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Effect of Aqueous-alcoholic Extract of *Aloe vera* on Retinal Tissue Changes in Rats with Experimental Multiple Sclerosis

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ABSTRACT

Multiple Sclerosis is a chronic inflammatory disease of the central nervous system and demyelination of the central and the peripheral nerves, axonal damage and their loss are considered as the most important symptoms of it. As a result, multiple neurological disorders including visual impairments are observed by incidence of the disease. Some have stated that retinal impairment occurs due to the production of free radicals in patients with MS. Therefore, use of antioxidants may reduce retinal damages caused by MS disease in these patients. So, the aim of this study is to investigate the effects of aqueous-alcoholic extract of Aloe vera on retinal tissue changes in rats with experimental MS. Methods: To do this research, 60 *male Wistar rats were randomly divided into 6 groups of 10 each, which included: control group (received water and food only), group 2 (received 200 mg/kgBW Aloe vera extract), group 3 (received 400 mg/kgBW Aloe vera extract), group 4 (the rats with myelin oligodendrocyte glycoprotein (MOG) peptide-induced MS), group 5 (the rats with experimental MS, which received 200 mg/kgBW of Aloe vera extract) and group 6 (the rats with experimental MS, which received 400 mg/kgBW of Aloe vera extract). At the end of the experiment, all of the rats were kept under deep anesthesia and the retinas were isolated for microscopic examinations. They were then placed in buffered formalin to carry out histological process and hematoxylin-eosin staining on them. Microscopic study includes measurement of retinal thickness and measurement of the thickness of retinal various layers including, PSL, ONL, INL, GCL, RNFL, IPL and OPL. Results: The results of this study showed that there was a significant decrease at 5% level of significance in the thickness of PSL, GCL, RNFL and the entire retina in the MS Groups compared to the Control Group. Also, there was a significant increase in the thickness of PSL, GCL, RNFL and the retina in the MS group received maximum dose of Aloe vera extract compared to the Control Group. There was also a significant decrease in PSL, RNFL and the retina thicknesses in the MS group received maximum dose of Aloe vera extract in comparison with the groups that received Aloe vera extract. Discussion and Conclusion: Finally, it can be said according to the research that thickness of the retina as well as its different layers is reduced by induction of MS, and treatment with Aloe vera gel extract will influence on the absence of change in these factors and on improvement of them.*

Key words: *Aloe Vera*, Retina, Experimental MS, Rat

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INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) and demyelination of the peripheral and the central nerves, axonal damage and their loss is considered as the main symptom of it and as a result, numerous neurological disorders appear proportional to incidence of the disease [1, 2]. Axon of nerve cells in CNS of adult mammalian has inherent ability to be repaired after injury [3]. Studies have stated that MS causes some nerve symptoms such as weakness in the extremities, vision changes, bladder dysfunction, pain and fatigue [4]. In addition, according to some studies, extensive neuronal death occurs in ganglion cells and in inner nuclear layer of the retina, which causes inflammation in it [5]. Generally, the relationship between inflammation and quality of neuronal function in MS has not been fully understood in relation to the onset of neuronal degradation [6]. However, some studies have

stated that myelin degradation in MS patients may occur in areas with mild inflammatory activity, leading to degradation of the retina [7]. Also, it has reported in some studies that induction of apoptosis and also oxidative stress occurs in the retina due to the production of various cytokines in MS patients, which ultimately leads to myelination disorder [8]. Therefore, use of antioxidants may reduce the risk of MS-related retina damage in these patients.

As an herbal medicine, Aloe Vera is a plant with thick fleshy leaves that belongs to the family *Liliaceae* [9]. Water soluble polysaccharides, prostaglandin precursors, vitamins A, C and E, lignin, saponin, plant sterols, phytoestrogens and amino acids are found in the chemical composition of the plant [10]. By penetrating into depth of cells and effecting on their DNA, Aloe vera prevents the cells from altering of their shapes due to external factors and also increases life of the cells, which affects the viral DNA causing changes in their function.

The plant has important therapeutic properties, including strengthening and modifying the immune system in inflammatory and viral diseases (such as herpes), wound healing, repairing burn injuries and anti-inflammation properties [11]. It has also reported that Aloe vera with its antioxidant properties has positive effects on intracellular enzymes, normal protein shapes and cell proliferation [12].

