

**Original article:**

**Protective Effect of Aloe Vera Gel Extract on Histopathologic Changes in Ovaries and Gonadotropin in MS Model of Rat**

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**Abstract:**

**Objective:** Multiple sclerosis (MS) is a self-immune demyelinating disorder of the CNS, followed by neuropathic discomfort, especially in young people in reproductive age. So, This research was conducted with the aim of assessing the protective effect of Aloe vera gel (AVG) extract on histopathologic changes of the ovary and gonadotropin in an experimental MS model of rat. **Methods:** In this research, 30 pieces of rats of Wistar race were randomly divided into 6 groups of 5. 20 pieces, 14µl of ethidium bromide was injected in their lateral ventricles to induce MS. In 5 pieces as control group 2, 14µL of saline was injected into the lateral ventricles, and the remaining 5 rats were considered as control group 1. Treatment of three groups of rats with MS was performed with AVG extract at 50 mg/kg BW, 100 mg/kg BW 100 and 200 mg/kg BW. At the end, blood sampling to measure serum FSH and LH, Their ovaries were isolated for microscopic examination. **Results:** The results of counting the number of ovarian follicles showed a significant decrease in the number of primary and secondary and graafian follicles in group 3, compared to the control group. There is a significant increase in the concentration of FSH and LH in group 3 in comparison to the control group. **Conclusion:** Finally, It can be said that by inducing MS, The number of ovarian follicles decreased, and the number of atretic follicles and the concentration of gonadotropin hormones increased, and treatment with AVG extract was effective in improving these factors.

**Keywords:** Aloe vera; Gonadotropin hormones; Ovaries; Experimental MS; Rats.

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**Introduction:**

Multiple sclerosis (MS) is a central nervous system disease, followed by neuropathy, especially in young people<sup>1</sup>. In MS, the immune system attacks the nerve fibers of the myelin sheath and destroys myelin in many areas and remains a fibrous tissue (scar) instead, which is called sclerosis and damaged

areas are called plaque. By destroying the myelin sheath or the nerve fiber itself, the ability to direct electrical impulses from the central nervous system to the organs and vice versa is disrupted, and this issue causes different symptoms of the disease in patient<sup>2</sup>. One of the most important problems with this disease is that a large number of people with MS are at reproductive age and the disease is more likely

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to develop in women than men<sup>3</sup>.

Common medications used to treat MS disease, despite of having a limited to modest benefits on improvement in some types of MS, have certain side effects ranging from mild to moderate and severe<sup>4</sup>. Studies in 2007 showed that many medications prescribed in MS had dangerous side effects in pregnancy, breast feeding and fertility<sup>5</sup>.

Most women with MS are concerned about the effects of disease on pregnancy and childbirth, as well as the effects of medications on the fetus<sup>6</sup>. Studies have shown that the improvement of symptoms usually decreases at the end of pregnancy but increases in the postpartum period, and the reasons for the decrease in the activity of the disease during this period are not well known, but factors such as a sudden eruption of estrogen after delivery and the loss of the immunosuppress or status of pregnancy can contribute to the disease<sup>7,8</sup>. It has also been reported that Gonadotropin hormones increase in patients with MS, and MS also reduces fertility and sexual cells in patients and causes sexual weakness<sup>9</sup>.

Nowadays, the use of alternative medicine or herbal remedies is more likely to be recognized due to the side effects of chemical drugs that are used to improve the disease and prevent the complications of patients with MS<sup>10,11</sup>, and it is estimated that of one of every three persons has used these treatments for various illnesses over his lifetime<sup>12</sup>. One of the herbs used in herbal medicine is Aloe Vera. Studies suggest that the extract of this plant not only increases estrogen levels, but also causes follicles to grow with its same effect as the sexual hormones<sup>13,14</sup>. It should also be noted that the most important chemical compounds of Aloe Vera are Poly Saccharides, anthraquinones, prostaglandins, phytoestrogens (such as beta-sitosterol), cholesterol, and fatty acids like campstrols<sup>15</sup>.

