Original Article

Protective Effects of Aloe Vera Alcoholic Extract Gel on Changes of Ovarian Hormones in Experimental Model of Multiple Sclerosis

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ABSTRACT

Background: Multiple Sclerosis (MS) is a demyelinating autoimmune inflammatory disorder of central neural system associated with neural disability in child bearing age. Studies show that common medicines in the treatment of MS have side effects especially on the reproduction system. Objectives: This study was conducted to examine the protective effects of Aloe Vera alcoholic extract gel on changes of ovarian hormones in experimental model of MS. Methods: In this experimental study, 30 female Wistar rat were randomly assigned to 6 groups (5 in each group). In 20 rat (4 groups) injections of 14 microliter of Anidiom Bromide in the lateral ventricles of brain were made. The 5 other rats were injected with 14 microliter of saline in the lateral ventricle of the brain, and the remaining 5 rats were considered control group. Then 3 groups of MS-induced rats were treated with the alcoholic extract of Aloe Vera gel in dosage of 50 mg/kg, 100 mg/kg, and 200 mg/kg. Finally, all the rats were anesthetized deeply, and then for measuring the serum density of LH, FSH, estrogen and progesterone hormones, blood was taken from their heart. Result: A meaningful increase in the density of LH and FSH hormones, and a meaningful decrease in the density of estrogen and progesterone in group 3 was observed compared with the control group. Also, the density of the progesterone hormone in group 6 compared with group 3 showed a meaningful increase. Conclusion: This study demonstrates that with induction of MS, there will be a decreases in the density of estrogen and progesterone hormones and an increase in the density of gonadotropin hormones; and treatment with extract of Aloe Vera gel has an improving effect on serum levels of sex hormones.

Keywords: Aloe vera, alcoholic extract, gel, ovarian hormones, experimental model.

Introduction

Multiple Sclerosis (MS) is a central neural system disease that creates neural disability especially in young people [1, 2]. No definite cure is found for this disease due to incomplete knowledge about it. One of the most significant problems created by this disease is that a lot of people suffering from MS are in childbearing age [3]. According to researches, estradiol decreases the size and number of brain injuries in the first stages of MS in pregnant women and increases their immune responses [4]. On the other hand, it has been shown that estradiol and progesterone influence the activity of MS disease, and it is reported that treatment with varying levels of these hormones can be effective in curing MS. Despite their limited to moderate benefits in treating some types of MS, common drugs for the treatment of MS have certain side effects ranging from mild to moderate to severe [5, 6]. This can be deduced even from a cursory look at the side effects of the most important common drugs for controlling MS, such as beta interferons, Glatiramer Estate and steroids. For instance, the most common side effects of interferon are reactions in the injection point, menstrual disorder, muscle stiffness, sweating and hypertension. A study in 2007 showed that a lot of drugs prescribed for MS are dangerous in the periods of pregnancy, breastfeeding and fertility. Today, because curing of MS has not been completely successful, researchers have turned towards complementary and traditional medicine, and are trying to take advantage of all existing potentials [7]. On the other hand, using complementary medicine in diseases such as Parkinson's, epilepsy and cancer has raised the potentiality of this kind of

medicine in curing MS [8]. One of the plants used in herbal medicine is Aloe Vera. Studies show that the extract of this plant not only causes an increase in the estrogen hormone, but

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also causes the growth of follicles with its similar effect on the stimulating follicle hormone (FSH) [9, 10]. It should be noted that the most important chemical compounds of Aloe Vera are poly saccharides, Anthraquinone, Prostaglandins, and Phytoestrogens such as Betasitostrol, cholesterol, and Fatty acids such as campstrol [11]. Therefore, since Aloe Vera has Phytoestrogen compounds and using plants with these compounds causes a decrease in LH and FSH hormones and also since in the MS patients there is usually an increase in FSH hormones, the conductors of this research tried to acquire detailed information about the influence of Aloe Vera gel on the changes in the Ovarian hormones in female flied rat afflicted with MS, assuming that this plant would be effective in curing the disease by decreasing the amount of the hormone.