As it has proposed, inflammation, oxidative stress and increased immune cell growth are some mechanisms of retinal damage in mouse models with MS. It is likely that Aloe vera, due to its anti-inflammatory and antioxidant properties, reduces retinal damages and their risks in mice with MS. Since there has not been any research on the effect of this extract on retinal changes in mouse with experimental MS, this study has been designed with the same purpose to be able to improve the retina of these patients through the Aloe vera herbal extract which has minimal or no therapeutic side effects.

MATERIAL AND METHODS

This study was conducted in a complete randomized manner and in a laboratory. All ethics of working with laboratory animals in this research had observed in accordance with the instructions of the Animal Protection Committee of Shiraz University. A total of 60 adult male Wistar rats weighing 200 ± 15 g and 100-120 days old were prepared from Razi Vaccine and Serum Research Institute, Shiraz, Iran. The rats were kept in metal cages with lattice doors in laboratory conditions including at 21±2 ° C and a cycle of 12 hours of light and 12 hours of darkness for 2 weeks in Animal House at Jahrom University of Medical Sciences. They fed from standard foods and water was also provided for them by special glass bottles. The cages were disinfected with 70% alcohol three times per week.

MS Induction Method

In order to induce MS, myelin oligodendrocyte glycoprotein (MOG) peptide, with M-E-V-G-W-Y-R-S-P-F-S-R-V-V-H-L-Y-R-N-G-K sequence and purity of 95% (prepared from Pharma Company) was prepared at a dose of 100 µg in saline solution (1: 1) and injected subcutaneously into the end of the tail region [13].

Extraction Method

The alcoholic extract was prepared from fresh leaves of Aloe vera plant. Briefly, the leaves were washed and their gel extracted. The gel was homogenized and placed in 4 times volume of 95% ethanol. The container containing the gel and alcohol was placed on a shaker for 4 days. The extract was filtered and then concentrated on a vacuum condenser at 45 ° C. The extract was completely dried at 40 ° C and powdered [14, 15].

Then, 60 male rats were divided into 6 groups of 10 each, which were as follows:
Group 1: 10 healthy rats which fed water and food freely
Group 2: 10 healthy rats that daily received 200 mg/kg body weight of Aloe vera extract by Gavage
Group 3: 10 healthy rats that daily received 400 mg/kg body weight of Aloe vera extract by Gavage
Group 4: 10 rats with MS, which received water and food, freely
Group 5: 10 rats with MS, which daily received 200 mg/kg body weight of Aloe vera extract by Gavage
Group 6: 10 rats with MS, which daily received 400 mg/kg body weight of Aloe vera extract by Gavage

After about a four-week period, the animals were anesthetized with intraperitoneal injection of high doses of ketamine and xylazine. Dissection process was performed in the next stage and the eyes and then the retinas were removed and

examined by optical microscopy. For microscopic examination, the specimens were first trimmed and placed in a container containing 4% glutaraldehyde. After washing, they were placed in a 0.4 M buffered formalin solution for 2 hours. The specimens were washed again and microscopic sections were prepared according to the methods of tissue preparation. Histomorphometric studies include thickness measurement of the retina and its sub-layers including, polarization scrambling layer (PSL), outer nuclear layer (ONL), inner nuclear layer (INL), ganglion cell layer (GCL), retinal nerve fiber layer (RNFL), inner plexiform layer (IPL) and outer plexiform layer (OPL). One-way analysis of variance was used to compare between the treatments. P values of less than 0.05 (P<0.05) was regarded as the level of significance. SPSS software version 18 was used to analyze the data and to perform statistical tests.

RESULTS

According to Diagram 1, PSL thickness in the MS group and in the MS groups treated with the minimum and the maximum doses of Aloe vera extract showed a significant decrease compared to the groups received the minimum and the maximum doses of the extract only. There was a significant decrease in the MS group and the MS groups received the maximum and the minimum doses of the extract compared to the control group. PSL thickness in the MS group received the maximum dose of Aloe vera, significantly increased than that of the MS group.