Therefore, considering that Aloe vera has phytoestrogenic compounds and the use of phytoestrogenic compounds reduces LH and FSH hormone levels<sup>5</sup>, and also in people with MS, there is usually an increase in gonadotropic hormones and assuming that Aloe vera plant can improve the disease by reducing these hormones, we decided to provide detailed information on the effect of Aloe vera extract on histomorphometric changes of ovaries and ovarian hormones in female rats with MS by this research.

## **Materials and Methods:**

The present study was conducted as an experimental laboratory and completely randomly research. This study was done on 30 female and matured Wistar rats weighing  $200 \pm 15$  gr and 100-120 days old, got from Razi Vaccine and Serology Research Center in Shiraz. The rats were placed in the animal's home of the Faculty of Veterinary Medicine, Shiraz University for two weeks under laboratory conditions ( $21 \pm 21$  ° C, 12 hours light and 12 hours darkness). During this period, the rats were kept in metal cages with cribriform doors and used standard food. Water was also provided by a special glass bottle. Their cages were disinfected with 70% alcohol three times a week.

### ***Same cycling of mature female rats***

Since in this study , matured female rats in the proestrus phase should be used and the rats prepared for this study were in different phases of the sexual cycle; a method should be used to put all of them in a same phase of sexual cycle. Therefore, vaginal smear were taken from all of them to ensure that they were all in same phase of the sexual cycle. In female rats, the sex cycle takes 4 to 5 days.

### ***The method of induction of disease***

MS disease is a chronic inflammatory disease of myelin degradation in the central nervous system, and myelin chemical destruction with the aid of ethidium bromide (EB) is one of the most commonly used models for inducing MS. Therefore, in this study, for induction of disease, intravenous injection of ethidium bromide in the brain peripheral ventricles of both sides of experimental group of rats was performed with a stereotaxy device and using the hamilton needle (Canula Guide 23, Dental needle 30). Etidium bromide was used as in the form of red powder from Merck Company and saline was used as solvent.

### ***Aloe vera alcoholic extract preparation method***

To prepare the Aloe Vera Alcoholic Extract, a number of fresh Aloe Vera leaves were prepared. After the scientific confirmation of botanical experts of the University of Shiraz, the leaves were washed and their gel was removed. The gel was placed in 95 degrees ethanol with four times of their volume. After placing the gel container on the shaker for four days, the solution was filtered by a flat filter and concentrated using the rotary evaporator device at 45 ° C. The final material was completely dried at 40 ° C and powdered<sup>16,17</sup>.

***Dividing 30 female rats into 6 groups of 5 was done as follows***

1. Control group 1: Without induction of MS and without receiving normal saline and Aloe Vera extract.
2. Control group 2: Without induction of MS and injection of 14  $\mu$ L, saline as ethidium bromide solvent to the lateral ventricle.
3. Experimental group 1: MS induction by injection of 14 $\mu$ l of ethidium bromide solution into ventricular region of the brain and receiving normal saline by gavage.
4. Experimental group 2: MS induction by injection of 14 $\mu$ l of ethidium bromide solution into the ventricular region of the brain and receiving the aloe vera extract at 50 mg / kg body weight per day using gavage.
5. Experimental group 3: MS induction by injection of 14 $\mu$ l of ethidium bromide solution into the ventricular region of the brain and receiving the aloe vera extract of 100 mg / kg daily using gavage.
6. Experimental group 4: MS induction by injection of 14  $\mu$ l of ethidium bromide solution into the ventricular region of the brain and receiving the aloe vera extract of 200 mg / kg daily by gavage.

