




Original Article

Effectiveness of the Aloe Vera extract in the treatment of fistula-in-ano



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ABSTRACT

Rationale: Considering that anal fistulae are still challenging regarding their treatment due to the risk of fecal incontinence in the most complex cases and the increasing use of phytotherapeutic drugs such as *Aloe Vera* in medicine, even with proven healing effectiveness, there is interest in researching this drug in the treatment of anal fistulae.

Objective: To evaluate the efficacy of *Aloe Vera* extract in the treatment of anal fistulae in rats. **Method:** Thirty male Wistar rats weighing 250–300 g were submitted to anal fistula and after 30 days were divided into three groups: Control Group (GCo) (n = 5), Carbopol Group (GCa) (n = 5), and *Aloe Vera* Group (GAV) (n = 10). In the GCo no treatment was performed, while in the others a daily infusion of 0.3 mL of solution (Carbopol only in GCa and Carbopol plus *Aloe Vera* extract in the GAV) was performed through the external orifice of the fistula for 30 days. Afterwards, euthanasia was performed and specimens were removed for histological study. It was evaluated the closure of the fistulous tract, the area of the remaining tract, the inflammatory infiltrate and the degree of vascular congestion. The results were submitted to statistical treatment by Kruskal–Wallis test, considering $p < 0.05$.

Results: There was no complete closure of the fistulous tract in any of the animals. The mean area of the remaining tract was 847.2 μm in the GCo, 565.6 μm in the GCa and 377.8 μm in the GAV ($p < 0.05$). The mean of the inflammatory infiltrate score was 2.4 in the GCo, 2.4 in the GCa and 2.3 in the GAV ($p > 0.05$), while in the evaluation of vascular congestion, we observed a mean of 1.6 in the GCo, 1.4 in GCa and 1.1 in GAV ($p < 0.05$).

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Conclusion: The extract of *Aloe vera* was able to reduce the lumen of the fistulous tract and reduce the degree of vascular congestion; however, it did not allow the complete closure of the fistulous tract nor diminished the inflammatory process.

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Eficácia do extrato de *Aloe Vera* no tratamento da fístula anal

R E S U M O

Palavras-chave:

Atos
Fístula retal
Drogas fitoterapêuticas
Aloe Vera

Racional: Considerando que o tratamento das fístulas anais tem risco de incontinência fecal e o crescente uso do *Aloe Vera* na medicina, há interesse em se pesquisar este fármaco.

Objetivo: Avaliar a eficácia do extrato de *Aloe vera* no tratamento das fístulas anais em ratos.

Método: Utilizou-se 30 ratos Wistar, os quais foram submetidos à criação de fístula anal e após 30 dias distribuídos em três grupos: Controle (GCo), Carbopol (GCa) e *Aloe Vera* (GAV). No GCo nenhum tratamento foi realizado, enquanto nos outros realizou-se infusão diária de 0,3 mL de Carbopol GCa e Carbopol mais extrato de *Aloe Vera* no GAV por 30 dias. Foram retirados os espécimes para estudo histológico, avaliou-se o fechamento do trajeto fistuloso, a área do trajeto remanescente, o infiltrado inflamatório e o grau de congestão vascular.

Resultados: Não houve fechamento completo do trajeto fistuloso em nenhum dos animais. A média da área do trajeto remanescente foi 847,2 μm no GCo; 565,6 μm no GCa e 377,8 μm no GAV ($p < 0,05$). A média do escore de infiltrado inflamatório foi 2,4 no GCo; 2,4 no GCa e 2,3 no GAV ($p > 0,05$), enquanto na avaliação da congestão vascular observou-se média 1,6 no GCo; 1,4 no GCa e 1,1 no GAV ($p < 0,05$).

Conclusão: O extrato de *Aloe Vera* foi capaz de diminuir o lumen dos trajetos fistulosos e reduzir o grau de congestão vascular, porém, não permitiu o fechamento completo dos trajetos fistulosos nem diminuiu o processo inflamatório.

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Introduction

Trans-sphincteric anal fistulas are increasingly common in coloproctology outpatient clinics and its treatment is a major challenge. Classical techniques such as fistulotomy and fistulectomy are not applicable to these cases due to the high risk of incontinence, and even the established mucosal flap advancement technique may cause fecal incontinence in up to 30% of cases.¹ More recently, the Ligation of the Intersphincteric Fistula Tract (LIFT) has emerged as a good option, but it cannot always be applied as it also has cases of failure and relapse.² The use of biological plugs and glue, while very interesting as it causes no incontinence, has yielded poor results and is costly.³ Therefore, despite the various techniques, the current state of treatment of trans-sphincteric fistulas demonstrates that there is still room for research into new options.

