

UNIVERSIDADE FEDERAL DE UBERLÂNDIA
INSTITUTO DE BIOTECNOLOGIA
CURSO DE BIOTECNOLOGIA

WOUND HEALING EFFECT OF ScLL LECTIN FROM SYNADENIUM
CARINATUM LATEX IN MURINE MODEL

Henrique Lara Peixoto Marques

Trabalho de Conclusão de Curso em formato de artigo científico, apresentado à coordenação do curso de Biotecnologia, da Universidade Federal de Uberlândia, para obtenção do grau de Bacharel em Biotecnologia.

Uberlândia – MG
Julho – 2019

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WOUND HEALING EFFECT OF LECTIN SCLL ISOLATED FROM
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Henrique Lara Peixoto Marques

Aprovado pela Banca Examinadora em: ____/____/____ Nota: _____

Fernanda Maria Santiago
Presidente da Banca Examinadora

Uberlândia, de de

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ABSTRACT

Wound healing is a physiological event that leads to the restoration of normal structure and in damaged tissues. Several natural products, such as lectins, have been shown to accelerate the healing process. Thus, in the present study, the *Synadenium carinatum* latex lectin (ScLL) was isolated and investigated for its healing properties that optimizes the restoration process of the injured tissue. Dorsal skin wounds were created in mice, which were divided in groups treated with ScLL at concentrations of 250 and 500 µg/ml and group treated with water (control). ImageJ program was used to measure the contraction of wounds and tissue sections were stained with Haematoxylin and Eosin for histological assay using Picro-Sirius Red for collagen determination content. Concerning about the results, ScLL lectin, in the macroscopic evaluation demonstrated better performance in the wound contraction, when compared to control. In the histological analysis, the ScLL presented an intense re-epithelialization level occurring faster than control group. In addition, we showed that ScLL 500 µg/ml presented a lower concentration of collagen than control group, however possesses anti-keloids properties by its ability to reduce exacerbated deposition of collagen.

Keywords: *Synadenium carinatum*, lectin, wound healing

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Wound healing effect of ScLL lectin from *Synadenium carinatum* latex in murine model

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1 INTRODUCTION

The wound healing process is a physiological process involving a variety of cells, cytokines, growth factors aiming to restore the structure and function of damaged tissues (CORIOLANO et al., 2014; PATEL *et al.*, 2019). The repair process starts immediately after injury and includes the phases such as inflammation (neutrophils, macrophages and lymphocytes recruitment), proliferation (characterised by formation of granulation tissue) and maturation (extracellular matrix remodelling) (PANG et al., 2017; CHUNCHARUNEE *et al.*, 2019). Moreover, all the stages are interdependent, overlapping and the healing is just complete when the disrupted surfaces are firmly knit by collagen (BUFFONI et al., 1993; MARTINS et al., 2006). However, many factors can affect one or more phases at wound healing such as age, obesity, stress, immunity status, patient's behaviour in face of a damaged tissue and microbial infections (GUO; DIPIETRO, 2010). Therefore, the pharmaceutical has increasingly been appealing to discovery of new sources-derived drugs that are able to modulate in a positive way the wound healing process (GHOSH; GABA, 2013; TCHOUYA et al., 2015).

Lectins are proteins, or glycoproteins of non-immune origin, that reversibly interact with carbohydrates and can be found in different organisms such as plants, animals, microorganisms, and virus. Some lectins participate in cell-cell communication, cancer metastasis, embryogenesis and tissue development (KARPOVA, 2016; HE et al., 2017). Their ability to interact with glycan's moieties present on the surface of immune cells resulting in immunomodulatory effects has been widely showed in previous studies. Some plant lectins, as Con A from *Canavalia ensiformis*; PHA-E and PHA-L from *Phaseolus vulgaris*, ScLL from *Synadenium carinatum* are able to induce Th1 immunity with high levels of IL-12 and IFN- γ production, while a few can stimulate Th2 immunity, inducing immune responses that could be beneficial against different pathogens and tumors (SOUZA et al., 2013; SANDER; CORIGLIANO; CLEMENTE, 2019).

Plant lectins may also improve the wound healing process in skin through the induction of epithelial reconstruction and increased keratin deposition, as demonstrated by Neto et al. (2011) with *Bauhinia variegata* seeds lectin. Thus, the present study aim was to investigate the healing potential of the topical administration of *S. carinatum* lectin on surgically induced wounds in a murine model.

2 MATERIAL AND METHODS

2.1 Plant material

Synadenium carinatum latex was collected of plants, which were grown under natural conditions in the University Campus localized in Uberlândia, Minas Gerais, Brazil (940 m altitude, 18°53'05.56" S and 48°15'36.54" W), from September to December of the years 2017 and 2018. A voucher specimen (HUFU 38354) was identified and deposited at the Herbarium of the Federal University of Uberlândia (UFU).

Crude extracts from *S. carinatum* were obtained from small incisions in the distal branches of plants and by mixing 15 mL aliquots with water at a ratio of 1:10. These extracts were then incubated 4°C for 24h and stored -80°C for 2 months. Afterwards, a rubber-like material was removed and the suspension was centrifuged at 12,000 g for 20 min at 4°C.

