

MARIÁUREA MATIAS SARANDY SOUZA

**Ação de extratos vegetais no reparo de feridas cutâneas em ratos  
diabéticos**

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Biologia Celular e Estrutural, para obtenção do título de Doctor Scientiae

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Reggiani Vilela Gonçalves  
(Orientadora)

"Que darei eu ao SENHOR por todos  
os benefícios que me tem feito?"  
(Salmo 116:12)

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## RESUMO

Souza, Mariáurea Matias Sarandy, D.Sc., Universidade Federal de Viçosa, outubro de 2016. **Ação de extratos vegetais no reparo de feridas cutâneas em ratos diabéticos.** Orientadora: Reggiani Vilela Gonçalves. Co-orientador: Rômulo Dias Novaes.

**Objetivo:** Investigar o efeito da aplicação tópica da pomada à base de *Strycnos pseudoquina* nas concentrações 5 e 10% na cicatrização de feridas cutâneas em ratos diabéticos. **Material e Método:** Amostras de *S. pseudoquina* foram coletadas no Município de Rio Verde, Goiás, Brasil e em seguida submetido a prospecção fitoquímica. O extrato foi emulsificado em lanolina nas concentrações 5% e 10%. Foram utilizados trinta ratos Wistar que após a indução do diabetes com estreptozotocina, foram divididos em 5 grupos de 6 animais: Sal: feridas tratadas com 0,9% de solução salina; VH (veículo da pomada): feridas tratadas com 0,6 g de creme de lanolina; SS (Sulfadiazina de Prata): feridas tratadas com 0,6 g de creme de Sulfadiazina de Prata (0,01%); ES5: feridas tratadas com pomada a base de *S. pseudoquina* (5%); ES10: feridas tratadas com pomada a base de *S. pseudoquina* (10%). Três feridas circulares de 12 mm de diâmetro foram realizadas no dorso dos animais e fragmentos das feridas foram retirados para análises histológicas e bioquímicas a cada 7 dias durante 21 dias. **Resultados:** Os grupos que receberam o extrato de *S. pseudoquina* nas concentrações 5 e 10% apresentaram maior taxa de fechamento das feridas, maior quantidade de células, vasos sanguíneos e aumento do colágeno III e I. Os marcadores de estresse oxidativo foram menores nos grupos ES5 e ES10, e os níveis de enzimas antioxidantes foram maiores nestes mesmos grupos. **Conclusão:** Os resultados deste trabalho demonstraram que a aplicação tópica de pomada à base de *S. pseudoquina* promove um reparo cutâneo rápido e eficaz em ratos diabéticos.

**Palavras-chave:** “Quina do Cerrado”; Cicatrização; Enzimas antioxidantes.

## ABSTRACT

Souza, Mariáurea Matias Sarandy, D.Sc., Universidade Federal de Viçosa, october 2016. **Action of plant extracts in the repair of skin wounds in diabetic rats.** Adviser: Reggiani Vilela Gonçalves. Co-Adviser: Rômulo Dias Novaes.

**Objective:** Investigate the effect of topical application of the ointment of *Strychnos pseudoquina* in concentrations of 5 to 10% in the skin wound healing in diabetic rats.

**Material and Methods:** Samples of *S. pseudoquina* were collected in Rio Verde, Goiás, Brazil, and then submitted to phytochemical prospection. The extract was emulsified in lanolin at concentrations of 5% and 10%. Thirty rats were used which after diabetes induction with streptozotocin, were divided into 5 groups of 6 animals: Sal: wounds treated with 0.9% saline solution; VH (Ointment vehicle): wounds treated with 0.6 g of lanolin cream; SS (Silver Sulfadiazine): wounds treated with 0.6 g of silver sulfadiazine cream (1%); ES5: wounds treated with ointment base *S. pseudoquina* (5%); ES10: wounds treated with ointment base *S. pseudoquina* (10%). Three circular wounds of 12mm in diameter were made on the animal's back and fragments of the wounds were removed for histological and biochemical analysis each 7 days for 21 days. **Results:** The groups that received concentrations of 5 and 10% of *S. pseudoquina* extract had higher closing rate of wounds and increase of cells, blood vessels, collagen III and I. The oxidative stress markers were lower in the groups ES5 and ES10 and levels of antioxidant enzymes were higher in the same groups. **Conclusion:** The results of this work have shown that topical application of *S. pseudoquina* ointment promotes fast and effective skin repair in diabetic rats.