Materials and Methods

Animals

The present study is conducted with an experimental, laboratory and completely random method. All the ethical principles of working with laboratory animals are respected according to the instructions of animal protection committee of Shiraz University. This research project is approved on December 21, 2015. The research was conducted on 30 mature female field rat of Wistar, weighing 200 ± 15 g, and 100 to 120 days old, acquired from Razi Vaccine and Serum-making Research Center in Shiraz. The rats were kept in the animal house of Faculty of Veterinary Medicine of Shiraz University. In this period, the rats were kept in metal cages with latticed doors and took standard food. Water was also given to them in special glass bottles. The cages were disinfected 3 times a week with 70% alcohol. Matching the Cycle of mature female rat. The rats in this research should have been in the same sex cycle because only mature female field rats in proestros phase, and the rats acquired for the research were in different sex cycles. To match their cycles, vaginal smear was taken from all of them to make sure all of them are in the same sex cycle. In female field rat, a sex cycle takes 4 to 5 days.

The method for induction of disease

MS is one of the chronic inflammatory diseases which destroys central neural system, therefore, chemical destruction of myelin with ethidium bromide (EB) is one of the most common practical models of inducing the disease. Therefore, in the present study, for inducing the disease in the rat in the experiment group, ethidium bromide was injected in lateral ventricles with a two-way method and by Stereotaxic apparatus by the help of Hamilton needle (guide canola 23, dentistry needle 30) [12]. Ethidium bromide was acquired from Merck company in the form of red powder. Salin was used for the solvent.

In order to prepare the alcoholic extract of Aloe Vera, a few fresh leaves of the plant were obtained. After their scientific verification by botanical experts of Shiraz University, they were washed and later their gel was extracted. The gel was placed in 95-degree ethanol in a volume of four times more. After placing the gel container on shaker for 4 days, the solution was cleared by filter and was condensed at 45 degrees by Rotary Evaporator. The resulting material was dried at 40 degrees Celsius and then powdered [13]. The classification of the 30 field rats into 6 groups of 5 was done as follows:

1. Control group: without the induction of MS and without taking any normal saline and Aloe Vera extract
2. Witness group: without the induction of MS, the injection of 14 microliter of saline as a solvent of ethidium bromide to the Lateral ventricle
3. Experiment group 1: induction of MS by the injection of 14 microliter of ethidium bromide solvent to the Lateral ventricle, and taking normal saline using gavage.
4. Experiment group 2: induction of MS by the injection of 14 microliter of ethidium bromide solvent to the Lateral ventricle, and taking of alcoholic extract of Aloe Vera as much as 50 mg/kg B.W using gavage.
5. Experiment group 2: induction of MS by the injection of 14 microliter of ethidium bromide solvent to the Lateral ventricle, and taking of alcoholic extract of Aloe Vera as much as 100 mg/kg B.W using gavage.
6. Experiment group 2: induction of MS by the injection of 14 microliter of ethidium bromide solvent to the Lateral ventricle, and taking of alcoholic extract of Aloe Vera as much as 200 mg/kg B.W using gavage.

It should be noted that Aloe Vera extract was prescribed for 30 days. After this period, all the rats were etherized and blood samples were collected from their hearts in order to measure LH, FSH, estrogen and progesterone hormones. Female sex hormones were measured using animal kits with ELISA method. To compare the groups, one-way analysis of variance (ANOVA) and Tukey test were used with the help of SPSS software, version 18. $P<0.05$ was taken as the significance level.

Results

The results of this study are shown in [charts 1 to 4]. As shown in [charts 1 and 2], the density of LH and FSH hormones in experiment groups 3 (without taking Aloe Vera extract) shows significant increase compared to the control group ($P\geq0.05$). The density of FSH hormone in group 6 (with the highest intake of Aloe Vera extract) and the density of LH hormone in groups 4, 5 and 6 (with the different doses of Aloe Vera intake) show significant decrease compared to the experiment group 3, which are almost similar to control group ($P<0.05$).

