

RESEARCH ARTICLE

Therapeutic approach by Aloe vera in experimental model of multiple sclerosis

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Abstract

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) that leads to an inflammatory demyelination, axonal damage, and progressive neurologic disability that affects ~2.5 million people worldwide. The aim of the present research was to test the therapeutic effect of Aloe vera in experimental model of MS. All experiments were conducted on C57BL/6 male mice aged 6–8 weeks. To induce the experimental autoimmune encephalomyelitis (EAE), 250 µg of the myelin oligodendrocyte glycoprotein 35–55 peptide emulsified in complete Freund's adjuvant was injected subcutaneously on day 0 over two flank areas. In addition, 200 ng of pertussis toxin in 100 µL phosphate buffered saline was injected intraperitoneally on days 0 and 2. The therapeutic protocol was carried out intragastrically using 120 mg/kg/day Aloe vera from 7 days before to 21 days after EAE induction. The mice were killed 21 days after EAE induction. The brains of mice were removed for histological analysis and their isolated splenocytes were cultured. The results indicated that treatment with Aloe vera caused a significant reduction in severity of the disease in experimental model of MS. Histological analysis showed 3 ± 2 plaques in Aloe vera-treated mice compared with 5 ± 1 plaques in control group. The density of mononuclear infiltration in the CNS of Aloe vera-treated mice (500 ± 200) was significantly less in comparison to 700 ± 185 cells in control group. Moreover, the serum level of nitric oxide in treatment group was significantly less than control animals. The level of interferon- γ in cell culture supernatant of treated mice splenocytes was lower than control group, whereas decrease in serum level of interleukin-10 in treatment group was not significant in comparison with control mice. These data indicate that Aloe vera therapy can attenuate the disease progression in experimental model of MS.

Keywords: Aloe vera; EAE; IFN- γ ; MS therapy; multiple sclerosis

Introduction

Multiple sclerosis (MS) is a chronic, immune-mediated demyelinating disease of the central nervous system (CNS) with unknown etiology which affects approximately two million people worldwide.^(1,2) The pathogenesis of the disease is characterized by the activation and infiltration of mononuclear cells, predominantly antigen-specific CD4⁺ and CD8⁺ T cells and B cells, in the CNS, reactivation by resident antigen presenting microglial cells, secretion of proinflammatory cytokines/chemokines along with generation of other inflammatory mediators, such as complement components, highly

reactive free radicals such as reactive oxygen and reactive nitrogen species (RONS) resulting in the demyelination of axons.⁽³⁾

Experimental autoimmune encephalomyelitis (EAE) is currently the most commonly used animal model for the study of MS. This model causes inflammation and demyelination that is similar to the disease manifestation seen in humans.⁽¹⁾ EAE is a T cell-mediated autoimmune disease of the CNS, in which the myelin oligodendrocyte glycoprotein (MOG) is an autoantigen recognized by autoreactive T cells. Migration of encephalitogenic T cells to the CNS plays an essential role in the development of EAE.⁽⁴⁾ The Th1 effector cell subset responsible

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for production of proinflammatory cytokines such as interferon- γ (IFN- γ) has traditionally been implicated in the pathogenesis of certain autoimmune diseases including EAE.⁽⁵⁾

The EAE is thought to be a T cell-mediated autoimmune disease, so that, both Th1 and Th17 cells were thought to be responsible for the inflammatory demyelination in MS and EAE. Th2 and Foxp3 regulatory T cells and related cytokines such as interleukin-5 (IL-5) and IL-10 have been shown to be important in the resolution stages of the disease.⁽⁶⁾ In addition, oxidative stress could also contribute in pathogenesis of a wide number of diseases including rheumatoid arthritis and some neurological disorders via the overproduction of ROS such as hydrogen peroxide, superoxide, and peroxynitrite.⁽⁷⁾