The use of plants for anal fistula treatment is not new, particularly in Indian medicine, but it has not been systematically adopted, perhaps due to the lack of publications demonstrating a truly safe and effective treatment in this situation. However, it would probably have the advantage of allowing cure without causing fecal incontinence.

The use of medicinal plants for anal fistula treatment has been increasing mainly in experimental research, but with great potential for use in clinical practice.⁴ There are few

publications in the literature on the use of *Aloe Vera* for anal fistula treatment, but, similar to what has been seen with other herbal medicines, there is also therapeutic potential for this plant. Because anal fistulas are believed to be the chronic phase of cryptoglandular abscess, local infection control may be helpful for treatment.⁵

Aloe Vera has been successfully used for a variety of purposes, particularly as an anti-inflammatory agent. Moreover, it has other properties, such as antibacterial and pro-healing, which would greatly assist the treatment of an anal fistula. Considering that there is little research using this herbal medicine for anal fistula treatment and addressing its real role in these clinical cases, this research aims to evaluate its effectiveness in this situation.

Objective

To evaluate the effectiveness of *Aloe Vera* in the treatment of anal fistulas in rats.

Method

The study design was approved by the Ethics Committee for Animal Experimentation of Anhanguera University – Uniderp.

We evaluated 20 adult male Wistar albino rats, about 300 g each, from the vivarium of the Universidade para o Desenvolvimento do Estado e da Região do Pantanal, where the experiment was conducted, following all ethical standards established by the Brazilian College of Animal Experimentation – COBEA.

For anal fistulas, the rats were anesthetized with intraperitoneal administration of ketamine and xylazine in the same syringe, with the solution containing 2 mL of 10% ketamine and 1 mL of 2% xylazine. For each 100 g of body weight, 0.1 mL of anesthetic solution was infused.

After anesthesia, the anal sphincter was transected with a #1 steel wire, introduced into the pectineal line and exteriorized 1 cm laterally to the right anal margin (Fig. 1A). The wire was cut, twisted and held in place for 30 days (Fig. 1B).

After 30 days, the rats were divided into three groups:

GCo Group (control): consisting of 5 rats, in which the steel wire was removed without therapeutic actions;

GCa Group (carbopol): consisting of 5 rats, in which the steel wire was removed and carbopol was injected into the fistulous tract;

GAV Group (Aloe Vera): consisting of 10 rats, in which the steel wire was removed and carbopol + Aloe Vera solution was injected into the fistulous tract.

In GCo Group, the animals received no treatment and were only observed for a period of 30 days. In GCa and GAV groups, the animals were treated with a daily injection of 0.3 mL of solution through the external fistula orifice using a 21 G plastic catheter for 30 days. In GCa Group the solution contained carbopol and in GAV Group the solution contained Aloe Vera extract plus carbopol.

After 30 days of observation/treatment, the animals received a new intraperitoneal anesthesia and were euthanized by deepening the anesthetic plane, followed by resection of a 2 × 2 cm cube of tissue surrounding the anal canal and the entire pathway from the fistula to the skin containing the external orifice, in order to obtain the entire fistulous tract. Specimens were identified and fixed in 10% buffered formalin. Subsequently, the material was processed in increasing concentrations of alcohol, diaphanized in xylol, and embedded in histological paraffin. Cross sections of 5 µm thickness were made with the aid of a rotating microtome (Microm, HM320). The sections were stained using the Hematoxylin-Eosin (HE) technique for qualitative histopathological analysis. Digital imaging of HE and TG stained slides was performed using a Carl Zeiss photo microscope attached to a Samsung micro-camera connected to a computer with an image capture card. The professional who read and interpreted the findings was blind to each animal's group designation.

Histological analysis was performed considering the following aspects:

- Persistence of fistulous tract;
- Area of remnant fistulous tract;
- Inflammatory infiltrate;
- Vascular congestion.

Persistence or closure of the fistulous tract visualized by microscopy: closure was considered only when the entire tract

Table 1 – Evaluation of the remaining fistulous tract area in the studied animals.

Rats	Groups		
	GCo	GCa	GAV
1	946	422	754
2	501	573	430
3	894	545	259
4	782	677	330
5	1113	611	244
6	–	–	221
7	–	–	432
8	–	–	403
9	–	–	368
10	–	–	347
Mean	847.2	565.6	377.8 ^a

^a p < 0.05.

was closed and the remnant, even if short, was considered persistence.

Remaining fistulous tract area: under optical microscopy, in a coronal section of the anal canal, in which the pixel area was measured and converted to square micrometers (µm²) after cursor marking the entire wall of the fistulous tract using the Bioestat software.