The density of estrogen and progesterone hormones in groups 2, 3 and 4 and also the density of progesterone in group 5 shows a significant decrease compared to the control group ($P\geq0.05$). While the density of estrogen in experiment groups 4, 5 and 6 (with different doses of Aloe Vera extract intake) and the density of progesterone in experiment group 6 (with the highest intake of Aloe Vera extract) compared to experiment group 3 (without any intake of Aloe Vera extract) show a significant increase ($P\geq0.05$), they are almost similar to control group [charts 3 and 4].

Therefore, this study demonstrates that by inducing MS, the density of gonadotropin hormone increases and the density of estrogen and progesterone decreases, and using Aloe Vera extract is fairly effective in the recuperation of these changes in hormones.

Discussion

Multiple sclerosis is a chronic disease of the central nervous system with a disorder in sex hormone, Cause decreased sexual performance [14, 15]. In the present research, the density of LH and FSH hormones in the rat with MS without any intake of Aloe Vera extract was significantly higher than the control group, which is in accordance with the previous researches.

Studies conducted by different researches show that in MS the density of gonadotropin hormones has a direct relationship with the severity of the disease, and an inverse relationship with the density of estradiol hormone. In patients with MS, a kind of environmental resistance in hypothalamicpituitary-adrenal axis is observable towards gonadotropins which results in an abnormal increase in the discharge of estradiols from the adrenal and consequently, gonadotropin is discharged wrongly from hypophysis [16]. Also, in the serum of patients with MS, the density of prolactin, LH and FSH is higher than normal people [17].

On the other hand, in patients with MS, cytokines would also cause inflammation in the hypothalamic-pituitary – gonadal path (HPG) and consequently, cause disturbance in this path and an increase in the discharge of LH in these patients [18]. According to the above cases, one of the ways of treating the sexual changes in MS patients is decreasing the gonadotropin hormones which have increased during the illness. In this research, following the use of Aloe Vera extract, the density of LH and FSH hormones in rat induced with MS significantly decreased compared to the control group.

Roberts et al. have reported that the amount of lutein hormone (LH) in field rat which have been subjected to a kind of phytoestrogen has decreased [19]. On the other hand, different studies on the rich diets of phytoestrogen in both humans and animals have shown that phytoestrogen inhibits the discharge of lutein hormones and by its effect on the hypothalamus and inhibiting the activity of cells that produce the hormone which releases gonadotropin, causes a stop in hypothalamic-pituitary – gonadal path [20].

In 2007, Monsefi et al. stated that Aloe Vera has phytoestrogenic compounds [9]. Also it was observed in a study on the effect of Aloe Vera on gonadotropin hormones in mature field rat that the hydro alcoholic extract of this plant has an anti-androgenic effect and can decrease the parameters related to androgen such as the discharge of gonadotropin [21]. Therefore, these researches are consistent with the present research about the changes in gonadotropin hormones in rat which are treated with Aloe Vera extract, and show that probably it is the Aloe Vera extract that with its phytoestrogenic effect, inhibits the pituitary - gonadal activity and consequently, causes a decrease in the discharge of gonadotropins.

In the present research, in rat induced with MS which have not taken any Aloe Vera extract, a decrease in the amount of estrogen and progesterone was observed compared to the control group, which is probably caused by the changes in the ovarian tissue after the induction of MS, and is consistent with the previous researches. On the other hand, the density of progesterone in the group with have undergone surgery but have not received any treatment shows a significant decrease compared to the control group. These changes are also more obvious when compared to the changes in estrogen hormone, which can be a result of research error and also the difference in the stability of these hormones in the serum.

Estrogen and progesterone are discharged from the ovary tissue, and the amount of this discharge is related to the changes in the tissue of the ovary (Guyton, 2006). Grinsted et al. in 1989 showed that in the serum of MS patients, the density of estrogen is significantly lower than normal people [17]. On the other hand, a research shows that MS affects the discharge of estradiol and progesterone, and treatment with varying levels of these hormones can be effective in treating MS [22]. Another study in 2004 shows other mechanisms of sex hormones in preventing the progress of inability in MS patients [23].