It should be noted that Aloe vera extract was prescribed for 30 days. After the termination of the period, all rats were anesthetized with ether and blood samples were taken from their hearts to measure LH and FSH hormones. Female sex hormones were measured using animal kit by ELISA test. The ovaries were also extracted for histological studies and sent to the histology laboratory to count the number of initial, primary, secondary, and graafian follicles, as well as the corpus luteum, corpus albicans, and the number of atretic follicles. One way ANOVA and Tukey test were used to compare the groups using SPSS version 18 software.  $P < 0.05$  was considered as a significant level.

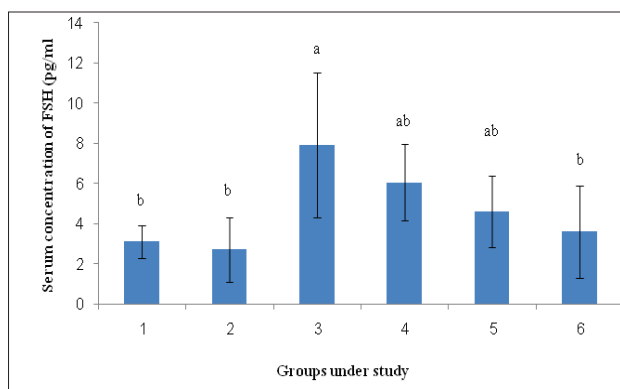
**Ethical clearance:** This study approved by Shiraz University. All ethics of working with laboratory animals in this research are based on the instructions of the Animal Protection Committee of Shiraz University. This research was approved at 22 December 2015.

**Results:**

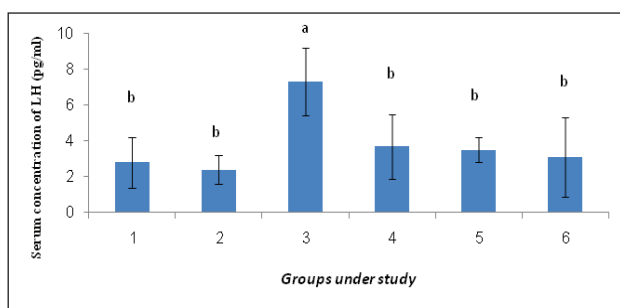
As shown in figures 1 and 2, the concentration of FSH and LH in the experimental group 3 (without receiving the extract of Aloe vera) showed a significant increase compared to the control group 1 ( $P < 0.05$ ). The concentration of FSH in group 6 (with the maximum dose of Aloe vera extract) and LH concentration in groups 4, 5 and 6 (with Aloe vera extract at different doses) decreased significantly compared to the experimental group 3, which was almost similar to that of the group Control 1 ( $P < 0.05$ ). Therefore, this study showed that by inducing MS, the concentration of gonadotropin hormones increases, and the use of Aloe vera extract will have some effect on the improvement of these hormonal changes.

Changes in the number of ovarian follicles in the six groups of rats are shown in figures 3 to 9. Although the decrease in the number of primordial follicles in the experimental group 3 without receiving the Aloe vera extract and their increase in the experimental group 6 with the maximum dose of Aloe vera extract comparing to the control group 1 and control group 2 ; but this difference was not significant ( $P > 0.05$ ) (Fig 3). However, according to Figure 4, the number of primary follicles in experimental group 3 without receiving Aloe vera extract and in the three treatment groups 4, 5 and 6 that received Aloe vera extract at different doses, was significantly decreased comparing to the control group 1 ( $P < 0.05$ ). Also, as shown in figures 5 and 6, the number of secondary and graafian follicles in the experimental group 3 (without receiving Aloe Vera extract) was significantly decreased comparing to the control group 1 ( $P < 0.05$ ). But this number increased in three treatment groups with Aloe Vera extract, which was not significant ( $P < 0.05$ ). On the other hand, there was a decrease in the number of corpus luteum and corpus albicans and increase in the number of atretic follicles in comparison with the control group 1 and this difference was not significant ( $P < 0.05$ ). In the treatment group 6, which received the maximum dose of Aloe Vera extract, significant increases in the number of corpus luteum and corpus albicans and significant reduction in atretic follicles were observed compared to the experimental group 3 without receiving the aloe vera extract (Figures 7, 8 and 9).