**Keywords:** “Quina do cerrado”; Healing; Antioxidant enzymes.

**Artigo 1: Effect of Topical Administration of Fractions and Isolated Molecules from Plant Extracts on Skin Wound Healing: A Systematic Review of Murine Experimental Models**

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## **Abstract**

**Background and Purpose.** Skin wound healing is a dynamic process driven by molecular events responsible by the morphofunctional repair of the injured tissue. In a systematic review, we analyzed the relevance of plant fractions and isolates on skin wound healing. By revising preclinical investigations with murine models, we investigated if the current evidence could support clinical trials. **Methods.** Studies were selected in the MEDLINE/PubMed and Scopus databases according to the PRISMA statement. All 32 identified studies were submitted to data extraction and the methodological bias was investigated according to ARRIVE strategy. **Results.** The studies demonstrated that plant fractions and isolates are able to modulate the inflammatory process during skin wound healing, being also effective in attenuate the oxidative tissue damage in the scar tissue and stimulates cell proliferation, neoangiogenesis, collagen synthesis, granulation tissue expansion, reepithelialization and the wound closure rate. However, we identified serious methodological flaws in all studies, such as the high level of reporting bias, absence of standardized experimental designs, analytical methods and outcome measures. **Conclusion.** Considering these limitations, the current evidence generated from flawed methodological animal studies makes difficult to determine the relevance of herbal medicines to treat skin wounds and derail conducting clinical studies.

**Keywords:** Healing process, herbal medicine, plant products, skin wound.

## **1. Introduction**

The skin wound healing is a dynamic and complex process divided into three complementary stages: inflammatory, proliferative and maturation. The inflammatory phase comprehends the intense leucocytes recruitment to the wound area, removal of cellular and extracellular matrix debris and syntheses of regulatory molecules such as cytokines and chemokines [1,2]. The proliferative phase progresses with an intense proliferation and migration of fibroblasts, endothelial cells and keratinocytes; as well as formation of the granulation tissue (rich in type III collagen) and progressive reepithelialization [1-3]. At the maturation phase, type III collagen is gradually replaced by type I collagen, which originates more thicker and resistant collagen fibers [2-4].

It has been demonstrated that flaws on the leukocyte recruitment and function can impair the healing process due to reductions in the synthesis of regulatory molecules that drives the extracellular matrix assembly [5-7] and neoangiogenesis [8]. In this way, the development of drugs and alternative treatments that favor the migration and cellular activity during the inflammatory and proliferation phases may enhance the skin wound repair.

Skin wounds represent a serious health problem worldwide frequently associated with high costs and inefficient treatments [9,10]. The use of herbal drugs is opening a new perspective for the treatment of skin wounds, mainly in developing countries. Once herbal strategies represent a simple pharmacological option, 80% of the population uses herbal drugs in their health care [1]. Although several plant species are currently used in the popular medicine to treat skin wounds worldwide [11-14], the scientific evidence that supports this practice is scarce. Thus, determining the security and efficiency of herbal drugs is an urgent and challenging task, which is essential to develop new technologies and products potentially applied in wound care.

In general, the healing properties of plant products are related to specific secondary metabolites, especially tannins, saponins, flavonoids, and alkaloids [11,15,16]. Plant products present a broad spectrum of biological functions such as astringent, antimicrobial, antioxidant and anti-inflammatory [17-22]; which has been systematically associated with the beneficial effects in stimulates the healing process [17,20,22]. Before extrapolates to the human condition, preclinical researches using animal models have been useful to test the toxicological security and biological effects of plants fractions and isolated molecules with potential applicability in the treatment of skin wounds [11,20].

