Research & Reviews: Journal of Microbiology and Biotechnology e-ISSN: 2320-3528

Investigating the Effect of Quercetin on Disease Progression and Recovery Process in Experimental Autoimmune Encephalomyelitis (EAE's) Rats

Ali Ebrahimi, Kazem Parivar*, Nasim Hayati-e Roodbari, Akram Eidi

Department of Biology, Faculty of Science, Science and Research Branch, Islamic Azad University, Tehran, Iran

Research Article

Received: 20-Apr-2023, Manuscript No. JMB-23-96618;
Editor assigned: 24-Apr-2023, PreQC No. JMB-23-96618(PQ);
Published: 22-May-2023, DOI: 10.4172/2320-3528.12.2.004.
***For Correspondence:**
Kazem Parivar, Department of Biology, Islamic Azad University, Tehran, Iran
E-mail: k.parivar1941@gmail.com
Citation: Parivar K, et al. Investigating the Effect of Quercetin on Disease Progression and Recovery Process in Experimental Autoimmune Encephalomyelitis (EAE's) Rats. RRJ Microbiol Biotechnol. 2023;12:004.

ABSTRACT

Background: It is now known that quercetin, a plant flavonoid, exerts numerous biological roles, such as anti-inflammatory, anti-cancer, antioxidant activity, and it also promotes the differentiation of neuronal precursor cells. Hence, in the present study, we aimed to investigate whether the administration of quercetin is able to impede the progression of Experimental Autoimmune Encephalomyelitis (EAE) in rats and incite neuronal repair in damaged areas. In order to induce EAE, an equal ratio of the spinal cord of guinea pig (as homogenate) and complete freunds adjuvant (CFA) was prepared. Then, 30 rats were assigned to three groups of ES (induced by EAE and treated with normal saline), EQI (induced by EAE and treated with Injectable quercetin), and EQO (induced by EAE and treated with quercetin orally) groups. Treatment started on the day after the onset of the disease and continued until the 21st day. The weight and clinical symptoms of rats were monitored daily, and the obtained data were statistically analysed.

Results: The results showed that quercetin had a positive effect on disease progression and enhanced recovery. In addition, the injectable form of quercetin was more effective than the oral one.

Conclusion: Based on the previous findings about the anti-oxidative and regenerative effects (proliferation and differentiation) of quercetin, this finding was confirmed. In addition, the effects of injectable quercetin are significant.

Keywords: Quercetin; Multiple sclerosis; Recovery; Regenerative; Disease; Flavonoid

INTRODUCTION

Quercetin is a common flavonoid found in various plant foods, such as onion, apple, and green leafy vegetables. Studies have shown that quercetin possesses various physiological properties, including anti-oxidative and anti-

inflammatory activity [1,2]. For instance, oral quercetin effectively decreases carbon tetrachloride-induced liver injury in mice and inhibits the depletion of glutathione peroxidase and superoxide dismutase enzymes [1]. Quercetin is known to inhibit the *in-vitro* proliferation of autoreactive T cells [3] and the expression of a proinflammatory cytokine named tumor necrosis alpha (TNF-α) [4] in human peripheral blood mononuclear cells. Quercetin is also a major member of the flavonoid family, which may be useful in the treatment of Multiple Sclerosis (MS) [5].

Multiple Sclerosis (MS) is a chronic inflammatory disease in which neuronal cells in the Central Nervous System (CNS) are targeted and destroyed by an autoimmune process, causing neurological and physiological deficits [6]. The most significant symptoms of this disease include motor paralysis, sensory impairment, especially visual impairment and cognitive defects [7].

Jerome, et al. indicated that flavonoids might be able to limit the demyelination process during Multiple Sclerosis. They showed that myelin uptake by macrophages is almost inhibited in response to flavonoids, such as quercetin. Ming, et al. demonstrated that quercetin promotes neuronal growth through enhancing intracellular cAMP levels and GAP-43 expression [8-13].

Objective

Regarding the beneficial effects of quercetin in biological systems, such as anti-oxidative effects [8], we hypothesized that the administration of quercetin to rats induced by EAE could halt the disease progression and even promote neuronal repair in paralyzed rats, thereby reducing oxidative stress.

MATERIALS AND METHODS

Treatment solution

Quercetin, a yellow powder with purity higher than 98% (Sigma Aldrich), was dissolved in ethanol and prepared at a concentration of 0.1 M as a storage compound. In order to prepare the required concentrations, it was diluent with saline solution.