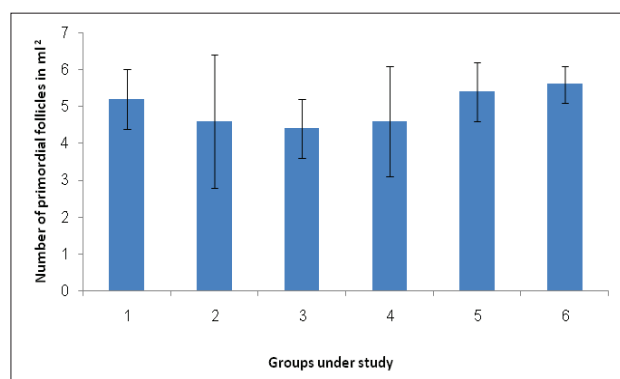
According to the above, It can be argued that induction of MS disease results in the reduction of normal follicles in the ovary, and therefore the increase of



**Fig 1.** Comparison of serum concentration of FSH (mean  $\pm$  standard error) in the studied groups (non-identical letters indicate a significant difference ( $P \geq 0.05$ )). 1: control 1, 2: control 2, 3: MS induction with normal saline, 4: MS induction with aloe vera extract at 50 mg / kg body weight, 5: MS induction with aloe vera extract of 100 Mg / kg body weight, 6: MS induction with aloe vera extract at 200 mg / kg body weight.

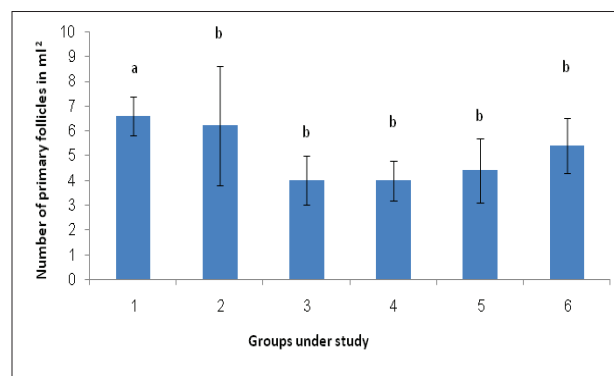


**Fig 2.** Comparison of serum levels of LH (mean  $\pm$  standard error) in the studied groups (non-identical letters indicate a significant difference ( $P \geq 0.05$ )). 1: control 1, 2: control 2, 3: MS induction with normal saline, 4: MS induction with aloe vera extract at 50 mg / kg body weight, 5: MS induction with aloe vera extract of 100 Mg / kg body weight, 6: MS induction with aloe vera extract at 200 mg / kg body weight.

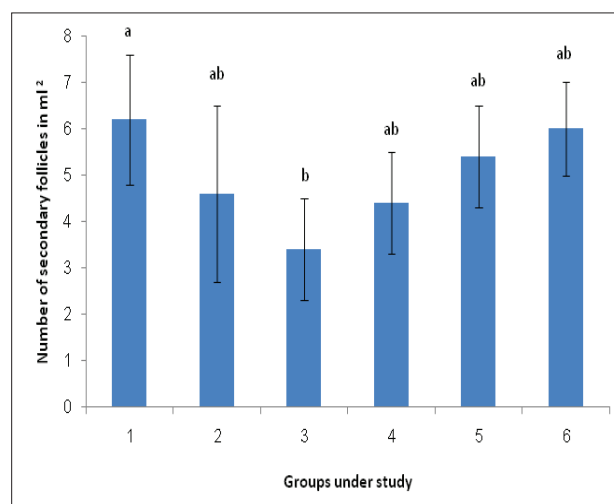


**Fig 3.** Comparison of the number of initial follicles (mean  $\pm$  standard error) in the studied groups (there was no significant difference between the groups ( $P < 0.05$ )).

