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

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ABSTRACT

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system, characterized by demyelination and neurodegeneration affecting the brain and spinal cord. Echinococcus granulosus the causative agent of Cystic echinococcosis induces profound immunomodulation effects that enable evasion of host anti-parasitic immune response. Curcumin is a natural polyphenol component with well-documented anti-inflammatory and antioxidant properties. The present study was conducted to investigate the immunomodulatory effects of *E. granulosus protoscolices* (PSCs) in experimental autoimmune encephalomyelitis (EAE), an established animal model of MS, and to compare their therapeutic effect with that of curcumin. 40 male Sprague Dawley rats were randomly divided into four groups (n = 10 per group): control, EAE, EAE treated with E. *granulosus* PSCs and EAE treated with curcumin. Levels of proinflammatory cytokines, brain nitric oxide (NO) concentration, serum myeloperoxidase (MPO) activity and serum malondialdehyde (MDA) were assessed. Based on our results, experimental autoimmune encephalomyelitis caused significant increase in proinflammatory cytokines, MPO activity, MDA levels and NO concentration as compared with controls. Treatment with either curcumin or PSCs reduced the release of inflammatory cytokines, MPO activity, MDA levels and NO concentration compared with EAE group. No difference was observed between curcumin and PSCs treated groups. The results of current study demonstrate that PSCs exert a therapeutic effect in EAE model by decreasing the production of pro-inflammatory cytokines and inhibiting oxidative stress. The similar role was also observed for curcumin. Notably, the efficacy of PSCs treatment was comparable to that of curcumin.

Keywords

Curcumin, Echinococcus granulosus, Experimental autoimmune encephalomyelitis (EAE), Multiple sclerosis, cytokine

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Abbreviations

MS: Multiple sclerosis

EAE: Experimental autoimmune encephalomy-

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Introduction

Multiple sclerosis (MS) is a chronic autoimmune neurodegenerative disease affecting approximately 2.8 million individuals worldwide. The disease is characterized by demyelination, axonal injury, the formation of lesions in the brain and spinal cord and progressive neurological dysfunction. MS represents as one of the main causes of neurobiological disability in young adults with wide psychological, financial, social, and health burdens. Certain regions around the world have experienced a notable rise in the prevalence of MS. In 2016, the global number of MS cases was estimated at 2,221,188, corresponding to a prevalence of 30.1 cases per 100,000 individuals [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 countries in the Eastern Mediterranean Region (EMR), Iran reported the highest prevalence rate, with 72.11 cases per 100,000 population. In Tehran, the capital and most populous city of Iran, MS prevalence increased from 79.3 cases per 100,000 people in 2006, to 162.38 cases per 100,000 by 2019 [95% confidence interval (CI): 160.27–164.52] [1].

Despite advances in the management of MS, current treatment options remain limited in their ability to halt disease progression or reverse neurological damage. Most approved treatments focus on immunomodulation to reduce the relapse frequency and severity; however, their effectiveness in preventing long-term disability, particularly in progressive forms of MS, is limited. In addition, these treatments are frequently associated with significant adverse effects, including increased susceptibility to infections, hepatotoxicity, cardiovascular complications, and reduced quality of life due to long-term treatment burden. Furthermore, the heterogeneity in patients' responses to disease-modifying therapies (DMTs) further complicates treatment strategies, underscoring the 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 multiple environmental and genetic factors have been implicated in MS susceptibility, the exact etiology of MS remains unknown. In experimental studies, the 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 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, oxidative stress has been closely linked to the progression of neuroinflammation. Previous studies found that, the CNS damage was induced by the excessive production of ROS including NO and hydrogen peroxide (H_2O_2) in MS patients [5,6].

Current pharmacological treatments for MS primarily rely on immunomodulatory or immunosuppressant drugs, aimed at attenuating immune reaction and reducing ROS production. However, these drugs are often accompanied by several undesirable side effects, such as headache and gastrointestinal disturbances [7]. Therefore, the identification of alternative treatment strategies with improved efficacy and fewer side effects has been the focus of recent studies.