Aloe vera is a perennial plant belonging to the family of Liliaceae, which includes about 360 species.⁽⁸⁾ Aloe vera is a stemless, drought-resisting succulent. It is indigenous to hot countries and has been used medicinally for over 5000 years by Egyptian, Indian, Chinese, and European cultures. Aloe vera gel is the mucilaginous aqueous extract of the leaf pulp of Aloe barbadensis Miller. It contains over 70 biologically active compounds and has been claimed to have anti-inflammatory, antioxidant, immune boosting, anticancer, antiageing and antidiabetic properties.⁽⁹⁾ Aloe plants have been used medicinally for centuries. Among them, Aloe barbadensis, commonly called Aloe vera, is one of the most widely used healing plants in the history of mankind.⁽¹⁰⁾ Aloe vera is one of the few substances known to effectively decrease inflammation and promote wound healing.^(11,12) Aloe vera gel has been widely promoted and used by patients for the treatment of a range of inflammatory digestive and skin diseases, including inflammatory bowel disease.⁽¹³⁾ Gel is derived from the pulp of the leaves and contains carbohydrate polymers such as glucomannanes or pectic acid.⁽¹⁴⁾ Because, the polysaccharides have diverse immunomodulatory activities *in vivo* as well as *in vitro*.⁽¹⁵⁾ Collectively, Aloe vera is a modulator of cellular and humoral immunity.⁽¹⁶⁾

Material and methods

Mice

C57BL/6 male mice, 6–8 weeks of age were obtained from the Pasture Institute of Iran. Mice were randomly separated into three groups; normal, control and treatment, with eight mice in each group. All mice were housed in cages in groups of four, under 12-h light–dark cycle and free access to food and water. All procedures involving animals were performed according to the guidelines of the Animal Ethic approved by Tehran University of Medical Science.

Preparation of Aloe vera gel extract

Aloe vera powder was prepared from Aloe vera leaf gel according to the published procedure⁽¹⁷⁾ with slight modifications. Mature, healthy and fresh leaves of Aloe vera having a length of ~75–90 cm were washed with fresh water. The leaves were cut transversely into pieces. The thick epidermis was selectively removed. The solid gel in the center of the leaf was homogenized. The prepared mucilaginous, thick and straw colored homogenate was lyophilized. The lyophilized sample was then extracted using 95% ethanol. The filtrate was collected and evaporated to dryness under reduced pressure in a rotary evaporator. The residue was stored in dry sterilized small containers at 4°C for future use. An aqueous suspension which is the form customarily used in folk medicine was prepared by dissolving suitable amount of ethanol free extract of Aloe vera leaf gel to get the desired concentration. The drug solutions were prepared freshly and administered intragastrically once per day, based on the designed program.⁽¹⁷⁾

EAE induction and therapeutic protocol

Initially, anesthesia was done by ether and each mouse then received a subcutaneous injection of 250 μ g synthetic MOG 35–55 (MEVGWYRSPFSRVVHLYRNGK; Diapharm Ltd, Russia) in 100 μ L phosphate buffered saline (PBS) mixed with 100 μ L of complete Freund's adjuvant (Sigma-Aldrich, St. Louis, MO). A 100 μ L volume was injected subcutaneously over two flank areas. Mice were also intraperitoneally injected with 250 ng of pertussis toxin (Sigma-Aldrich) in 400 μ L of PBS. A second, identical injection of pertussis toxin was given after 48 h.^(1,6)

Aloe vera was administered intragastrically at dose 120 mg/kg/day.^(18,19) Control animals received PBS. Daily treatment was carried out from 7 days before EAE induction to day 21 postinduction. Clinical assessment was performed daily from day 9 postimmunization until day 21. Mice were monitored daily and assessed by clinical score. The clinical assessment was scored as follows: 0, no clinical sign; 1, partial loss of tail tonicity; 2, complete loss of tail tonicity; 3, flaccid tail and abnormal gait; 4, hind leg paralysis; 5, hind leg paralysis with hind body paresis; 6, hind and foreleg paralysis; 7, moribund or death.⁽²⁰⁾

