Original Article

Treatment of rats anal fistula with glycerin Aloe barbadensis Miller extract

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ARTICLE INFO

Article history:
Received 17 April 2018
Accepted 26 May 2018
Available online 6 July 2018

Keywords:
Rats
Rectal fistula
Phytotherapy
Aloe
Therapy

ABSTRACT

Rationale: There is a need for a low cost treatment for complex anal fistulas that can heal without causing fecal incontinence, with room for the study of herbal medicines.

Objective: To evaluate the effect of the glycerin Aloe barbadensis Miller extract in the treatment of anal fistula in rats.

Method: Thirty Wistar rats underwent peritoneal anesthesia with ketamine and Xylasine, followed by transfixation of the anal sphincter with a steel wire, which remained for 30 days to develop the anal fistula. After this period, the steel wire was removed and three groups with 10 animals were formed: A (control), without treatment; B (seton), in which a cotton seton was introduced; C (therapeutic seton), in which a cotton thread was introduced as a seton, and Aloe barbadensis Miller extract was daily dripped in the seton; after 30 days, the setons of groups B and C were removed, and after two weeks without a seton euthanasia and removal of specimens was conducted, and the fistula closure and the inflammatory process were analyzed.

Results: Fistula persistence was observed in all animals in Group A, six in Group B and three in Group C (\(p = 0.015\)). The mean local inflammation levels were 0.9 in Group A; 0.8 in Group B, and 0 in Group C (\(p = 0.015\)).

Conclusion: The use of a seton soaked in a glycerin Aloe barbadensis Miller extract was effective in the healing of anal fistulas in rats, in addition to causing less inflammatory process than the non-phytotherapeutic seton.

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https://doi.org/10.1016/j.jcol.2018.05.009
2237-9363/© 2018 Sociedade Brasileira de Coloproctologia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
In Crohn’s Seton can be used as a step of the treatment, as in the case of the Ligation of Intersphincteric Fistula Tract (LIFT) technique where it can be placed to induce more intense fibrosis of the walls of the fistulous tract, facilitating the second step that consists of the section and ligation of the tract.

The therapeutic approach with the use of seton proposed here is inspired by ancient descriptions of the application of setons soaked in drugs, usually herbal medicines, used mainly in India, Brazil, for its vast flora, has a large number of publications showing the therapeutic benefits of herbal remedies, but there are no reports of their use in anal fistulas.

Herbal medicines are synthesized drugs based on plant extracts. In general, there has been growing scientific support for its beneficial effects on the body. In addition, their use is consecrated, especially among some populations that have little or no access to the traditional medicine, quite common in several developing regions in Brazil. Among these, Aloe vera stands out, specifically the subtype Aloe barbadensis Miller, popularly known as aloe. Aloe belongs to the Liliaceae family, native to North Africa, to which more than 300 species belong in several localities, including Brazil. This plant has several documented properties, such as anticarcinogenic, antiallergic, immunostimulating and healing effects. Other studies also highlight, with increasing evidence, other biological effects, such as antibacterial, anti-inflammatory, antiviral and antifungal activities.

Considering that there is no research with the use of this herbal drug in the treatment of anal fistulas, this research aims to evaluate its action in this situation.

Objective

To evaluate the effect of glycerin Aloe barbadensis Miller extract on the treatment of anal fistula in rats.
Method

The project was approved by the Animal Experiment Ethics Committee of the Federal University of Mato Grosso do Sul (UFMS).

Thirty Wistar male, adult and albino rats weighing approximately 300 g each were studied, from the Vivarium of the Federal University of Mato Grosso do Sul, where the experiment was performed, observing all the ethical norms established by the Brazilian College of Animal Experimentation – COBEA.

The rats were anesthetized for the production of anal fistulas. Anesthesia was performed by intraperitoneal administration of ketamine and xylazine in the same syringe. The solution consisted of 2 mL of 10% ketamine and 1 mL of 2% xylazine. For each 100 g of body weight, 0.1 mL of the anesthetic solution was infused.