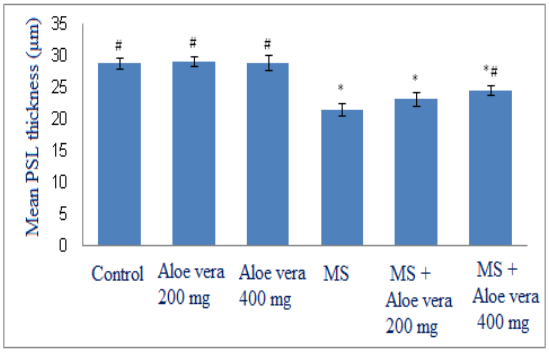

Diagram 1: Comparison of the mean PSL thickness (µm) among different groups

** Significant difference (P <0.05) with the control group; # Significant difference (P <0.05) with MS group.*

According to Diagram 2, ONL thickness in the MS group and the MS group received the maximum and the minimum doses of Aloe vera extract reduced compared to the control group. An increase in ONL thickness was observed in the MS group received the maximum and the minimum doses of the extract compared to the MS group. These changes were not statistically significant at 5% level of significance.

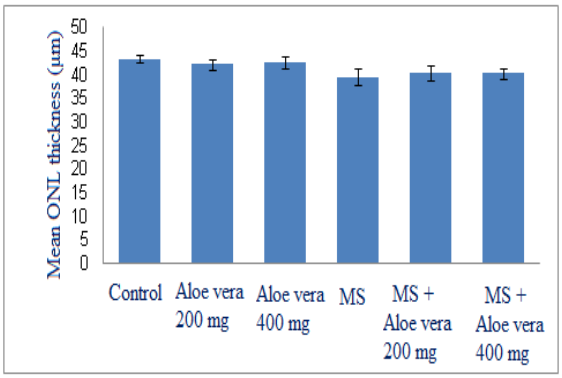

Diagram 2: Comparison of the mean ONL thickness (µm) among different groups

** Significant difference (P <0.05) with the control group; # Significant difference (P <0.05) with MS group.*

According to Diagram 3, thickness of INL in the various groups did not show a significant difference compared to that of the control group and in relation to each other.

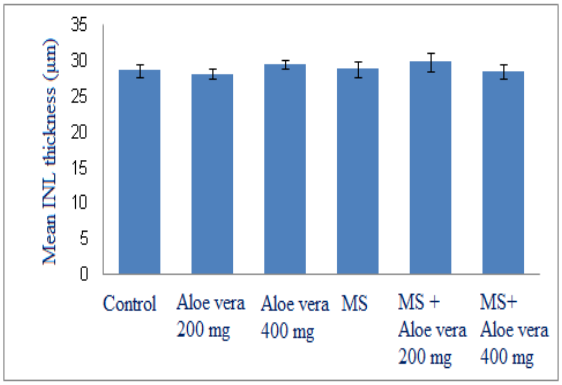

Diagram 3: Comparison of the mean INL thickness (µm) among different groups

** Significant difference (P <0.05) with the control group; # Significant difference (P <0.05) with MS group.*

According to Diagram 4, thickness of GCL in the MS group and in the MS group received the minimum dose of Aloe vera extract decreased significantly compared to that of the control group and the group received different doses of Aloe vera alone. A significant increase in GCL thickness was also observed in the MS group received the maximum dose of the extract compared to that of the MS group.

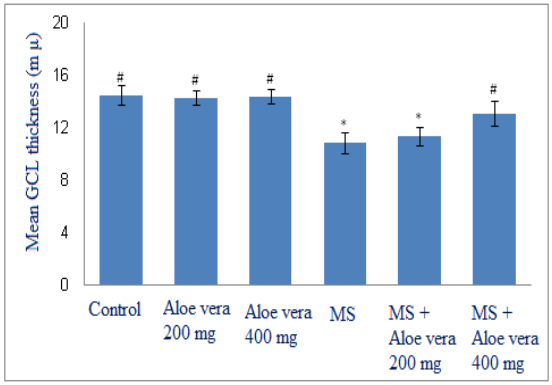

Diagram 4: Comparison of the mean GCL thickness (m µ) among different groups

** Significant difference (P <0.05) with the control group; # Significant difference (P <0.05) with MS group.*

According to Diagram 5, thickness of RNFL in the MS group and in the MS group received the maximum and the minimum doses of Aloe vera extract was significantly lower than that of the control group and the group received different doses of the extract alone. A significant increase in GCL thickness was also observed in the MS group received the maximum dose of the extract compared to that of the MS group.

