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The Positive Effect of *Atropa belladonna* on Inflammatory Cytokines in the Animal Model of Multiple Sclerosis

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Abstract

Background: Multiple sclerosis (MS) is a chronic autoimmune disease characterized by inflammation and demyelination of the central nervous system. Given the role of inflammation in the pathogenesis of MS and the anti-inflammatory effect of *Atropa belladonna* (AB), the aim of this study was to determine the effect of AB on inflammatory and anti-inflammatory factors in MOG_{35-55} induced experimental autoimmune encephalomyelitis (EAE).

Methods: Thirty-two purebred C57BL/6 mice, weighing (20±2g) were randomly assigned to the 4 groups: control, and three experimental groups: EAE, EAE+AB100, and EAE+AB300 that after EAE induction received 0, 100, and 300 mg/kg AB daily. AB was dissolved in PBS (phosphate-buffered saline) and the volume of gavage in all groups was 100 µL. After 30 days, the mice were weighed, anesthetized with ether and blood was collected directly from the heart. Specific animal ELISA kits measured the inflammatory cytokines (IL-10, IL-17, IL-4, and TNF-α). One-way ANOVA with Duncan post hoc test was used for comparison between groups.

Results: EAE increased serum concentrations of TNF-α, IL-17, and decreased IL-10 and IL-4 compared to the control group. AB significantly decreased the mean level of TNF-α, IL-17 and increased IL-10 and IL-4 compared with EAE group. The effect of 300 mg/kg was clearly better than 100 mg/kg. There was also a significant difference between the control group and the 300 mg/kg group.

Conclusion: In the present study, AB plant extract increased serum levels of anti-inflammatory cytokines and decreased pro-inflammatory cytokines in the MS animal model.

Keywords: *Atropa belladonna*; Experimental; Autoimmune; Encephalomyelitis; Inflammation; Mice; Multiple sclerosis.

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Introduction

Multiple Sclerosis (MS) is a chronic autoimmune disease characterized by inflammation and demyelination of the central nervous system.[1] Despite the importance of this disease and the great efforts, its etiology is still unknown and its pathogenesis is somewhat known. Many studies indicate the key role of the immune system (autoimmune process) in the pathogenesis of it.[2-4] Studies of inflammatory cells and their mediators show that the main cause of inflammation and destruction of neurons is autoimmune T cell activation against myelin basic protein associated with the production of inflammatory cytokines.[5,6] Therefore, MS is an inflammatory disease and inflammation can be seen in various models of it.[6] Immunologically, MS is associated with increased Th17 and Th1 responses.[7] Th17 plays an important role in the development and progression of inflammation by secreting proinflammatory cytokine IL-17. High levels of IL-17 in the cerebral plaques and cerebrospinal fluids (CNFs) of patients with MS indicates the role of this cytokine in the pathogenesis of the MS. The main function of IL-17 in the experimental autoimmune encephalomyelitis (EAE) is to break the blood-brain barrier.[8] Th1 response leads to overproduction of TNF-α and IFN-γ. TNF-α is a proinflammatory factor that is secreted in acute inflammation[9] and facilitates the secretion of pro-inflammatory cytokines such as IL-1b, IL-6 and IL-8.[9] According to studies, TNF-α plays an important role in the immunopathogenesis of MS and its animal model (EAE).[10] The number of TNF-α-secreting cells and its concentration in serum and CSF of patients with MS are higher than healthy individuals.[11]

Interleukin 4 (IL-4) is produced by $CD4^+$Th2 cells and plays a role in the growth and development of B cells. In vitro, IL-4 also blocks Th1 cell activation, which may reduce the production of IL-1 and TNF-α cytokines.

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Evidence suggests that an increase in serum IL-4 levels is associated with a decrease in serum TNF-α, which may be due to the effect of IL-4 on Th1 cells.[12]

Interleukin 10 (IL-10) plays a key role in limiting, terminating inflammatory responses and preventing tissue damage. [13] It has been shown that in patients with MS, increased production of IL-10 by CD4 lymphocytes has been associated with decreased symptom severity of MS. [14]