Animal handling

In this experiment, 40 female lewis rats with the mean weight of 200 gr were purchased from darupakhsh company and kept in the animal room of the Islamic Azad University, science and research branch, under standard conditions of temperature, light, and nutrition. Also, in order to isolate the spinal cords of guinea pigs, four guinea pigs were procured from the centre for animal resources and research of the pasteur institute, Iran.

Induction of EAE

In this study, the induction of EAE was performed based on a method established by schneider. In this method, a homogenate containing an equal ratio of the freshly extracted spinal cord samples and complete freunds adjuvant was injected into lewis rats.

Preparation of samples

The isolated spinal cord specimens were transferred to the phosphate buffer solution to prepare the emulsion. In order to prepare a homogeneous and injectable emulsion, the spinal cord was first washed several times with saline and then transferred to a nitrogen tank for 15 minutes. The frozen spinal cord was cut into smaller pieces by sterile scissors and phosphate saline and was completely ground in a sterile porcelain mortar inside an icebox. The ground spinal cord was weighed and combined with concentrated adjuvant at a ratio of 1:1. In order to homogenize

the mixture, it was transferred into a 20 ml syringe connected to another syringe using a tube. The mixture was moved several times between the two connected syringes.

Induction and treatment

In this experiment, 10 specimens were examined to confirm the EAE induction. For this purpose, 0.2 ml of the prepared emulsion was intraperitoneally injected into lewis rats. After the emergence of EAE symptoms, the animals were divided into three groups of 10 rats, namely the ES group, which was induced and treated with normal saline, the EQ group, which was induced and treated with injectable quercetin, and the EO group induced and treated with oral quercetin. The injection time was considered day 0, and treatment started from the next day. All groups were weighed before the injection, and the measurement was recorded daily. In addition, the behavioural and physiological changes, as well as disease severity, were recorded as displayed in Tables 1A and 1B. The disease process took up to 21 days.

Table 1A. Weight changes during the induction process in groups from 1 to 10.

Groups	Days									
	1	2	3	4	5	6	7	8	9	10
EOQ	185	185	186	187	189	192	192	190	190	189
ES	194	194	195	195	197	198	198	200	200	200
EIQ	201	202	203	205	205	205	204	204	200	200

Table 1B. Weight changes during the induction process in groups from 11 to 21.

Groups	Days										
	11	12	13	14	15	16	17	18	19	20	21
EOQ	189	186	186	185	185	184	183	183	183	182	182
ES	197	194	190	187	184	184	180	177	177	177	177
EIQ	198	198	195	195	199	200	200	201	200	200	200

EAE severity

In different studies, various types of methods were used to grade the severity of EAE in animals. In all methods, the degree of EAE in each group on a certain day represents the average severity of EAE in whole specimens. It should be noted that the severity of EAE must be measured at a specific time of day.

Statistical analysis

The analysis of the obtained data was carried out by the SPSS software version 16.0. The obtained values were expressed as the means and standard errors of the means (mean ± SD). The difference between the experimental groups was analysed by one-way Analysis of Variance (ANOVA) followed by tukeys post hoc test. The accepted level of significance was set at $p<0.05$.

RESULTS

Tables 2A and 2B and Figures 1A and 1B show the records of weighting in experimental groups. According to the result, changes in the weight of EOQ and ES groups are very similar. Besides, the alterations in the weight of the EIQ group are different from the previous two groups. These changes can also be statistically visualized in Figure 2.

Table 2A. Changes in disease severity of experimental groups from 1 to 10.

Groups	Days									
	1	2	3	4	5	6	7	8	9	10
EOQ	0	0	0	0	0	0	0	0	0.1	0.2
ES	0	0	0	0	0	0	0	0	0.1	0.1
EIQ	0	0	0	0	0	0	0	0	0	0.1

Table 2B. Changes in disease severity of experimental groups from 11 to 21.

Groups	Days										
	11	12	13	14	15	16	17	18	19	20	21
EOQ	0.4	1	1.4	1.9	2.3	2	2	1.9	1.9	1.5	1.5
ES	0.4	1.5	2.1	2.1	2.1	1.8	1.7	1.7	1.7	1.7	1.7
EIQ	0.4	0.3	0.5	0.7	0.8	0.8	0.7	0.6	0.5	0.5	0.5

Figure 1A. The disease progression in EOQ and EIQ groups. The severity of EAE in the EIQ group was significantly lower than the EOQ shown in linear graph. **Note:** ES; EOQ; EIQ.