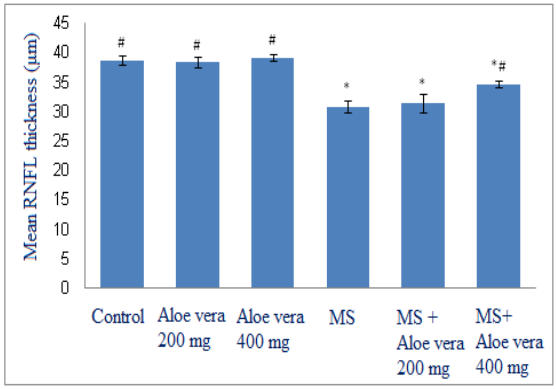

Diagram 5: Comparison of the mean RNFL thickness (µm) among different groups

** Significant difference (P <0.05) with the control group; # Significant difference (P <0.05) with MS group.*

According to Diagram 6, thickness of the entire retina in the MS group and the MS group received the maximum and the minimum doses of the extract decreased significantly compared to the control group and to the groups received the extract alone. Thickness of the entire retina in the MS group received the maximum dose of the extract showed a significant increase compared to the MS group which received no treatment.

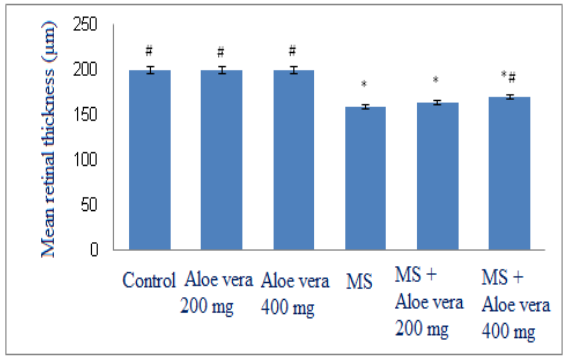

Diagram 6: Comparison of the mean retinal thickness (µm) among different groups

** Significant difference (P <0.05) with the control group; # Significant difference (P <0.05) with MS group.*

According to Diagram 7, thickness of IPL in the studied groups did not show a significant change compared to that of the control group and the other groups.

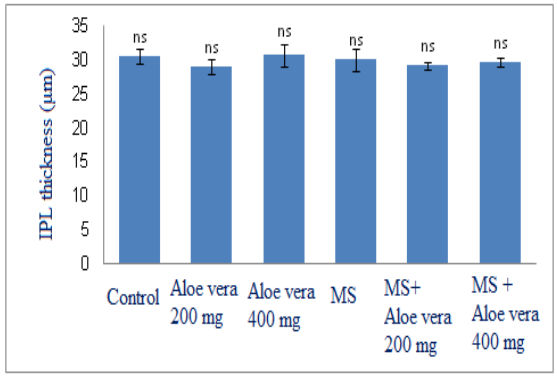

Diagram 7: Comparison of the mean IPL thickness (µm) among different groups

**ns: signifies lack of significant difference (P <0.05) with the other studied groups.*

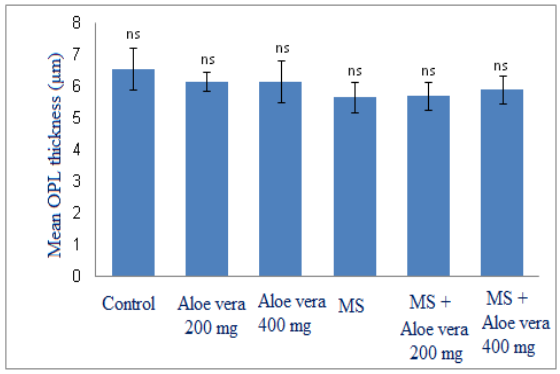

Diagram 8: Comparison of the mean OPL thickness (µm) among different groups

**ns: signifies lack of significant difference (P <0.05) with the other studied groups.*

According to Diagram 8, OPL thickness in the MS group and the MS group treated with Aloe vera

was lower than that of the control group. OPL thickness in the MS groups treated with the minimum and the maximum doses of the extract increased in comparison to the MS group that received no treatment. These changes were not statistically significant at 5% level of significance.

In general, MS reduced thickness of the entire retina and its various layers and Aloe vera extract somewhat improved the effects in the treated groups and increased thickness of various layers of the retina and also the thickness of the entire retina.

DISCUSSION

The results of this study showed that there was a significant decrease in the overall thickness of the entire retina and PSL, GCL and RNFL sub-layers in the MS group compared to the control group, which indicates negative effects of MS on changes of thickness of different layers of the retina in the studied groups.