Atropa belladonna (AB) is a perennial herbaceous plant, native to Europe, North Africa, and Western Asia. It also grows in the north of Iran. [15] The family of potatoes (Solanaceae), including AB, are rich in tropane alkaloids. Other active components of the plant include hyoscine, atropine and scopolamine.[16] Atropine and hyoscine have anticholinergic effects and can affect the CNS, body temperature, eyes, digestive tract and cardiovascular system.[17,18] Atropine, an antagonist of muscarinic receptors, modulates leukocyte infiltration, inhibits antibody response, and prevents T lymphocyte proliferation.[19] The anti-inflammatory and antioxidant effects as well as the analgesic and antidepressant effects of the plant have been proven in various studies.[20] Given the role of inflammation in the pathogenesis of MS and the anti-inflammatory effect of AB, we aimed to determine the effect of AB on inflammatory and anti-inflammatory factors in animal model of MS.

Materials and Methods

Animals

No human participant was involved in this study. All stages of research were conducted under the supervision of Ethics Committee of Jahrom University of Medical Sciences. Moreover, all procedures conducted adhered strictly to the ethical guidelines for use of animals in research. In this experimental study, thirty-two (4 groups of 8) purebred C57BL/6 mice aged 6-8 temperature of 23 ± 1 °C and humidity of 50% to 55% and free access to food and water.

Preparation of Extract

Plant material – *Atropa belladonna* L. (AB) (Solanaceae, code: HBR 52956)[21] was collected in July 2021 from the vicinity of Jahrom. The plant was unequivocally identified by Dr. Amir Barjian, from Islamic Azad University of Jahrom. Herb (leaves) of the plant was dried at room temperature in the dark. A voucher specimen (KO-30301) was deposited in the Herbarium. Preparation of the aqueous extract of AB The water extract was prepared by pouring 10 g of a powder of dried AB leaves into 100 mL of boiling distilled water. The suspension was then left for 10 minutes at room temperature. Consecutively, the extract was filtered (0.2 µm).[22] For the animal study two dose were used: the original solution obtained by extraction (AB100 mg/kg) and a (AB-300 mg/kg).

EAE Induction

After one week for adaptation, to induce EAE, 200 µg of MOG_{35-55} (Sigma-Aldrich) dissolved in 100 µL of phosphate buffer saline was mixed with 100 µL of complete Freund's adjuvant and injected subcutaneously. The disease course was evaluated on the basis of the clinical score test as below and score 2 was considered as EAE:

Zero (no disease), 1 (tail movement disorder), 2 (tail paralysis), 3 (mild walking disorder), 4 (paralysis of hind leg and severe walking disorder), 5 (paralysis of hind leg plus partial paralysis of the lower half of the body), 6 (quadriplegia) and 7 (death from MS).[23]

We evaluated the clinical score in two consecutive stages at first day and thirtieth day of MS course and reported them as first score and thirty score.

Experimental Groups and Blood Sampling

Mice were randomly assigned to the following groups: control, and three experimental groups: EAE, EAE + AB100 and EAE + AB300 that after EAE induction received 0, 100, and 300 mg/kg AB daily. AB was dissolved in PBS (phosphate buffered saline) and volume of gavage in all groups was 100 µL. After 30 days, the mice were weighed, anesthetized with ether and blood was collected directly from the heart. Serum samples were prepared immediately by centrifugation (3000 rpm for 15 minutes) and stored in freezer -20 °C. The inflammatory cytokines (IL-10, IL-17, IL-4, and TNF-α) were measured by specific animal ELISA kits (Diametra, Italy).

Statistical Analysis

Data are shown as mean ± SD and $P < 0.05$ is considered significant. One-way ANOVA with Duncan post hoc test was used for comparison between groups. To check for normality, Kolmogorov test was used and data were analyzed using SPSS software, version 26.

Results

The weight gain in the control group was significantly higher than the other groups and there was no difference among other groups (Figure 1). Based on Figure 2a, b, the pro-inflammatory cytokines of TNF-α (232.01 ± 22.92 pg/mL) and IL-17 (212.96 ± 27.35 pg/mL) were significantly ($P < 0.001$) increased under the influence of MOG_{35-55} compared to the control group (163.99 ± 7.78 pg/mL and 145.93 ± 10.41 pg/mL) respectively. 300 mg/kg of AB significantly reduced ($P < 0.001$) TNF-α (199.14 ± 4.02 pg/mL) and IL-17 (178.36 ± 8.54 pg/mL) levels, although it was still significantly different from the control group.