Histology assessment

To evaluate histopathology, mice were killed after 21 days following anaesthetization by perfusion ketamine & Zylasin through the heart. Brains were dissected out and fixed with neutral 10% formalin overnight and routinely embedded in paraffin wax. Brain sections (8 μ m thick) were stained with Hematoxylin and Eosin (H&E) that used to highlight areas of inflammation by darkly staining the

nuclei of mononuclear cells. Immune cells stained with H&E were counted in a blinded manner under a light microscope using a $\times 10$ magnification with 12 sections examined per animal. Each section was counted manually before being combined to give a total for the animal.⁽²¹⁾

Nitrite assay

Nitric oxide (NO) was assayed by measuring nitrite as the end product of reaction, which was determined by a colorimeter assay based on the Griess reaction. Griess reagent was prepared by solving 1g sulfanilamide in 100 mL phosphoric acid 5% mixed with 0.1g naphthyl ethylene diamine HCl in 100 mL distilled water. Serum sample (100 μ L) was mixed with 100 μ L of Griess reagent at room temperature for 10 min. Absorbance was measured using microplate reader at 550 nm. Concentration of nitrite was determined by standard curve of sodium nitrite prepared in distilled water.⁽²²⁾

IFN- γ and IL-10 evaluation

Single cell suspensions were obtained from spleens 21 days postimmunization. Splenocytes isolated from mice were treated with lysis buffer to remove red blood cells. Cells were resuspended in PBS and a cell count was performed. 2×10^6 cells/well were cultured in 24-well culture plates (Greiner Bio-One, Germany) in Roswell Park Memorial Institute medium supplemented with 10% FCS, 2 mM L-glutamine, 50 μ g/mL gentamicin-penicillin (Sigma), in the presence of 10 μ g/mL MOG 35-55 peptide. Cultures were incubated for 72 h in 5% CO₂ at 37°C. To assay cytokine, cells culture supernatants were collected after stimulation with antigen. IFN- γ and IL-10 have been quantified by two-site sandwich enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (Bender Med. Systems, Austria) on cell culture supernatants. Absorbance was read at 450 nm using microplate ELISA reader (Stat Fax 2100, Awareness US). The concentration of cytokine was estimated from a standard curve generated with each run.

Statistics

Statistical analyses were performed by Mann-Whitney nonparametric test and the Student's *t*-test for unpaired data. Results were reported as mean \pm SD; $P < 0.05$ were considered to be statistically significant.

Results

Clinical findings

All immunized mice with MOG 35-55 developed EAE. The clinical course and severity of the disease differed

consistently between treatment and control groups (Figure 1). The mean severity score of disease was higher in control mice (4.87 ± 0.83) than in treated animals (2 ± 1.41), ($P < 0.05$). These effects were led to significant clinical improvement and delayed disease progression during 21 days of observation, indicating that Aloe vera can inhibit the progression of EAE.

Histology findings

We examined whether it could be a correlation between the clinical symptoms of EAE with histopathology of CNS in control and treated mice. Histology analysis showed 3 ± 2 plaques in group of Aloe vera-treated mice whereas 5 ± 1 plaques (in each section) were detected in control group as shown in Figure 2 ($P < 0.001$). The density of mononuclear infiltration in the CNS of Aloe vera-treated mice was 500 ± 200 whereas it was 700 ± 185 ($P < 0.001$), in the control group, as shown in Figure 2. The mice treated with Aloe vera displayed a less inflammation in the CNS compared with control animals. These results indicate that the histopathology of CNS correlates with the clinical severity of EAE in treated and control mice.

NO production findings

Griess reaction was performed on serum sample. As shown in Figure 3, NO production was significantly reduced in treated mice ($8/07 \pm 2/02$ μ M, $P < 0.05$), compared to control mice (11.14 ± 2.8 μ M). In the normal group NO production was 4.7 ± 1.25 μ M.

