

IMMUNOLOGICAL AND HISTOPATHOLOGICAL CHANGES AFTER TOPICAL APPLICATION OF ALOE VERA LEAF EXTRACT DURING VENTRAL HERNIOPLASTY IN RAMS

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ABSTRACT

The implication of *Aloe vera* was determined using histopathological and immunohistochemical examinations. Eighteen adult rams were randomly assigned to two equal groups. Under sedation and local anaesthesia, ventrolateral abdominal wall hernias were induced and left untreated for one month. Subsequently, hernioplasty was performed using polypropylene mesh. The animals were categorized into two groups, the control group and the *Aloe vera* gel-treated group. Clinical, immunohistochemical, and histopathological were investigated during the days 7, 30, and 45 post-treatment. The results of clinical monitoring represented unspecified secondary health problems in all animals with no signs of recurrence or rejection. Immunohistology results indicated strong positive expression of IL-6 in both groups, but its expression declined at the next 45 days in the *Aloe vera* G. VEGF expression at 7 days indicated a negative reaction, whereas at 15 to 45 days indicated a positive expression close to the mesh. The histopathological assessment indicated an early improved healing process in *Aloe vera* G, which was represented by the presence of a high number of new blood vessels and collagen deposition compared to the control group. The results of the inflammatory score indicated responses between the highest score in the control group and less degree in the *Aloe vera* group; accompanied by the highest granulation tissue in the control group but a high number in new vessels in the *Aloe vera* group. In conclusion, using *Aloe vera* gel prevented infection and abscessation and enhanced the healing process. The histopathological and immunohistochemistry changes emphasize this outcome.

Keywords: *Aloe vera*, Hernioplasty, Histopathological, Immunohistochemistry.

INTRODUCTION

Hernias treatment is considered the most common surgical correction, which is applied for repairing the defective

abdominal muscle wall. There is no perfect method or implant used for every procedure, thus it is still a crucial challenge for the successful repairing and prevention of recurrent hernias. Ventral hernia caused by a significant abdominal wall muscle defect, which constitutes a real clinical problem, especially in ruminants, needs surgical reconstitution (Haidar *et al.*, 2023). Tension sewing to close and approximate the defect runs the risk of causing infection, recurrent hernias, wound dehiscence, and

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delayed wound healing (Subburaj, 2023). The hernial ring size that exceeds 3 cm in diameter mandates surgical correction using prosthetic material for hernioplasty procedure by using polypropylene mesh (Mitura *et al.*, 2021; Zedan *et al.*, 2022). The implantation of mesh was performed in several methods including inlay and sublay techniques. In the sublay technique, the mesh is implanted below the repaired muscle, which is known as sub-lay implantation. This could be between the back of the peritoneum and the rectus muscle or between the rectus sheath and the muscle sheath or muscle of the posterior rectus (preperitoneal) (Messer and Rosen 2023).

Aloe Vera is a plant, which clinically utilized for various therapeutic applications. It naturally contains a variety of valuable chemicals with the potential to be used in the treatment of numerous ailments. It is used as an antifungal and antibacterial bioactive material against different pathogenic fungi and bacteria (Danish *et al.*, 2020). It has beneficial value, including those as oxidative agents against diabetes, cancer, bacteria, hypertension, lipid-lowering, inflammation, immunological control, liver protection, viruses, and ulcers (Ogidi *et al.*, 2023). It is concerned with bioactive material, which increases the expression IL-6 gene similar to other bioactive materials material as hyaluronic acid (Mohammed *et al.*, 2022), so this study is designed to analyze the role of adding *Aloe vera* leaf extract during ventral hernioplasty in rams to improve the healing process and to reduce infection and adhesion.

MATERIALS AND METHODS

Eighteen clinically healthy adult Iranian breed rams with a mean (\pm SE) weight of 45 ± 0.7 kg and mean (\pm SE) age of 16 ± 0.8 months were included in this study. All experimental animals were in the same state of accommodation and feeding. This

experiment was approved by the Ethics Committee of the Veterinary Medicine College / University of Mosul No: UM.VET.2021.055.