Curcumin ($C_{21}H_{20}O_6$) is a polyphenolic substance extracted from turmeric (Curcuma longa) and belongs to the Zingiberaceae family. It has been shown that curcumin can play pharmacological roles in various autoimmune and neurodegenerative disorders such as dementia, Alzheimer's disease, Parkinson's disease, Huntington's disease, and MS. the anti-inflammatory mechanism of curcumin are largely attributed to its ability to suppress proinflammatory cytokines production, inhibit TNF-α and NO release and prevent the 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 belonging to

Abbreviations-Cont'd

elitis
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

the taeniidae family. Beyond its parasitic pathology, *E. granulosus* has garnered increasing 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 central to the development of autoimmune diseases such as MS. Recent experimental evidence suggests that parasitic infections, including E. granulosus, can attenuate autoimmune and inflammatory conditions by downregulating pathological immune responses and promoting immune tolerance. Accordingly, the therapeutic potential of *E. granulosus* and its derivatives has been explored in diseases such as colitis, type 1 diabetes, and EAE. Previous work demonstrated 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].

Also, previous studies showed that the *E. granulosus* exhibits enhanced survival in pregnant mice, likely mediated through increased secretion of Th2 associated 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 their therapeutic efficacy with curcumin, a well-established natural anti-inflammatory components.

Results

Effect on body weight

In the present study, the EAE model reproduced several characteristic with MS. All immunized animals displayed clinical signs of EAE and reached at least grade 2 of disease, confirming the successful induction of the model. 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; $p < 0.05$). In contrast, treatment with curcumin (303.3 ± 7.49 versus 340.3 ± 9.93) or PSCs (291 ± 11.46 versus 340.3 ± 9.93) significantly attenuated body weight loss, compared to EAE animals ($p < 0.05$). These findings suggest that *E. granulosus* infection may be able to protect rats against EAE.

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 significantly in curcumin and PSCs treated groups ($p < 0:05$) compared with EAE group, indicating the notable effect of treatments on proinflammatory cytokines. The difference between the Cur and PSCs was not statistically significant ($p > 0.05$). These findings demonstrate the anti-inflammatory activities of curcumin and PSCs against EAE-induced neurodegeneration.

Effect on oxidative stress markers

In this study, EAE induced ROS generation and lipid peroxidation, as reflected by elevations in 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 ($p < 0.05$), as presented in Table.

Treatment with both curcumin or PSCs significantly reduced serum MPO activity and MDA levels compared to EAE group ($p < 0.05$), indicating attenuation of lipid peroxidation and neutrophil infiltration indicating attenuation of lipid peroxidation and neutrophil infiltration indicating attenuation of lipid peroxidation and neutrophil infiltration. No significant difference were observed between the curcumin and PSCs treated groups ($p > 0.05$).

Furthermore, EAE rats showed a significant elevation in brain NO level compared to controls (According yo the table). Importantly, both curcumin and *E. granulosus* significantly decreased NO production compared with the EAE group (According yo the table) ($p < 0.05$).

Discussion

Recent advances in helminth-based therapies have introduced innovative strategies for the management of immune-related disorders by exploiting the immunomodulatory properties of parasitic organisms. Several studies have highlighted the beneficial effects of helminths and their secreted products in reducing the severity and 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 their efficacy with that of curcumin in EAE as an animal model of MS. The EAE model has the similar characteristic with MS to some extent. Successful induction of EAE was confirmed, as all immunized animals developed clinical disease of at least grade 2 severity. 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). Importantly, treatment with either curcumin (303.3 ± 7.49 versus 340.3 ± 9.93) or PSCs (291

Table 1.
Effects of Curcumin and E. granulosus PSCs on Biochemical Parameters of EAE affected rats. Values Expressed are Mean ± SD.

Parameters	Animal treatments			EAE + PSCs
	Control	EAE	EAE + Curcumin	
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 ± 0.44^{b}	750 ± 0.44b	656 ± 0.44^{a}
NO (μMOL/L)	785.7 ± 0.44a	1047.42 ± 0.44^{b}	882.6 ± 0.44^{c}	785.7 ± 0.44^{a}
MPO (mU/mL)	1106.22 ± 0.44a	976.8 ± 0.44^{b}	974.04 ± 0.44^{b}	1106.22 ± 0.44^{a}
MDA (N mol/Ml)	1.6 ± 0.2a	12.4 ± 0.1^{b}	5.5 ± 0.3^{b}	7.4 ± 0.1^{a}
SOD (U/L)	1250.45 ± 0.44a	1346.67 ± 0.44^{b}	1364.09 ± 0.44^{b}	1250.45 ± 0.44^{a}

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 ($p < 0.05$).

