory or immunosuppressant drugs, designed to diminish the immune reaction and ROS production. However, these therapies showed several side effects, such as headache and gastrointestinal complications [7]. Finding alternative treatment strategies with improved efficacy and fewer side effects has been the focus of recent studies.

Curcumin (C21H20O6) is a polyphenolic substance extracted from turmeric (*Curcuma longa*) and belongs to the Zingiberaceae family. It has been shown that curcumin could play pharmacological roles in various autoimmune and neurodegenerative disorders such as dementia, Alzheimer's disease, Parkinson's disease, Huntington's disease, and MS. Its anti-inflammatory mechanism is related to the suppression of proinflammatory cytokines, inhibition of TNF-α and NO release and inhibiting differentiation and proliferation of Th17 cells [8].

Echinococcosis is a globally distributed zoonotic disease caused by infection with the larval stage of Echinococcus granulosus, a cestode of the taeniid family. Beyond its parasitic pathology, E. granulosus has drawn attention for its unique immunomodulatory capabilities. The parasite employs sophisticated strategies to manipulate the host immune system, prominently through the induction of regulatory immune pathways and a skewed T-helper type 2 (Th2) response. This shift suppresses proinflammatory T-helper type 1 (Th1) and Th17 pathways, which are known to be central in the pathogenesis of autoimmune diseases such as multiple sclerosis (MS). Recent experimental evidence suggests that parasitic infections, including those by E. granulosus, can attenuate autoimmune and inflammatory conditions by downregulating pathological immune responses and promoting immune tolerance. In this context, the therapeutic potential of E. granulosus or its derivatives has been explored in diseases such as colitis, type 1 diabetes, and experimental autoimmune encephalomyelitis

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(EAE)—the most commonly used animal model of MS. These findings support the hypothesis that E. granulosus may exert beneficial effects in MS by modulating immune responses and limiting neuroinflammation. Therefore, investigating its therapeutic effect in an MS model is not only scientifically justified but also contributes to the broader understanding of parasite-based immunotherapy [9,10].

Our previous study showed that the *E. granulosus* significantly survived in pregnant mice due to the secretion of Th2 related cytokines [11]. Current study was conducted to investigate he immunomodulator effect of *E. granulosus* on cytokine production and oxidative stress markers in EAE model of MS and to compare its efficacy with curcumin as a naturally occurring components.

2.Results:

2.1. Effect on body weight

In the present study, the EAE model has the similar characteristic with MS to some extent. All immunized animals displayed EAE, achieving at least grade 2 of the disease, which was considered successful. The mean body weight of EAE rats was significantly reduced, compared to the normal control rats (188.2 ± 8.89 versus 340.3 ± 9.93; $p < 0.05$). However, the extent of weight loss was significantly restricted in curcumin (303.3 ± 7.49 versus 340.3 ± 9.93) and PSCs (291 ± 11.46 versus 340.3 ± 9.93) treated rats, compared to the EAE animals ($p < 0.05$). These findings indicate that E. granulosus infection may be able to protect rats against EAE.

2.2. Effects on inflammatory cytokines

The results showed a significant increase in the levels of proinflammatory cytokines in EAE group as compared to control group ($p < 0.05$). However, their levels decreased markedly in curcumin and PSCs treated groups ($p < 0:05$) compared to EAE group showing the notable effect of treatments on proinflammatory cytokines. The difference between the Cur and PSCs was not statistically significant ($p > 0.05$). These findings outline the anti-inflammatory activities of curcumin and PSCs against EAE-induced neurodegeneration.

2.3. Effect on oxidative stress markers

In this study, EAE induced ROS formation and lipid peroxidation as indicated by elevation of MDA, MPO activity and NO levels. The detection results of blood biochemical indicators showed that EAE induced a significant increase of the contents of serum MDA and MPO compared to control group ($p < 0.05$) (Based on the Table).

Treatment with both curcumin and PSCs ameliorated neutrophil infiltration and lipid peroxidation as evidenced by suppression of serum MPO and MDA (Based on the Table) compared to EAE group ($p < 0.05$). No significant difference was observed between curcumin and PSCs treated groups ($p > 0.05$).