For inflammatory infiltrate determination, a score system was used to verify the local inflammatory process and its intensity, which was classified as: (0) absence of inflammatory infiltrate; (1) mild inflammatory infiltrate; (2) moderate inflammatory infiltrate; and (3) intense inflammatory infiltrate.

Vascular congestion was also assessed by light microscopy applying the following score: (0) absent; (1) mild; (2) moderate; and (3) intense.

Data analysis and comparison between experimental groups were performed using the nonparametric Kruskal-Wallis test considering a significance level of 5%.

Results

There was no complete closure of the fistulous tract in any of the animals. When assessing the area of the remaining fistulous tract, an average of 847.2 µm was observed in GCo; 565.6 µm in GCa, and 377.8 µm in GAV (p < 0.05) (Table 1).

Fig. 2 illustrates the persistence of fistulous tracts in two of the animals studied.

The mean inflammatory infiltrate score was 2.4 in GCo; 2.4 in GCa, and 2.3 in GAV (p > 0.05) (Table 2).

In the assessment of vascular congestion an average of 1.6 was found in GCo; 1.4 in GCa, and 1.1 in GAV (p < 0.05) (Table 3).

Discussion

The use of herbal medicines has increased greatly in recent years in the treatment of various diseases. Regarding fistulas, the use is very limited mainly in the West. Nevertheless, in experimental research there is already a growing interest on the subject, which may lead to the future incorporation of such products in clinical situations.

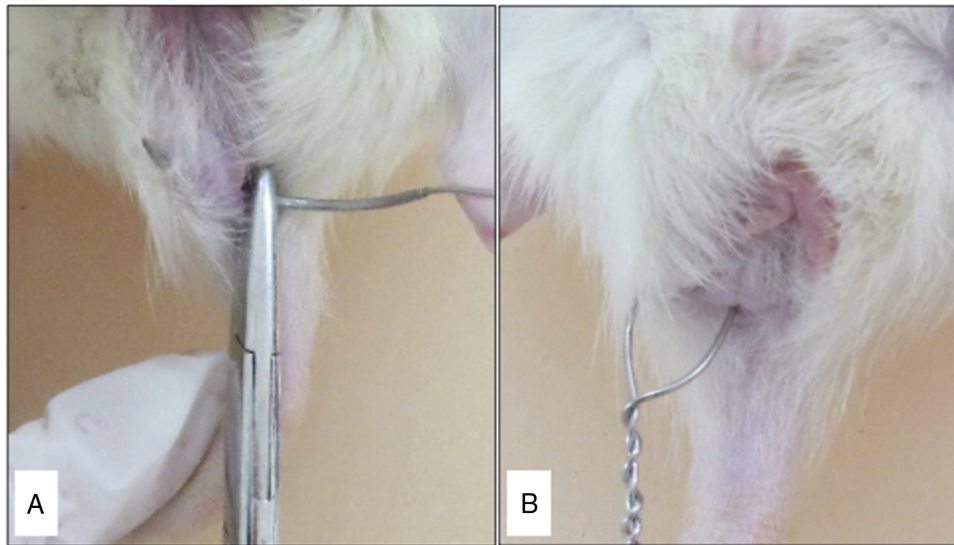


Fig. 1 – (A) Transection of the anal sphincter with steel wire; (B) Steel wire positioned to create a fistula.

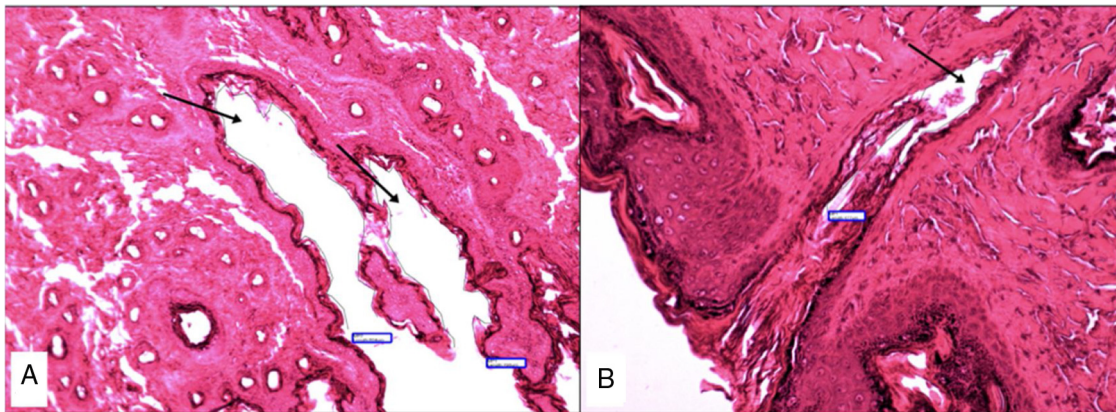


Fig. 2 – Photomicrograph demonstrating the measurement of the fistulous tract area after treatment. (A) GCo animal; (B) GAV animal; arrows indicate the patent fistulous tract (HE 400x).