A protocol of local anaesthesia via an inverted (L) infiltration using 2% lidocaine HCl (Johnlee Pharmaceuticals, India) at 3.4 mg/kg B.Wt. (Simpson *et al.*, 2022). A large abdominal defect of 10 cm was induced to create a hernia. The hernia was left untreated for one month, postoperatively. The hernia was then repaired by using polypropylene mesh in a modified sublay suture technique.

The *Aloe vera* gel was prepared by using fresh *Aloe vera* leaf at the plant's base and was cut off before a fresh *Aloe vera* leaf was plucked. It was then thoroughly cleaned, free of any dirt, and set upright in a cup or bowl for ten to fifteen minutes. This enables the yellow resin to come loose from the leaf. The latex found in the resin has the potential to irritate the tissues. Any remaining resin on the paper was washed off after the resin had fully dried, and the thick layer was removed using a small knife or vegetable peeler. When the leaf was removed, the pure natural *Aloe vera* gel became visible. The gel was then scooped and added to the blender with a small spatula. The gel blended until became a foamy liquid (Pattnaik *et al.*, 2022). The gel becomes ready for local application at the borders of the repaired hernia (Figure 1).

Tissue processing and sectioning

The specimen was collected at 7-, 15-, and 45 days post-surgery under the same protocol of anesthesia for surgery. The collected specimen was fixed in 10% neutral buffered formalin, later these samples were washed in running tap water for one hour, the specimen was put in paraffin to get a paraffin block, then sliced by rotary microtome and transferred to the water bath, then transferred to a labeled glass slide, and dried overnight then dried for 60 min. Slides were then

stained by routine stain hematoxylin and eosin (Luna, 1968).

Immunohistochemistry was done according to (Post *et al.*, 2016) for interleukin-6 (IL-6) and vascular endothelial growth factors (VEGF-A) expression (Maae *et al.*, 2011). Histopathological and immunological changes are scored to provide easily

understandable numerical data that can be used in statistical analyses to compare groups. The elements in Table 1 (Yurtçu *et al.*, 2011) are explained. The healing process that had the highest scores indicated an optimized one, with features like maturation granulation tissue formation, newly formed blood vessels, collagen fiber deposition, and the expression of IHC markers like IL-6 and VEGF-A.



Figure 1: Intraoperative image shows the adding *Aloe vera* gel at the borders of repaired hernia.

RESULTS

Following surgical induction of hernia, all experimental rams underwent clinical and physical examinations, which revealed minor, non-specific secondary health problems such as lethargy, moderate depression, mild reduction in activity, and partial anorexia. The area of operation displayed the classic inflammatory signs such as swelling, redness, heat, and pain. The surgical wounds healed without complications except for signs of seroma in 2 animals of first G. which subsided during 20-30 days, post-surgery. There were local abscesses and inflammatory signs in 3 animals of first G., the abscess was observed at the fold created by the polypropylene mesh, which was located directly above the implanted mesh. At the same time, there were no inflammatory reactions or abscess formation in *Aloe vera* G. No signs of recurrence or rejection of mesh were seen in any operative ram. Regarding this experiment, it was indicated that the using polypropylene mesh, which was fixed by using a modified sublay technique reduced the incidence of hernia recurrence.

The histopathological results of the 7th-day post-hernioplasty in Control G and *Aloe vera* indicated the presence of space represented the remaining surgical mesh at the site of insertion. These vacuoles are surrounded by hyperplasia of fibroblasts in addition to the initial deposition of collagen fibers as crude bundles surrounding these vacuoles, which are flooded by granulation tissues that contain activated fibroblast, with infiltration of mononuclear inflammatory cells especially macrophages and lymphocytes (Fig. 2A , B).

