The healing effect of allantoin on skin wounds induced in goats

Efeito cicatrizante da alantoína em feridas induzidas em caprinos

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ABSTRACT

The aim of the present study was to demonstrate the healing effect of allantoin in skin wounds experimentally induced in goats. Fifteen healthy 12-18-month-old crossbread females goats were divided into three experimental groups: group treated with a commercial formulation of allantoin 3% associated to zinc oxide 3g (GA); control group treated with saline solution (GC) and group treated with a commercial formulation of 35% lauryl dimethyl benzyl ammonium chloride (GP). The wounds were daily treated for 14 days and evaluated on 2nd, 6th, 10th and 14th days by macroscopic characteristics observations and histological analysis. It was observed a significant increase of wound contraction from the 2nd to 6th post-operative day on the group treated with allantoin while for the microscopic evaluation the collagen deposition was higher on GA groups compared to GC and GP, as well as the inflammatory process was less intense in the allantoin-treated group. In conclusion, it was observed that the allantoin-treated group had a positive effect on inflammation response, collagen deposition and wound contraction, demonstrating that allantoin may be prescribed as an alternative treatment for skin wounds in goats.

Keywords: Collagen; Wound Treatment; Picrosirius red; Small ruminants

RESUMO

O objetivo do presente estudo foi demonstrar o efeito cicatrizante da alantoína em feridas cutâneas induzidas experimentalmente em caprinos. 15 cabras mestiças sadias com idade entre 12 e 18 meses foram divididas em três grupos experimentais: grupo tratado com formulação comercial de alantoína 3% associada a óxido de zinco 3g (GA); grupo controle tratado com solução salina (GC) e grupo tratado com formulação comercial de cloreto de lauril dimetil benzil amônio 35% (GP). As feridas foram tratadas diariamente por 14 dias e avaliadas no 2º, 6º, 10º e 14º dias por observações das características macroscópicas e análise histológica. Observou-se aumento significativo da contração da ferida do 2º ao 6º dia de pós-operatório no grupo tratado com alantoína, enquanto na avaliação microscópica a deposição de colágeno foi maior nos grupos GA em relação ao GC e GP, assim como já que o processo inflamatório foi menos intenso no grupo tratado com alantoína. Em conclusão, observou-se que o grupo tratado com alantoína teve um efeito positivo na resposta inflamatória, deposição de colágeno e contração da ferida, demonstrando que a alantoína pode ser prescrita como tratamento alternativo para feridas cutâneas em caprinos.

Palavras-chave: colágeno; tratamento de feridas; Picrosirius red; pequenos ruminantes
INTRODUCTION

The skin is the first barrier against external agents and the wound healing process involves a set of chemical, physical and biological responses that aim to re-establish tissue integrity after a trauma or as a result of a pathological process (SORG et al., 2017; KAMARAZAMAN et al, 2022).

The process of tissue reparation is divided in steps, although the limits of each phase are not very different. According to Nayak, Sandiford and Maxwell (2009), the process begins with the inflammation, characterized by migration of leukocytes and polymophonuclear cells mediated by growth factor which lead to the proliferation phase, in which occurs the processes of angiogenesis, fibroplasia, collagen deposition and epithelialization. The third phase is responsible for reorganization, degradation and maturation of the extracellular matrix and can last years.

The allantoin has been cited in literature by its numerous pharmacological properties, such as anti-inflammatory and keratolytic activity, fibroblast proliferation and collagen synthesis (JORGE et al., 2008). Besides that, allantoin is the base of wound products normally used in farms, even though it only has prescription for equines, dogs and cats. However, it is not very clear the effects of allantoin on the wound healing process in small ruminants, despite it is used for this purpose.

Therefore, the aim of this study was to investigate the wound healing process induced by allantoin in goats through macroscopic and microscopic evaluations.

MATERIALS AND METHODS

The experiment was approved by the Ethics and Animal Testing Committee from State University of Maranhao (Protocol number 18/2018/CEEA/UEMA). Fifteen healthy 12-18-month-old crossbread females goats were housed in a farm located in Sao Luis, Maranhao, Brazil, 30 days prior to the experiment in order to allow the animals to adapt to the experimental conditions and then submitted to clinical evaluation, deworming and pregnancy diagnosis.

The animals were fed daily with forage of Pennisetum purpureum and Cynodon plectostachyus and premixture composed of 20% soybean meal, 15% urea, 19% ground corn, 30% common salt and 15% mineral salt and the drinking water was ad libitum. The goats were randomly distributed in three stalls: group treated with a commercial
formulation of allantoin 3% associated to zinc oxide 3g in a vehicle q.s. GA); control group treated with saline solution (GC) and group treated with a commercial formulation of 35% lauryl dimethyl benzyl ammonium chloride (GP).