MOG_{35-55} induced EAE decreased ($P < 0.001$) serum concentrations of IL-10 (79.98 ± 9.45 pg/mL) compared to control (114.78 ± 7.25 pg/mL) (Figure 2c). According to Figure2c, AB significantly increased ($P < 0.001$) the mean level of IL10 compared with EAE group. However,

the effect of 300 mg/kg (99.29 ± 8.99 pg/mL) was clearly better than 100 mg/kg (86.31 ± 11.87 pg/mL).

Group EAE 300AB showed a significant increase ($P < 0.001$) in cytokines IL4 (50.61 ± 8.21 pg/mL) compared to EAE 100AB (39.96 ± 7.01 pg/mL) and EAE groups (35.21 ± 2.08 pg/mL) and no significant difference was observed between them (Figure 2d). There was also a significant difference between the control group (76.57 ± 4.36 pg/mL) and other groups (Table 1).

AB could not significantly change the clinical score between first and thirtieth days of experiment (Table 1).

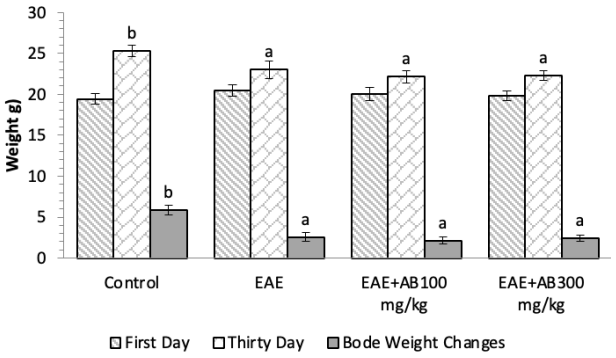

Figure 1. Body Weight Gain (g) in Different Groups 30 Days After EAE Induction. EAE, EAE AB100 and EAE AB300 that after EAE induction received 0, 100, and 300 mg/kg Atropa Belladonna daily. Columns with common letters are not statistically significant in accordance with Duncan test. AB: *Atropa belladonna*, EAE: experimental autoimmune encephalomyelitis

Discussion

In this study, the effect of AB was investigated on EAE mice (induced by MOG_{35-55}). In summary, AB reduced serum concentrations of TNF-α and IL-17 (pre-inflammatory cytokines) and increased serum concentrations of IL-4 and IL-10 (anti-inflammatory cytokines).

It has been shown that increased anti-inflammatory cytokines such as IL-10, IL-4, TGF-β can be helpful in inflammatory diseases such as MS.[24] IL-4 is made by Th2 $CD4^+$ cells and is involved in the growth and development of B cells. The IL-4 acts as a suppressor cytokine in the EAE model, and its mRNA is also expressed during remission.[25] In addition, resistance to EAE induction is related to Th2 cells by secretion of IL-4.[26,27] Some studies have shown the role of IL-4 in reducing the symptoms and severity of the EAE model.[28] Liblau and colleagues showed that disease duration was slightly longer than control groups in IL-4 deficient mice. Therefore, it may play a role in completing the disease process.[29] Based on the results of the present study, AB increased serum IL-4 concentrations, which could be due to its effect on $CD4^+$.

Monocytes, macrophages, B cells, and Th2 cells make up IL-10. Its primary role is to suppress cytokines made by macrophages.[30] One study found that IL-10 deficient mice were more prone to EAE and showed more severe EAE compared to normal and IL-4 deficient mice.[31] In addition, in these IL-10 deficient models, T cells secrete

