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Effects of aloe vera gel on the induction of endometriosis and regression of endometrial explants in a rat model

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Summary

Purpose of investigation: To evaluate the preventive and reducing effect of aloe vera gel on surgically-induced endometrial foci in rats. *Materials and Methods:* Twenty-four reproductive aged female non-pregnant, nulligravid Sprague-Dawley albino rats were used. The rats were randomly divided to three groups (Group 1: control, Group 2: aloe vera endometriosis formation, and Group 3: aloe vera endometriosis treatment). A peritoneal lavage using one-ml saline was taken at all the operations for determination of superoxide dismutase (SOD), malondialdehyde (MDA), and catalase (CAT). Forty-eight horns were implanted in 24 rats. *Results:* All the implants were properly formed after implantation. In Group 3, before aloe vera application, the sum of the volumes was 87.2 ± 20.4 mm^3 and after treatment the volumes dropped to 28.9 ± 14.9 mm^3 ($p = 0.01$). As evaluation of aloe vera on the formation of endometriosis in the second operation in Group 2, the sum of the volumes was 2.9±1.4 mm^3 and in Group 1, 118.9 ± 20.0 mm^3 ($p = 0.001$). Likewise, similar changes were observed in the histopatological scores. *Conclusion:* The application of aloe vera was seen to raise antioxidant levels in the peritoneal fluid and to reduce oxidative stress markers. Aloe vera is effective in the inhibition of formation and regression of endometriotic lesions.

Key words: Aloe vera; Endometriosis; Oxidative stress.

Introduction

Approximately 60% of all women of reproductive age are affected by endometriosis, which is defined as the presence of endometrial tissue outside the endometrium and the myometrium [1]. The exact etiology of endometriosis has not been fully clarified but it has been suggested that reactive oxygen species (ROS) may increase the implantation, growth, and adhesion of endometrial cells in the peritoneum [2]. Oxidative stress occurs when there is an imbalance in the oxidant-antioxiant system, either through over-production of ROS or a decrease in the antioxidant defense. Following activation of immune cells, especially polymorphonuclear leucocytes and macrophages, the production of ROS is known to increase [3]. Superoxide anion (O_2^-) appears to be of importance in ROS. Superoxide dismutase (SOD) rapidly decomposes superoxide anion into hydrogen peroxide and oxygen and catalase (CAT) is another enzyme involved in the detoxification of hydrogen peroxide (H_2O_2), a molecule in ROS [4]. It has been extensively reported that Malondialdehyde (MDA) can be used to estimate the effect of oxidative stress on lipids [5]. Oxidative stress has been reported to play an important role in the development and progression of endometriosis [6]. In women with endometriosis, evidence of oxidative stress has been observed in the peritoneal cavity and ectopic tissue. The peritoneal fluid of endometriosis patients has been found to contain high concentrations of lipid peroxidation products [7].

Aloe vera (synonym: Aloe barbedensis Miller) is a plant with yellow flowers and triangular leaves, similar to cactus, belonging to the Liliaceal family, which comprises 360 species [8]. The plant leaves contain abundant amounts of mucilaginous fluid of high viscosity, known as aloe vera gel [8]. Various reports have shown that aloe vera gel stimulates wound-healing and skin hydration, induces hematopoiesis, and possesses anti-diabetic, anti-carcinogenic, antimicrobial, anti-oxidant, and anti-inflammatory properties [9, 10]. The antioxidant properties of aloe vera render it suitable for use in conditions of enhanced oxidative stress which may have significant implications for the prevention of diseases [11].

This study aimed to evaluate the effect of aloe vera gel on endometriotic implants in a rat endometriosis model. An investigation was made of the effect of aloe vera gel on the formation and regression of endometriosis.

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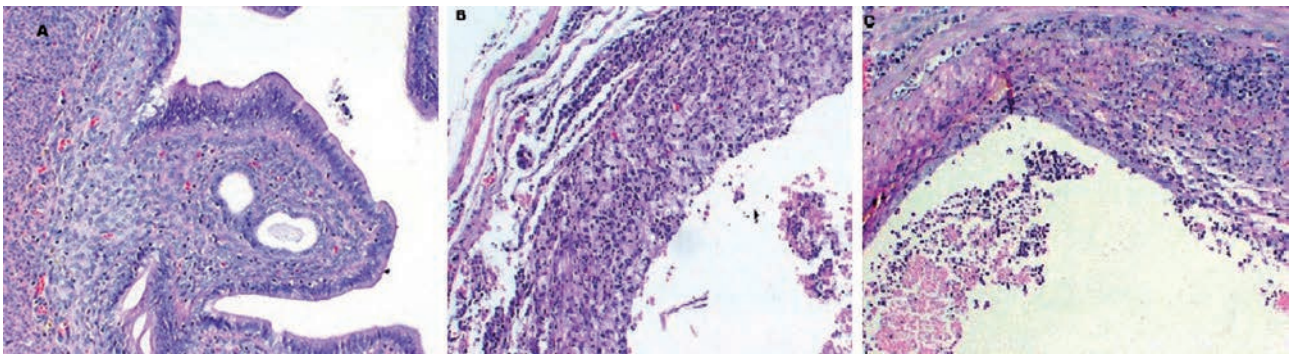