To carry out the wounds, the trichotomy of the right and left paralumbar fossa was performed to evaluate macroscopy and microscopy healing process, respectively. The animals were submitted to local anesthesia inverted L block with 2% lidocaine hydrochloride with vasoconstrictor 6mg/kg BW (CLARKE K., TRIM C., HALL L., 2014) and a circular excision of 2.5 cm in diameter. The wounds were treated daily during 14 days or until completed healing process.

For the macroscopic evaluation, the wound areas were measured once a day with a caliper and the wound contraction was calculated as follow: $A = \pi \times R \times r$, where $\pi = 3.14$, $R =$ greater radius and $r =$ smaller radius of the wound. It was also observed macroscopic characteristics such as: hemorrhage (presence or absence), crust (partial or total, exuberant or not exuberant, dry or moist), granulation tissue (presence or absence), swelling, color and hair growth in the área adjacent to the wound and epithelialization.

For the microscopy evaluation, tissue fragments with a margin of normal skin were collected with a punch of 0.6 centimeters in diameter on the 2nd, 6th, 10th and 14th days of the experiment and preserved in 10% formaldehyde for 24h. Afterward, 5μm thickness sections were performed and stained in Weigert Hematoxilin and Eosin for morphological analysis and Picrosirius Red Staining for morphometric analysis of fiber collagen.

The slides were studied by an analyzer of computer images Leica Qwin D-1000, version 4.1 (Cambridge, UK) from the Microscopy Laboratory of UEMA, where 10 areas of 5.900 μm² /slide were selected, in order to evaluate the healing process characteristics, such as neutrophils, inflammation, congestion, fibroblast proliferation, neovascularization and epithelization, classified according to the intensity (+ discrete, ++ moderate, +++ intense). To evaluate the collagen fibers based on the biber density, it was used the image processor ImageJ, distributor FIJI, version Madison.

The data of the macroscopic and morphometric evaluation was presented in mean values and performed by T-test paired with $p<0.05$, using the Minitab 19 program and the experimental outline was in randomized blocks.
RESULTS AND DISCUSSION

Macroscopic analysis

In the macroscopy evaluation, it was observed that on the 2nd day, both three groups had shown similar characteristics. Animals from GA presented wounds with regular borders, absence of exudate, bleeding points and re-epithelization and presence of partial crusts, as shown in figure 1 (A and B). The wounds from GP presented irregular borders, bleeding points, absence of re-epithelization and partial crusts, according in figure 1 (C and D). Finally, in the GC, partial crust, bleeding points and absence of exudate and irregular borders were observed, as in figure 1 (E and F). In all groups evaluated, there was not edema, swelling and changes on skin color near the wounds.

Figure 1. Wound contraction of allantoin (A, B), oitment (C, D) and control (E, F) groups on the 2nd, 6th, 10th and 14th post-operative day

On the 6th post-operative day, the same pattern of wound healing process were observed in all three groups (Figure 1) and hair growth was noticed around the wound. On the 10th day, it was observed decreases wounds area and remains of irregular borders, redness on the wound surface, absence of foreign bodies and bleeding points in the GA (Figure 1, A and B). Both GC and GP presented redness on the wound surface, presence of foreign bodies and bleeding points and absence of exudate (Figure 1, C, D, E and F).

In all groups, the epithelialization process occurred in a centripetal manner, however, this process was more intense on GC and GP, causing irregular edges in wounds, contributing to the findings of Van Wikle Júnior (1967) and Corsi (1994). In addition, it was observed that centripetal retraction was more uniform on the group.
treated with allantoin. According to these authors, wound contraction is characterized by centripetal movements of wound edges, contributing to its closure.

On 14th of evaluation it was observed that there was parcial reepitelization, total crusts, absence of bleeding points, exudate and foreign bodies on GA (Figure 1, A and B). Both GP and GC presented parcial reepitelization, presence of crusts, exudate and bleeding points, as well as foreign bodies adhered to the wound surface (Figure 1, C, D, E and F). The formation of crusts on GA, GP and GC is explained through the process where fibrina and leukocites remain are deposited, resulting in a thick look. On oitment and control group those crusts were more exuberant, suggesting that the fibrin deposition were more dense and contributed to atract polymorphonuclear cells, such as neutrophils, to the wound area.

These results showed that the macroscopic characteristics were similar in all groups, regarding the group treated with allantoin presented better results on inflammatory and remodeling phases. According to Paller et al. (2017), allantoin is responsible for the healing and adstringent effect as it stimulates inflammation cells activity and granulation tissue formation, which is important for the wound healing process.

The mean values of the contraction of the wounds is presented on table 1. Gradual reduction was observed in all experimental groups during the experiment. The wound contration rate was 87,27% in the allantoin group, 89,73% in the control group and 83,95% in the oitment group.