Table 2 – Evaluation of the degree of inflammatory infiltrate around the fistulous tract in the studied animals.

Rats	Groups		
	GCo	GCa	GAV
1	2	2	3
2	2	3	2
3	3	2	3
4	2	2	2
5	3	3	3
6	-	-	2
7	-	-	1
8	-	-	2
9	-	-	2
10	-	-	3
Mean	2.4	2.4	2.3

p < 0.05.

Table 3 – Evaluation of the degree of vascular congestion around the fistulous tract in the studied animals.

Rats	Groups		
	GCo	GCa	GAV
1	0	1	2
2	1	2	1
3	3	2	2
4	1	0	1
5	3	2	2
6	-	-	2
7	-	-	1
8	-	-	0
9	-	-	0
10	-	-	0
Mean	1.6	1.4	1.1 ^a

^a p < 0,05.

Aloe Vera has gained prominence as a healing agent. In a recent publication, Oryan et al.⁶ demonstrated in an experimental study that the plant was able to promote rapid burn wound healing in rats. It also had anti-inflammatory and healing effects in the treatment of lichen planus, including superiority and less adverse effects than corticosteroids, considered the standard treatment.⁷

In a systematic review of the prophylactic and therapeutic effects of *Aloe Vera* on radiotherapy, there was a beneficial effect on the skin lesions of individuals undergoing lung cancer radiotherapy and also in the treatment of actinic proctitis.⁸ Soares et al.⁹ also observed that *Aloe Vera* in combination with stem cells developed better bone repair and reduced inflammatory cascade than mesenchymal cells in an experimental rat study.

Although in the present study there was no complete closure of any of the fistulous tracts, the group of animals treated with *Aloe Vera* obtained significant lumen reduction compared to the other groups, which leads us to infer that the closure was in progress and probably if we extended the treatment for a longer period there could be total closure in these animals. As the number of publications on the use of herbal medicines in anal fistulas is very small, the treatment period has been stipulated based on literature data,⁴ but the result obtained here serves as a stimulus for further research to be carried out evaluating the effect of its application over longer periods as well as at different concentrations. Regardless of the projection made here about what could have happened, the fact is that the lumen has been reduced, confirming what had already been reported in similar researches⁶⁻⁹ regarding the healing potential of *Aloe Vera*.

Another positive finding observed in the present study was the fact that there was a significant reduction in vascular congestion, which was also observed by other authors.^{4,6,7} Vascular congestion triggered by the inflammatory cascade is one of the early stages of the inflammatory process, whereas when the infectious/inflammatory condition is resolved, decreased vascular congestion is also one of the early stages of the end of inflammation.¹⁰ Although there was no decrease in inflammatory infiltrate in the animals treated by *Aloe Vera* compared to the other groups, there was a decrease in vascular congestion, i.e., the initial stage of inflammation reduction occurred, which brings us back to the issue of treatment time. It is possible that a longer treatment could also lead to a decrease in the inflammatory infiltrate, and this possibility should be verified in future research.

Despite the many publications on *Aloe Vera* demonstrating its ability to accelerate healing, decrease the inflammatory process and even act as an antimicrobial agent, its efficacy is not unanimous, and there are also experimental and clinical situations in which such effects have not been confirmed. Coelho et al.¹¹ investigated oral wound healing in rats with and without the use of hydroalcoholic extract of *Aloe Vera* and found no difference between groups. They found that there was also no reduction in the inflammatory process, similar to that observed in the present research, which leads us to question whether the vehicle used could influence the results, since Comparin et al.⁴ found a reduction in the inflammatory process of anal fistulas in rats using the same plant for treatment, but with glycerin as a vehicle. In the present research,

carbopol was used as a vehicle and no decrease in the inflammatory process was observed. Thus, with conflicting results for the same plant but different vehicles, one may question whether this could influence the outcome, perhaps because some vehicles allow greater product availability in the treated area than others.