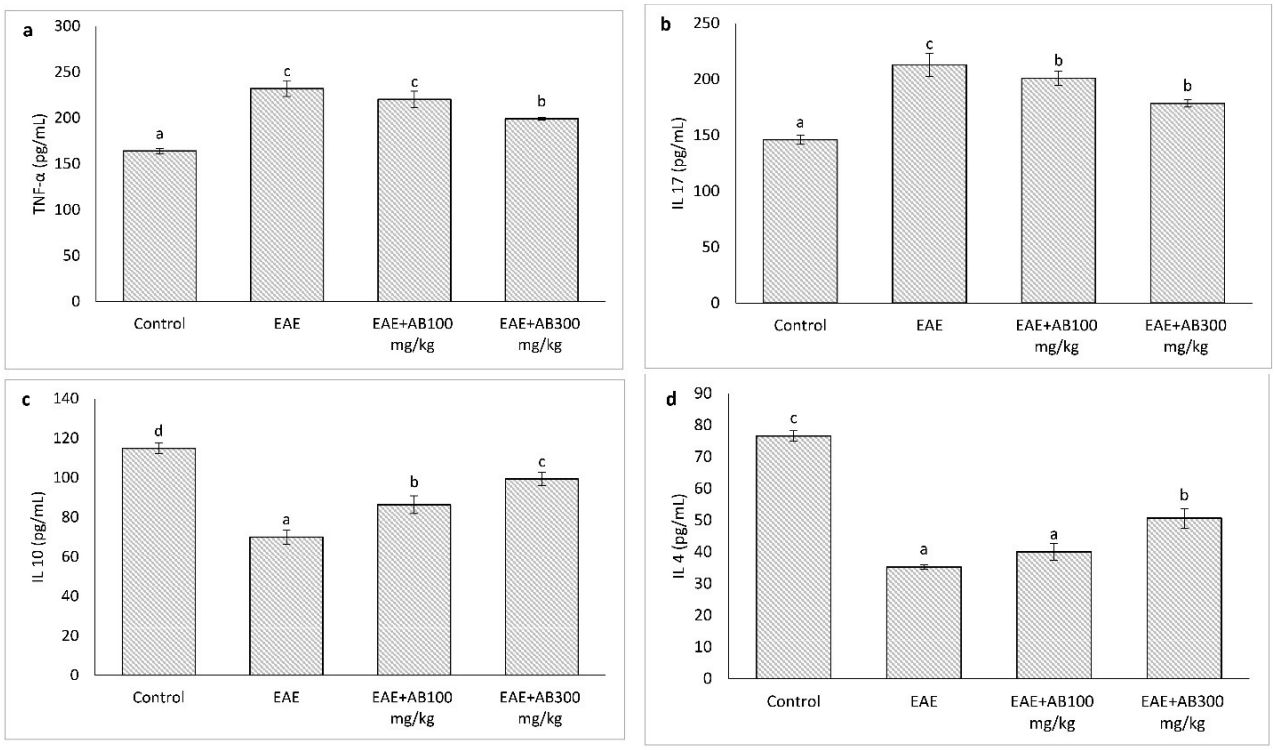

Figure 2. Mean Serum Concentration (pg/mL) of TNF-α (a), IL-17 (b), IL-10 (c) and IL-4 (d) in Different Groups 30 Days After EAE Induction. EAE, EAE AB100 and EAE AB300 that after EAE induction received 0, 100, and 300 mg/kg *Atropa belladonna* daily. Columns with common letters are not statistically significant in accordance with Duncan test. AB: *Atropa belladonna*, EAE: experimental autoimmune encephalomyelitis

Table 1. Multiple Sclerosis Treatment Approaches Using *Atropa belladonna* in Comparison With EAE Model or Control in Accordance With Duncan Test

Parameters	Groups				*P* Value
	Control	EAE	EAE+AB100 mg/kg	EAE+AB300 mg/kg	
First day weight (g)	19.43±1.72	20.42±1.81	20.01±2.16	19.86±1.58	0.785
Thirty day weight (g)	25.29±1.89 b	23.01±2.88 a	22.14±2.08 a	22.29±1.49 a	0.040
Body weight gain (g)	5.86±1.57 b	2.58±1.51 a	2.14±1.07 a	2.43±0.97 a	0.001
TNF-α (pg/mL)	163.99±7.78 a	232.01±22.92 c	220.33±23.35 c	199.14±4.02 b	0.001
IL-17 (pg/mL)	145.93±10.41 a	212.96±27.53 c	200.91±17.39 c	178.36±8.54 b	0.001
IL-10 (pg/mL)	114.78±7.25 d	79.98±9.45 a	86.31±11.87 b	99.29±8.99 c	0.001
IL-4 (pg/mL)	76.57±4.36 c	35.21±2.08 a	39.96±7.01 a	50.61±8.21 b	0.001
First score	0.00±0.00 a	2.57±0.98 b	2.71±0.76 b	2.86±0.69 b	0.001
Thirty score	0.00±0.00 a	2.85±0.70 b	2.62±0.49 b	2.28±0.95 b	0.001