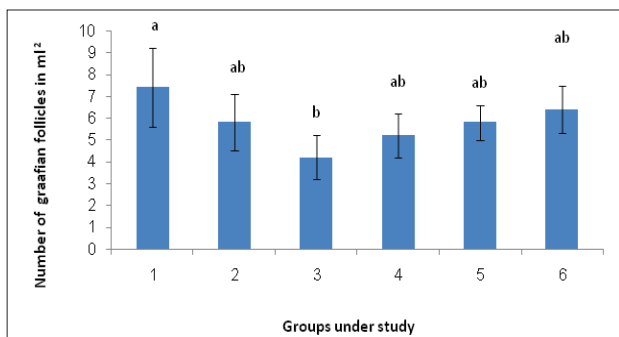
1: control 1, 2: control 2, 3: MS induction with normal saline, 4: MS induction with aloe vera extract at 50 mg / kg body weight, 5: MS induction with aloe vera extract of 100 Mg / kg body weight, 6: MS induction with aloe vera extract at 200 mg / kg body weight.



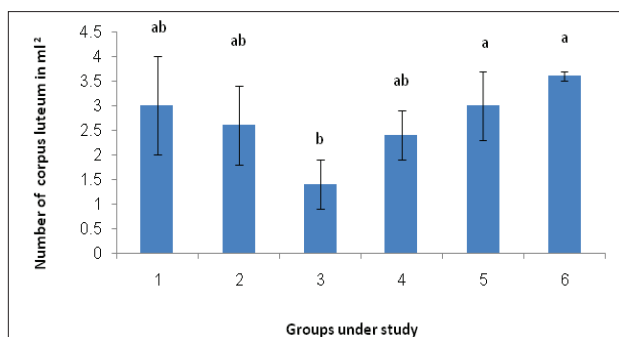
**Fig 4.** Comparison of the number of primary follicles (mean  $\pm$  standard error) in the studied groups (non-identical letters indicate a significant difference ( $P \geq 0.05$ )). 1: control 1, 2: control 2, 3: MS induction with normal saline, 4: MS induction with aloe vera extract at 50 mg / kg body weight, 5: MS induction with aloe vera extract of 100 Mg / kg body weight, 6: MS induction with aloe vera extract at 200 mg / kg body weight.



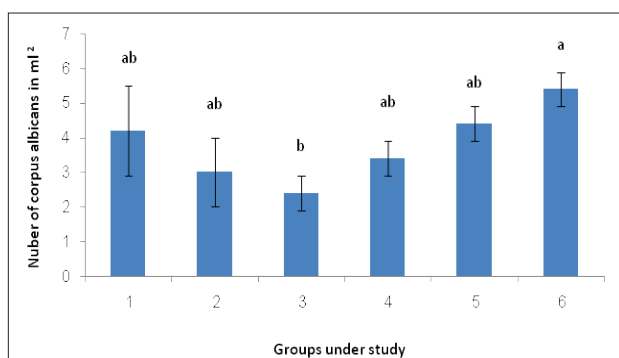
**Fig 5.** Comparison of the number of secondary follicles (mean  $\pm$  standard error) in the studied groups (non-identical letters indicate a significant difference ( $P \geq 0.05$ )). 1: control 1, 2: control 2, 3: MS induction with normal saline, 4: MS induction with aloe vera extract at 50 mg / kg body weight, 5: MS induction with aloe vera extract of 100 Mg / kg body weight, 6: MS induction with aloe vera extract at 200 mg / kg body weight.



**Fig 6.** Comparison of the number of graafian follicles (mean ± standard error) in the studied groups (non-identical letters indicate a significant difference ( $P \geq 0.05$ )). 1: control 1, 2: control 2, 3: MS induction with normal saline, 4: MS induction with aloe vera extract at 50 mg / kg body weight, 5: MS induction with aloe vera extract of 100 Mg / kg body weight, 6: MS induction with aloe vera extract at 200 mg / kg body weight.