2.2 Animals

Six to eight -week-old Female Balb/c mice weighing 25-30g were kept in the Animal Facility Center (CBEA) of Federal University of Uberlândia, MG, Brazil in standard animal cages (21Hx32Lx20W cm) under conventional laboratory conditions (12-h light/dark cycle, dark cycle starts at 19.00 h and 25°C), and supplied with water and food ad libitum. All procedures were conducted according to guidelines for animal ethics and this study had been approved by the Ethics Committee for Animal Experimentation of the UFU (protocol number 135/12).

2.3 Purification and determination of the hemagglutinating activity of ScLL

D-galactose-binding lectin (ScLL) was purified on immobilized D-galactose agarose column (Pierce, Rockford, Illinois, USA), balanced with 0.05 M borate-buffered saline (BBS), pH 7.2. The ScLL was eluted with 0.4 M D-galactose in BBS (BBS-D-gal) and dialyzed against Tris buffer (pH 7.2). The protein concentration was determined (BRADFORD, 1976) using bovine serum albumin as standard protocol. The electrophoretic profile of this lectin was visualized by SDS-PAGE (12%) (LAEMMLI, 1970). Hemagglutination assays were performed to confirm ScLL lectin activity, as previously described (SOUZA et al., 2005). ScLL aliquots were stored at -20°C until using *in vitro* and *in vivo* assays.

2.4 Hemolytic assay

Balb/c mice erythrocytes were used to evaluate the hemolytic activity of ScLL lectin according to Silva et al. (2015) with some modifications. First the red blood cells were collected and washed twice with 0.9% NaCl (w/v). Next 0.5% erythrocytes (v/v) were incubated at 37 °C in the presence of ScLL in the concentrations ranging from 0.39 to 200 µg/mL for 1 hour. After that, the samples were centrifuged (450g for 5 min) and cell lysis determined by spectroscopically as absorbance at 540 nm. The absorbance measured from lysed red blood cells in presence of 1% (v/v) Triton X-100 was considered as 100%. All results were obtained from three independent experiments made each one in triplicate.

2.5 Cytotoxicity assay

In order to determine ScLL cytotoxic activity, we performed the MTT assay by using Human Foreskin Fibroblasts (HFF) (ATCC SCRC-1041.1) and peritoneal murine macrophages, as previously described by Mosmann (1983) with some modification. The cells were cultured separately in 96-well plates (1×10^5 cells/well) in triplicate and treated with ScLL lectin in different concentrations (0.39 to 200 µg/mL) for 18h at 37°C and 5% CO₂. As control the HFF and macrophages were treated with only complete RPMI medium. This experiment was performed in triplicate wells and all results obtained of three independently observations.

2.6 Evaluation of healing effect

The evaluation of the healing effect was based on macroscopic and microscopic criteria, of a circular excision wound model.

Female of Balb/c mice (n=45) were randomly allocated into 9 groups (n=5) and submitted to one dorsal skin wound each. Briefly, the animals were anesthetized with xylazine (15 mg/Kg IM/intramuscular) and ketamine (100 mg/Kg, IM/intramuscular). After the hair was removed from the back of each mice, the surgical procedure was carried out in the skin area marked by a 7.5 mm-metal "punch", and the fragment was taken out preserving muscle fascia underneath. After one hour of the surgery, the lesions' mice belonging to Groups 1, 2 and 3 were treated with ScLL in the concentrations of 250 µg/ml by 5, 10 and 15 days respectively; Groups 4, 5 and 6 were treated ScLL in the concentration of 500 µg/ml by 5, 10 and 15 days respectively and Groups 7, 8 and 9 the lesions were treated with only vehicle (water) by 5, 10 and 15 days respectively. The local

treatment of the lesions and the measurement (photos) were conducted once a day, at the same time. During the course of the treatment, the scars were photographed using an high digital camera (Sony Cyber-shot) and the wound areas calculated by ImageJ program. The percentage wound contraction was calculated using the following equation:

$$\% \text{ of contraction} = \frac{\text{wound area on day 0} - \text{wound area on day n}}{\text{wound area on day 0}} \times 100$$

2.7 Histopathological analysis

For histological analysis purpose, mice were euthanized at day 5, 10, and 15 post-treatment with Thiopental (150mg/kg) injected intraperitoneally followed by cervical dislocation. After euthanize, the tissue samples were fixated in 10% formalin buffer for 24h, embedded in paraffin and tissue sections stained with hematoxylin-eosin (HE), full evaluation of tissue sections, and Picro-Sirus Red was used to quantify collagen fibers deposition by using ImageJ software.

The HE findings were analyzed according with the following criteria:

Re-epithelialization - classified as absent when there was no visible epithelium in the optical field; discreet or moderate, when it appeared incomplete or partial; and intense when fully or completely visualized on the connective tissue.

Granulation Tissue - classified as absent when there was no visible tissue; discreet when it appeared only on the border, moderate when it appeared incomplete; and intense when present on the entire extent of the wound.