In MM patients the decrease in the amount of estrogen and progesterone cause an intensification in the effects of this disease and also infertility; Therefore, using a medical plant which causes an increase in the estrogen and progesterone can be effective in the treatment of the sexual diseases caused by this disease [24]. According to the present research, although the density of estrogen and progesterone in the rat which are induced with MS and are receiving Aloe Vera extract decrease compared to the control group, it shows a significant increase compared to the rat which are induce with MS but do not receive any Aloe Vera extract [25, 26]. Therefore, according to the results, there is a probability that phytoestrogenic material and the antioxidant properties of Aloe Vera extract would cause an increase in these hormones and consequently, cause recuperation in the effects of MS. These results can be consistent with the results acquired by other researchers. As they stated in the study of the effect of Aloe Vera on the changes in female sex hormones that the extract of this plant cause an increase in the estrogen compared to the control group [27]. What is considered significant in the treatment of MS is that the increase in estrogen and progesterone causes a recuperation in MS, and a decrease in these hormones causes an increase in the symptoms of this disease [28].

Telefo et al. in 2004 showed that the extract of the plants which have similar compounds to Aloe Vera cause an increase in the ovarian steroidogenes and also an increase in the serum density of estrogen [29]. Since Aloe Vera extract has similar effects to the effect of follicle stimulating hormone on the ovary, it functions like this hormone and cause an increase in the growth and evolution of follicles, and consequently, an increase in the discharge of sex hormones from follicle cells [21]. It has also been shown that Aloe Vera extract has a biological activity of estrogen formation because of its β- Estradiol compound (Phytoestrogen) [28].

Bansil et al. also stated that the increase of sex hormones of estrogen and progesterone causes a recuperation in MS because they increase the myelin formation by oligodendrocytes and affect CNS [22]. Eventually, it can be stated that phytoestrogens are natural herbal ingredients which have estrogenic effects and are found in some plant such as Aloe Vera. The similarity of the structure of these materials with estrogen has given them the ability to connect to the estrogen receptors and bring about every kind of estrogenic effects [27, 28, 30]. According to previous researches about the characteristics of the effective materials in Aloe Vera, and according to the present research, it can be stated that the extract of Aloe Vera gel has an effect on the prevention of ovarian hormone changes caused by MS. Therefore, the extract of Aloe Vera gel as an herbal medicine has a good potential in the recovery of gonadotropin, estrogen and progesterone hormones and also fertility in MS patients, which can be attributed to phytoestrogenic properties in the plant.

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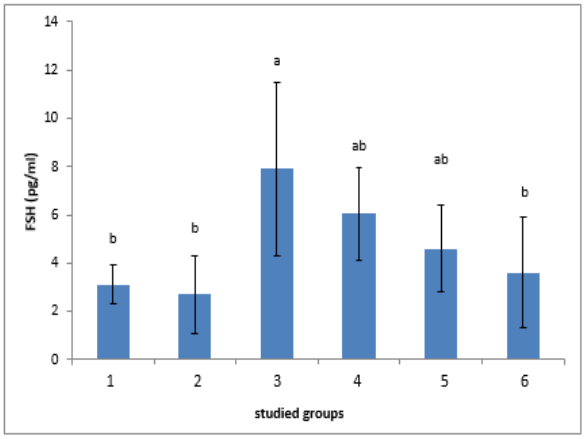

Chart 1: The comparison of serum density of FSH (average ± the standard error) in groups under study

(non-similar letters show significant statistical difference (P≥0.05).)

(1) control, (2) witness, (3) inducing MS with normal saline intake, (4) inducing MS with the intake of alcoholic Aloe Vera extract as much as 50 milligrams per one kilogram of the body weight, (5) inducing MS with the intake of alcoholic Aloe Vera extract as much as 100 milligrams per one kilogram of the body weight, (6) inducing MS with the intake of alcoholic Aloe Vera extract as much as 200 milligrams per one kilogram of the body weight.