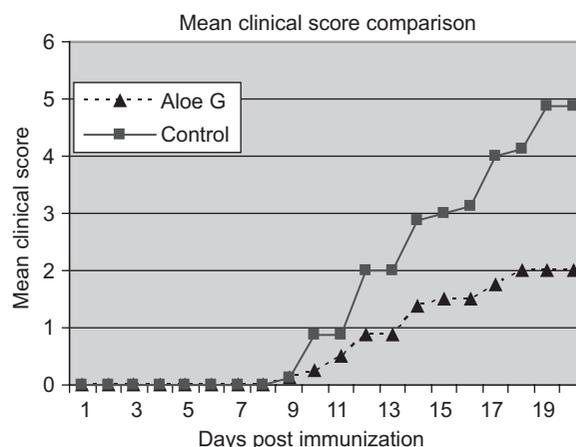


Figure 1. Mean clinical scores of experimental autoimmune encephalomyelitis (EAE) induced by myelin oligodendrocyte glycoprotein (MOG) 35-55 in control mice and in mice treated with Aloe vera. All mice were immunized with 250 μ g MOG 35-55 emulsified in complete freund's adjuvant and screened every other day for the presence of clinical signs of EAE.

IFN- γ and IL-10 assessment

Splenocytes were obtained from mice and cultured for 72 h in the presence of MOG 35–55. Treatment group exhibited significantly lower levels of IFN- γ production in comparison with patient group (Figure 4, 313 ± 187 $\mu\text{g}/\text{mL}$ and 1492 ± 443 $\mu\text{g}/\text{mL}$, respectively, $P < 0.001$), whereas the level of IL-10 production in treatment group had not significantly changed in comparison with control group (Figure 5, 1151 ± 426 $\mu\text{g}/\text{mL}$ and 1187 ± 548 $\mu\text{g}/\text{mL}$, respectively)

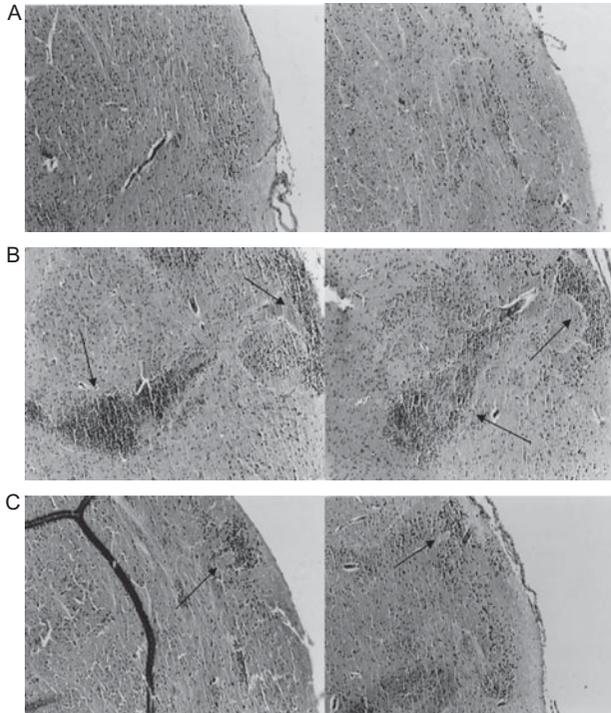


Figure 2. Brain sections from (A) normal, (B) control, (C) treated mice were stained with H&E. Leukocyte infiltration are less evident in the Aloe vera-treated mice in comparison with control mice.

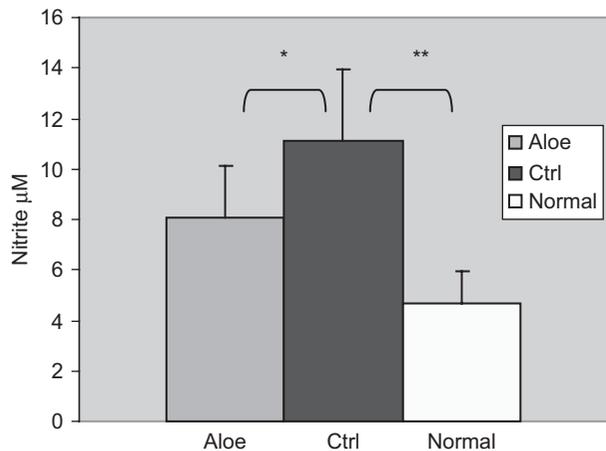


Figure 3. NO production was measured by the Griess reagent. Data are presented as the mean \pm SD of duplicate samples from 5 to 10 mice. * $P < 0.05$, ** $P < 0.01$.