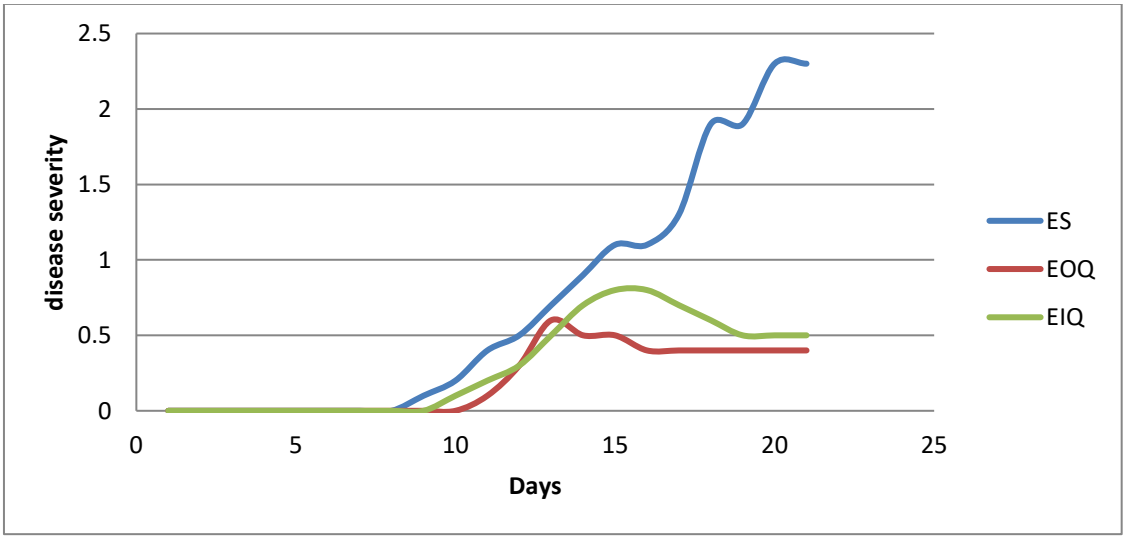


Figure 1B. The disease progression in EOQ and EIQ groups. The severity of EAE in the EIQ group was significantly lower than the EOQ shown in Column diagram. **Note**: *=Statistically significant difference.

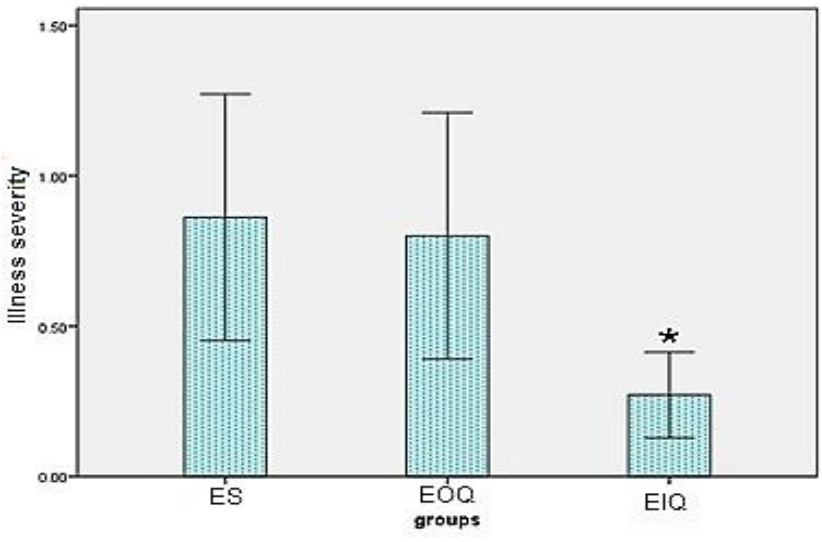
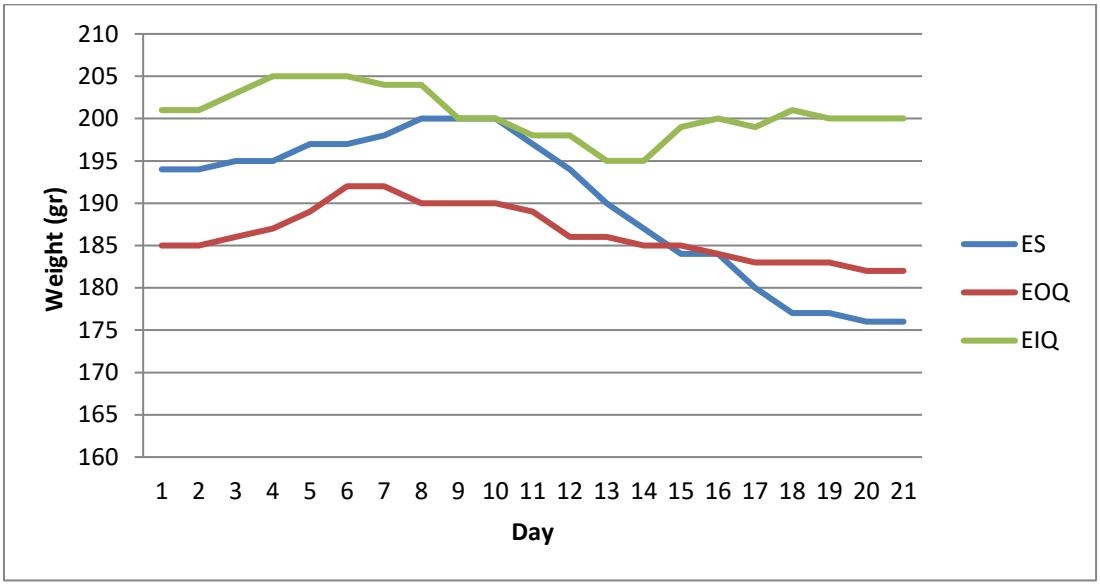