Intensity of Inflammatory Infiltrate - classified as absent when there was no visible inflammatory infiltrate; discreet when cells appeared isolated; moderate when not involved the entire extent of the wound; and intense when present on the entire extent of the wound.

Types of Inflammatory Cells - classified as absent when there was no visible inflammatory cells; discreet when had predominance of polymorphonuclear cells; moderate when there were polymorphonuclear and mononuclear cells; and intense when there were predominance of mononuclear cells.

Vascular Proliferation - classified as absent when there was no visible vascular proliferation; discreet when there were few vases on the edge; moderate when not involved the entire extent of the wound; and intense when present on the entire extent of the wound.

2.8 Statistical analysis

Statistical analysis was carried out using GraphPad Prism v.5.0 (GraphPad Software, Inc., La Jolla, CA). To compare all groups (control; ScLL in the concentrations of 250 and 500 µg/ml) in relation to treatment days (5, 10 and 15), two-way ANOVA test was used followed by Bonferroni post-test. To analyze the cell viability and cell hemolysis data were used nonparametric Kruskal Wallis test, followed by Dunn's test. All data are expressed as mean ± S.D. Statistical values $p < 0.05$ were considered significant.

3 RESULTS

3.1 Characterization of ScLL lectin

Electrophoretic by SDS-PAGE profile of *S. carinatum* crude extract showed several peptide components, with relative molecular masses (M_s) ranging from 28 to 98 kDa (Figure 1B). These proteins after purification on galactose affinity chromatography demonstrated galactose non-binding proteins (void) corresponded to fractions 2-10 and resin binding proteins (lectin) were eluted with 400 mM of galactose between fractions 20–22 (Figure 1A). The electrophoretic profile of the lectin demonstrated a protein with two subunits and apparent molecular weights of 28 and 30 kDa (Figure 1B). Moreover, this lectin was incubated with 2% murine erythrocytes and showed hemagglutinating activity (25.0 - 1.55 µg) (Figure 1C), thus confirming lectinic activity and identified as ScLL, a lectin previously isolated by Souza et al. (2005).

The effects of ScLL lectin on the hemolysis of Balb/c mice erythrocytes and on cytotoxicity of cells lines such as fibroblasts and peritoneal murine macrophages were assessed at different concentrations. After incubating 1 and 18 hours respectively, the ScLL lectin did not change the viability of these cells (Figure 1D and E).

3.2. Evaluation of healing effect

The macroscopic analyses were used to determine to wound healing activity of the ScLL lectin in 15 days of the treatment. Photographs of the wounds of a representative mouse from each group were taken on day 1, 5, 8, 10, 13, and 15, respectively, after wound induction, at the end of the inflammatory phase, during formation of granulation tissue, during re-epithelialisation phase and including the day of sacrifice .

The wounds of the mice of all groups on the first day showed bright red colour corresponding to the blood that covers the wound. At day 5, wound aspects differ across

the treated groups with ScLL and vehicle used. In the control group, wounds showed a dark red colour while those from 250 and 500 µg/ml ScLL treated groups exhibited a brown color due to fibrin deposition on the surface of the scars. In the eighth day, the mice treated with 500 µg/ml ScLL lectin demonstrated an apparent decrease in the wound contraction area when compared with control group and 250 µg/ml ScLL lectin and at day 10, we noticed a complete healing in the group treated with 500 µg/ml ScLL. (Figure 2A)

Wound reduction results of control group and treated groups with ScLL in 250 and 500 µg/ml in 15 days were demonstrated in Figure2B. Groups treated with 500 µg/ml showed significant difference ($p<0.05$) in the wound reduction area when compared to the control group (untreated group) and treated group with 250 µg/ml during the first eleven days treatment. However, the group treated with 250 µg/ml demonstrated at no time showed significant difference in the wound reduction area when compared to the control group. Then, due the animals treated with the highest concentration of ScLL demonstrated a better performance in wound reduction, the following experiments was performed only with the 500 µg/ml ScLL lectin.

3.3. Histological analysis

For the histological evaluation of the injured tissue, samples of control group and treated group with ScLL lectin were collected from each group on 5, 10 and 15 days after treatment. Five histological parameters were defined for analysis in HE staining, being scored according to their intensity and transformed into quantitative variables, as showed in table 1.

3.3.1 Re-epithelization

In the first five days of treatment, the ScLL treated group demonstrated a more advanced reepithelization stage compared to the control group but in discreet intensity. At 10 days after surgical wound, the ScLL treated animals showed a intense reepithelialization while the control group demonstrated moderate reepithelialization. On day 7, both groups showed a level of intense reepithelialization (figure 3A).

3.3.2 Granulation Tissue

Concerning the presence of granulation tissue, in the first five days, the ScLL treated group showed a intense presence of granulation tissue while control group demonstrated a moderated presence. At 10 days of treatment, presence in both groups there were a reduced to discrete intensity. Finally, on day 15 after treatment, there was no visible granulation tissue for both groups (Figure 3B).