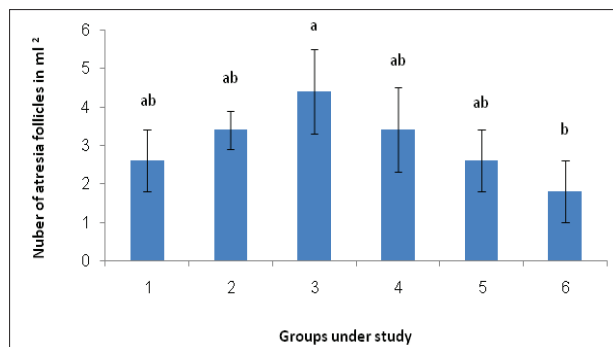


**Fig 7.** Comparison of the number of corpus luteum (mean ± standard error) in the studied groups (non-identical letters indicate a significant difference ( $P \geq 0.05$ )). 1: control 1, 2: control 2, 3: MS induction with normal saline, 4: MS induction with aloe vera extract at 50 mg / kg body weight, 5: MS induction with aloe vera extract of 100 Mg / kg body weight, 6: MS induction with aloe vera extract at 200 mg / kg body weight.



**Fig 8.** Comparison of the number of corpus albicans (mean ± standard error) in the studied groups (non-identical letters indicate a significant difference ( $P \geq 0.05$ )). 1: control 1, 2: control 2, 3: MS induction with normal saline, 4: MS

induction with aloe vera extract at 50 mg / kg body weight, 5: MS induction with aloe vera extract of 100 Mg / kg body weight, 6: MS induction with aloe vera extract at 200 mg / kg body weight.