At 15 days post-surgery, in first G and second G (*Aloe vera* G), the site of operation infiltrated with focal mononuclear cells around the polypropylene mesh, also the result exhibited the presence of collagen fibers deposition, and the presence of granulation tissue (Fig. 2D and E). One month after the operation in the first G, there was fibrocyte hyperplasia. The mesh became infiltrated by mononuclear inflammatory cells. Edema caused collagen fiber deposition and new blood vessels emerged as granulation tissue. (Fig. 2G). Whereas, in the second group at the same period, the

result revealed infiltration of mononuclear inflammatory cells around the mesh, hemorrhages with the formation of new blood vessels, presence of mature collagen fibres, and granulation tissue formation (Fig. 2H). Forty-five days, in the first group, besides the presence of new blood vessels and

inflammatory cells, there was the maturation of granulation tissue and immature collagen fibers (Fig. 2J). Whereas in the second group, there were mature collagen fibers and granulation tissue in the maturation phase (Fig. 2K).

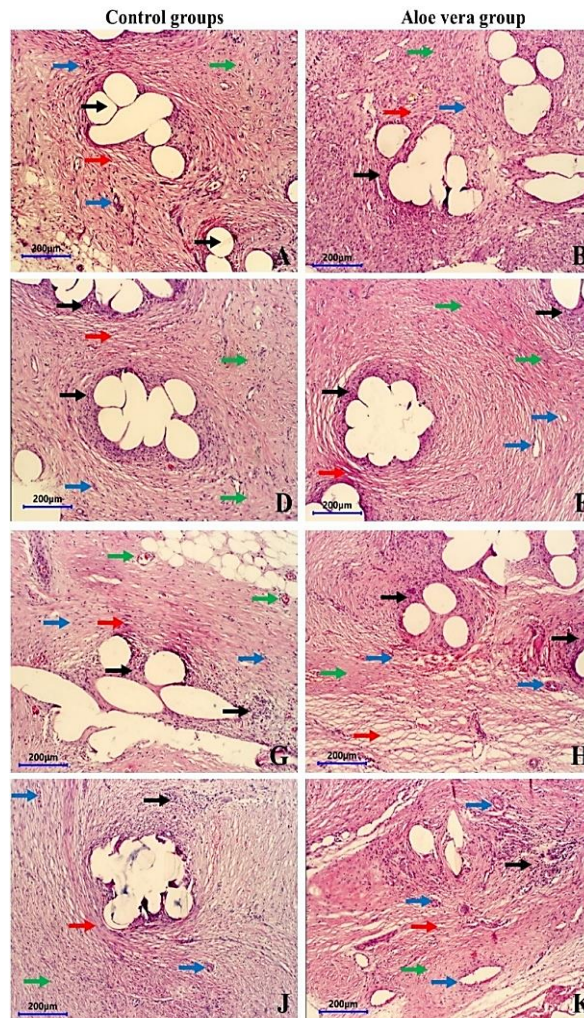


Figure 2: Photographic image, A, B: Control and Aloe Vera group at 7 days exhibited the site of mesh or space (**arrow**), fibrocytes hyperplasia (**arrow**), collagen fiber synthesis (**arrow**), and granulation tissue (**arrow**). Fig. D and E: control and Aloe Vera group at 15 the site of operation infiltrated with focal mononuclear cells around the polypropylene mesh(**arrow**), also the result exhibited the presence of collagen fibers deposition and edema(**arrow**), and the presence of granulation tissue(**arrow**). **Fig. G and H: In control group Aloe Vera group at 30 days** there was fibrocytes hyperplasia. The mesh became infiltrated by mononuclear inflammatory cells(**arrow**). Edema and mature collagen fiber deposition(**arrow**) granulation tissue(**arrow**), new blood vessels(**arrow**). Fig. J: in control group at 45 days indicated the presence of inflammatory cells around the mesh (**arrow**), new vascularization (**arrow**), immature collagen fibers (**arrow**), and granulation tissue (**arrow**). **Fig. K: Aloe Vera group at 45 days** exhibited mononuclear inflammatory cells around the mesh (**arrow**), new vascularization (**arrow**), mature collagen fibers (**arrow**), and granulation tissue in maturation phase (**arrow**). H&E.