Researchers have stated that MS is a chronic inflammatory disease of the CNS. Demyelination of the peripheral and the central nerves, axonal damages and their loss are the most important signs of the disease and as a result, numerous neurological disorders appear proportional to it [1, 2]. Some nerve symptoms are included weakness in the extremities, bladder dysfunction, pain and fatigue, and most importantly, visual changes [4].

It has also stated that myelination is considered as a necessary process in the development of vertebrates. Nerve signals are transferred faster by myelinated axons. Myelin protects the axon and supports its proper function [16]. Demyelination is a phenomenon in which myelin sheath is degraded and can be seen in both primary and secondary forms in the nerve tissues. Damage in primary demyelination is caused by removal of myelin and this process is associated with loss of damage or low damage to the axon [17].

However, the myelin sheath in the secondary demyelination disappears after direct damage to the axon and its disconnection [18]. Therefore, it is likely that myelination disorder in the group of the rats with MS affects the function of the axons in the visual area, resulting in visual impairment and reduced diameter of the retina.

Studies have reported that myelin sheath is damaged in MS disease and its producing cells called, oligodenrocytes, are also damaged, leading to disturbance in guidance of neural message of the CNS [19]. In fact, it has reported that some of the brain damages in MS disease are remyelinated by oligodendrocytes and schwann cells [20, 21]. Some evidences suggest that the differentiation of oligodendrocyte precursor cells (OPCs) into adult oligodendrocytes is inhibited by the presence of myelin inhibitors at the site of injury, which can be one reason for the failure of remyelination in diseases such as MS [22]. Previous studies on MS have also stated that deficiency in neurons causes myelination disorder and, consequently, disturbance in optic nerve and damage to the retina [23]. As in the present research, the results of retinal photomorphometric studies showed reduced thickness in different layers of the retina in the MS groups in comparison to that of the control group, which is probably due to disruption of myelination process and failure of remyelination in MS disease.

By studying on MS disease and retinal changes, some researchers also noted that extensive neuronal death occurs in ganglion cells and in the retina's inner nuclear cell layers, which led to inflammation in the retina [5]. Generally, the relationship between inflammation and quality of neuronal function in MS disease has not been fully understood in relation to the onset of neuronal degradation [6]. Some studies, however, have stated that myelin degradation may occur in areas with mild inflammatory activity in MS patients, leading to degradation of the retina [7]. Therefore, a possible mechanism that cause damage to the various layers of the retina and also visual impairment is inflammation which caused by extensive neuronal death.

Myelin oligodendrocyte glycoprotein (MOG) was used in the present study to induce MS disease. It has stated in researches that use of MOG for induction of MS causes retinal inflammation in 90% of animals due to the destruction and demyelination of optic nerves and destruction of ganglion networks [24, 25]. It has also suggested in examining the effects of MOG on the retina of rats that the substance is able to alter and also to degenerate various layers of the retina, especially GCL. This effect is applied by MOG due to impairment of optic nerves [26]. It is also stated that MOG causes inflammation in the neural parenchyma of the eye and also penetration of T

cells into that region [27]. The probable mechanism for retinal degradation in rats with experimental MS is likely to be such that intrinsic immunity and cellular immunity are both activated in somehow, which in turn activates microglia and causes breakdown of the blood-retinal barrier (BRB) and degrades the neurons. MOG, on the other hand, causes destruction of GCL and this process was not observed in animals available in the control group that did not receive this material. It is likely that a specific MOG antibody induces neuronal degradation and activates microglya and T- Cells available in the optic nerve and the retina [28]. So, it is probable in the present study that destruction of retinal neurons, failure of the blood barrier, activation of microglia, as well as destruction of various layers of the retina, especially GCL, occurred in the groups received MOG for induction of MS. This was observed as a decrease in the diameter of this layer, indicating a disorder in the function of the retinal neurons.

Some studies have also reported that induction of apoptosis in the retina is occurred in MS patients due to production of various cytokines [27]. As it has shown in researches, MS has been responsible for thinning the macular layer, as well as the retinal nerve fiber layer in the case of autopsy [27], which suggests that these changes are not likely to be independent of the brain atrophy and also of optic nerve damages in these patients [29, 30]. So, it is likely in the present study that apoptosis of the retinal cells and its various layers occurred in the groups received MOG. This is in agreement with the results of previous studies. Also, the results of measuring the diameter of different layers of the retina have showed a decrease in diameter of these layers in the group of the rats with MS compared to the control group.