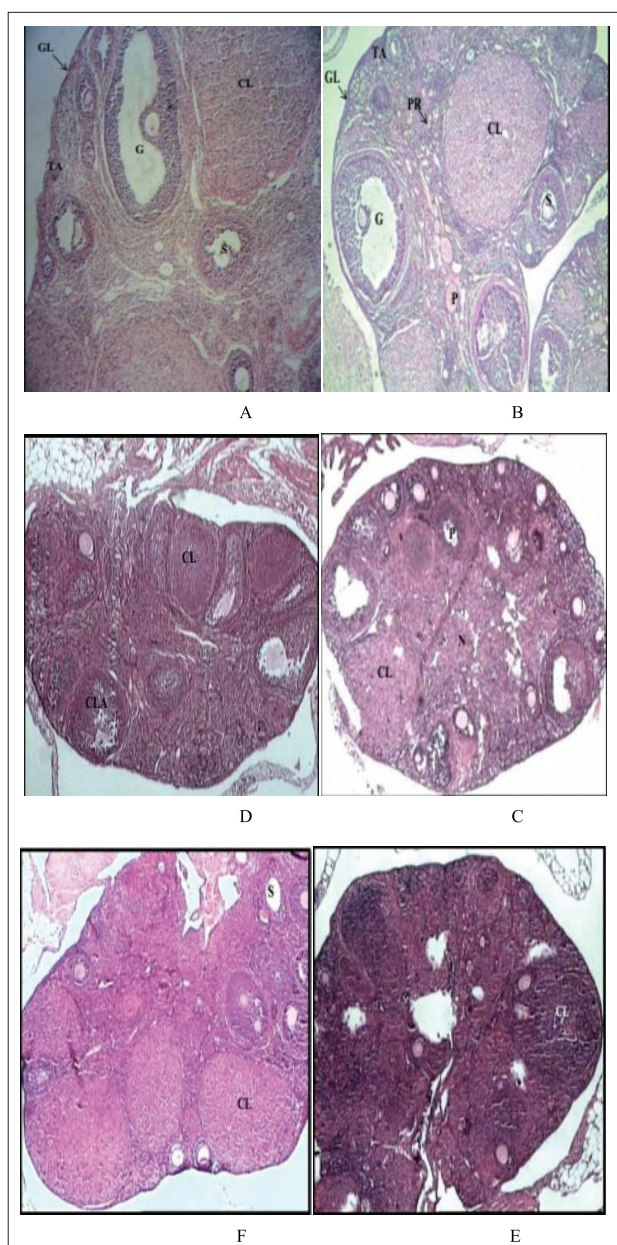


**Fig 9.** Comparison of the number of atretic follicles (mean ± standard error) in the studied groups (non-identical letters indicate a statistically significant difference ( $P \geq 0.05$ )). 1: control 1, 2: control 2, 3: MS induction with normal saline, 4: MS induction with aloe vera extract at 50 mg / kg body weight, 5: MS induction with aloe vera extract of 100 Mg / kg body weight, 6: MS induction with aloe vera extract at 200 mg / kg body weight.

atretic or extinct follicles and the use of aloe vera extract will be beneficial for its improvement. In Figure 10, it has been shown that ovarian tissue has been degraded in ms groups and the number of follicles has decreased, but in the group receiving Aloe vera extract, the ovarian tissue was recovered dose-dependent.

### Discussion:

In the present study, the concentration of LH and FSH hormones in the rats with MS without receiving Aloe Vera extract significantly increased compared with the control group, which is consistent with the results of previous studies. Studies by various researchers have shown that in MS, the concentration of gonadotropin hormones has a direct correlation with the severity of the disease and has a diverse relation with the concentration of estradiol hormone. In patients with MS, there is a peripheral resistance to the gonadotropins in hypothalamic pituitary adrenal gland axis, Which leads to an abnormal increase in the secretion estradiol from the adrenal gland, resulting in an incorrect increase in the secretion of gonadotropins from the pituitary<sup>18</sup>, as well as in the serum of patients with MS, prolactin, LH and FSH concentration is higher than normal people<sup>19</sup>. Increasing FSH due to decreased ovarian secretion inhibitor causes lack of inhibition of FSH



**Fig 10.** A part of the ovary photomicrograph in female rats showed histomorphometric changes in various ovarian follicles, corpus luteum and corpus albicans in control group 1, control group 2, MS induction with normal saline (C), MS Induction with aloe vera extract at 50 mg / kg body weight (D), MS induction of with aloe vera extract at 100 mg / kg body weight (E), MS induction with aloe vera extract of 200 mg / kg body weight (F). (Coloration of hematoxylin and eosin, magnification 72) CL: corpus luteum, PR: initial follicle P: primary follicle, S: secondary follicle, G: follicle graafian, TA: tunica albuginea GL: germ cell, N: tissue necrosis, CLA: extinct corpus luteum.

secretion<sup>20</sup>. On the other hand, in patients with MS, cytokines also cause inflammation in the pathway of the hypothalamus-pituitary-gonadal (HPG) and, as a result, disrupt the pathway of HPG and increase the secretion of LH in MS patients<sup>21</sup>.

Regarding the mentioned issues, one of the ways to treat sexual dysfunction in patients with MS is to reduce gonadotropin hormones that have increased during the disease. In this study, following the use of Aloe vera extract, the concentration of FSH and LH hormones in the rats with MS was significantly decreased compared to control group.

Roberts et al reported that the level of LH in rats exposed to phytoestrogens was reduced<sup>22</sup>. On the other hand, studies on a diet rich in phytoestrogens in humans and animals showed that phytoestrogens inhibited the secretion of luteinizing hormones, and phytoestrogens, with the effect on the hypothalamus and inhibition of the activity of the cell producing the gonadotropin releasing hormone, stopped the hypothalamus-pituitary - Gonadal pathway<sup>23</sup>.

In 2007, Monsefi et al. stated that aloe vera had phytoestrogenic compounds<sup>5</sup>, and also in a study on the effect of aloe vera plant on gonadotropin hormones in matured rats, it was found that the hydroalcoholic extract of this plant had an anti-androgenic effect and It can reduce the parameters of androgen dependent, including secretion of gonadotropins<sup>24</sup>.