Despite the increasing number of experimental trials in the last decade, few advances were observed in the treatment of skin wounds, especially in humans. Considering that studies using animals models are conceived to support clinical investigations, there is a clear limitation in translate to the human context the findings obtained from animal models of wound healing. Considering that herbal drugs are extensively used in the popular medicine, we still do not know where is the gap that hinders the implementation of experimental findings for the development of innovations and technologies potentially useful in the clinical management of skin wounds. Thus, we systematically revised preclinical studies with murine models that investigated the effects of plant fractions and isolated molecules in the treatment of skin wounds. Beyond determine the relevance of plant derivatives in the skin repair, we analyzed the methodological quality of all preclinical studies identified, especially considering that the quality of evidence generated from flawed methodological studies could compromise the generalizability of the findings and derail conducting clinical studies.

## **2. Materials e Methods**

### **2.1. Search strategy**

Research papers that investigate the action of plant fractions an isolated molecules in murine models of skin wound healing, published until 09/04/2015 (15:05:23), were recovered and independent analyzed by three researchers (FBL, MMS and RVG). The search strategy was constructed by four components: “animals (filter)”, “injury (wounds)”, “organ (skin)” and “plants extract (isolates and fractions)”. The filters were developed from PubMed database according to the hierarchical distribution of Medical Subject Headings [MeSH Terms]. A standardized search filter for animals studies was applied in PubMed database [27]. The same search strategy was adapted and used to recover studies in the Scopus platform. The standard animal filter provided by Scopus was used. The complete search strategy is described on Table S1. Language restrictions were applied to recover only articles in English, Spanish and Portuguese.

### **2.2. Selection strategy**

An initial selection based on title and abstract [TIAB] was independently conducted by the researchers (FBL, MMS, RDN). Duplicate studies were removed and only studies investigating the effect of fractions and isolated from plants extracts in murine models of skin wound healing were considered. After the initial search, all relevant studies were recovered in full text and evaluated by eligibility criteria. Works containing unrefined extracts; commercial isolates; in vitro assays; humans; non traumatic injuries; other or animal models, first intention wounds, metabolic diseases associates, secondary studies (i.e. letter to the editor, note, review, editorial) were excluded (Figure 1).

### 2.3. Data extraction

Data were extracted and tabulated in a descriptive way (Table 1A, 1B and Table 2A and Table 2B). The characteristics investigated were: publication characteristics (author, title, publication year, country); research methods (control group, randomization, experimental procedures, blind evaluation of the results); experimental model (animal, number of animals, sex, age, weight, specie, acclimation period, animal's housing, number of animals per cage and experimental groups, food supply, temperature, light cycle); plants (plant's species, isolates, fractions, dose, toxicity test, exotic/native plant, popular name, utilized part of the plant, popular indication); wounds description (wound area, measurement interval, and treatment duration) (Table 1A, 1B, Table 2A and 2B). In a comprehensive approach, ethnobotanical/ethnopharmacological aspects were also investigated as follows: plant's species investigated (Geographic distribution and existence or not of bioprospecting), popular indication, reports of toxicity tests (Figure 2).

### 2.4. Analysis of bias

The articles quality was analyzed by the criteria described on the ARRIVE platform (Animal Research: Reporting of In Vivo Experiments). These criteria are based in short descriptions that indicate essential characteristics of all studies with animal models, such as: theoretical and methodological basis, research objective, refinement of the analytical methods, statistical design and sample calculations, measure outcomes [28]. Recently there has been an increasing interest in the systematic reviews of research involving animals [29]. Considering the purpose of the systematic review on evaluating important aspects of the referenced publications, we built a table summarizing all the aspects investigated as well as their relevance, describing positive and negative characteristics of the recovered studies (Table 2A and 2B).

### 3. Results

#### 3.1. Included studies

From the PubMed and Scopus database, 1008 articles were recovered. 164 studies duplicated and 489 with thematic inadequacy were excluded after reading the title and abstract. After recovery of 329 articles in full text, 303 studies were excluded were excluded for not meeting the eligibility criteria. Thus, 26 studies were included on the systematic review. The reference list all included studies was carefully analyzed to ensure the identification of additional relevant studies. Thus, six studies were additional identified and recovered, completing 32 works added on this review. From these studies, 19 utilized fractions, 12 plant isolates and 1 study used both fractions and isolates for the treatment of cutaneous wounds (Figure 1).

