Protective Effect of Aloe Vera Alcoholic Extract Gel on Changes of Estrogen and Progesterone in Experimental Model Rats with MS

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Introduction:
Multiple Sclerosis (MS) is a disease of central nervous system. Researchers have turned to traditional medicine for the treatment of MS. The present article aimed to investigate the protective effect of Aloe Vera alcoholic extract gel on changes of estrogen and progesterone in experimental MS models of female rats.

Materials and Methods:
A total number of 30 female Wistar rats were randomly divided into 6 equal groups. The experimental group 1 received 14 micro liters of ethidium bromide in lateral ventricles and received normal saline gavage; the experimental group 2 received 14 micro liters of ethidium bromide in lateral ventricles and received 50 mg/Kg of Aloe Vera; the experimental group 3 received 14 micro liters of ethidium bromide in lateral ventricles and received 100 mg/Kg of Aloe Vera, the experimental group 4 received 14 micro liters of ethidium bromide in lateral ventricles and received 200 mg/Kg of Aloe Vera. The control group did not receive any drugs. The sham group received 14 micro liters saline in lateral ventricles. Finally, blood samples were taken from their hearts in order to measure the serum concentration of estrogen and progesterone.

Results:
The serum concentration of estrogen in the sham group, experimental groups 1 and 2 was significantly lower than that in the control group. The concentration of progesterone in the sham group, experimental groups 1, 2 and 3 was significantly lower than that in the control group.

Conclusion:
The results show that MS decreased estrogen and progesterone, and that the active ingredients of Aloe Vera increased the concentration of the estrogen and progesterone in rats with MS.

Keywords: Aloe Vera, Estrogen, Progesterone, MS

Introduction
Multiple sclerosis (MS) is a central nervous system disease resulting in neurological disabilities, especially among young adults (1). It causes some neurological symptoms, such as weakness and lethargy in the extremities, visual impairment, bladder dysfunction, pain, and fatigue (2). There is no definite treatment for MS as our knowledge about its nature is incomplete. An important problem with MS is the large number of people acquiring the disease during their reproductive ages (3).
Women are affected with MS 1.77 times as men, with the peak incidence in ages 20-40 years. Moreover, its clinical course varies between a totally benign condition and progressive disabling condition (4). According to Iran MS Society, there are almost 40000 MS patients in Iran (5), and most of the women with MS are worried about its effects on pregnancy and childbirth and also drug side effects on the embryo (6). Studies have shown that MS exacerbates in final stages of pregnancy and improves after childbirth (7).

According to studies, estradiol reduces the size and number of cerebral lesions in early stages of MS in pregnant women and increases their immune responses (8). It is not clearly known why MS is less active during pregnancy, but factors such as sudden drop in estrogen after childbirth and disappearance of immunosuppression in pregnancy may affect the disease improvement (9).

The medications commonly used to treat MS cause specific mild, moderate, and even severe side effects despite their little to moderate advantages in improvement of the disease (10). Today, researchers have turned to the complementary and traditional medicine and try to use all available capacities to treat the disease as modern medicine has failed to treat it completely (11).

Studies show that 67% of MS patients use different complementary and pharmacological therapies (12), and 38.4% of patients use herbal medicine to treat the disease (13).

Aloe vera is one of the herbs used to treat MS. The most important chemicals in aloe vera are polysaccharides, anthraquinones, prostaglandins, phytoestrogens, such as betasitosterol, cholesterol, and fatty acids, such as campesterol, which affect the reproductive system (14).

As mentioned before, the decreased level of estrogen in MS patients exacerbates symptoms of the disease and also causes infertility, and thus, medicinal herbs increasing estrogen may be effective in treatment of MS. The involvement of young adults with MS and their disability in reproductive ages give special importance to the disease and studies revealing different aspects of the disease. This study was conducted mainly to examine protective effects of the aloe vera extract on changes in estrogen and progesterone in female rats with MS.

Materials and Methods
This experimental study was performed completely randomly. All ethical principles for working on laboratory animals were complied with instructions of Animal Protection Committee of Shiraz University. This research project was approved on Dec. 22nd, 2015 by Ethics Committee of the School of Veterinary Medicine, Shiraz University and registered as A/P/1451. This study was performed on 30 female Wistar adult rats weighing 200±15 g and aged 100-120 days supplied from Razi Vaccine and Serum Research Institute in Shiraz. The rats were housed in the animal house of the School of Veterinary Medicine, Shiraz University, at temperature of 21 ± 2 °C and 12/12 light-dark cycles for two weeks. The rats were housed in metal cages with gridded doors and received standard feed. They also received water in specific glass bottles. The cages were disinfected with 70% ethanol three times a week.