± 11.46 versus 340.3 ± 9.93) significantly attenuated EAE-associated weight loss ($p < 0.05$). These findings indicate that *E. granulosus* infection may be able to protect rats against EAE.

Excessive production pro-inflammatory cytokines plays a central role in mediators cause remarkable tissue damage. In this study, 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 these cytokines in EAE group as compared with controls ($p < 0.05$). However, their levels decreased markedly in curcumin and PSCs treated groups ($p <$ 0:05) compared with 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. During early disease stages, T cells that recognize myelin, along with peripheral immune cells like macrophages, traverse the compromised blood–brain barrier and infiltrate the central nervous system (CNS). Once inside the CNS, these activated T cells release high levels of inflammatory cytokines including IL-1β, IL-6, and TNF-α, which contribute to myelin damage and axonal degeneration. Among these cytokines, IL-6 plays a critical role by promoting Th17 differentiation in peripheral lymphoid tissues, thereby enhancing disease severity and spinal cord lesions. These Th17 cells drive ongoing neuroinflammation and demyelination, particularly in the experimental autoimmune encephalomyelitis (EAE) model. IL-1β stimulate astrocytes to produce cytokines, chemokines, adhesion molecules, and matrix-degrading enzymes, while, TNF-α sustains 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 attributed to the regulation of key pro-inflammatory cytokines, including IL-12, TNF-α, IL-1β, IL-6, and IFN-γ. In 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 can downregulate IL-6 gene expression in astrocytes [8] and inhibit the differentiation and development of Th1 and Th17 cells in EAE [12].

At the molecular level, curcumin modulates cytokine expression by downregulating pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-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 pathway and modulation of the Janus kinase/signal transducers and activators of tran-

scription (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); while simultaneously reducing 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 been reported to exert immunomodulatory effects on host immune response and has been investigated in the treatment of allergic and autoimmune conditions including allergic airway inflammation, asthma and bacterial sepsis. Previous investigations on murine bacterial sepsis [9] demonstrated that administration of E. granulosus cyst fluid increases 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 findings are consistent 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 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 investigations in EAE models have shown that E. 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, the laminated layer isolated from E. granulosus cyst wall have been reported to inhibit IL-1β, IL-6, and TNF-α production, potentially through modulation of NF-κB signaling cascade and the IRAK pathway [24].

Recent studies have also highlighted the immunoregulatory potential of E. granulosus protoscoleces (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 pathogenic Th1/Th17-driven inflammation characteristic of MS. In addition, PSC may influence oxidative stress pathways by reducing ROS production and enhancing antioxidant defenses. Although the precise molecular mechanisms remain incompletely defined, 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 regulates antioxidant response elements (AREs) [25].

Oxidative stress is a critical contributor to both the initiation and progression of EAE and MS. Immune cells infiltration into affected tissues triggers excessive ROS production, 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 the present 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

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. MPO is produced not only by neutrophils but also by activated microglia, astrocytes, and neurons, may contribute to the development of neuropathological changes. Moreover, MPO activity and neutrophil accumulation have been implicated in blood–brain barrier disruption and neuroinflammation [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 these findings, the present study showed a significant increase MPO and MDA levels 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 ($p > 0.05$).

It is well established that EAE is associated with elevated levels of inducible nitric oxide synthase (iNOS) and enhanced NO production. 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, DNA damage, and neuronal cell death [29]. In this study, brain NO levels were significantly elevated in EAE model and MS patients. Importantly, curcumin and E. granulosus significantly decreased the brain NO production compared with EAE group ($p < 0.05$).

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].

Similarly, E. granulosus cyst fluid has been reported to suppress iNOS expression in the liver, lungs, and kidneys of septic mice [9]. Consistent with our results, E. granulosus infection has been shown to alleviate clinical manifestations in a dextran sulfate sodium-induced colitis model, accompanied by reduced production of 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 NO in oxidative stress pathways, the observed decline in MPO activity and MDA concentrations may reflect reduced ROS generation, likely mediated by the immunomodulatory properties of E. granulosus.