Table 1. Mean and standard deviation of the area of experimentally induced wounds in goat skin in the Allantoin (GA), Oitment (GP) and Control groups (GC) on the 2nd, 6th, 10th and 14th post-operative day.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2º</td>
<td>6º</td>
<td>10º</td>
</tr>
<tr>
<td>Allantoin</td>
<td>Mean</td>
<td>4,164&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3,145&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1,937&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Standard deviation</td>
<td>(0,439)</td>
<td>(0,367)</td>
<td>(0,326)</td>
</tr>
<tr>
<td>Oitment</td>
<td>Mean</td>
<td>3,576&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2,873&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2,024&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Standard deviation</td>
<td>(0,666)</td>
<td>(0,364)</td>
<td>(0,468)</td>
</tr>
</tbody>
</table>
The summary of the average and standard deviation of wound area the on the 2nd, 6th, 10th and 14th post-operative day of the GA, GC and GP are presented on table 1. It was observed that from the second to the 6th post-operative day, there was significant increase on wound contraction on Allantoin group compared to the others groups.

The results of wound contraction showed that despite there was no statistical difference between the groups, the numerical difference demonstrated that the wound contraction was higher on the wounds treated with ointment when compared to the other groups. However, it was observed that from the second to the sixth day, there was a significant increase on wound contraction on Allantoin group, which was similar to the findings of Araújo et al. (2010).

It was noticed that on Allantoin Group, the wounds were covered by a thick layer that minimizes the contact with the external environment. This characteristic is responsible for the regular edges of the wounds, potencializing the process of fibroplasia, which was observed in this group. According to Hussein (2018), the amount of myofibroblasts in the center of the wound compress the granulation tissue, stimulating the centripetal movement on the wound. It suggests that the effect of allantoin may have improved the stage of fibroplasia.

In addition, in this work, the use of allantoin was satisfactory as it seemed to present repellent features and, consequently, avoiding possible parasites that could cause tissue damage, delaying the healing process. Furthermore, the allantoin solution was adhered to the wound, when compared to the other groups, which ease the management of farm animals. Thus, more studies should be carried out in order to check those properties of repellency and adhesion.
Microscopic analysis

The results obtained in this work indicated that both three groups evaluated presented satisfactory wound healing process, evidenced by the decreased inflammation, collagen fiber deposition and re-epithelization.

On the second post-operative day, inflammation process was observed in both three groups, evidenced by the presence of inflammatory cells, congestion, necrosis and absence of fibroblasts and epithelial cells. On allantoin-treated group it was observed the process of angiogenesis whereas on GC and GP there was presence of bleeding points and the inflammation process was more intense on GP group (Figure 2, Table 2).

Table 2. Histological characteristics on Allantoin Group (GA), Oitment Group (GP) and Control Group (GC).

<table>
<thead>
<tr>
<th>Group</th>
<th>Day</th>
<th>Inflammation</th>
<th>Neutrophils</th>
<th>Fibroplast proliferation</th>
<th>Neovascularisation</th>
<th>Epithelialization</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA</td>
<td>2</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>GC</td>
<td>2</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>GP</td>
<td>2</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

Source: Author’s own production

Six days after the injury, the inflammation process was still intense on control and oitment group and focal bleeding points were also evidenced on control group. In this phase, the neutrophils cells decreased only on the wounds treated with allantoin (Figure 2, Table 2).
Figure 2. Photomicrographs of the skin of goats from the three groups evaluated on the 2nd, 6th, 10th and 14th post-operative day, showing the presence of neutrophils (black arrows), fibroblast proliferation (white arrow), angiogenesis (A) and epithelialization (R).

Despite acute inflammation process was observed in both three groups, characterized by polymorphonuclear cells, vascular congestion and edema (CORSI et al., 1994; CURI et al., 2005), a constant decrease of neutrophils was observed with the allantoin-treated group (GA) unlike GC and GP as time goes by (Figure 2, Table 2). This result may indicate that the group treated with allantoin presented less intense inflammation process than the other groups, which was similar to the findings described by Rahal et al. (2003) e Potmes et al. (1997). According to Maldebaum et al. (2003) and Sorg et al. (2017), although the inflammation plays an important role in the wound healing process, prolonged inflammatory phase might delay the tissue repair as the other steps depend on the previous phase. Allantoin is a wound healing substance that stimulates new tissue formation due to its astringent and keratolytic properties and has shown to have anti-inflammatory effects.

On 10th post-operative day, the fibroblast proliferation was moderate in both three groups as well as the migration of epithelial cells, except on allantoin group, which presented intense epithelization and more uniform fibrobast arrangement, as shown in figure 2 and table 2.