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EAE caused significant elevation in brain NO level as compared to control group (According yo the table). Importantly, curcumin and E. granulosus decreased significantly the production of brain NO compared to the EAE group (According yo the table) ($p < 0.05$).

Table 1.

Table. Effects of Curcumin and E. granulosus PSCs on Biochemical Parameters of EAE affected rats. Values Expressed are Mean ± SD.

Parameters	Animal treatments			
	Control	EAE	EAE + Curcumin	EAE + PSCs
IL-1β (pg/mL)	130.07±0.44	148.3±0.44	129.42±0.44	130.07±0.44
IL-6 (pg/mL)	370.43±0.44	342.86±0.44	342.86±0.44	370.43±0.44
TNF-α (pg/mL)	656±0.44a	759.03±0.44b	750±0.44b	656±0.44a
NO (µMOL/L)	785.7±0.44a	1047.42±0.44b	882.6±0.44c	785.7±0.44a
MPO (mU/mL)	1106.22±0.44a	976.8±0.44b	974.04±0.44b	1106.22±0.44a
MDA (N mol/Ml)	1.6±0.2a	12.4±0.1b	5.5±0.3b	7.4±0.1a
SOD (U/L)	1250.45±0.44a	1346.67±0.44b	1364.09±0.44b	1250.45±0.44a

MPO: Myeloperoxidase; NO: Nitric oxide; IL: Interleukin; TNF-α: Tumor necrosis factor-α; SOD: Superoxide dismutase, Cur: Curcumin, PSCs: Protoscolices, EAE: Experimental autoimmune encephalomyelitis. a denotes significant difference compared to control group ($P < 0.05$), b denotes significant difference compared to EAE group ($P < 0.05$).

3. discussion

Recent progress in helminth-based therapies has paved the way for innovative approaches in managing immune-related disorders, primarily through leveraging the immunomodulatory properties of these parasites. Several studies have highlighted the beneficial effects of helminths and their secreted products in reducing the severity or incidence of allergic reactions and other inflammatory conditions [19].

In the present study, we investigated the anti-inflammatory and anti-oxidant effects of PSCs, and to compare it with curcumin in EAE as an animal model of MS. The EAE model has the similar characteristic with MS to some extent. All immunized animals displayed EAE, achieving at least grade 2 of the disease, which was considered successful. The mean body weight of EAE rats was significantly reduced, compared to the normal control rats (188.2 ± 8.89 versus 340.3 ± 9.93; $p < 0.05$). However, the extent of weight loss was significantly restricted in curcumin (303.3 ± 7.49 versus 340.3 ± 9.93) and PSCs (291 ± 11.46 versus 340.3 ± 9.93) treated rats, compared to the EAE animals ($p < 0.05$). These findings indicate that *E. granulosus* infection may be able to protect rats against EAE.

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Pro-inflammatory cytokines overproduction and mediators cause remarkable tissue damage. In this experiment, the production of proinflammatory cytokines TNF-α, IL-6 and IL-1β in the supernatant of stimulated spleenocytes in spleen of individual rats were measured by sandwich ELISA. The results showed a significant increase in the levels of proinflammatory cytokines in EAE group as compared to control group ($p < 0.05$). However, their levels decreased markedly in curcumin and PSCs treated groups ($p < 0:05$) compared to EAE group showing the notable effect of treatments on proinflammatory cytokines. The difference between the Cur and PSCs was not statistically significant ($p > 0.05$). These findings outline the anti-inflammatory activities of curcumin and PSCs against EAE-induced neurodegeneration.