3.3.3. Inflammatory Infiltrate

The inflammatory infiltrate analyzes on the fifth day demonstrated in the both groups a moderate intensity. When evaluated at day 10, there was a decrease of the inflammatory infiltrate for both groups, leading to the discrete intensity. On 15 days of treatment, control group still demonstrated minimum intensity, while ScLL treated group did not show the presence of inflammatory infiltrate (Figure 3C).

3.3.4 Types of Inflammatory Cells

Inflammatory cells types were verified and showed in Figure 3D. In the first 5 days, the ScLL treated group and control group were considered with discrete intensity. At 10 days, ScLL treated group changes to intense intensity while control group was to moderated intensity. Finally, in 15 days, only control group maintained a discrete intensity of inflammatory cells.

3.3.5 Vascular Proliferation

In the early 5 days of treatment, ScLL treated group showed a moderated to intense vascular proliferation, while the control group demonstrated a moderated intensity . On days 10, both ScLL treated group and control group had values considered discrete. In 15 days, none of the groups showed presence of vascular proliferation (Figure 3E).

3.4 Histopathological analysis of the lesions

Histopathological analysis were based on histologicals views of scars from treated group and control group with a comparison of representative animals for each group after 5, 10 and 15 days of treatment, as shown in figures 4, 5 and 6.

With 5 days of treatment it was observed in the control group a very evident and thick crust and the granulation tissue poorly formed. In the treated group, a more

advanced healing process was observed with lower crust thickness, early re-epithelization and well-formed granulation tissue, as shown in figure 4.

In 10 days of treatment, the control group still presents a thick crust, but with formation of an exuberant granulation tissue in the connective tissue and initiation of re-epithelialization. In the treated group, an accelerated healing process is observed, with complete closure of the wound and formation of epidermal attachments, as shown in figure 5.

Finally, with 15 days of treatment, in the control group, it is possible to note a small crust with discrete tissue of underlying granulation, but with formation of epidermal attachments. In the treated group was observed a complete skin healing with fully epidermis regeneration and restoration of the connective tissue, as shown in figure 6.

3.5 Quantification of collagen

Effect of the ScLL lectin on density and differentiation of collagen was verified and shown in Figure 7. The individual analyzes for the presence of total collagen showed a gradual increase of collagen fibers in control group. However, ScLL treated group demonstrated a decrease of collagen fibers on 15 th day (figure 7A). The presence of type 1 collagen for the control group also showed a gradual increase during the analyzed days of treatment, while in the ScLL treated group there was similar values of type 1 collagen on day 5 and on day 10, demonstrated a decrease in day 15 (figure 7B). Concerning about the type 3 collagen, the ScLL treated group showed a more marked increase of type 3 collagen when compared to the control group, maintaining a similar value for 5 and 10 days and a more visible increase in 15 days. Control group had no significant increase from day 5 to day 15. Both increases where observed in smaller values than increases of total collagen and type 1 collagen, as shown in figure 7C. Representative microscopic images of these data are showed in figure 7D.

4. DISCUSSION

Although only a small portion of all plants in the world are being used as medicinal agents, their importance should not be disregarded, since almost 65% of the world's population has already incorporated this alternative into their primary health modality. Admirably, about one third of all traditional herbal medicines are intended for the

treatment of skin wounds and in modern medicines, this value is between 1 and 3%. The benefits of plant extracts in the healing of cutaneous wounds have been increasingly studied and gained a lot of support from several experimental studies. (BUDOVSKY, et al., 2015).

ScLL lectin did not change the viability of these cells, and similar results were demonstrated by Souza et al. (2005) using J774.A1 cells incubated with ScLL in concentrations ranging from 1 to 200 µg/ml. Recently, lectins purified of members of the Euphorbiaceae (Eutirucallin lectin from the *Euphorbia tirucalli* latex) did not show any effect on cell viability (PALHARINI et al., 2017). Cytotoxicity tests with natural products are important because they are a potential source for isolating compounds that can be used to develop new therapeutic methods.

The animals treated with the highest concentration of ScLL demonstrated a better performance in wound contraction when compared to control group and treated group with smaller concentration. The wound contraction is an essential parameter to the progress of cutaneous wound healing because it decreases the wound size, repairs the functional barrier and facilitates reepithelization (CHATTOPADHYAY et al., 2002). It is known that the skin fragment, when withdrawn, induces a formation of a solution consisting of fibrin, clot and inflammatory exudate, resulting in the formation of the leukocyte fibrous crust (SIMÕES et al., 1986; MARCHINI, 1994), which indicates the beginning of the wound healing process. In addition, the wound healing effect of *O. ficus-indica* flower was evidenced by the early formation (between 3 and 5 of post-wounding) of scabs (provisional matrix), which protects against irritations and infections, resulting in faster healing (AMMAR et al., 2015). Based on these facts, a faster wound contraction in group treated with 500 µg/ml when compared with 250/ml ScLL and control groups, may be related to the formation of the crust in the early stage. Similar results have been demonstrated by other herbal extracts. For instance, the methanolic extract showed faster healing with earlier wound contraction when compared with the control group (SHIVHARE et al., 2010).