Therefore, the results of these studies are consistent with changes in gonadotropin hormones in Aloe vera treated rats in this study. The aloe vera extract, probably due to its phytoestrogenic properties, can inhibit the activity of pituitary- gonads and thus decrease the secretion of gonadotropins.

Regarding the results, it was found that the number of normal ovarian follicles in female rats with MS and without treatment was significantly decreased and the number of atretic or extinct follicles was significantly increased and therefore this finding was consistent with previous studies by the researchers who reported that the reduction of sexual cells and the reduction of puberty of these cells could be due to neurological impairment in MS, as the puberty of the sexual cells is happened by the secretion of various and specific glycoproteins from the epithelial cells caused by neural stimulation. What is certain is MS disease is a condition with an impairment of the nervous system<sup>9,25</sup>. On the other hand, MS causes activation of inflammatory cytokines in other organs and the destruction of neurons and myelin cells in the nerve

tissue<sup>21</sup>. Therefore, in the present study, decreasing the number of ovarian follicles, especially matured follicles and increasing the number of atretic follicles in the rats, can be due to neurological disorders in MS, resulting in reduced glycoproteins and activation of inflammatory cytokines in ovarian tissue.

The present study showed that induction of MS without receiving Aloe Vera extract also significantly reduced the number of corpus albicans compared to the control group, which is consistent with changes in corpus luteum. Also, in this study, Aloe vera extract was effective in improving the number of ovarian follicles, corpus luteum and corpus albicans, especially with the maximum dosage. This finding is consistent with the research by Kosif et al. who stated that the aloe vera extract has a similar effect as the follicular stimulating hormone of the ovary and acts like this hormone, which increases and develops the ovarian follicles<sup>26</sup>.

Another study found that the Aloe vera has a beneficial effect on the reproductive system and ovarian tissue of the rat, and this plant increases folliculogenesis by increasing the vein around the ovarian follicles and ovarian stroma<sup>27</sup>.

The protective effect of Aloe vera on the testis histological changes in male rats has also been reported and stated that Aloe vera increases the number of spermatogonia, spermatocytes, spermatids and sertoli cells significantly<sup>28</sup>. Many previous studies have highlighted the role of phytoestrogens in protecting cells, which stated that phytoestrogenic compounds inhibited the process of apoptosis and thus reduced atretic follicles<sup>29,30</sup>, and also reported that phytoestrogens with their anti-oxidant properties have positive effects on intracellular enzymes, normal form of protein, growth factor and cell proliferation<sup>31,32</sup>. Therefore in this research, it can be concluded that the Aloe vera extract can inhibit apoptosis and reduces atretic follicles and increases ovarian follicles in rats receiving Aloe vera

extract due to its antioxidant properties and having phytoestrogenic compounds which is similar to estradiol formula.

### **Conclusion:**

According to previous researches on the properties of effective ingredients in Aloe Vera, and considering the results of this research, we can express the protective effect of aloe vera gel to prevent changes in gonadotropin and histopathologic ovarian hormones caused by MS disease. Therefore, Aloe vera plant gel extract as a medicinal plant has good potential for improving gonadotropin hormones, sexual cells and fertility in patients with MS that can be attributed to phytoestrogenic properties of this plant.

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### **Declaration of Interests:**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported

### **Author's contribution:**

Data gathering and idea owner of this study: Elham Rahmanian Koshkaki

Study design: Fatemeh Meshkini, Mina Tajali, Amir Ali Rafati

Data gathering: Abolghasem Abasi Sarcheshmeh, Vida Samiei, Farzad Rahmanian Koshkaki

Writing and submitting manuscript: Fatemeh Mohseni, Elham Rahmanian Koshkaki, Mina Tajali

Editing and approval of final draft: Fatemeh Mohseni, Elham Rahmanian Koshkaki

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