Following anesthesia, the anal fistulas were made. They were performed with the rats in the supine position, with the four limbs abducted. A #1 steel wire (Aciflex®) was introduced into the jagged line, crossing the anal sphincter, which was exteriorized 1 cm lateral to the anal margin on the left. After piercing the skin with the needle, the steel wire was cut and a loose knot was made through rotational movements.12

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

1 Control group (A): consisting of 10 rats that were only observed after the withdrawal of the steel wire, without other therapeutic actions.
2 Seton group (B): consisting of 10 rats in which the steel wires were replaced by a cotton seton 0.
3 Seton + glycerin Aloe barbadensis Miller extract Group (C): also consisting of 10 rats in which the steel wires were replaced by cotton seton 0 soaked in the extract, which was applied daily, followed by its rotation, so that the product reached the inside portion of the fistula. This second part of the study also lasted 30 days.

After this period, the setons were removed from all animals. After two weeks, new intraperitoneal anesthesia, euthanasia by deepening of the anesthetic plan and removal of the pieces for histological study were performed. A cube was resected, including the rectal canal and the distal rectum, and the area where the steel wire or seton was, to observe whether the fistula was persistent or not. The specimens were washed in saline solution and placed in properly identified flasks containing 10% formaldehyde solution for further histological analysis. Subsequently, hematoxylin- and eosin-stained slides were prepared. The reading and interpretation of the findings were done with the professional not being aware of each animal’s group.

Histological analysis was performed with the following aspects being observed:

Persistence of the fistula: observation of persistence or not of the patent fistulous tract.

Inflammatory process: verification of local inflammatory process and its intensity, being classified in Grade 0 (absence of inflammation), Grade 1 (mild inflammation) and Grade 2 (moderate inflammation).

Table 1 – Evaluation of persistence of the fistulous tract in the specimens.

<table>
<thead>
<tr>
<th>Rats</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
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<tbody>
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<td>1</td>
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<tr>
<td>Total</td>
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<td>6</td>
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</table>

+, Persistence of fistula; –, absence of fistula. *p = 0.015.

Fistula tract area: in those specimens where fistula persistence was observed, its areas were measured by means of the software CT capture program in micrometers and compared to the means of the groups.

The results were submitted to statistical treatment by the Kruskal–Wallis test followed by the Dunn test. The level of significance was set at 5% and the Statistical Package for Social Sciences 24 (SPSS 24) software was used.

Results

Regarding the closure of the fistula, it was observed that in the control group (Group A), there was no closure of any of the 10 fistulas. In the seton group (Group B), four of the 10 fistulas were closed, and in Group C, seven of the 10 fistulas were closed, demonstrating that in this group there was less persistence of the fistulous tract than in the others; with p = 0.015 (Table 1).

Regarding the evaluation of the specimens inflammatory process, it was observed that in Group A four animals had no inflammation, three had mild inflammation and three had moderate inflammation, resulting in a mean of 0.9. In Group B, three animals had no inflammatory process, six had mild inflammation and only one had moderate inflammation, resulting in a mean of 0.8. In Group C, no animal had an inflammatory process. Thus, this group had the lowest degree of inflammation when compared to the others (p = 0.015) (Table 2).

By evaluating the area of the persistent fistulous tracts, a mean of 29.6 μm was obtained in Group A; 17.5 μm in Group B, and 8.16 μm in Group C; with p = 0.015 (Table 3) (Fig. 1A and B).

Discussion

According to Gokulakkrishna, Hippocrates was the first one to detail the seton method in the anal fistula, when he used horse hair for it. However, it is believed that its use dates from the Egyptians, more than four thousand years ago.8

In about 500 BC, the Indian physician Sushruta, a pioneer of plastic surgery, described in his book, Sushruta Samhita, the
use of seton with medications or extract of natural products for treating anal fistulas. Such treatment was named Kshara-sutra. The word derives from the Sanskrit kshar, that means something that is corrosive and sutra, that is translated as wire.⁸ Although not widespread in the Western world, the Kshara-sutra is widely used in the East, with emphasis on India. This country is known to have several families of plants that are born exclusively in their soil, justifying the Indian pioneering in this area.