On the 14th day the wound healing process was significantly advanced and the inflammatory cells had decreased on GA, whereas it remained moderate on control and
ointment groups. The epithelization process was intense in both three groups and collagen fibers aspect were more numerous and differentiated.

It was observed that on 10th and 14th post-operative day, the fibroblast amount increased on Allantoin and Control groups, indicating that the wound healing process was in the proliferative phase. According to Grinnel et al. (1981); Guildli Neto (1992); Franco et al., (2003); Eckersley e Dudley (1988), this phase is characterized by the presence of macrophages, fibroblasts and neoformation, which constitutes the granulation tissue, leading to the remodeling phase.

In addition to the intense presence of fibroblasts, the allantoin group had higher mean collagen fiber deposition values when compared to the control and oitment groups on all days evaluated, as shown on table 3 and figure 3. There was no statistical difference between the groups on 2nd, 6th, 10th post-operative day, however, on 14th day it was noticed statistical difference. It was observed increase of collagen fibers deposition in all groups, specially from the second to the sixth day with consecutive decrease on oitment and control groups from the 10th and 14th post-operative day, according to table 3.

**Figure 3.** Photomicrographs of the skin of goats from the three groups evaluated on the 2nd, 6th, 10th and 14th post-operative day showing the disposition and amount of collagen fibers (200μm).
These results suggest that besides the wound healing process was in the remodeling or maturation phase, the deposition of these fibers in the extracellular matrix provided the epithelialization of the wounds, since epithelial migration is guided by the migration of keratinocytes, variety of cytokines and growth factors (SORG et al., 2017).

In addition, the mean values of collagen deposition on allantoin group presented were greater than on GC and GP in all four days of evaluation. Furthermore, the mean values of collagen deposition remained higher on GA when compared to the other groups, even though it decreased on 10th post-opertive day (Table 3).

**Table 3.** Mean and standard deviation of collagen deposition of experimentally induced wounds on the skin of goats on Allantoin (GA), Ointment (GP) and Control (GC) groups on the 2nd, 6th, 10th and 14th post-operative day.

<table>
<thead>
<tr>
<th>Days</th>
<th>Groups</th>
<th>GA</th>
<th>GC</th>
<th>GP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
</tr>
<tr>
<td>2nd</td>
<td></td>
<td>40.84</td>
<td>29.61</td>
<td>35.67</td>
</tr>
<tr>
<td></td>
<td>Standard deviation</td>
<td>(10.49)</td>
<td>(1.14)</td>
<td>(8.90)</td>
</tr>
<tr>
<td>6th</td>
<td></td>
<td>51.85</td>
<td>33.84</td>
<td>37.05</td>
</tr>
<tr>
<td></td>
<td>Standard deviation</td>
<td>(8.66)</td>
<td>(19.66)</td>
<td>(7.9)</td>
</tr>
<tr>
<td>10th</td>
<td></td>
<td>49.55</td>
<td>25.99</td>
<td>29.23</td>
</tr>
<tr>
<td></td>
<td>Standard deviation</td>
<td>(17.21)</td>
<td>(14.40)</td>
<td>(10.32)</td>
</tr>
<tr>
<td>14th</td>
<td></td>
<td>61.72</td>
<td>13.66</td>
<td>35.62</td>
</tr>
<tr>
<td></td>
<td>Standard deviation</td>
<td>(17.81)</td>
<td>(13.66)</td>
<td>(11.55)</td>
</tr>
</tbody>
</table>

Means followed by the same letter in the lines do not differ statistically with 95% (p<0.05).
The amount of collagen fiber deposition promoted by allantoin show its potential to stimulate extracellular matrix synthesis, accelerate connective tissue growth and promote the wound epithelization (FEDOSOV et al., 2017; XIE et al., 2022). According to Araujo et al. (2010) and Paller et al. (2017), the wound healing process induced by allantoin takes places through the response to the regulation of inflammatory cells by inhibiting the chemotaxis of immune cells in the wound area, stimulation of fibroblast proliferation and extracellular matrix synthesis, which results in the acceleration of skin healing (YAŞAYAN et al., 2020). Thereby, it was observed that our study corroborates with the findings of those author.

Thus, as allantoin had shown efficacy for the wound healing process in this work, it is necessary that future studies are carried out in order to develop a allantoin-based drug with therapeutic prescription for wound healing in goats.

CONCLUSION

Allantoin had shown satisfying effect on wound healing process induced in goats due its potential to stimulate inflammation, fibroblast proliferation, fiber collagen deposition, epithelialization and wound contraction, indicating that allantoin may be prescribed as an alternative treatment for skin wounds in goats.
ACKNOWLEDGEMENTS

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REFERENCES