The development of multiple sclerosis (MS) is influenced by various factors, including Th1 cell activation and the excessive release of pro-inflammatory mediators. In the early stages of the disease, T cells that recognize myelin, along with peripheral immune cells like macrophages, traverse the compromised blood–brain barrier and enter the central nervous system (CNS). Once inside, these activated T cells release large amounts of inflammatory cytokines such as IL-1β, IL-6, and TNF-α, contributing to myelin damage and, ultimately, axonal loss. Among these, IL-6 plays a critical role by enhancing disease severity and spinal cord lesions through the induction of Th17 cells in peripheral lymphoid tissues. These Th17 cells drive ongoing neuroinflammation and demyelination, particularly in the experimental autoimmune encephalomyelitis (EAE) model. IL-1β has been shown to rapidly stimulate astrocytes to produce cytokines, chemokines, adhesion molecules, and matrix-degrading enzymes. Similarly, TNF-α facilitates continued demyelination and promotes macrophage infiltration during EAE-associated neuroinflammation [20].

Curcumin has been previously recognized for its neuroprotective properties and its potential to enhance cognitive function within the central nervous system (CNS). Its beneficial effects in autoimmune disorders have been linked to its ability to regulate key pro-inflammatory cytokines, including IL-12, TNF-α, IL-1β, IL-6, and IFN-γ. In experimental autoimmune encephalomyelitis (EAE), curcumin's anti-inflammatory actions appear to be primarily mediated through its influence on cytokine and growth factor signaling pathways. Furthermore, curcumin has demonstrated the capacity to suppress astrocyte activation and reduce the secretion of inflammatory molecules by microglial cells [21]. Curcumin could down-regulate the expression of IL-6 gene in astrocytes [8] and prevent the differentiation and development of Th1 and Th17 cells in EAE [12].

Mechanistically, curcumin modulates cytokine expression by downregulating pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-

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1β), and interleukin-6 (IL-6), while promoting the production of anti-inflammatory cytokines like interleukin-10 (IL-10). This immunomodulatory profile is primarily mediated through the inhibition of nuclear factor-kappa B (NF-κB) signaling and modulation of the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway. In parallel, curcumin exerts antioxidant effects by enhancing the activity of endogenous antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). It also reduces levels of reactive oxygen species (ROS) and lipid peroxidation products, thereby mitigating oxidative damage to neuronal structures. These combined effects contribute to the restoration of redox balance and suppression of oxidative stress-driven demyelination in MS [22].

E. granulosus has immunomodulatory effects on host immune response and has been used to treat allergic and autoimmune conditions such as allergic airway inflammation, asthma and bacterial sepsis. A recent investigation on murine bacterial sepsis [9] demonstrated that administration of Echinococcus granulosus cyst fluid led to an increase in anti-inflammatory cytokines such as IL-10 and TGF-β, while simultaneously decreasing levels of pro-inflammatory markers including TNF-α and IFN-γ. These observations align well with the results obtained in our study. Infection with E. granulosus has been shown to promote a shift in the immune profile from a Th1-dominant to a Th2-dominant response, thereby suppressing the activity of Th1 and Th17 cells and reducing the associated inflammatory cytokine production—an effect that supports the modulation and downregulation of immune-mediated inflammation [23]. Earlier research has shown that administration of Echinococcus granulosus cyst fluid in an EAE mouse model of multiple sclerosis led to a marked reduction in the expression of pro-inflammatory cytokines such as IFN-γ, IL-1β, and TNF. This treatment also promoted a shift in the immune profile from a Th1-dominated response toward a Th2 phenotype, as evidenced by decreased IFN-γ and elevated IL-4 levels. Similarly, another study reported that the laminated layer isolated from the cyst wall of E. granulosus suppressed the production of IL-1β, IL-6, and TNF-α, likely through modulation of the NF-κB signaling cascade and the IRAK pathway [24].

Recent studies have also highlighted the immunoregulatory potential of Echinococcus granulosus protoscolex (PSC), which may provide a novel helminth-based approach to modulating immune responses in autoimmune diseases such as MS. PSC components are thought to exert immunosuppressive effects through the induction of regulatory T cells (Tregs) and the shift of the immune response toward a Th2-dominant profile, thereby attenuating the pathogenic Th1/Th17-driven inflammation typical of MS. Moreover, PSC may influence

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oxidative stress pathways by reducing the production of ROS and enhancing antioxidant defenses in host immune cells. Although the precise molecular mechanisms remain to be fully elucidated, helminth-derived antigens, including those from E. granulosus, have been shown to upregulate nuclear factor erythroid 2–related factor 2 (Nrf2), a key transcription factor that governs the expression of antioxidant response elements (AREs) [25].