Immunohistochemistry results

The results at 7 days post-surgery indicated strong positive expression of IL-6 in both groups. At 45 days the expression declined in the *Aloe vera* group as compared to the control group (Figure 3). The VEGF expression at 7 days recorded a weak expression in both groups, whereas at 15 days the expression was positive in *Aloe vera* G, and in weak positive reaction in first G. At 45

days the VEGF expression was positive in both groups *Aloe vera* and first G. The results in the 7th and 15th-day post-operation showed that the most prominent inflammatory response was recorded in *Aloe vera* G compared to the first G, while in 30th, and 45th days post-operation, the inflammatory reaction was significantly ($P<0.05$) high in the control group compared to *Aloe vera* group (Table 1).

Table 1: Immunohistochemical feature scores (mean \pm st) of hernia sites in control and *Aloe vera* groups of rams (n=18) at 7,15,30 and 45 days after surgery.

Feature	Group	7 days	15 days	30 days	45 days
Inflammatory reaction	Control	0.87 \pm 0.01 C	2.08 \pm 0.02 C	3.18 \pm 0.01 A	1.72 \pm 0.02 A
	<i>Aloe vera</i>	1.00 \pm 0.02 B	2.57 \pm 0.04 B	2.94 \pm 0.13 B	0.97 \pm 0.04 B
Granulation Tissue Formation	Control	0.89 \pm 0.01 C	1.25 \pm 0.01 C	2.34 \pm 0.02 A	1.97 \pm 0.01 A
	<i>Aloe vera</i>	1.58 \pm 0.01 B	1.98 \pm 0.03 B	2.01 \pm 0.02 B	1.02 \pm 0.01 D
Angiogenesis	Control	1.55 \pm 0.01 C	1.90 \pm 0.01 C	2.21 \pm 0.01 C	2.01 \pm 0.01 A
	<i>Aloe vera</i>	1.67 \pm 0.02 B	2.45 \pm 0.01 B	2.98 \pm 0.02 B	1.51 \pm 0.11 B
Fibrous tissue	Control	0.13 \pm 0.01 C	0.97 \pm 0.02 C	1.10 \pm 0.02 C	0.87 \pm 0.01 A
	<i>Aloe vera</i>	0.32 \pm 0.01 B	1.21 \pm 0.01 B	1.34 \pm 0.11 B	0.77 \pm 0.01 B
Expression of IL-6	Control	1.20 \pm 0.01 C	1.75 \pm 0.01 C	1.92 \pm 0.02 A	1.77 \pm 0.01 A
	<i>Aloe vera</i>	2.82 \pm 0.01 B	2.10 \pm 0.02 B	1.73 \pm 0.02 B	1.02 \pm 0.01 B

Different vertical letters mean the presence of significant differences on the same day within different groups at $P<0.05$.

On the 7th day post-operation, results indicated a high amount of granulation tissue formation in the *Aloe vera* group compared to the control group at the final stage. However, in the 30th and 45th days post-operation, this image was reflected; in which the highest granulation tissue formation was recorded in the control group compared to the *Aloe vera* group significantly at $P<0.05$ (Table 1).

Concerning the new tissue formation, results at the 7th, 15th, and 30th days showed that initial new blood vessels increased in *Aloe vera* and control G during all post-operation days and then decreased during 45 days, post-surgery. Later this criterion was decreased to

the lowest level on the last day of the experiment to end the healing process (Table 1).

On the 7th, 15th, and 30th, 45th days post-operation, the results indicated an increase in fibrous tissue formation in *Aloe vera* compared to the first G (Table 1).

The score expression of IL-6 on the 7th and 15th day post-operation showed an increase in the expression of a pro-inflammatory agent in *Aloe vera* and first G. However, expression of IL-6 decreased significantly ($P<0.05$) during 30 and 45 days in the *Aloe vera* group compared to control at $P<0.05$. (Table 1).