Figure 1. — A) Histopathological evaluation of endometriotic implants stained with hematoxylin and eosin stain, original magnification: ×200 (endometriosis control group second operation). B) Impaired endometriosis formation, epithelial layer intermediately protected with leukocytes infiltrating (aloe vera formation group second operation). C) The prominent loss of epithelium in the neighborhood of endometriotic foci stained with hematoxylin and eosin stain, original magnification: ×200. (Aloe Vera treatment group third operation).

Materials and Methods

Animal model

Approval for the study was granted by the Local Ethics Committee for Experimental Animals and was performed at the Experimental Animal Production and Research Laboratory of Yeditepe University (YUDETAM), Yeditepe University, Turkey. All experiments were performed in compliance with international guidelines on the ethical use of animals.

Twenty-four reproductive aged female non-pregnant, nulligravid Sprague-Dawley albino rats weighing 200-250 grams bred at YUDETAM were used in this study. The animals were fed ad libitum and housed in pairs in steel cages in a temperature-controlled environment (22°±2°C) with 12-hour light–dark cycles.

Experimental design and surgical procedures

The rats were randomized into three groups. Group 1 was the endometriosis control group (n=8), Group 2 was the aloe vera endometriosis formation group (n=8), and Group 3 was the aloe vera endometriosis treatment group (n=8).

The induction of endometriosis was performed in the first operation for all three groups. In the first operation, 0.1 ml of aloe vera gel was applied to the implantation sites of Group 2. After two weeks of estradiol treatment, the second operations were performed and 0.1 ml of aloe vera gel was applied to the implantation sites of Group 3. The third operations were performed for assessment of the effects of aloe vera on the endometriotic foci regression (Group 3) after two weeks of estradiol treatment following the second operation.

First operation: endometriosis induction

All the rats were anesthetized with an intramuscular administration of 60 mg/kg ketamine hydrochloride with seven mg/kg xylazine hydrochloride. Endometriosis was surgically induced using the method described by Vernon and Wilson [12]. After the administration of general anesthesia, the abdominal cavity was opened using a vertical incision. A peritoneal lavage using one-ml saline was taken. The uterine horns were ligated at the uterotubal junction and the cervical end was subsequently removed. The uterine horns were placed in phosphate-buffered saline at 37°C. Four pieces of graft measuring 6 x 3 x 1 mm were made by division of the uterine horns. Without removing the myometrium, two of these pieces were implanted onto the peritoneal surface of the right abdominal wall so that the endometrium was in contact with the peritoneal surface. Both ends of the implants were secured with non-absorbable polypropylene 6-0 suture to the inner part of the abdominal wall. The remaining two pieces were placed in the left inner part of the abdominal wall with same methodology. All tissues were implanted just opposite both vascular bifurcations. In Group 2 rats, the areas of implantations were covered with 0.1 ml aloe vera gel. In Group 1 and 3 rats, the areas of implantations were covered with 0.1 ml saline solution. The midline abdominal incision was closed with the continuous suture technique using 3-0 vicryl sutures. All rats were given 50 mg/kg/day cefazolin sodium intramuscularly for three days after the operation, to prevent any intraperitoneal infection. All rats were given 50 µg/kg estradiol twice a week intramuscularly until the second operation.

Second operation: assessment of the endometriotic foci and the effect of aloe vera on the formation of endometriosis

Two weeks after the first operation, the second one was performed to assess the endometriotic lesions for all groups. Exogenous high-dose estrogen created a hyper-estrogenic state and resulted in well-defined endometriotic lesions. The second operations were performed using the aforementioned methodologies. A peritoneal lavage using one-ml saline was applied. Before the endometriotic lesions were biopsied, all the implants were measured in three dimensions (length - width - height in millimeters) with a ruler by the same author.