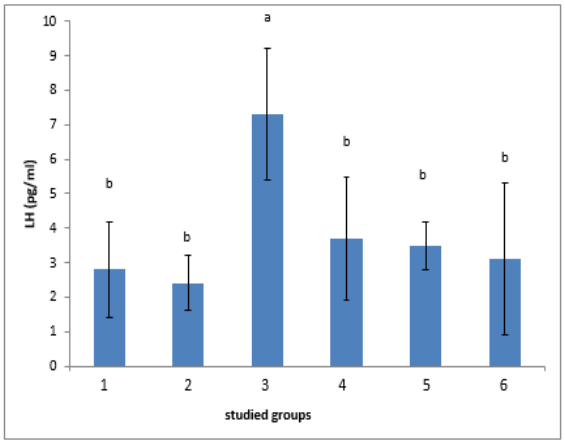

Chart 2: The comparison of the amount of serum density of LH hormone (average ± the standard error) in groups under study

(non-similar letters show significant statistical difference (P≥0.05).)

(1) control, (2) witness, (3) inducing MS with normal saline intake, (4) inducing MS with the intake of alcoholic Aloe Vera extract as much as 50 milligrams per one kilogram of the body weight, (5) inducing MS with the intake of alcoholic Aloe Vera extract as much as 100 milligrams per one kilogram of the body weight, (6) inducing MS with the intake of alcoholic Aloe Vera extract as much as 200 milligrams per one kilogram of the body weight.

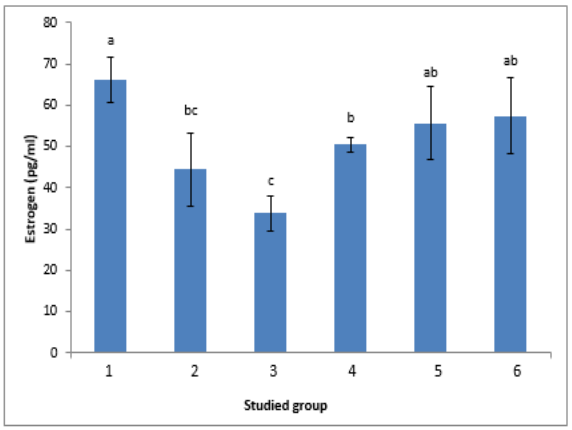

Chart 3: The comparison of the amount of serum density of estrogen hormone (average ± the standard error) in groups under study

(non-similar letters show significant statistical difference (P≥0.05).)

(1) control, (2) witness, (3) inducing MS with normal saline intake, (4) inducing MS with the intake of alcoholic Aloe Vera extract as much as 50 milligrams per one kilogram of the body weight, (5) inducing MS with the intake of alcoholic Aloe Vera extract as much as 100 milligrams per one kilogram of the body weight, (6) inducing MS with the intake of alcoholic Aloe Vera extract as much as 200 milligrams per one kilogram of the body weight.

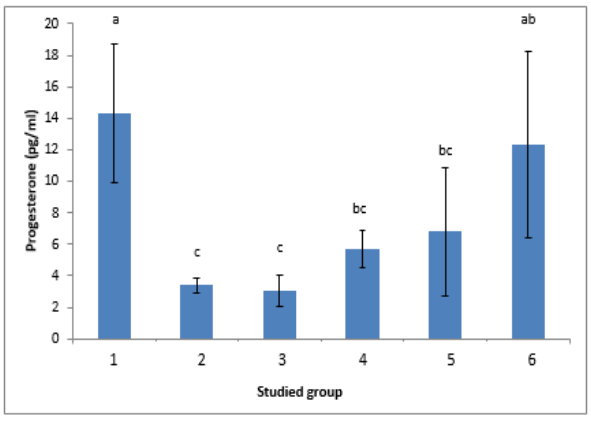

Chart 4: The comparison of the amount of serum density of progesterone hormone (average ± the standard error) in groups under study

(non-similar letters show significant statistical difference (P≥0.05).)

(1) control, (2) witness, (3) inducing MS with normal saline intake, (4) inducing MS with the intake of alcoholic Aloe Vera extract as much as 50 milligrams per one kilogram of the body weight, (5) inducing MS with the intake of alcoholic Aloe Vera extract as much as 100 milligrams per one kilogram of the body weight, (6) inducing MS with the intake of alcoholic Aloe Vera extract as much as 200 milligrams per one kilogram of the body weight.