In the five parameters evaluated on histological analysis, treated group manage to get an intense level of re- epithelization sooner than control group, a more intense formation of granulation tissue in the beginning of treatment, and "eliminate" the inflammatory infiltrates before 15th day, while in the control group these infiltrates were still present. On "types of inflammatory cells", treated group with 500ul/ml evolved from 5th to 10th day from presence of polymorphonuclear infiltrates to presence of only

mononuclear infiltrates, while the control group remained with polymorphonuclear infiltrates throughout the treatment evaluation process, and both groups showed similar values for vascular proliferation, with a higher concentration of treated group 500ul/ml only in the five first days of treatment. Also, control group showed presence of a thick fibrin clot during most part of the treatment, while treated group showed a much less thick fibrin clot during the beginning of analysis, which disappeared with 10 days of treatment. Healing is a process involving cellular and biochemical responses of the body to recovery the function and structure of the damaged tissue (SANTORO; GAUDINO, 2005; 11 KAPOOR et al., 2006). The process of wound healing is divided in three phases, with limits not very distinct: inflammatory phase with two subphases that are homeostasis and inflammation itself, proliferate phase and finally the remodeling phase (SUMITRA; MANIKANDAN; SUGUNA, 2005). The first step healing process is the control of hemorrhage (hemostasis) (LAURENS; KOOLWIJK; DE MAAT, 2006). Hemostasis consists of two main processes: platelet deposition, which results in the formation of a thrombus platelet, and the activation of the coagulation cascade that results in the conversion of fibrinogen into a network of insoluble fibrin filaments, leading the formation of the fibrin clot that minimizes the loss of blood, fluids and also provides a provisional extracellular matrix for the beginning of the wound organization (SANTORO; GAUDINO, 2005; LAURENS; KOOLWIJK; DE MAAT, 2006; DIEGELMANN; EVANS, 2004). It is important to emphasize that the inflammatory response itself, starting from the conclusion of hemostasis (MOORE, 1999; TSIROGIANNI; MOUTSOPOULOS NM; MOUTSOPOULOS HM., 2006). Also the inflammation plays a significant role, for stimulating cell migration, proliferation and also recruiting fibroblasts to the proliferative phase (MULISA; ASRES; ENGIDAWORK, 2015). In addition, according to Zekavat et al. (2016), the group treated with Carboxymethyl cellulose (CMC) showed a minor degree of haemorrhage and tissue necrosis, which accelerates the healing process of diabetic and nondiabetic wounds. In this context, on the 5th day of treatment, areas with hemorrhage were observed only in treated group with 250ul/ml which may indicate that hemostasis was still in process and the healing process may have been more impaired in this group, when compared to control and treated group with 500ul/ml. Some studies have demonstrated that the topical treatment of natural plant components may delay or hinder the healing process, as observed in the study conducted by Araújo (1998) and Contrera (1985).

The proliferative phase is the second stage of healing and it is characterized by the formation of granulation tissue, which depends primarily on the fibroblast, for synthesizing new components of the extracellular matrix, such as collagen fibers type I and III, and depends on the formation of new capillary vessels, since they provide nutrients for the proliferation and development of cells (GUO; DIPIETRO, 2010; CARVALHO, 2002; SILVA, 2014). This tissue consists of cellular elements, including fibroblasts, inflammatory cells, and neovascular and matrix components, such as fibronectin and proteases (WERNER; GROSE, 2003). It was observed at 5 days that both groups already possessed characteristics of granulation tissue. Similar phenomena were observed in the study led by Santos et al. (2006), which it was also found on the 7th day in both groups studied, the presence of granulation tissue. Moreover, at 15 days were observed a reduction of this granulation tissue in animals treated with lectin and control. This result is in agreement with Candido (2001) which reports that the proliferative phase lasts for 12 to 14 days and occur cell migration, mostly keratinocytes, promoting re-epithelialization.

Previous studies demonstrated that deletion of polymorphonuclear cells (PMN) results in acceleration of wound closure of full-thickness dermal wounds. (QIAN et al., 2016), so a persistence of inflammatory cells in the proliferative phase may have impaired the performance of the control group in the stages of wound healing.

An important stage of wound healing is the regeneration of the blood system to restore nutrient perfusion. If this restoration does not happen, the healing process can be disrupted or drastically delayed (SORG, Heiko *et al*). Based on these facts, a higher neovascularization rate found on treated group when compared to the control group may have been a contributing factor to a faster wound closure process. Collagen is the most abundant protein in the human body and it is of fundamental importance in the constitution of the skin (EULÁLIO et al., 2007). In the healing process, the collagen synthesized by the fibroblasts, initially to compose the extracellular matrix, is degraded more actively and a thicker collagen is deposited and organized parallel to the tension lines, that is, the collagen fibers undergo a remodeling that contributes to the tissue reconstitution and provides tensile strength (MYLLYHARJU; KIVIRIKKO, 2001; ISAAC et al., 2010). Taking these factors into consideration the control group, because of the higher concentration of collagen at 14 and 21 days after wounding, may have contributed to a faster healing of the lesion when compared with the group treated with ScLL, giving in the end of the process a greater resistance to the scar. The present findings

are similar to those of Abu-albasal (2001) who studied the effect of several plants on wound healing (*Trigonella foenumgraecum*, *Artemisia herba-alba*, *Anchusa strigosa*, *Nigella sativa* and *Punica granatum*) and observed a correlation between the acceleration of the wound healing process and the production of collagen.