Discussion

EAE as experimental model of MS is an autoimmune disease of CNS mediated by CD4⁺ T lymphocytes specific for autoantigens of the myelin sheath, including myelin basic protein, proteolipid protein and MOG peptide.^(23,24) Remitting/relapsing-EAE (RR-EAE) induced by active immunization with the immunodominant epitope of MOG 35–55 is characterized by an initial acute phase, followed by a series of remissions and relapses.⁽²⁵⁾ CD4⁺ Th1 cells and their proinflammatory cytokines are suspected to be important in the pathogenesis of MS, and necessary for the induction of RR-EAE.⁽²⁶⁾

The cytokine profile and the nature of cellular infiltrate are markedly different during the clinical course of RR-EAE, so that the maximal expression of the proinflammatory cytokines IFN- γ and tumor necrosis factor- α

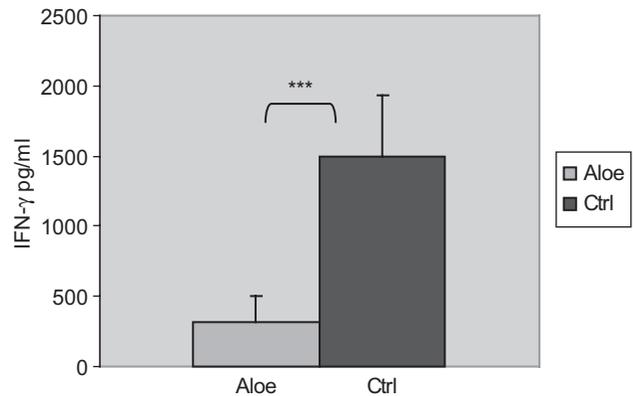


Figure 4. Spleen cells were isolated from all mice on day 21 following immunization with MOG 35–55 and cultured. The culture supernatants were collected after 72 h, and the levels of interferon- γ was measured by enzyme-linked immunosorbent assay. The values are mean of triplicates, and the error bars represent SD. *** $P < 0.001$.

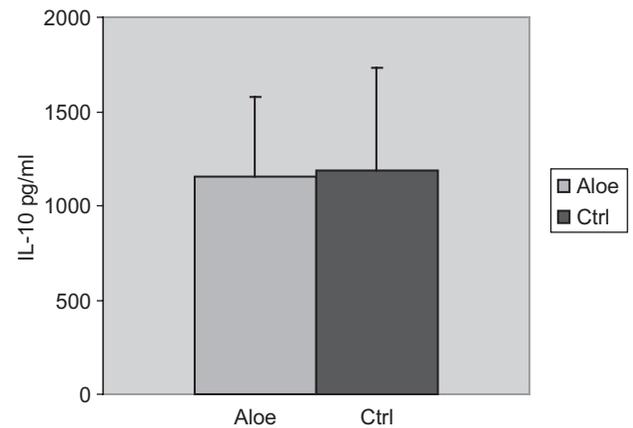


Figure 5. Spleen cells were isolated from all mice on day 21 following immunization with MOG 35–55 and cultured. The culture supernatants were collected after 72 h, and the levels of IL-10 was measured by enzyme-linked immunosorbent assay. The values are mean of triplicates, and the error bars represent SD.

are important mediators for disease induction. Th2 cell clones specific for encephalitogenic peptides are unable to induce the disease and can inhibit Th1 autoimmune clones, presumably by secreting IL-4, IL-10, and transforming growth factor- β .⁽²⁷⁾

Our findings suggest that Aloe vera is capable of suppressing a preactivated immune system in the late effector phase leading into disease eruption. We assume that several mechanisms involve in therapeutic effect of Aloe vera.