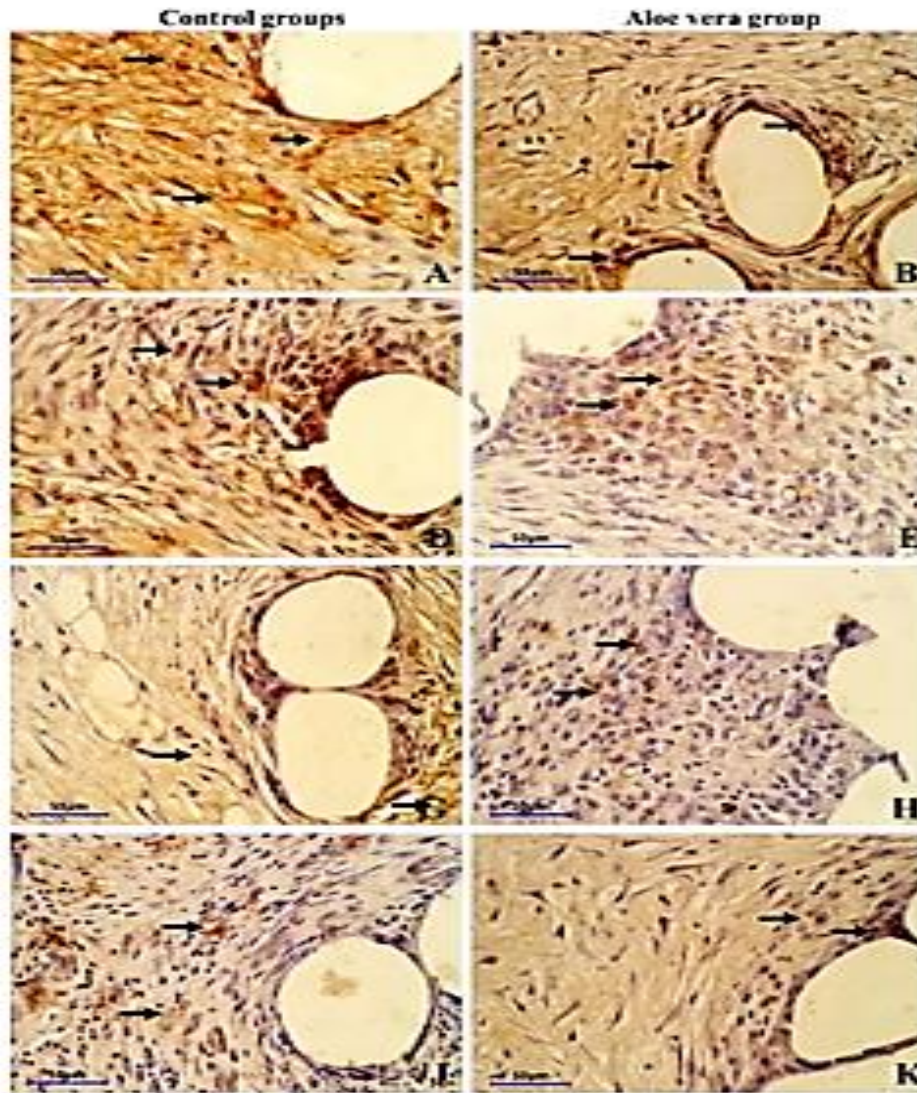


Figure 3: A: In first G, and Figure, B: *Aloe vera* G at 7 days, Figure, D Golden-brown spots in the cytoplasm of cells around the surgical mesh (arrow) suggest a strong positive reaction with IL-6 in the first G and Figure E: *Aloe vera* G at 15 days. Figure H: *Aloe vera* G showed a favourable reaction with IL-6 appearing as golden-brown granules in the cytoplasm of cells surrounding the surgical mesh (arrow) at 30 days, while Figure G: control group showed the same thing. On the 45th day, Figure J shows a positive reaction with IL-6 appearing as golden-brown granules in the cytoplasm of cells surrounding the surgical mesh in the first G (arrow), and Figure K shows a weak positive reaction in a small number of cells in the *Aloe vera* G (arrow). Histology-confirmed IL-6 antibody.