According to TREDGET et al. (1997), in some situations an abnormal scarring of the skin may occur, causing several disorders, such as keloids. These constantly extend beyond the boundaries of the original wound and hardly regress over time (BRAN et al., 2009). Moreover, the keloids occur in individuals with a familial predisposition and its characterized by causing persistent pain, itching and have an unappealing appearance (BAYAT et al., 2002; OGAWA et al., 2016). The keloids are characterized by an intense function and proliferation of fibroblasts, an exorbitant accumulation of extracellular matrix, exaggerated deposition of collagen (SU et al., 2010; SHIN; MINN, 2004), absence of equilibrium between synthesis and resorption, causing a continuous increase of collagen production by being superior to the amount that is degraded, and fibroblast resistance to apoptosis (KREISNER et al., 2005; ISAAC et al., 2011). In agreement with this, the group treated with ScLL 500 µg/ml may contribute as a form of treatment or prevention in the formation of keloids in the healing process, by minimizing the accumulation of collagen, since it presented lower collagen deposition at 15 days of treatment. A similar result was demonstrated in a study with *Calotropis latex*, suggesting that it that can be used in the treatment of keloids, because of the ability to inhibit intense response of fibroblasts, accumulation of collagen in the matrix and to induce florid granulation tissues (ADEROUNMUA et al., 2013).

5. CONCLUSION

In conclusion, the present investigation describes the healing potencial of topical administration of the *S. carinatum* lectin on surgically induced wounds. The properties of the ScLL lectin here reported indicate that it demonstrates hemagglutinating activity, without hemolytic and cytotoxic effects. Concerning the macroscopic evaluation, ScLL lectin presented better performance in the wound contraction and re-epithelialization faster than control group, however showed lower deposition of collagen. Despite this, the lectin (ScLL 500 µg/ml) could be a potential source of therapeutic agents in the prevention and treatment of keloids, due the ability of inhibit exaggerated accumulation of collagen in the matrix.

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Figure 1. Purification of ScLL lectin from *S. carynatum* and its hemagglutinating, hemolytic and cytotoxic activity (A) ScLL purification by galactose affinity chromatography. The first peak (a) corresponds to galactose non-binding proteins (void) and the second peak (b) is related to galactose binding proteins (GBP) eluted with 400 mM of galactose. (B) SDS-PAGE from *S. carynatum*: line 1) crude extract; line 2) non-binding proteins; line 3) ScLL lectin analyzed under non-reducing conditions revealed two proteins of approximately 28 and 30 kDa. (C) ScLL lectin presented hemagglutinating activity (25-1.55 µg), (D) Percentage of hemolysis in Balb/c mice erythrocytes, indicating that ScLL lectin is not hemolytic; and (E) Percentage of cell viability using Human Foreskin Fibroblasts (HFF) and peritoneal murine macrophages demonstrated that in different concentrations the ScLL lectinn is not cytotoxic.

Figure 2. Photographic representation of wound contraction on different post wounding days of the control group and ScLL lectin treated groups, demonstrated better result in 500 µg/ml concentration when compared with 250 µg/ml and control.

Figure 3. (A) Graph showing the intensity of reepithelialization, (B) intensity of granulation tissue, (C) intensity of Inflammatory Infiltrate, (D) intensity of different Types of Inflammatory Cells and (E) intensity of Vascular Proliferation.

Figure 4. Representative photomicrography of the skin of control group and ScLL treated group at 5-day after surgical injury. In the control group, was observed that granulation tissue is still poorly developed with the presence of a thick fibrin clot. In the Scll treated group, notice a much less thick fibrin clot, the granulation tissue is well developed, and the epidermis shows an accelerated reepithelization, but without epidermal appendages formation.

Figure 5. Representative photomicrography of the skin of control group and ScLL treated group at 10-day after surgical injury. In the control group is still evident the presence of a thick fibrin clot; the granulation tissue is already formed in the connective tissue, and began the epidermis reepithelization. Comparing the ScLL treated group, notice an advanced process of healing with complete re-epithelization of the epidermis.

Figure 6. Representative photomicrography of the skin of the control group and ScLL-treated group at 15-day after surgical injury. In the control group, note permanence of fibrin clot and an underlying granulation tissue; the epidermis appendage regeneration is already observed in the connective tissue. In the ScLL treated group, see a complete skin healing with fully epidermis regeneration and restoration of the connective tissue.

Figure 7. Concentration collagen analyzes after 5, 10 and 15 day of treatment with ScLL from *S. carinatum*. (A) Concentration of total collagen; (B) Concentration of type 1 collagen.; (C) Concentration of type 3 collagen. Values are expressed in mean $\pm$ S.D. * $P > 0.05$ compared with the control group. (D) Photomicrograph of histological sections showing the density of the different types of collagen by polarized microscopy of the control group and ScLL treated group after 5, 10 and 15 days of treatment (Sirius Red – 20X).