In 2017, the Indian Council for Medical Research published a review of the country’s studies on traditional and phytotherapeutic treatments, highlighting those established over time due to their safety and good results presented, among them the treatment of anal fistulas with Kshara-sutra, or ayurvedic medicinal seton.¹³ Using the same term for the treatment, Kshara-sutra, several herbal medicines have been used in medicinal setons in India, such as Apamarga Kshara, Snuhi Ksheera Sutra and Tilanala K.S, most of them not available in our settings.

There are no publications available in the literature of experimental studies with the use of medicinal setons such as the present research, which makes it difficult to compare and analyze the results presented here.

It was observed that the group treated with medicinal setons had closure of the fistula and absence of a local inflam-
Aloe barbadensis Miller, already demonstrated in other situations, but not yet tested in the treatment of anal fistulas. 

In the present study, none of the animals in the drug-sedentary group presented an inflammatory process after two weeks of treatment, in sharp contrast with the seton and sedentary group presented an inflammatory process after two weeks. At the end of the treatment, all rats showed healing of the fistula without fecal incontinence, with a follow-up ranging from six months to a year and a half.

Another herbal medicine used in the treatment of anal fistulas was ginseng. Ohya et al. evaluated 22 children with anal fistula who had already been treated with the conventional method without success, obtaining healing in 21 of them, of which 5 presented recurrence after the end of the treatment; however, according to the authors, all had healing after the reintroduction of the treatment.

Recently, Mena et al. published a small series of cases of patients treated with medicinal seton of a compound of five herbal medicines. The authors pointed out that no patient had any adverse effects and all patients had healed fistulas in a one-year follow-up, proved by clinical and ultrasonographic evaluation.

Yokogawa et al. compared different herbal medicines in the treatment of anal fistula, and found that Capsicum Annuum tincture was superior to Ficus carica latex, Aquirantus fauriei Kshara and long Turmeric rhizome, for promoting greater inhibition in nitric oxide production.

The comparison of conventional treatment with fistulotomy and medicinal seton was made in the publication by Dutta et al., in which the authors randomized 26 patients to the kshara-sutra group and 24 to the fistulotomy group, with the same number of intersphincteric and trans-sphincteric fistulas between the two. The phytotherapic group had a longer healing time, but less pain throughout the process, while the other factors compared, such as healing rate and incontinence, were similar. Similar results had already been obtained by Ho et al., with the difference that the healing time was the same, with only more pain in the medicinal seton group in the first days.

The comparison that is intended to be made of the medicinal seton is with the techniques used in the treatment of complex fistulas, since the simple ones have quite satisfactory results with the surgical treatment. In a recent study with medicinal seton, there was absence of fecal incontinence, and 3.3% recurrence of anal fistulas, while surgical treatment had fecal incontinence of up to 40%, and recurrence of up to 26.5%, with it being even greater with high anal fistulas. In a follow-up of 50 patients with anal fistula treated with medicinal seton, there was 100% cure after 29 weeks, with 56% of patients having achieved healing in less than 12 weeks. It should be added that 18% of these patients had between six and ten years of disease, 20% had high anal fistula, 28% had two or more concomitant fistulas and 64% had fistulas of more than 10 cm, with some cases reaching 20 cm. After six months of post-treatment follow-up there was no case of fecal incontinence or recurrence.

Considering all the data, the study with medicinal setons should be stimulated. Since its advantages in the treatment of anal fistulas, especially in complex fistulas, are known, because it is less invasive and more efficient, with less chance of complications and recurrence, the medicinal seton should gain more and more space in the scientific settings. Additionally, Aloe has also found growing evidence of medical use, with important antioxidant and healing activities. The bacterial and anti-inflammatory activities of aloe would have an important impact on the evolution of anal fistulas, because their pathophysiology is based on chronic inflammatory and anti-inflammatory processes, which, in principle, reinforced the healing and anti-inflammatory effects of Aloe barbadensis Miller, already demonstrated in other situations, but not yet tested in the treatment of anal fistulas.