Mean serum concentration of inflammatory parameters in different group 30 days after EAE induction. EAE, EAE AB100 and EAE AB300 that after EAE induction received 0, 100, and 300 mg/kg *Atropa Belladonna* daily. Columns with common letters are not statistically significant in accordance with Duncan test. AB: *Atropa belladonna*, EAE: experimental autoimmune encephalomyelitis.

more inflammatory cytokines (interferon alpha or TNF-α).[31] Therefore, it can be concluded that increasing IL-10 due to AB plays an important role in controlling and reducing symptoms and reducing the secretion of pre-inflammatory cytokines. A decrease in pre-inflammatory factors such as IL-17, 1L-1, TNF-α, MCP-1, NF-kb can be a sign of the recovery process of MS disease.[24]

TNF-α is made by single-stranded phagocytes, natural killer (NK) cells, B cells, and T cells, as well as by astrocytes and microglia in the CNS. The inflammatory role of TNF-α in reducing the symptoms of the disease in EAE models has been demonstrated in several studies.[30,32-34] TNF-α is directly involved in the apoptosis of oligodendrocytes and the demyelination of neurons.[30] In addition, it plays a direct and indirect role in inducing cell death in Oligodendrocyte progenitor cells.[35] Therefore, a decrease in serum concentrations of TNF-α can reduce the progression of the disease, myelin degradation, and apoptosis of oligodendrocytes. In this study, AB reduced the average serum concentration of TNF-α. One of the reasons for the decline in TNF-α is probably the increase in IL-4 under the influence of AB. Because IL-4 can prevent the activation of Th1 cells, which is the maker of IL-4. [12]

IL-17 can increase the expression of pre-inflammatory factors through its effect on a range of cells.[36] IL-17 also plays a major role in the development of EAE models.[37] It seems that reducing the serum concentration of this factor (such as what happened in this study by AB) can play an important role in reducing the activation and production of pre-inflammatory factors, thereby controlling the disease to some extent.

We could not access some effects of AB on cells in CNS, due to deficit of fee and fasciitis, so we suggested to search some points like these: investigation of AB effect on histology of neurons, glial cells, BBB and the rate of AB that can transfer of this barrier.

Conclusion

In the present study, AB plant extract increased serum levels of anti-inflammatory cytokines and decreased pre-inflammatory cytokines in the MS animal model.

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Authors' Contribution

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Competing Interests

The authors declare that they have no conflicts of interest.

Ethical Approval

This procedure was approved by the ethical committee of Jahrom University of Medical Sciences under the number of IR.JUMS.REC.1394.225

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References

1. Lassmann H, van Horssen J. The molecular basis of neurodegeneration in multiple sclerosis. FEBS Lett. 2011;585(23):3715-23. doi: 10.1016/j.febslet.2011.08.004.
2. Panitch HS, Hirsch RL, Schindler J, Johnson KP. Treatment of multiple sclerosis with gamma interferon: exacerbations associated with activation of the immune system. Neurology. 1987;37(7):1097-102. doi: 10.1212/wnl.37.7.1097.
3. Gandhi R, Laroni A, Weiner HL. Role of the innate immune system in the pathogenesis of multiple sclerosis. J Neuroimmunol. 2010;221(1-2):7-14. doi: 10.1016/j.jneuroim.2009.10.015.
4. Steinman L. Multiple sclerosis: a coordinated immunological