Table 1.

Histological Parameters	0	1	2	3
Reepithelialization	Absent	Discreet	Moderate	Intense
Granulation Tissue	Absent	Discreet	Moderate	Intense
Intensity of Inflammatory Infiltrate	Absent	Discreet	Moderate	Intense
Types of Inflammatory Cells	Absent	Discreet	Moderate	Intense
Vascular Proliferation	Absent	Discreet	Moderate	Intense

Figure 1.

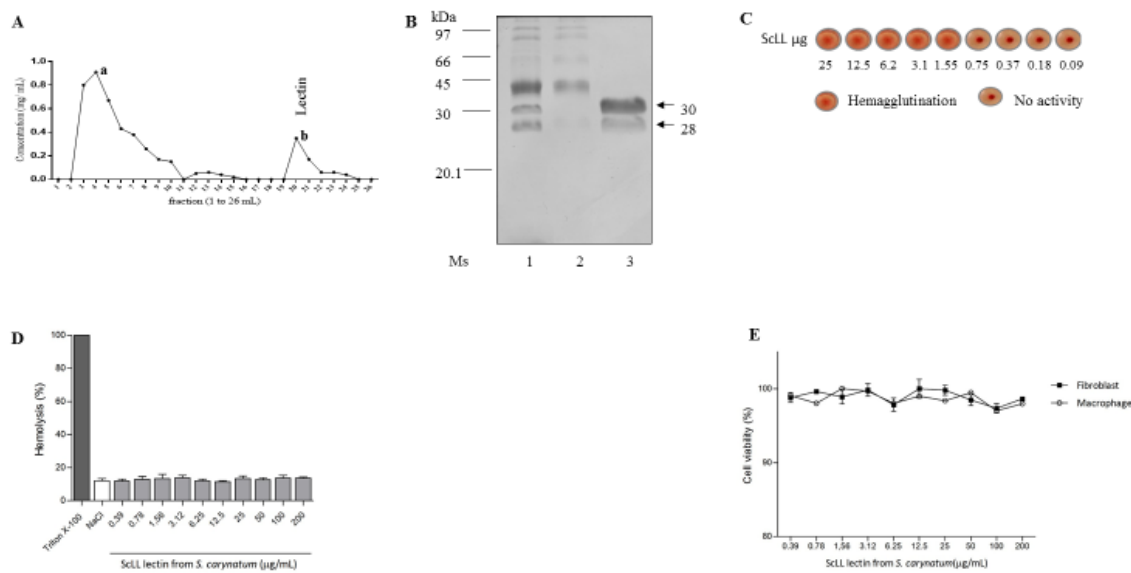


Figure 2.

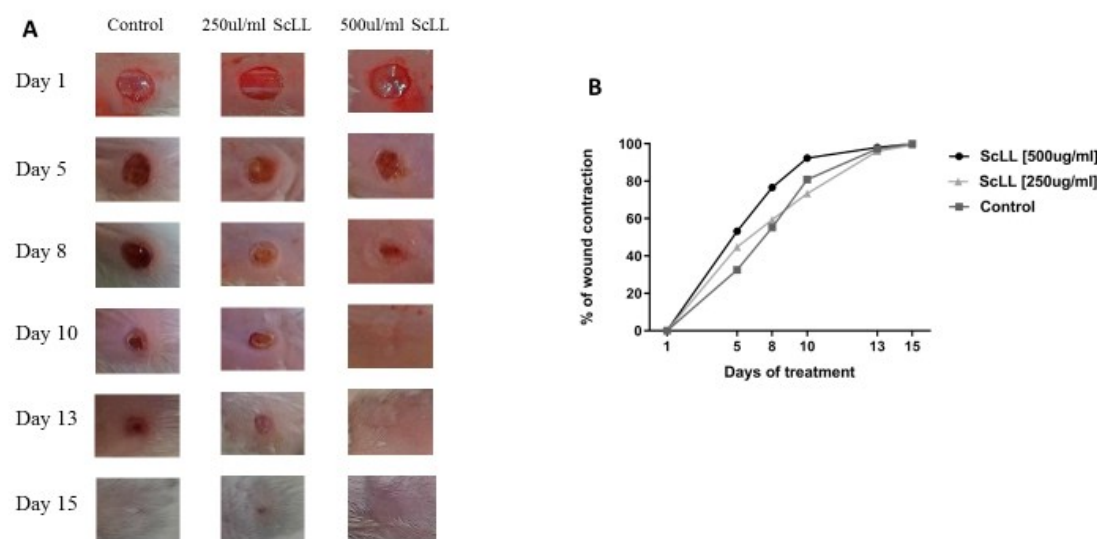


Figure 3.