The pathogenic processes in EAE are mainly related to the secretion of IFN- γ .^(28,29) This study showed that treatment with Aloe vera can inhibit a proinflammatory Th1-based cytokine response and attenuate clinical signs in EAE. The results obtained in this research clearly show that Aloe vera therapy effectively reduces the severity of EAE-associated clinical symptoms. Moreover, Aloe vera therapy showed an appropriate cytokine response based on decreasing the proinflammatory Th1 cytokine, IFN- γ production.

In addition, the results showed that the level of serum NO was significantly less than control group, because the pathway over which IFN- γ induces NO production might be defeated through decreasing the production of this cytokine.⁽⁵⁾

NO is a free radical gas which acts as an important mediator/messenger in neuroprotection, neurotransmission, memory, and synaptic plasticity under physiological conditions. However, it has been reported that high amounts of NO have antiproliferative and/or cytotoxic effects in rat oligodendrocyte cultures leading to damage. Also, in an experimental model the inhibition of inducible NO Synthase expression and NO production prevented development of allergic encephalomyelitis. These reports indicate the neurotoxicity of excessive amounts of NO in MS. In particular lysis of oligodendrocytes by cytotoxic T lymphocytes leading to demyelination can be the result of NO secreted by monocytes, macrophages, and microglia. This indicates the modulatory action of NO on T-cell function.⁽³⁰⁾

NO may be involved in the development of several pathological features of MS, the hallmark of which is the demyelinated plaque with reactive glial scar formation.⁽³¹⁾ NO has two major effects on cerebral vessels, both of which may be involved in the pathogenesis of MS lesions—namely, vasodilation and a disturbance of the blood brain barrier. NO can arise in lesions from various sources, including nerve terminals.^(32,33) NO can promptly and reversibly block axonal conduction⁽³¹⁾ and demyelinated axons are particularly vulnerable to this effect.⁽³⁴⁾ The simple exposure of axons to low micromolar NO can result in persistent conduction block⁽³⁵⁾ due (at least *in vitro*) to axonal degeneration.^(31,36) It has been noted that NO inhibits T lymphocyte proliferation, preferentially Th1 cells.^(37,38)

These results suggest that Aloe vera modulates EAE, at least in part, by suppressing NO and IFN- γ production.