Oxidative stress is recognized as a critical contributor to both the onset and progression of experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis (MS). The infiltration of immune cells into affected tissues initiates the overproduction of reactive oxygen species (ROS), which in turn target essential cellular components. These free radicals compromise cellular integrity by damaging membrane lipids, altering protein structures, and inducing mutations or breaks in DNA molecules [26]. In this study, EAE induced ROS formation and lipid peroxidation as indicated by elevation of MDA, MPO activity and NO levels.

The detection results of blood biochemical indicators showed that EAE induced a significant increase of the contents of serum MDA and MPO compared to control group ($p < 0.05$) (Table1).

MDA is a product of oxygen free radicals triggering lipid peroxidation of unsaturated fatty acids on cell membranes

Myeloperoxidase (MPO) is widely recognized as a reliable indicator of neutrophil infiltration and is frequently used to assess inflammatory responses in both human and animal studies. Growing evidence indicates that MPO produced not only by neutrophils but also by activated microglia, astrocytes, and even neurons, may contribute to the development of neuropathological changes. Moreover, MPO activity and neutrophil accumulation have been implicated in the disruption of the blood–brain barrier, further exacerbating central nervous system inflammation [27].

Previous studies have reported the increased levels of serum MPO and MDA as confirmed by blood–brain barrier disruption damage, infiltration of inflammatory cells into CNS and demyelination. These findings are observed in mice models of EAE , as well as patients with MS [28]. In agreement with previous works, the present study showed a significant increase in MPO and MDA content in the EAE group. On the other hand, treatment with both curcumin and PSCs ameliorated neutrophil infiltration and lipid peroxidation as evidenced by suppression of serum MPO and MDA (Table) compared to EAE group ($p < 0.05$). No significant difference was observed between curcumin and PSCs treated groups ($p > 0.05$).

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It is well established that experimental autoimmune encephalomyelitis (EAE) is associated with elevated levels of inducible nitric oxide synthase (iNOS) and enhanced production of nitric oxide (NO). Notably, iNOS expression has been observed in astrocytes located within active demyelinating lesions, indicating that astrocyte-derived NO, generated through iNOS activity, may play a significant role in promoting neurotoxicity driven by oxidative stress. [29]. During oxidative stress, nitric oxide (NO) interacts with superoxide anions to generate peroxynitrite ($ONOO^-$), a highly reactive oxidant responsible for neuronal injury observed in both EAE models and multiple sclerosis patients. In the present study, brain NO levels were significantly increased in EAE mice compared to controls (Table 1). The NO produced by activated astrocytes contributes to mitochondrial dysfunction and DNA strand breaks, ultimately resulting in neuronal cell death [29]. This elevation is in harmony with the previous finding that NO is widely expressed in the brain of EAE model and MS patients. Importantly, curcumin and *E. granulosus* decreased significantly the production of brain NO compared to the EAE group (Table) ($p < 0.05$).

Curcumin possesses a molecular structure characterized by a central carbon linker connecting two aromatic rings. These aryl rings bear phenolic hydroxyl and methoxy substituents, which enhance the compound's ability to neutralize reactive oxygen species (ROS), thereby contributing to its antioxidant activity. Remarkably, curcumin also demonstrates neuroprotective properties by preventing oligodendrocyte apoptosis and inhibiting axonal damage induced by nitric oxide [21].

A prior study reported that treatment with Echinococcus granulosus cyst fluid markedly suppressed iNOS expression in the liver, lungs, and kidneys of septic mice [9]. Consistent with our results, Echinococcus granulosus infection has been shown to alleviate clinical manifestations in a dextran sulfate sodium-induced colitis model, accompanied by reduced production of nitric oxide (NO) and TNF-α, as well as downregulation of iNOS and nuclear factor-κB expression in the colon [30]. Given the established involvement of TNF-α and nitric oxide (NO) in oxidative stress pathways, the observed decline in MPO activity and MDA concentrations may reflect reduced reactive oxygen species (ROS) generation, likely mediated by the immunomodulatory properties of Echinococcus granulosus.