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In the present study, none of the animals in the drug-sedentary group presented an inflammatory process after two weeks of treatment, in sharp contrast with the seton and control groups, in which, in fact, the presence of inflammation was expected, since the fistula is a chronic inflammatory process, and multifilament wire setons induce a greater inflammatory response. In a recent study investigating the activities of the Aloe vera extract, its anti-inflammatory activity was proven to treat skin wounds. Additionally, the healing of the group treated with this extract was superior, since only the animals treated by this drug achieved total re-epithelialization. Thus, the results of this research are in line with the publications on this plant with regard to healing and anti-inflammatory effects.

Mohite et al. used a seton soaked in an herbal medicine in 114 patients with anal fistula for three weeks. All had local purulent secretion, 35 reported anal pain for two to three days, and 18 had local induration, and the mean time of elimination of the seton was eight weeks. At the end of the treatment, all patients showed healing of the fistula without fecal incontinence, with a follow-up ranging from six months to a year and a half.

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infectious conditions. Thus, a seton with aloe extract tends to reach excellent results, and may even alter the paradigms of the treatment of anal fistulas.

An observation that should be carefully considered refers to the fact that even in the three animals of the medicinal seton group that persisted with fistula no inflammation was observed, demonstrating the anti-inflammatory effect of Aloe barbadensis Miller. Moreover, in these three cases, the area of the fistulous tract was smaller than in the other groups, showing that they could probably heal after a few days, opening the field for future research of this herbal medicine with different treatment times, in order to evaluate the optimal duration of aloe application.

Several publications have tried to identify the mechanisms by which this plant could offer the good medical results observed. The aloe parenchyma is rich in several substances, among them phenolic compounds. These compounds have a high antioxidant activity and reduce the synthesis of Reactive Oxygen Species (ROS). ROS are known to generate cell aging and are at the basis of various organic diseases, causing damage to DNA or oxidizing lipids and proteins. Among ROS, 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) stands out. A recent study confirmed the in vitro sequestering action of the ethanol extract of the aloe gel of this compound, strengthening the antioxidant evidence of this plant.10

In addition to the aforementioned phenolic compounds, the aloe gel has enzymes, vitamins, mineral salts, and amino acids. In addition, it has anthraquinones, such as aloenine, barbaloin and isobarbaloin, which have been proven to be healers.10

Thus, it is clear that the use of medicinal setons is not new, which is shown by the large number of publications for several years demonstrating satisfactory results with the method. The greatest difficulty in their analysis, however, lies in the diversity of plants used in these setons, in particular the fact that many of them are not found in all countries, as most of those cited in Indian publications do not exist in our country. On the other hand, considering our rich flora and also the national publications that show positive effects of several plants in the treatment of many diseases, the combination of these two therapeutic proposals was lacking. This was the intention of this research, where the technique of the medicinal seton was added with the aloe, perhaps the phytotherapeutic with the greatest number of publications demonstrating healing and anti-inflammatory efficacy, exactly what is desired in the treatment of the anal fistula. The results obtained here showed a greater number of healed tracts with aloe, and there was no inflammatory process in the parts of any animal after two weeks of removal of the seton, which did not occur in the other groups, confirming that in this model Aloe barbadensis Miller was effective.

**Conclusion**

The use of a seton soaked in a glycerin Aloe barbadensis Miller extract was effective in the healing of anal fistulas in rats, and led to lower inflammatory process than the non-phytotherapeutic seton.

**Conflicts of interest**

The authors declare no conflicts of interest.

**REFERENCES**

17. Nema A, Gupta SK, Dudhamal TS, Mahanta V. Transrectal ultra sonography based evidence of ksharasutra therapy for