For histopathological analysis, one of the four implants was removed using a randomization table. In Group 3 rats, the areas of implantations were covered with 0.1 ml aloe vera gel. In Group 1 rats, the areas of implantations were covered with 0.1 ml saline solution. Group 2 rats were euthanized under anesthesia and all measurements and tissue collections were performed as described above.

Third operation-necropsy: evaluation of the effects of aloe vera gel

A third laparotomy was performed at the end of week 4 to measure the implant sizes and to perform peritoneal lavage using one-ml saline. The third operations were performed during estrus. All the rats in Groups 1 and 3 were euthanized under anesthesia and all measurements and tissue collections were performed as described above.

Volume analysis

The spherical volume of each ectopic uterine tissue was calculated using the prolate ellipsoid formula: V (mm3) = 0.524 x W x T x L, in which W = width, T = thickness, and L = length (all in millimeters) [13].

Histopathological analysis

All tissue samples were embedded in paraffin after routine dehydration, and five-mm thick sections were prepared via microtome. The paraffin-embedded sections from each autograft were stained with hematoxylin and eosin (HE) and semi-quantitatively examined under a light microscope (Figure 1). The persistence of epithelial cells in endometrial implants was scored as follows: no epithelium, 0; poorly preserved epithelium, 1; moderately preserved epithelium with leukocyte infiltration, 2; and well-preserved epithelial layer, 3 [14]. All histological chemical measurements were performed by the same histologist who was blinded to the treatment groups.

Biochemical analysis

All biochemical analyses were made on the peritoneal lavage samples which were taken during the surgical procedures.

MDA determination

For determination, 500 µl of peritoneal fluid sample was added to 750 µL of 440 mM H_3PO_4 and 250 µL of 42 mM and thiobarbutiric acid (TBA) solution to have a final volume of 1.5 ml. This solution was incubated for one hour at 100°C, and then an aliquot of 500 µl was added to 500 µl of methanol: 1M NaOH (91:9, v:v) mixture. Mixture was centrifugated at 4,000 rpms for five minutes, 30 µl of supernatant was injected to the high-performance liquid chromatography system.

Measurement of fluorescence of the butanol extract was made at an excitation wavelength of 539 nm and emission wavelength of 533 nm. As the standard solution 1,1,3,3 tetraethoxypropane was used and the values were calculated as micromoles per liter.

SOD activity determination

Total (Cu-Zn and Mn) SOD activity was determined according to the method of Durak *et al.* [15]. This method is based, on the inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine/xanthine oxidase system as a superoxide generator. After 1.0 ml ethanol/chloroform mixture (5/3, v/v) was added to the same volume of sample and centrifuged, activity was assessed in the ethanol phase of the supernatant. One unit of SOD definition was made as the enzyme amount, causing 50% inhibition in the NBT reduction rate. Activity was presented as units per liter.

CAT activity determination

For determination of CAT activity, Aebi method was used [16]. The assay principle was based on the determination of the rate constant k (dimension: s-1, κ) of hydrogen peroxide decomposition. The rate constant of the enzyme was determined by measurement of absorbance change per minute, Activities are presented as κ units (rate constant) per liter (κU/L).

Table 1. — *The comparison of endometriotic volumes and histopathological scores between the groups.*

	Endometriosis control (Group 1) (n=8)	AV endometriosis treatment (Group 3) (n=8)	*p*
2nd operation (mean ± SEM)			
Volume (mm^3)	118.9±20.0	87.2±20.4	0.28
Histopathological score	2.1±0.3	2.2±0.3	0.79
3rd operation (mean ± SEM)			
Volume (mm^3)	136.6±21.6	28.9±14.9	0.01
Histopathological score	2.5±0.4	0.8±0.2	0.004

(Test: Mann Whitney U Test)

Statistical analysis

The statistical analysis was performed using SPSS version 11.5. All variables are expressed as mean and standard error. Differences between groups were evaluated by Kruskal–Wallis variance analysis followed by a post hoc Mann–Whitney U-test. A value of $p < 0.05$ was considered statistically significant.

Results

All laparotomy sites were intact and none of the animals had an incisional hernia. No deaths resulted in any of the groups.

Forty-eight horns were implanted in 24 rats. All of the implants were properly formed after implantation. In Group 1 (endometriosis control group) and Group 3 (aloe vera endometriosis treatment), there was no significant difference between endometriotic foci and lesions were well vascularized at the second operation (Table 1). There was no statistically significant difference between the two groups ($p = 0.28$). Histopathological scores during this operation were 2.1 ± 0.3 in Group 1 and 2.2 ± 0.3 in Group 2 ($p = 0.79$).