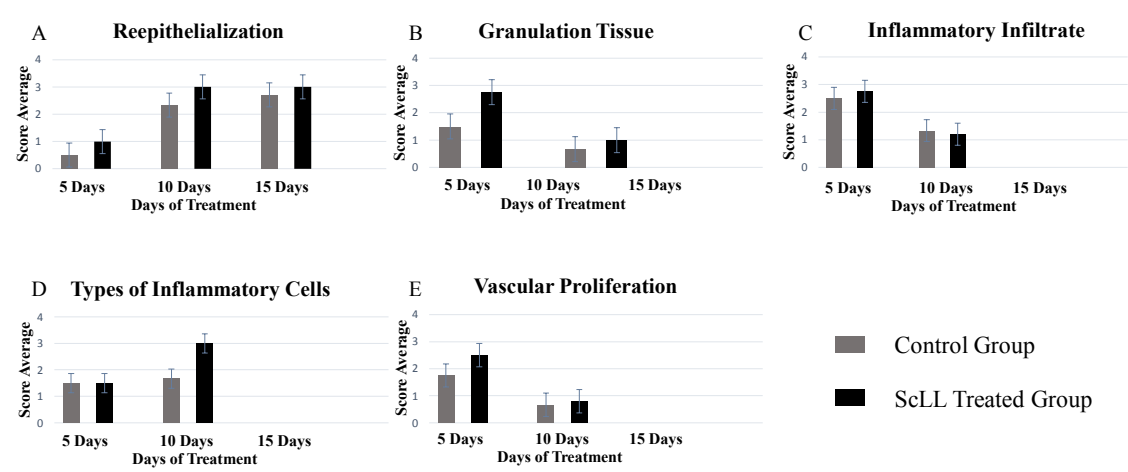


Figure 4.

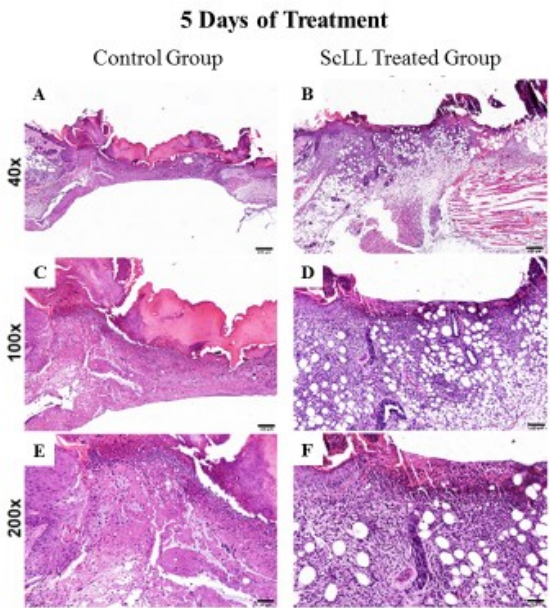


Figure 5.

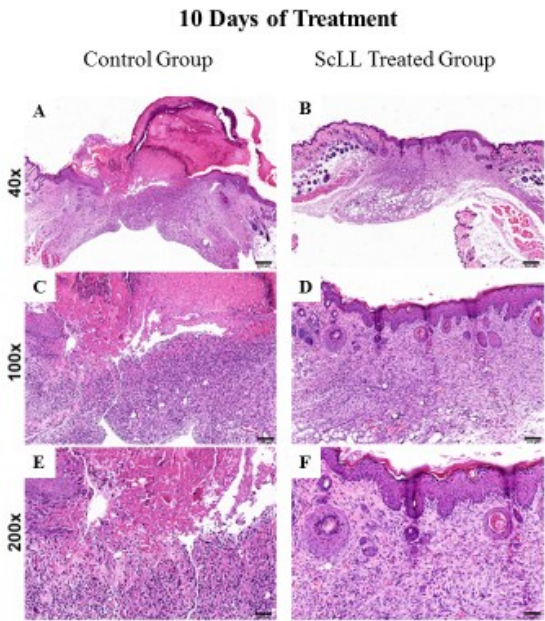


Figure 6.

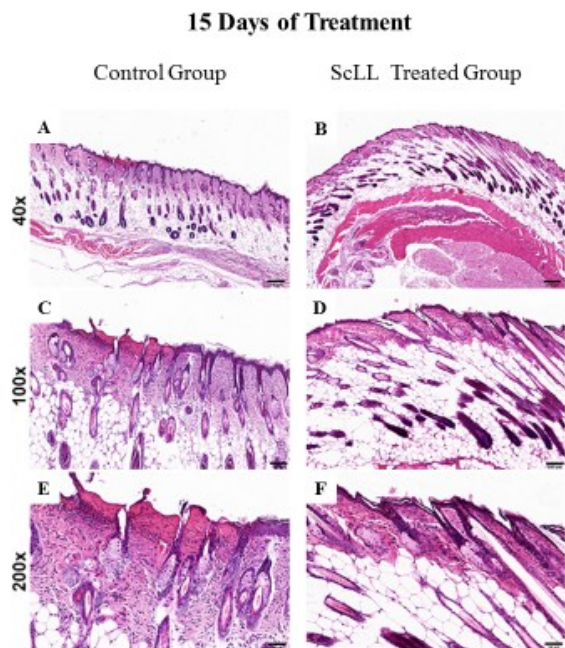


Figure 7.