This finding encourages further investigations on Aloe vera and its role as a potential candidate for developing more efficient therapeutic strategies against multiple sclerosis.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Peiris, M., Monteith, G.R., Roberts-Thomson, S.J., Cabot, P.J. A model of experimental autoimmune encephalomyelitis (EAE) in C57BL/6 mice for the characterisation of intervention therapies. *J. Neurosci. Methods.* 2007, 163, 245–254.
2. Furlan, R., Kurne, A., Bergami, A., Brambilla, E., Maucchi, R., Gasparini, L., Butti, E., Comi, G., Ongini, E., Martino, G. A nitric oxide releasing derivative of flurbiprofen inhibits experimental autoimmune encephalomyelitis. *J. Neuroimmunol.* 2004, 150, 10–19.
3. Zaheer, S., Wu, Y., Bassett, J., Yang, B., Zaheer, A. Glia maturation factor regulation of STAT expression: a novel mechanism in experimental autoimmune encephalomyelitis. *Neurochem. Res.* 2007, 32, 2123–2131.
4. Wang, Y., Kai, H., Chang, F., Shibata, K., Tahara-Hanaoka, S., Honda, S., Shibuya, A., Shibuya, K. A critical role of LFA-1 in the development of Th17 cells and induction of experimental autoimmune encephalomyelitis. *Biochem. Biophys. Res. Commun.* 2007, 353, 857–862.
5. Xiao, B.G., Ma, C.G., Xu, L.Y., Link, H., Lu, C.Z. IL-12/IFN-gamma/NO axis plays critical role in development of Th1-mediated experimental autoimmune encephalomyelitis. *Mol. Immunol.* 2008, 45, 1191–1196.
6. Jiang, H.R., Al Rasebi, Z., Mensah-Brown, E., Shahin, A., Xu, D., Goodyear, C.S., Fukada, S.Y., Liu, F.T., Liew, F.Y., Lukic, M.L. Galectin-3 deficiency reduces the severity of experimental autoimmune encephalomyelitis. *J. Immunol.* 2009, 182, 1167–1173.
7. Fisher, A.E., Maxwell, S.C., Naughton, D.P. Superoxide and hydrogen peroxide suppression by metal ions and their EDTA complexes. *Biochem. Biophys. Res. Commun.* 2004, 316, 48–51.
8. Klein, A.D., Penneys, N.S. Aloe vera. *J. Am. Acad. Dermatol.* 1988, 18, 714–720.
9. Grindlay, D., Reynolds, T. The Aloe vera phenomenon: a review of the properties and modern uses of the leaf parenchyma gel. *J. Ethnopharmacol.* 1986, 16, 117–151.
10. Eamlamnam, K., Patumraj, S., Visedopas, N., Thong-Ngam, D. Effects of Aloe vera and sucralfate on gastric microcirculatory changes, cytokine levels and gastric ulcer healing in rats. *World J. Gastroenterol.* 2006, 12, 2034–2039.
11. Davis, R.H., Leitner, M.G., Russo, J.M., Byrne, M.E. Wound healing. Oral and topical activity of Aloe vera. *J. Am. Podiatr. Med. Assoc.* 1989, 79, 559–562.
12. Shelton, R.M. Aloe vera. Its chemical and therapeutic properties. *Int. J. Dermatol.* 1991, 30, 679–683.
13. Langmead, L., Chitnis, M., Rampton, D.S. Use of complementary therapies by patients with IBD may indicate psychosocial distress. *Inflamm. Bowel Dis.* 2002, 8, 174–179.
14. Sampedro, M.C., Artola, R.L., Murature, M., Murature, D., Ditamo, Y., Roth, G.A., Kivatinitz, S. Mannan from Aloe saponaria inhibits tumoral cell activation and proliferation. *Int. Immunopharmacol.* 2004, 4, 411–418.