When all the groups were evaluated in the second operation, the mean lesion volumes were 118.9 ± 20.0 mm^3 in Group 1, 87.2 ± 20.4 mm^3 in Group 3, and 2.9 ± 1.4 mm^3 in Group 2 (endometriosis formation group) (Table 2). In the determination of the effect of aloe vera on the formation of the endometriosis, statistically significant differences were determined between endometriotic volumes (between Group 1

Table 2. — *The comparison of endometriotic volumes and histopathological scores between the groups during the second operation.*

	Endometriosis control (Group 1) (n=8)	AV endometriosis treatment (Group 3) (n=8)	AV endometriosis formation (Group 2) (n=8)	*p*
2nd operation (mean ± SEM)				
Volume (mm^3)	118.9 ± 20.0	87.2 ± 20.4	2,9 ± 1.4	0.001*
Histopathological Score	2.1 ± 0.3	2.2 ± 0.3	0.8 ± 0.3	0.01**

2nd operation (Kruskal Wallis Test, Post Hoc Mann Whitney U Test);

* Between Group 1 and Group 3: $p = 0.19$, between Group 1 and Group 2: $p = 0.001$ and between Group 2 and Group 3: $p = 0.002$)\;

** Between Group 1 and Group 3: $p = 0.80$, between Group 1 and Group 2: $p = 0.01$, and between Group 2 and Group 3: $p = 0.008$).

Table 3. — *The mean volume of endometriotic lesions and histopathological scores within the groups.*

	2nd operation (mean ± SEM)	3rd operation (mean ± SEM)	*p*
Group 1 (n=8)			
Volume (mm^3)	118.9 ± 20.0	136.6 ± 21.6	0.04
Histopathological score	2.1 ± 0.3	2.5 ± 0.4	0.47
Group 3 (n=8)			
Volume (mm^3)	87.2 ± 20.4	28.9 ± 14.9	0.01
Histopathological score	2.2 ± 0.3	0.8 ± 0.2	0.04

(Wilcoxon Test)

and Group 2: $p = 0.001$ and between Group 2 and Group 3: $p = 0.002$) and histopathological scores (between Group 1 and Group 2: $p = 0.01$, and between Group 2 and Group 3: $p = 0.008$).

At the third operation, mean lesions volumes were 28.9 ± 14.9 mm^3 in Group 3 and 136.6 ± 21.6 mm^3 in Group 1. The difference between the two groups was significant ($p = 0.01$). Histopathological scores during this operation were 2.5 ± 0.3 in Group 1 and 0.8 ± 0.2 in Group 3 ($p = 0.004$).

In Group 1, pre-treatment volume was 118.9 ± 20.5 mm^3 and post-treatment volume was 136.6 ± 21.6 mm^3. A slight increase was observed in the lesions ($p = 0.04$). The changes in histopathological scores were not statistically significant ($p = 0.47$) (Table 3).

In Group 3, before aloe vera implantation, the sum of the volumes was 87.4 ± 20.4 mm^3 and after aloe vera implementation this dropped dramatically to 28.9 ± 14.9 mm^3 ($p = 0.04$). Likewise, similar changes were observed in the histopathological scores, which were 2.2 ± 0.3 and 0.8 ± 0.2 before and after aloe vera treatment, respectively ($p = 0.04$) (Table 3).

At the first operation, the values MDA were as follows: Group 1 0.44 ± 0.22 mmol/L, Group 2 0.43 ± 0.09 mmol/L, and Group 3 0.49 ± 0.08 mmol/L with no statistically significant difference between the groups. In Group 1, MDA increased to 0.65±0.12 mmol/L ($p = 0.001$) at the second operation. The MDA levels of Group 1 were 0.63 ± 0.09 mmol/L at the third operation, but no statistically significant changes were observed between the second and third operations ($p = 0.20$). In Group 2, the values of MDA slightly increased to 0.46 ± 0.20 mmol/L at the second operation but this was not statistically significant ($p = 0.56$). When the values MDA of Groups 1 and 2 were compared, the difference was statistically significant at the second operation ($p = 0.001$). In Group 3, the MDA levels statistically significantly increased at the second operation ($p = 0.001$) and decreased at the third one ($p = 0.02$).