Figure 2. The weight changes during the induction and treatment in experimental groups. Minimum weight change was observed in the EIQ group, and maximum weight change was observed in the ES group. **Note:** ES; EOQ; EIQ.



DISCUSSION

In an *in-vitro* study conducted on peripheral blood mononuclear cells from MS patients, it was shown that the treatment with quercetin reduced their proliferation and modulated the levels of IL-1 beta, matrix metalloproteinase-9, and TNF-α in cell culture supernatants [9]. Voet, et al. suggested that chronic microglial activation can result in the production of inflammatory and neurotoxic mediators, including nitric oxide, iNOS, and ROS, which are all closely associated with the pathogenesis and development of MS [10-16]. Mrvova, et al. indicated that a derivative compound from quercetin named 3-O-(3-chloropivaloyl) quercetin (CPQ) reduced the expression of iNOS in lipopolysaccharide-activated BV-2 microglia and inhibited the activation of NF-kB [15]. In another study conducted on a murine model of EAE, it was demonstrated that the treatment of EAE mice with quercetin inhibited the IL-12-induced activation of JAK2, TYK2, STAT3, and STAT4, as well as Th1 differentiation [10].

It is now known that mast cells are involved in inflammatory processes and allergic responses in which immunologic stimulation causes the production of inflammatory mediators. It has been suggested that mast cells are the immune gate of the brain and are likely associated with neuropathological processes such as MS [11-16]. Quercetin has been associated with a reduction in the release of tryptase and IL-6, as well as the inhibition of histidine decarboxylase mRNA in human mast cells. Kempuraj, et al. studied the histological changes in different parts of the spinal cord in EAE-induced animals and showed the extent of myelin sheath loss in various regions of the spinal cord [14]. They indicated that most pathological changes and loss of myelin sheath occurred in the lumbar region of the spinal cord, as confirmed by our research. Therefore, one of the methods to identify the effects and severity of EAE in induced animals is the assessment of pathological alterations in the spinal cord and brainstem of animals induced by EAE.

Suganthy, et al. investigated the impact of quercetin on the repair of the spinal cord and brain injuries using histological and molecular methods [8]. They presented two hypotheses in this regard:

1. Regarding the ability of quercetin in iron-chelating (Fe^{2+}) and reducing the intensity of inflammatory processes, the administration of this flavonoid significantly improves neurological deficits.
2. The co-administration of quercetin in the acute phase following the injury process is neuroprotective, while no neuroprotective effect was reported in the chronic phase of neurodegeneration.

Finally, they showed that quercetin reduces the formation of edema and cell death (apoptosis) at a certain dose level (5 mg/kg per body weight). Quercetin is also one of the potent iron chelators. This study suggests a high therapeutic potential for the treatment of acute CNS trauma by quercetin.