15. Reynolds, T., Dweck, A.C. *Aloe vera* leaf gel: a review update. *J. Ethnopharmacol.* 1999, 68, 3–37.
16. Wooles, W.R., Diluzio, N.R. Reticuloendothelial function and the immune response. *Science.* 1963, 142, 1078–1080.
17. Rajasekaran, S., Sivagnanam, K., Subramanian, S. Antioxidant effect of *Aloe vera* gel extract in streptozotocin-induced diabetes in rats. *Pharmacol. Rep.* 2005, 57, 90–96.
18. Cory-Slechta, D.A., Weiss, B. Efficacy of the chelating agent CaEDTA in reversing lead-induced changes in behavior. *Neurotoxicology.* 1989, 10, 685–697.
19. Meldrum, J.B., Ko, K.W. Effects of calcium disodium EDTA and meso-2,3-dimercaptosuccinic acid on tissue concentrations of lead for use in treatment of calves with experimentally induced lead toxicosis. *Am. J. Vet. Res.* 2003, 64, 672–676.
20. Skundric, D.S., Zakarian, V., Dai, R., Lisak, R.P., Tse, H.Y., 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, 34–45.
21. Natarajan, C., Muthian, G., Barak, Y., Evans, R.M., Bright, J.J. Peroxisome proliferator-activated receptor-gamma-deficient heterozygous mice develop an exacerbated neural antigen-induced Th1 response and experimental allergic encephalomyelitis. *J. Immunol.* 2003, 171, 5743–5750.
22. Phizackerley, P.J., Al-Dabbagh, S.A. The estimation of nitrate and nitrite in saliva and urine. *Anal. Biochem.* 1983, 131, 242–245.
23. Tuohy, V.K., Lu, Z., Sobel, R.A., Laursen, R.A., Lees, M.B. Identification of an encephalitogenic determinant of myelin proteolipid protein for SJL mice. *J. Immunol.* 1989, 142, 1523–1527.
24. Mendel, I., Kerlero de Rosbo, N., Ben-Nun, A. A myelin oligodendrocyte glycoprotein peptide induces typical chronic experimental autoimmune encephalomyelitis in H-2b mice: fine specificity and T cell receptor V beta expression of encephalitogenic T cells. *Eur. J. Immunol.* 1995, 25, 1951–1959.
25. McRae, B.L., Kennedy, M.K., Tan, L.J., Dal Canto, M.C., Picha, K.S., Miller, S.D. Induction of active and adoptive relapsing experimental autoimmune encephalomyelitis (EAE) using an encephalitogenic epitope of proteolipid protein. *J. Neuroimmunol.* 1992, 38, 229–240.
26. Beck, J., Rondot, P., Catinot, L., Falcoff, E., Kirchner, H., Wietzerbin, J. Increased production of interferon gamma and tumor necrosis factor precedes clinical manifestation in multiple sclerosis: do cytokines trigger off exacerbations? *Acta Neurol. Scand.* 1988, 78, 318–323.
27. Miller, A., al-Sabbagh, A., Santos, L.M., Das, M.P., Weiner, H.L. Epitopes of myelin basic protein that trigger TGF-beta release after oral tolerization are distinct from encephalitogenic epitopes and mediate epitope-driven bystander suppression. *J. Immunol.* 1993, 151, 7307–7315.
28. Chen, Y., Kuchroo, V.K., Inobe, J., Hafler, D.A., Weiner, H.L. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science.* 1994, 265, 1237–1240.
29. Racke, M.K., Bonomo, A., Scott, D.E., Cannella, B., Levine, A., Raine, C.S., Shevach, E.M., Röcken, M. Cytokine-induced immune deviation as a therapy for inflammatory autoimmune disease. *J. Exp. Med.* 1994, 180, 1961–1966.
30. Acar, G., Idiman, F., Idiman, E., Kirkali, G., Cakmakçi, H., Ozakbas, S. Nitric oxide as an activity marker in multiple sclerosis. *J. Neurol.* 2003, 250, 588–592.
31. Smith, K.J., Lassmann, H. The role of nitric oxide in multiple sclerosis. *Lancet. Neurol.* 2002, 1, 232–241.
32. Rand, M.J. Nitroergic transmission: nitric oxide as a mediator of non-adrenergic, non-cholinergic neuro-effector transmission. *Clin. Exp. Pharmacol. Physiol.* 1992, 19, 147–169.
33. Toda, N., Tanaka, T., Ayajiki, K., Okamura, T. Cerebral vasodilation induced by stimulation of the pterygopalatine ganglion and greater petrosal nerve in anesthetized monkeys. *Neuroscience.* 2000, 96, 393–398.
34. Redford, E.J., Kapoor, R., Smith, K.J. Nitric oxide donors reversibly block axonal conduction: demyelinated axons are especially susceptible. *Brain.* 1997, 120 (Pt 12), 2149–2157.
35. Kapoor, R., Davies, M., Smith, K.J. Temporary axonal conduction block and axonal loss in inflammatory neurological disease. A potential role for nitric oxide? *Ann. N. Y. Acad. Sci.* 1999, 893, 304–308.
36. Garthwaite, G., Goodwin, D.A., Batchelor, A.M., Leeming, K., Garthwaite, J. Nitric oxide toxicity in CNS white matter: an in vitro study using rat optic nerve. *Neuroscience.* 2002, 109, 145–155.
37. Allione, A., Bernabei, P., Bosticardo, M., Ariotti, S., Forni, G., Novelli, F. Nitric oxide suppresses human T lymphocyte proliferation through IFN-gamma-dependent and IFN-gamma-independent induction of apoptosis. *J. Immunol.* 1999, 163, 4182–4191.
38. Dietlin, T.A., Hofman, F.M., Gilmore, W., Stohlman, S.A., van der Veen, R.C. T cell expansion is regulated by activated Gr-1+ splenocytes. *Cell. Immunol.* 2005, 235, 39–45.

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