In summary, the present findings indicate that administration of Echinococcus granulosus protoscoleces (PSCs) effectively mitigates neurodegeneration in the EAE rat model by attenuating both inflammatory and oxidative processes. These results suggest that helminth-based interventions using E. granulosus may represent a promising therapeutic approach for multiple sclerosis and other autoimmune disorders characterized by chronic inflammation.

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Limitations

This study has several limitations that should be acknowledged. Firstly, the assessment of therapeutic efficacy was primarily based on clinical scoring, without incorporating detailed histopathological analyses of neural tissues to evaluate demyelination or axonal damage. Secondly, the absence of immunohistochemical and molecular evaluations limited our understanding of the underlying mechanisms, particularly concerning cytokine profiles and oxidative stress markers. Additionally, the study did not assess the long-term effects and potential side effects of curcumin and Echinococcus granulosus protoscolex treatments. Future research should address these aspects to provide a more comprehensive understanding of the therapeutic potential and safety profile of these interventions in multiple sclerosis models.

4. Materials and Methods

4.1. Experimental animals

A total of 40 healthy male adult Sprague-Dawley rats (250~300 g), were purchased from the Pasteur Institute of Iran, Tehran. They were kept in laboratory cages (10 animals per cage). Food and water were freely available. The rats were kept in a room with a 12:12 hour light-dark cycle and a constant temperature of 21±2ºC. The animal experiments were carried out according to the Ethics Committee of University of Tabriz (Ethical code number: IR.TABRIZU.REC.1398.004) for the care and use of laboratory animals and the National Research Council's Guide for the Care and Use of Laboratory Animals.

The efforts were made to minimize unnecessary pain and suffering within labs. After one week acclimatization, the animals were randomly divided into four groups (ten animals per group) and treated as follow:

1. Control group (normal saline administered orally every day until the end of the study)

2. Experimental autoimmune encephalomyelitis (EAE) group (non-treated as positive control)

3. EAE+ curcumin group, EAE affected animals in this groups received curcumin (100 mg/kg) by the gavage method every day from the onset of clinical symptoms for 60 days.

4. EAE + PSC group, EAE affected animals in this group were inoculated with PSCs of *E. granulosus* intraperitoneally from the time the first sign of disease was observed.

4.2. Induction of EAE as an experimental model of MS

The procedure was conducted based on previous study protocol [12]. According to the

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procedure described by Chen et al. (2010), an emulsion was prepared using homogenized guinea pig spinal cord tissue (SICBD, Karaj, Iran) mixed in equal parts with Freund's complete adjuvant (FCA; Sigma Co., Burlington, MA, USA), which contained 10 mg/kg of heat-killed Mycobacterium tuberculosis. The experimental rats were anesthetized using a combination of ketamine (100 mg/kg) and xylazine (10 mg/kg) (Alfasan, Woerden, The Netherlands). A total of 400 µL of the antigen-adjuvant emulsion was injected subcutaneously into the dorsal area, and an additional 100 µL was administered into the footpad using a 25-gauge needle. After 48 hours, 109 colony-forming units of Brucella parapertussis (IBRC-M 10710, ATCC 15311) were injected intraperitoneally in 300 µL of phosphate-buffered saline (PBS). Disease progression was evaluated daily by assessing motor deficits in each rat. Clinical scoring and body weight measurements were recorded from day 0 to day 12 post-induction based on previously established evaluation scales[13]. The EAE model is considered successful when the score exceeds 2.