Tumay et al.¹² also question whether the vehicle, product concentration, and method of *Aloe Vera* application may interfere with the results. These authors observed that when using the plant by gavage there was a higher risk of intestinal anastomosis rupture in rats, inferring that this was due to lower collagen production, contrary to the vast majority of *Aloe Vera* publications, which reported the opposite, higher collagen production and faster healing. This may also be the reason why there was no decrease in inflammation or complete fistula closure as opposed to that reported by Comparin et al.⁴ — so far the only publication on *Aloe Vera* for anal fistula treatment and therefore our only source of direct comparison. These authors, in addition to using a different vehicle than the one used in the present study, applied the product by dripping on cotton setons that remained in the fistulous tract during treatment, rotating it so that the product was in contact with the interior of the fistulas. In the present study, we chose not to use seton and the product was injected through the external orifice. It is possible that the *Aloe Vera*-soaked cotton seton may have allowed for greater availability of the product as opposed to the method used here, thus indicating another need for future research to determine the best means of using *Aloe Vera*. Retrospectively evaluating the results, we understand that the introduction of a plastic catheter into the external orifice to inject the product may also have been a cause of no decrease in inflammation, which could perhaps be circumvented by maintaining an untreated period for specimen collection. Future research may provide answers to this question as well.

Thus, these results show a promising finding for the treatment of anal fistulas with *Aloe Vera*, taking into account the significant reduction in lumen and vascular congestion. The main limiting factor of the study is based not on the product, but on the lack of standardization of both the application technique and the vehicle to be used, which opens a field of research to be explored.

Conclusion

Aloe Vera extract associated with carbopol as a vehicle was not able to completely close the fistulous tracts or reduce the inflammatory process of the animals studied, but it promoted lumen reduction and decrease in vascular congestion.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

1. Balciscueta Z, Uribe N, Balciscueta I, Andreu-Ballester JC, García-Granero E. Rectal advancement flap for the treatment

- of complex cryptoglandular anal fistulas: a systematic review and meta-analysis. *Int J Colorectal Dis.* 2017;32:599-609.
2. Osterkamp J, Gocht-Jensen P, Hougaard K, Nordentoft T. Long-term outcomes in patients after ligation of the intersphincteric fistula tract. *Dan Med J.* 2019;66(4), pii: A5537.
 3. Narang SK, Keogh K, Alam NN, Pathak S, Daniels IR, Smart NJ. A systematic review of new treatments for cryptoglandular fistula in ano. *Surgeon.* 2017;15:30-3.
 4. Comparin BC, Santos CHM, Dourado DM, Reichel PMG, Paola RS, Pontes ERJ. Treatment of rats anal fistula with glycerin Aloe barbadensis Miller extract. *J Coloproctol.* 2018;38:283-9.
 5. Arakaki MS, Santos CHM, Falcão GR, Cassino P, Nakamura RK, Gomes NF, et al. Experimental model of anal fistula in rats. *J Coloproctol.* 2013;33:135-8.
 6. Oryan A, Alemzadeh E, Mohammadi AA, Moshiri A. Healing potential of injectable Aloe vera hydrogel loaded by adipose-derived stem cell in skin tissue-engineering in a rat burn wound model. *Cell Tissue Res.* 2019;377:215-27.
 7. Ali S, Wahbi W. The efficacy of Aloe vera in management of oral lichen planus: a systematic review and meta-analysis. *Oral Dis.* 2017;23:913-8.
 8. Farrugia CE, Burke ES, Haley ME, Bedi KT, Gandhi MA. The use of Aloe vera in cancer radiation: An updated comprehensive review. *Complement Ther Clin Pract.* 2019;35:126-30.
 9. Soares IMV, Fernandes GVO, Larissa Cordeiro C, Leite YKPC, Bezerra DO, Carvalho MAM, et al. The influence of Aloe vera with mesenchymal stem cells from dental pulp on bone regeneration: characterization and treatment of non-critical defects of the tibia in rats. *J Appl Oral Sci.* 2019;27:e20180103.
 10. Colombo PC, Ganda A, Lin J, Onat D, Harxhi A, Iyasere JE, et al. Inflammatory activation: cardiac, renal, and cardio-renal interactions in patients with the cardiorenal syndrome. *Heart Fail Rev.* 2012;17:177-90.
 11. Coelho FH, Salvadori G, Rados PV, Magnusson A, Danilevicz CK, Meurer L, et al. Topical Aloe vera (Aloe barbadensis Miller) extract does not accelerate the oral wound healing in rats. *Phytother Res.* 2015;29:1102-5.
 12. Tumay LV, Kılıçturgay S, Savcı V, Saraydaroglu O, Anarat R. Effects of Aloe vera on healing of colonic anastomoses: experimental rat study. *J Coloproctol.* 2019;39:33-40.