If the SOD and CAT levels were taken into account at the first operation, the values were as follows: Group 1 64.5 ± 2.25 U/L, 2,149 ± 13 U/L, Group 2 67.05 ± 1.15 U/L, 2, 228 ± 54 U/L, and Group 3 65.4 ± 4.5 U/L, 2,154 ± 46 U/L with no statistically significant difference between the groups. In Group 1, SOD and CAT levels decreased to 54.1 ± 2.94 U/L, 2,010 ± 24 U/L ($p = 0.01$, $p = 0.01$) at the second operation but there were no statistically significant changes between the second and third operations ($p = 0.74$, $p = 0.82$). In Group 2 the levels of the SOD and CAT increased significantly to 86.97 ± 1.97 U/L, 2,978 ± 183 U/L, respectively, at the second operation ($p = 0.001$, $p = 0.001$).

In Group 3, the values of SOD and CAT significantly decreased to 52.34 ± 7.4 U/L, 1,997 ± 143 U/L, at the second operation ($p = 0.001$, $p = 0.001$). The SOD and CAT levels significantly increased to 74.17 ± 5.97 U/L, 2,478 ± 156 U/L at the last operation when compared to the second one ($p = 0.001$, $p = 0.001$).

Discussion

One of the most enigmatic and problematic diseases affecting women of reproductive age is endometriosis, for which there is no ideal medical treatment as yet. Oxidative stress occurs when there is an imbalance in the oxidant-antioxidant system as a result of either over-production of ROS or a reduction in antioxidant defence [17].

With a profound alteration of ROS detoxification pathways, endometriotic cells display high endogenous oxidative stress. In stromal endometriotic cells, the O_2^- level has been shown to significantly increase and is produced by the cytosolic NAD(P)H oxidase [18].

In the metastatic potential of tumor cells, ROS play a role in the proliferation and control of tumor growth [19]. Just as in tumor cells, the increased production of endogenous ROS is associated with an increase in the proliferation rate in endometriotic cells. The oxidative stress regulation in endometriotic cells is also close to that described in tumor cells [19]. Therefore as in tumor cells, a significant decrease in intracellular H_2O_2 concentration inhibits the proliferation of endometriotic cells both in vitro and in vivo [19].

In endometriosis patients, local tissue destruction and disease aggressiveness may be caused by oxidative stress [20]. Aloe vera possesses many pharmacological properties, including anti-inflammatory, immune-stimulant, wound healing, and antitumor, which could be involved in the mediation of ROS levels [21]. It has been claimed that aloe vera protects against pro-oxidant-induced membrane and cellular damage [22].

Altincik *et al.* reported no toxic effects from the application of aloe vera gel to peritoneal surfaces and in a rat peritonitis model also demonstrated an anti-oxidant and anti-inflammatory effect of aloe vera [22].

In the current study, endometriosis formation was prevented by the application of aloe vera during the endometriosis implantation period. In a study by Kang *et al.*, it was shown that aloe vera decreased the oxidative stress markers in rats with oxidative stress [23].

After the formation of endometriosis based on an evaluation of the application of aloe vera, endometriotic foci were observed to have declined two weeks later, which was shown

by the significant decrease in volume and the histopathological scores compared to the control group.

At the end of the second operation, examination of the peritoneal fluid of the rats in the two endometriosis formation groups (Groups 1 and 3) revealed higher oxidative stress markers and lower anti-oxidative markers, which was consistent with the endometriosis hypothesis. However, at the end of the third operation, the oxidative stress markers in Group 3 were seen to have significantly decreased. The oxidative stress marker and anti-oxidative stress marker values in the control group remained at relatively unchanged levels at the third operation. This suggests that aloe vera not only prevents the formation of endometriosis, but also prevents the progression of the endometriosis due to oxidative stress.

In a study investigating the effect of aloe vera on peritoneal trauma, fewer peritoneal adhesions were seen to have formed following the pre-trauma application of aloe vera [24]. This effect was thought to have resulted from the anti-inflammatory properties of aloe vera. In the current study group, both in the formation of endometriosis (Group 2) and the endometriosis treatment group (Group 3), the application of aloe vera was found to be effective, which suggests that it enabled the endometriosis via the anti-oxidative pathway. Oxidative stress is known to play a role in the pathogenesis of development and progression of endometriosis [19].

Conclusion

The results of this study have shown that aloe vera gel is effective in inhibiting the formation of endometriotic lesions and in their regression. This effect of aloe vera is thought to be a result of reducing oxidative stress markers and increasing anti-oxidative stress markers, which are known to play a role in the pathogenesis of endometriosis.

Further experimental studies are required in different animal models using other methodologies and doses to confirm the safety and efficacy of aloe vera gel in the prevention and treatment of endometriosis.

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