The results of VEGF expression at 7 days negative status in both control and *Aloe vera* groups. (Fig.4, A, B). At the 15 days the expression was positive *Aloe vera* G, and

weak positive reaction in first G Fig.4, D, E). In the 30th and 45 days post-surgery the expression of VEGF in both groups indicated positive expression

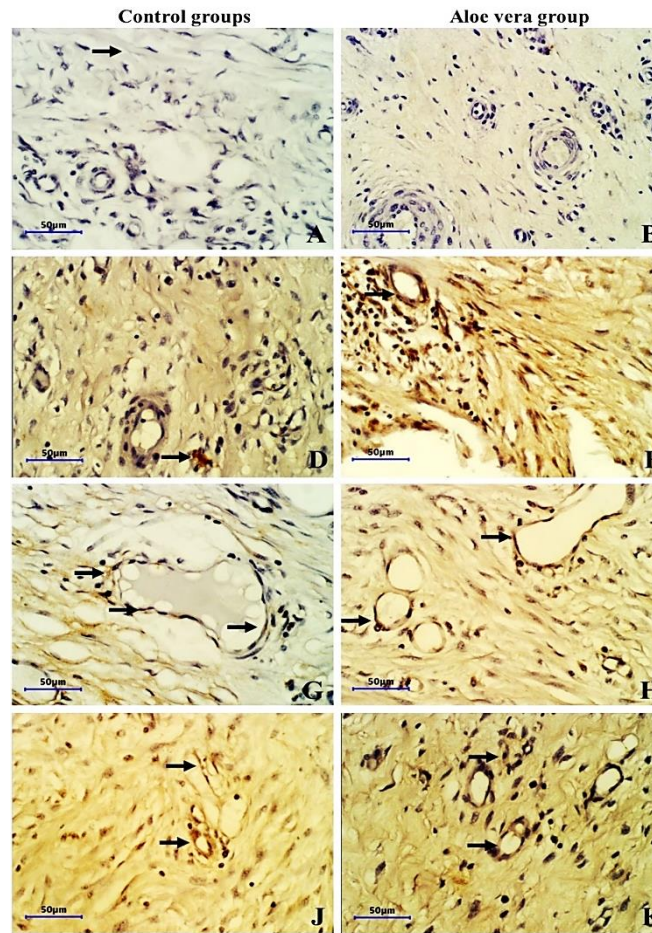


Figure 4: A: In the control group and, B: *Aloe vera* G at 7 days indicated negative reaction with VEGF, B: indicated negative reaction with VEGF, C: Showed strong positive reaction (**arrow**). D: in the first G at 15 days indicated a weak positive reaction E: In *Aloe vera* G at 15 days indicated a positive reaction (**arrow**). G: In the control group and H: In the *Aloe vera* group at 30 days indicated positive reaction (**arrow**). J: In first G and, K: In *Aloe vera* group at 45 days indicated positive reaction with VEGF, in different periods in all groups VEGF reaction appeared as golden-brown granules in the cytoplasm of cells around surgical mesh VEGF antibody IHC.

DISCUSSION

Regarding the obtained outcomes, the components of hernia subsided after using a modified sublay technique in contrast to the results of other studies (Liang *et al.*, 2023; Kitessa *et al.*, 2021) that used another technique of suturing onlay, which associated with recurrence and development of incisional hernia in goat (Eesa *et al.*, 2007) and sheep (Abass 2008).

There were no inflammatory reactions or abscess formation in *Aloe vera* G indicating that this agent has an antibacterial effect in

animals of the treatment G. This effect of *Aloe vera* helped in infection tolerance and decreased the possibility of bacterial contamination at the surgical site and improved wound healing this coincided with (Hekmatpou *et al.*, 2019; Atiyah *et al.*, 2023).

Many studies pointed to the great effect of *Aloe vera* gel as a good medium for wound healing, but the exact mechanism that induces and accelerates wound healing is still unclear (Chelu *et al.*, 2023). Its good angiogenic material has three angiogenic materials β -sitosterol, β -sitosterol glucoside, and aloemodin (Risau, 1997).