attack against myelin in the central nervous system. Cell. 1996;85(3):299-302. doi: 10.1016/s0092-8674(00)81107-1.
5. Fletcher JM, Lalor SJ, Sweeney CM, Tubridy N, Mills KH. T cells in multiple sclerosis and experimental autoimmune encephalomyelitis. Clin Exp Immunol. 2010;162(1):1-11. doi: 10.1111/j.1365-2249.2010.04143.x.
6. Frischer JM, Bramow S, Dal-Bianco A, Lucchinetti CF, Rauschka H, Schmidbauer M, et al. The relation between inflammation and neurodegeneration in multiple sclerosis brains. Brain. 2009;132(Pt 5):1175-89. doi: 10.1093/brain/awp070.
7. Danikowski KM, Jayaraman S, Prabhakar BS. Regulatory T cells in multiple sclerosis and myasthenia gravis. J Neuroinflammation. 2017;14(1):117. doi: 10.1186/s12974-017-0892-8.
8. Jadidi-Niaragh F, Mirshafiey A. Th17 cell, the new player of neuroinflammatory process in multiple sclerosis. Scand J Immunol. 2011;74(1):1-13. doi: 10.1111/j.1365-3083.2011.02536.x.
9. Zandi-Esfahan S, Fazeli M, Shaygannejad V, Hasheminia J, Badihian S, Aghayerashti M, et al. Evaluating the effect of adding fish oil to fingolimod on TNF-α, IL1β, IL6, and IFN-γ in patients with relapsing-remitting multiple sclerosis: a double-blind randomized placebo-controlled trial. Clin Neurol Neurosurg. 2017;163:173-8. doi: 10.1016/j.clineuro.2017.10.004.
10. Lim SY, Constantinescu CS. TNF-α: a paradigm of paradox and complexity in multiple sclerosis and its animal models. Open Autoimmun J. 2010;2(1):160-70.
11. Ozenci V, Kouwenhoven M, Huang YM, Kivisäkk P, Link H. Multiple sclerosis is associated with an imbalance between tumour necrosis factor-alpha (TNF-alpha)- and IL-10-secreting blood cells that is corrected by interferon-beta (IFN-beta) treatment. Clin Exp Immunol. 2000;120(1):147-53. doi: 10.1046/j.1365-2249.2000.01175.x.
12. Imitola J, Chitnis T, Khoury SJ. Cytokines in multiple sclerosis: from bench to bedside. Pharmacol Ther. 2005;106(2):163-77. doi: 10.1016/j.pharmthera.2004.11.007.
13. Saraiva M, O'Garra A. The regulation of IL-10 production by immune cells. Nat Rev Immunol. 2010;10(3):170-81. doi: 10.1038/nri2711.
14. Perrella O, Sbreglia C, Perrella M, Spetrini G, Gorga F, Pezzella M, et al. Interleukin-10 and tumor necrosis factor-alpha: model of immunomodulation in multiple sclerosis. Neurol Res. 2006;28(2):193-5. doi: 10.1179/016164105x39879.
15. Sedaghat S, Hajiaghaee R, Taghizad Farid R, Kadkhoda Z, Ghasemi SV, Naghdi Badi HA, et al. Identification and determination of different alkaloids from *Atropa belladonna* L. by gas chromatography method. J Med Herb. 2011;2(3):203-10. [Persian].
16. Sarker SD, Nahar L. An introduction to natural products isolation. Methods Mol Biol. 2012;864:1-25. doi: 10.1007/978-1-61779-624-1_1.
17. Rajput H. Effects of *Atropa belladonna* as an anti-cholinergic. Nat Prod Chem Res. 2013;1(1):104. doi: 10.4172/npcr.1000104.
18. Miraldi E, Masti A, Ferri S, Barni Comparini I. Distribution of hyoscyamine and scopolamine in *Datura stramonium*. Fitoterapia. 2001;72(6):644-8. doi: 10.1016/s0367-326x(01)00291-x.
19. Razani-Boroujerdi S, Behl M, Hahn FF, Pena-Philippides JC, Hutt J, Sopori ML. Role of muscarinic receptors in the regulation of immune and inflammatory responses. J Neuroimmunol. 2008;194(1-2):83-8. doi: 10.1016/j.jneuroim.2007.11.019.
20. Owais F, Anwar S, Saeed F, Muhammad S, Ishtiaque S, Mohiuddin O. Analgesic, anti-inflammatory and neuropharmacological effects of *Atropa belladonna*. Pak J Pharm Sci. 2014;27(6):2183-7.
21. Ferreira RA, Silva CK, Lucinda-Silva RM, Branco JO. Leaf morphoanatomy of *Solanum capsicoides* All. (Solanaceae) from Restinga area. Lat Am J Pharm. 2013;32(2):287-91.