Conclusion

In conclusion, the present findings indicate that administration of 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.

Limitations

This study has several limitations. First, therapeutic efficacy was primarily assessed using clinical scoring, without incorporating detailed histopathological analyses of neural tissues to evaluate demyelination or axonal damage. Second, the lack of immunohistochemical and molecular evaluations limited mechanistic insights into cytokine signaling and oxidative stress pathways. Finally, long-term effects and potential side effects of curcumin and PSC treatments were not evaluated.

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

Materials & Methods

Declaration of Generative AI and AI-assisted technologies in the writing process

In the process of writing this article, no AI was used.

Ethical Approval

All animal experiments were approved by the Ethics Committee of the University of Tabriz (Ethical code number: IR.TABRIZU.REC.1398.004) and conducted in strict accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals.

Experimental animals

A total of 40 healthy adult male Sprague-Dawley rats (weighing 250~300 g), were obtained from the Pasteur Institute (Tehran, Iran). Animals were housed in laboratory cages (10 rats 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±2°C. The efforts were made to minimize animal 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: Received normal saline orally once daily until the end of the study)
2. EAE group: Rats with EAE that received no treatment (positive control)
3. EAE+ curcumin group: EAE induced rats received curcumin at a dose of 100 mg/kg via oral gavage, starting from the onset of clinical symptoms and continuing for 60 days.
4. EAE + PSC group: EAE induced rats were intraperitoneally inoculated with PSCs of E. granulosus at the onset of the first clinical signs of disease.

Induction of EAE as an experimental model of MS

The procedure was conducted based on previous study protocol [12]. According to the 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. 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 region, followed by an additional 100 µL injection into the footpad using a 25-gauge needle. After 48 hours, 1 ×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 monitored daily by assessing motor deficits in each rat. Clinical scores and body weight measurements were recorded from day 0 to day 12 post-induction according to established evaluation scales[13]. Successful induction of EAE was defined as the attainment of a clinical score exceeds 2.

Preparation and Viability Assessment of PSCs:

Fresh hydatid cysts were obtained from the livers of naturally infected sheep slaughtered at the Tabriz industrial abattoir (East Azerbaijan province, Iran). These samples were transferred to the Parasitology Laboratory, 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 PSCs concentration, 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 mean count was used to calculate 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. Viability was calculated as the percentage of unstained PSCs relative to total count. This assessment was also conducted in triplicate [15].

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

Induction of E. granulosus infection

Experimental infection with E. granulosus was performed according to a previously published protocol [11]. Following PSCs preparation and viability confirmation, 500 PSCs were injected intraperitoneally to each rat.

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 vortexed thoroughly to ensure proper dispersion and gently warmed at 37°C to enhance solubility. The final concentration was adjusted to deliver a dose of 100 mg/kg body weight. Fresh suspensions were prepared daily under aseptic conditions, maintained at room temperature, and protected from direct light. Prior to administration, the suspension was mixed thoroughly to ensure uniformity and administered orally using a sterile gavage needle.

Tissue preparation and biochemical analysis

At the end of the study, body weights were recorded, and rats were euthanized under ether anesthesia. Spleen tissues were harvested for cytokine analysis. Spleen cells were stimulated, and levels of TNF-α, IL-1β, IL-6 were quantified using Commercially ELISA kits (Bender Med Co., Austria) according to the manufacturer's described protocol [16]. Cytokine concentrations were expressed as 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 MPO were measured using a colorimetric activity assay kit (BioVision, USA), following the manufacturer's protocol. Additionally, the activity of SOD in the serum was assessed using an established method as previously described. [17]. The preparation of brain tissue and measurement of NO levels was performed following previously described protocol [18], and NO levels were expressed as µmol/L.

Statistical analysis

All data are presented as mean ± standard deviation (SD). Statistical comparisons among experimental groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. A p-value less than 0.05 was considered statistically significant. All analyses were carried out using Stata software version 14 (StataCorp, Texas, USA).

Authors' Contributions

Y.J. conceived and planned the experiments. Y.J., A.IB. SJ.E. carried out the experiments. Y.J. planned and carried out the simulations. Y.J., A.IB. contributed to sample preparation. Y.J., SJ.E., A.IB., S.A. contributed to the interpretation of the results. S.A. took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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