The β -sitosterol has a great ability to induce plasminogen activator, which has a direct effect on the VEGF gene by increasing its expression which leads to an increase in endothelial cell proliferation and an increase in the number of new blood vessels (Ismail *et al.*, 2021; Akbarian *et al.*, 2022). In this study, there were no inflammatory reactions and the reepithelization process, where wound healing improved the process as compared to the control group. This finding might be attributed to aloe-emodin which exhibits an anti-inflammatory response alone and with β -sitosterol causing an increase in epithelial cell proliferation, which helps in the reepithelization of wound healing this concerned with (Dong *et al.*, 2020).

Besides, *Aloe vera* contains many bioactive materials such as anthraquinones, hormones, sterols, vitamins, and proteins with organic compounds (Maan *et al.*, 2018). All of these agents represented to have an effect on the IL-6 gene and increase their expression in the site of healing after systemic or general administration (Kistner *et al.*, 2022).

The early and rapid appearance of IL-6 indicated a rapid decrease in its concentration, while post-inflammatory cytokines (IL-12) rapidly elevated in concentration, which will accelerate the formation of granulation tissue, collagen deposition, and maturation. This is because IL-6 plays a crucial role in the healing process during the first three days, and a decrease in IL-6 concentration may lead to an increase in IL-12 concentration in the injured area, which might play a crucial role in the healing process (Johnson *et al.*, 2020).

CONCLUSION

We concluded that using *Aloe vera* gel prevents bacterial infection, and abscess formation, and enhances the healing process of the wounded area. The immunohistochemistry and histopathological changes emphasize these results.

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التغيرات المناعية والنسجية المرضية بعد الاستخدام الموضعي لمستخلص أوراق الصبار أثناء عملية اصلاح الفتق البطني في الكباش

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اعتماداً على التغيرات النسيجية المرضية والمناعية الكيميائية. تم اختيار ثمانية عشر كباشاً بالغاً بشكل عشوائي في مجموعتين متساويتين خضعت حيوانات التجربة لبروتوكول من التسدير العميق والتخدير الموضعي، وتم عمل فتق جدار البطن الجانبي ثم تركه لمدة شهر واحد، ثم تم اصلاح الفتق باستخدام شبكة البولي بروبيلين. اعتبرت المجموعة الأولى كمجموعة ضابطة بدون اي معاملة والمجموعة الثانية مجموعة المعاملة بإضافة جل الصبار المحضر. تم إجراء فحوصات سريرية ومناعية ونسجية مرضية بفترات ٧ و ٣٠ و ٤٥ يوماً بعد العملية. أظهرت النتائج السريرية بعد عملية الفتق بعض العلامات الصحية الغير متخصصة في جميع الحيوانات التي خضعت للعملية حيث لم يسجل وجود علامات على اعاده ظهور الفتق او الرفض في كلتا المجموعتين. دلت نتائج الفحص المناعي النسيجي الى تفاعل قوي للانترلوكين ٦ في كلتا المجموعتين لكن بدا بالانخفاض في اليوم ال ٤٥ في مجموعة هلام الصبار اما عامل النمو لبطانة الوعاء الدموي فكانت النتيجة سالبة في اليوم السابع مع وجود تفاعل ايجابي في اليوم ١٥ وال ٤٥ على شكل بقع قهوانية/ذهبية اللون بالقرب من الشبكة المستخدمة اما نتائج الفحص النسيجي المرضي فقد بينت تحسن عمليات الالتئام في مجموعة هلام الصبار تمثلت بوجود أوعية دموية كثيرة وكولاجين مقارنة بالمجموعة الضابطة. أشارت نتائج تحليل التفاعل الالتهابي إلى استجابة مختلفة بين أعلى تفاعل التهابي في المجموعة الضابطة تليها درجة أقل في مجموعة هلام الصبار وأعلى نسيج حبيبي في المجموعة الضابطة وعدد كبير من الأوعية الجديدة في مجموعة الصبار. وخلاصة ذلك ، فإن استخدام جل الصبار يمنع الخمج وتكوين الخراج ويعزز عملية الشفاء، وتؤكد التغيرات النسيجية والمناعية هذه النتيجة.