Muthian, et al. evaluated the role of exercise and physical activity in myelin sheath repair in mice intoxicated by cuprizone [10]. They analyzed the expression of restorative markers and histological alterations in the corpus callosum region of the brain, such as counting the number of oligodendrocytes in control and treatment groups, and concluded that physical activity could be effective in the repair process of the myelin sheath and the number of oligodendrocytes as a preparatory factor in coping with stress [12].

CONCLUSION

Based on the previous findings about the anti-oxidative and regenerative effects (proliferation and differentiation) of quercetin, this finding was confirmed. In addition, the effects of injectable quercetin are significant. A vast number of studies reported that quercetin might be useful as a complementary treatment for the treatment of MS, as our results confirmed this fact. Ming, et al. showed that the oral administration of quercetin at doses of 20 mg/kg and 100 mg/kg per body weight, 3 times per week, failed to enhance the antitumor effect of tumor-specific antigens, while the intraperitoneal administration of quercetin increased its total levels in tumor tissues. They suggested this result is associated with the metabolic conversion of quercetin *in-vivo*. We also observed a similar effect on the ineffectiveness of the oral method.

FUNDING

Not applicable.

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable.

INFORMED CONSENT STATEMENT

Not applicable.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

This research was carried out under the ethics code number IR.IAU.SRB.REC.1397.126 of expert Committee on Ethics in Biomedical Research, Islamic Azad University. All the ethical principles of working with animals, such as the conditions of keeping, the minimum number that can be used, the conditions of anesthesia and painless injections, etc., were observed.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Cui Y, et al. Protective effects of quercetin and quercetin-5',8-disulfonate against carbon tetracholoride-caused oxidative liver injury in mice. Molecules. 2013;19:291-305.
2. Bureau G, et al. Resveratrol and quercetin, two natural polyphenols, reduce apoptotic neuronal cell death induced by neuroinflammation. J Neurosci Res. 2008;86:403-410.
3. Verbeek R, et al. The flavones luteolin and apigenin inhibit *in vitro* antigen-specific proliferation and interferon-gamma production by murine and human autoimmune T cells. Biochem Pharmacol. 2004;68:621-629.
4. Nair MP, et al. The flavonoid quercetin inhibits proinflammatory cytokine (tumor necrosis factor alpha) gene expression in normal peripheral blood mononuclear cells *via* modulation of the NF-kappa beta system. Clin Vaccine Immunol. 2006;13:319-328.
5. Hohlfeld R, et al. Immunological update on multiple sclerosis. Curr Opin Neurol. 2001;14:299-304.
6. Blakemore WF. Regeneration and repair in multiple sclerosis: The view of experimental pathology. J Neurological Sci. 2007;125:1-4.
7. McQualter JL, et al. Multiple sclerosis: A battle between destruction and repair. J Neurochem. 2007;100:295-306.
8. Suganthy N, et al. Bioactive effects of quercetin in the central nervous system: Focusing on the mechanisms of actions. Biomed Pharmacother. 2016;84:892-908.
9. Sternberg Z, et al. Quercetin and Interferon-beta modulate immune response(s) in peripheral blood mononuclear cells isolated from multiple sclerosis patients. J Neuroimmunol. 2008;205:142-147.
10. Muthian G, et al. Quercetin, a flavonoid phytoestrogen, ameliorates experimental allergic encephalomyelitis by blocking IL-12 signaling through JAK-STAT pathway in T lymphocyte. J Clin Immunol. 2004;24:542-552.
11. Ribatti D, et al. Mast cells and angiogenesis in multiple sclerosis. Inflammation Res. 2020;69:1103-1110.
12. Ming MC, et al. Quercetin promotes neurite growth through enhancing intracellular cAMP level and GAP-43 expression. Chin J Nat Med. 2015;13:667-672.
13. Jerome J, et al. Flavonoids inhibit myelin phagocytosis by macrophages; a structure-activity relationship study. Biochem Pharmacol. 2003;65:877-885.
14. Kempuraj D, et al. Inhibitory effect of quercetin on tryptase and interleukin-6 release, and histidine decarboxylase mRNA transcription by human mast cell-1 cell line. Clin Exp Med. 2006;6:150-156.
15. Mrvova N, et al. Modulation of BV-2 microglia functions by novel quercetin pivaloyl ester. Neurochem Int. 2015;90:246-254.
16. Voet S, et al. Microglia in central nervous system inflammation and multiple sclerosis pathology. Trends Mol Med. 2019;25:112-123.