4.3:Preparation and Viability Assessment of PSCs:

Fresh hydatid cysts were obtained from the livers of naturally infected sheep slaughtered at the Tabriz industrial abattoir in East Azerbaijan province, Iran. These samples were transferred to the Parasitology Laboratory at the Faculty of Veterinary Medicine, University of Tabriz. To begin, the surface of each cyst was disinfected twice using 70% ethanol. Following disinfection, the cyst fluid was aspirated using a 50 ml syringe, collected in glass tubes, and centrifuged at 1500 rpm for 3 minutes. The supernatant was carefully discarded, and the collected protoscoleces (PSCs) were washed several times with phosphate-buffered saline (PBS; pH 7.2).

To determine the concentration of PSCs, 5 µl of the suspension was placed on a microscope slide, covered with a coverslip, and examined under a light microscope at 40× magnification. This procedure was performed in triplicate, and the average count was used to calculate the PSCs density per milliliter. The suspension was then adjusted to contain 500 PSCs/mL in 0.9% normal saline, ensuring a viability of over 90% for subsequent inoculation into mice.

Viability was assessed according to the method described by Smyth and Barrett (1980)[14]. Briefly, 20 µl of the PSC suspension was mixed with an equal volume of 0.1% eosin solution (prepared by dissolving 1 g eosin powder in 1 L of distilled water) in a clean microtube. After 15 minutes of staining, the PSCs were observed under a light microscope: unstained (colorless) PSCs were considered viable, while stained (red) ones were considered non-viable. The viability percentage was calculated by dividing the number of live PSCs by the total number

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of PSCs and multiplying by 100. This assay was also conducted in triplicate [15].

As a control, a portion of the PSC suspension was heat-killed by incubation at 60°C for 30 minutes in an oven (Fan Azma Gostar, Karaj, Iran), then stained with 1% eosin using the same method described above.

4.4. Induction of *E. granulosus* infection

Induction of *E. granulosus* infection was performed based on protocol conducted previously by our team [11]. After PSCs preparation and viability assessment, 500 PSCs were injected into each rat intraperitoneally.

4.5. Preparation of Curcumin Suspension for Oral Gavage:

Curcumin powder (≥94% purity) was suspended in sterile olive oil to prepare a uniform suspension suitable for oral gavage. The mixture was thoroughly vortexed to ensure proper dispersion and gently warmed at 37°C to enhance solubility. The final concentration was adjusted to provide a dose of 100 mg/kg body weight. The suspension was freshly prepared each day under aseptic conditions, stored at room temperature, and protected from direct light. Before administration, the suspension was shaken well to maintain homogeneity, and administered orally to the animals using a sterile feeding gavage needle.

4.6. Tissue preparation and biochemical analysis

The body weight of rats in each group was obtained at the end of the study. At the end of the study, the rats were scarified under ether anesthesia. The spleen tissue was used for cytokine measurements. Stimulation of spleen cells and measurements of cytokines concentration (TNF-α, IL-1β, IL-6) was conducted using Commercially ELISA kits (Bender Med Co., Austria) based on protocol described previously [16]. The results were presented as average (pg/mL). Following anesthesia, blood samples were collected directly from the heart. To obtain serum, the samples were centrifuged at 1500 rpm for 10 minutes. Serum levels of myeloperoxidase (MPO) were assessed using a colorimetric activity assay kit (BioVision, USA), following the manufacturer's protocol. Additionally, the activity of superoxide dismutase (SOD) in the serum was determined based on a previously established method. [17]. The preparation of brain tissue and measurement of nitric oxide (NO) levels was performed based on previously described protocol [18]. The results were presented as µmol/L.

4.7. Statistical analysis

Statistical values are expressed as the mean ± standard deviation (SD). To compare differences among experimental groups, a one-way analysis of variance (ANOVA) was conducted, followed by Tukey's post hoc test for pairwise comparisons. A p-value less than 0.05 was

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considered statistically significant. All analyses were carried out using Stata version 14 (StataCorp, Texas, USA).

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5. Ethics:

The animal experiments were carried out according to the Ethics Committee of University of Tabriz (Ethical code number: IR.TABRIZU.REC.1398.004) for the care and use of laboratory animals and the National Research Council's Guide for the Care and Use of Laboratory Animals.

6. Data Availability

The data that support the findings of this study are available on request from the corresponding author.

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