22. Gál P, Vasilenko T, Kováč I, Kostelníková M, Jakubčo J, Szabo P, et al. *Atropa belladonna* L. water extract: modulator of extracellular matrix formation in vitro and in vivo. Physiol Res. 2012;61(3):241-50. doi: 10.33549/physiolres.932223.
23. Skundric DS, Zakarian V, Dai R, Lisak RP, Tse HY, James J. Distinct immune regulation of the response to H-2b restricted epitope of MOG causes relapsing-remitting EAE in H-2b/s mice. J Neuroimmunol. 2003;136(1-2):34-45. doi: 10.1016/s0165-5728(03)00005-5.
24. Hauser SL, Oksenberg JR. The neurobiology of multiple sclerosis: genes, inflammation, and neurodegeneration. Neuron. 2006;52(1):61-76. doi: 10.1016/j.neuron.2006.09.011.
25. Begolka WS, Vanderlugt CL, Rahbe SM, Miller SD. Differential expression of inflammatory cytokines parallels progression of central nervous system pathology in two clinically distinct models of multiple sclerosis. J Immunol. 1998;161(8):4437-46.
26. Karpus WJ, Gould KE, Swanborg RH. CD4+suppressor cells of autoimmune encephalomyelitis respond to T cell receptor-associated determinants on effector cells by interleukin-4 secretion. Eur J Immunol. 1992;22(7):1757-63. doi: 10.1002/eji.1830220714.
27. Cua DJ, Hinton DR, Stohlman SA. Self-antigen-induced Th2 responses in experimental allergic encephalomyelitis (EAE)-resistant mice. Th2-mediated suppression of autoimmune disease. J Immunol. 1995;155(8):4052-9.
28. Shaw MK, Lorens JB, Dhawan A, DalCanto R, Tse HY, Tran AB, et al. Local delivery of interleukin 4 by retrovirus-transduced T lymphocytes ameliorates experimental autoimmune encephalomyelitis. J Exp Med. 1997;185(9):1711-4. doi: 10.1084/jem.185.9.1711.
29. Liblau R, Steinman L, Brocke S. Experimental autoimmune encephalomyelitis in IL-4-deficient mice. Int Immunol. 1997;9(5):799-803. doi: 10.1093/intimm/9.5.799.
30. Begolka WS, Miller SD. Cytokines as intrinsic and exogenous regulators of pathogenesis in experimental autoimmune encephalomyelitis. Res Immunol. 1998;149(9):771-81. doi: 10.1016/s0923-2494(99)80004-2.
31. Bettelli E, Das MP, Howard ED, Weiner HL, Sobel RA, Kuchroo VK. IL-10 is critical in the regulation of autoimmune encephalomyelitis as demonstrated by studies of IL-10- and IL-4-deficient and transgenic mice. J Immunol. 1998;161(7):3299-306.
32. Issazadeh S, Ljungdahl A, Höjeberg B, Mustafa M, Olsson T. Cytokine production in the central nervous system of Lewis rats with experimental autoimmune encephalomyelitis: dynamics of mRNA expression for interleukin-10, interleukin-12, cytolysin, tumor necrosis factor alpha and tumor necrosis factor beta. J Neuroimmunol. 1995;61(2):205-12. doi: 10.1016/0165-5728(95)00100-g.
33. Issazadeh S, Lorentzen JC, Mustafa MI, Höjeberg B, Müssener A, Olsson T. Cytokines in relapsing experimental autoimmune encephalomyelitis in DA rats: persistent mRNA expression of proinflammatory cytokines and absent expression of interleukin-10 and transforming growth factor-beta. J Neuroimmunol. 1996;69(1-2):103-15. doi: 10.1016/0165-5728(96)00076-8.

34. Issazadeh S, Navikas V, Schaub M, Sayegh M, Khoury S. Kinetics of expression of costimulatory molecules and their ligands in murine relapsing experimental autoimmune encephalomyelitis in vivo. J Immunol. 1998;161(3):1104-12.
35. Arnett HA, Mason J, Marino M, Suzuki K, Matsushima GK, Ting JP. TNF alpha promotes proliferation of oligodendrocyte progenitors and remyelination. Nat Neurosci. 2001;4(11):1116-22. doi: 10.1038/nn738.
36. Moseley TA, Haudenschild DR, Rose L, Reddi AH. Interleukin-17 family and IL-17 receptors. Cytokine Growth Factor Rev. 2003;14(2):155-74. doi: 10.1016/s1359-6101(03)00002-9.
37. Komiyama Y, Nakae S, Matsuki T, Nambu A, Ishigame H, Kakuta S, et al. IL-17 plays an important role in the development of experimental autoimmune encephalomyelitis. J Immunol. 2006;177(1):566-73. doi: 10.4049/jimmunol.177.1.566.