Studies have shown that oxidative stress and also myelination disorder are considered as MOG mechanisms [31]. Therefore, MOG in the present study resulted in development of disorders in nerves, as well as in various layers of the retina due to the production of oxidative stress.

The results of the study on the group of the rats with MS, which received Aloe vera extract showed that it could somewhat improve the complications of retina caused by MS disease in these rats. As there was a significant increase in the thickness of PSL, GCL, RNFL and the entire retina in the group of the rats with MS received the maximum dose of Aloe vera compared to the MS group. This indicates the positive effects of the extract in reduction of MS complications in this group. There was also an increase in the thickness of various layers of the retina in the MS group received the minimum dose of Aloe vera compared to the MS group. This is not statistically significant at 5% level of significance. On the other hand, a significant decrease in the diameter of PSL, RNFL, and the entire retina in the MS groups received the minimum and the maximum doses of the extract compared to the groups received the extract alone showed that MS has damaging effects on the retinal tissue and the extract reduced the complications to some extent.

Studies have shown that medicines used to treat MS symptoms have their own known side effects [31]. Therefore, use of traditional medicines to treat various diseases, especially MS, is increasing day by day.

Due to lack of complete success in the treatment of MS disease, attention of researchers is drawn to complementary and traditional medicine and they try to use all available capacities to solve this problem [10]. Treatment with alternative medicine or herbal remedies is becoming more and more acceptable day by day, and it is estimated that one out of every three people used these treatments for various illnesses during their lifetime [32].

It has also stated that Aloe vera as an herbal medicine penetrates into depth of cells and influences on their DNA. So, it prevents the cells from altering of their shapes due to external factors and also increases cell life. This herb has important therapeutic properties, including strengthening and modifying the immune system in inflammatory and anti-inflammatory diseases [11]. It has stated in examining the thickness of the retina in patients with MS that it causes inflammation in the retina, thereby reducing the thickness of its' various layers [33]. It has also argued that localized inflammation in the retina in people with MS leads to the destruction of retinal cells, as well as the activation of inherent immunity and secretion of lymphocytes [33]. So, it is likely that Aloe vera, due to its anti-inflammatory and modulating properties, reduces the risk and damages to the retina in rats with MS, and immunomodulatory activity of the plant has proven in the animal models of the disease [13].

It has also found that another mechanism in retinal injury in rats with experimental MS is appearance of oxidative stress and also reduction of myelination. On the other hand, studies have shown that MOG is used as an effective ingredient for experimental induction of MS, as well as experimental autoimmune encephalomyelitis (EAE). Due to the production of oxidative stress and also disorders in the process of myelination, this substance causes apoptosis in neurons and incidence of MS and EAE [34].

Several studies have shown antioxidant and also anti-oxidative stress effects of Aloe vera extract in other tissues, and this property of the extract has proved in numerous studies. They have also reported that Aloe vera with its antioxidant properties on intracellular enzymes has positive effects on normal shape of protein and cell proliferation [12]. As discussed above, apoptosis occurs in retinal cells of the rats with MS and it is likely that Aloe vera, due to its antioxidant and proliferative properties, prevents from development of apoptosis in the retina of these animals. Previous researches on investigation of effect of Aloe vera extract on retinal thickness in male rats with diabetes have showed that the thickness of the retina and its layers maintained its natural histological structure in the group treated with it, which was dependent on the antioxidant properties of the extract [35]. Studies have also shown that Aloe vera can act as an antioxidant to reduce radical oxygen and thus can have its protective effects on vulnerable tissues [9, 35 and 36]. So, it is determined that Aloe vera extract, due to the antioxidant properties, may improve diameter of the entire retina and its various layers in the groups received the extract.

CONCLUSION

It can be concluded that thickness of the retina and its different layers is changed and decreased by MS disease, which is due to impairment in remyelination, inflammation, impaired function of the retinal neurons, as well as induction of apoptosis in nerve cells and production of oxidative stress due to the MOG peptide. Aloe vera extract reduces the complications of MS on the retina by its anti-inflammatory and anti-oxidant properties. So, the extract can probably reduce the complications of MS disease on the retina of these patients.

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