Induction of the disease
MS is a chronic inflammatory disease demyelinating the central nervous system, and chemical destruction of myelin with ethidium bromide (EB) is one of the most common models applied for induction of MS. In this study, EB was injected into lateral ventricles of the rats in the experimental group using stereotaxic apparatus and Hamilton syringe (needle gauge of 30) in order to induce MS (15). The red powder of EB, supplied by Merck Company, was dissolved using saline.
Preparation of aloe vera alcoholic extract
The aloe vera alcoholic extract was prepared from fresh leaves of aloe vera. Upon scientific approval of the botanists in Shiraz University, the leaves were washed, and their gel was removed and put into 95% ethanol at a ratio of 1:4. Then the gel container was placed on the shaker for four hours, and the resulting solution was filtered and concentrated at temperature of 45 °C using a rotary evaporator. The obtained substance was completely dried and milled at temperature of 40 °C (16).

The 30 female rats were randomly divided into six groups of five rats as follows:

- **Experimental group 1** received 14 µl of EB solution in lateral ventricles along with normal saline by gavage.
- **Experimental group 2** received 14 µl of EB solution in lateral ventricles along with 50 mg/kg bw of the aloe vera alcoholic extract by gavage (17).
- **Experimental group 3** received 14 µl of EB solution in lateral ventricles along with 100 mg/kg bw of the aloe vera alcoholic extract by gavage.
- **Experimental group 4** received 14 µl of EB solution in lateral ventricles along with 200 mg/kg bw of the aloe vera alcoholic extract by gavage (17 & 18).
- **Sham group** received 14 µl of saline as the solvent of EB in lateral ventricles using a Hamilton syringe (7 µl in each ventricle).

The control group was kept under normal conditions without receiving anything. After 35 days, all rats were anesthetized using ether, and blood samples were drawn from their hearts in order to measure estrogen and progesterone. The female sex hormones were measured using an animal kit and ELISA test. The groups were compared through one-way ANOVA and Tukey’s test in SPSS18 software. The P<0.05 was determined as the significance level.

**Results**
Based on the results, the concentration of estrogen in the sham group, experimental group 1, and experimental group 2 decreased significantly compared with that in the control group. Furthermore, the concentration of estrogen in experimental groups 2, 3, and 4 increased significantly compared with that in experimental group 1 (P < 0.05) (Figure 1).

The results showed that the concentration of progesterone in the sham group and experimental groups 1, 2, and 3 decreased significantly compared with that in the control group. The concentration of progesterone in experimental group 4 increased significantly compared with that in experimental group 1 (P < 0.05) (Figure 2).

![Figure 1: Changes in estrogen in experimental groups](image-url)
Discussions

Regarding the results, the concentration of estrogen and progesterone in the experimental groups decreased compared with that in the control group, which shows the adverse effects of MS on changes in sex hormones. It has been argued that the concentration of estrogen in MS patients’ serum is significantly lower than that in normal people’s serum (19), and this conforms to the results of this study.

Moreover, some studies have reported that estradiol and progesterone affect the activity of MS and that the use of different concentrations of these hormones may be effective in its treatment (20). According to the studies performed in 2004, inhibiting the progression of disability in MS patients is another mechanism for sex hormones (21). Therefore, the decrease in the concentration of estrogen and progesterone in the experimental groups of this study is completely reasonable and consistent with previous studies.

According to the results, the concentration of estrogen in experimental groups 2, 3, and 4 increased significantly compared with that in experimental group 1. Moreover, the concentration of progesterone in experimental group 4 increased significantly compared with that in experimental group 1, which probably shows the effectiveness of aloe vera in treatment of MS and changes in sex hormones.

A study on the effect of aloe vera on changes in female sex hormones has shown that the aloe vera extract increases estrogen in experimental groups (22). Obviously, the increase in estrogen improves MS, and the increased estrogenic hormones in the present study probably reduced adverse effects of the disease on fertility in MS patients.

A study on the effects of aloe vera on pregnant mice’s ovaries revealed that aloe vera increased vascularization around the secondary follicles and had an effect similar to that of estrogen and the follicle-stimulating hormone (23).

As the aloe vera extract has similar effects to that of the follicle-stimulating hormone on ovaries (24), it acts like that hormone and increases the growth and development of follicles and thus the secretion of estrogen from follicle cells. Studies have shown that the steroidal ligand initiates estrogen receptor gene transcription that results in further formation of specific
messenger RNAs. Estrogen and progesterone receptors exist in many proteins produced in most estrogen-responsive cells (22). It has been determined that the betasitosterol in aloe vera has the biological activity of estrogen production, and thus, phytoestrogens probably improve the changes in the concentration of estrogen and progesterone in groups receiving aloe vera.

**Conclusion**

Based on the results, the aloe vera extract may improve the changes in the concentration of estrogen and progesterone in a dose-dependent manner in MS patients due to its phytoestrogens. Therefore, the aloe vera extract is recommended for improvement of changes in estrogen and progesterone in MS patients.

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**Conflict of Interest**

The authors declare no conflicts of interest regarding the compilation/publication of this article.

**References:**