#### 3.2. Qualitative analysis

The analyzed studies were conducted in 13 different countries, especially India (40.62%, n=13), followed by Brazil and Turkey (12.5%, n=4 each). The most utilized animal model on the experiments were rats (75%, n=24), followed by mice (12.5%, n=4) and both (12.5%, n=4). Considering the animal strain, 65.7% were Wistar rats, 17.14% Sprague-dawley rats, 11.42% Swiss mice and 5.71% were Hairless mice. Half of the experimental models used male animals (n=16), 15.62% (n=5) used females and 18.75% (n=6) studies used both sex. 15.62% (n=5) of all studies did not report this information. The animals' age ranged from 2 to 5 months for rats and from 8 to 12 weeks for mice, however, 71.8% (n=23) of the studies did not relate this information. The weight of rats ranged from 150 to 400g and the mice weighted between 18 and 40g, and only 2 studies (6.25%) did not report this data.

More than half of the studies did not describe the popular name of the plant species

investigated (59.37%, n=19). The first treatments utilized on the control group were: 25% (n=8) ointment base (which did not have its formulation described), 15.6% (n=5) used saline solution, 9.4% (n=3) Nitrofurazone and 6.2% (n=2) utilized distilled water. Only 3.1% (n=1) did not present the treatment for the control group. The others works utilized miconazole and non-ionic cream; gentamicin; natrigel solution; soft paraffin (85%), setosteryl alcohol (5%), hard paraffin (5%) and wool fat (5%); framycetin ointment; PBS; sodium alginate; vaseline; Tween 80; tragacanth; povidone iodine ointment; madecassol and ointment base; chlorocresol BP 0.1% were mentioned only once, representing 40.6% of all included studies (n=13). 62.5% (n=20) of the plant species were native, 12.5% (n=4) were exotic and 25% (n=8) of the studies did not describe this characteristic.

About wound area investigated presented a large variation (5mm<sup>2</sup> to 600mm<sup>2</sup>), and 9.37% (n=3) of the studies did not describe this data. The calculations used to measure the wound area were described in only 59.37% (n=19) of the studies. All the works described the interval in which the wound area was measured, and the most common interval was daily, 31.25% (n=10), followed by measurements taken each 4 days, 28.12% (n=9) (Table 1A, 1B). From the 32 species of plants, 23 different families were reported, and the main ones are Asteraceae 18.75% (n=6), Euphorbiaceae 9.37% (n=3), Leguminosae 6.25% (n=2), Fabaceae 6.25% (n=2), and the other families Liliaceae, Boraginaceae, Scrophulariaceae, Ranunculaceae, Apiaceae, Myrsinaceae, Mimosae, Malpighiaceae, Tiliaceae, Crassulaceae, Martyniacae, Rutáceas, Araliaceae, Piperaceae, Solanaceae, Caprifoliaceae, Dipterocarpaceae, Oleaceae, Combretaceae were mentioned once and represent 59.37% (n=19) of the included studies. The regions of the plant that were mostly were the leaves representing 37.5% (n=12), followed by the flowers 12.5% (n=4), bole bark (12.5% (n=4), and seeds 6.25% (n=2). The fruit, the

whole plant and the latex were mentioned once, representing 3.12% (n=1) each. However, 21.87% (n=7) of the studies did not mention this information. Considering the popular indication, healing effects were described in 46.87% (n=15) of the studies, followed by anti-inflammatory effects 34.37% (n=11), treatment of gastrointestinal diseases 28.12% (n=9), burns 18.75% (n=6), anti-rheumatic 12.5% (n=4), ophthalmological diseases 6.25% (n=2). 18.75% (n=6) of the studies did not report the popular indication. Only 33.4% (n=11) of the studies report toxicity tests (Figure 2).

### 3.3. Bias Analysis

Among the analyzed works, 78.12% presented a title coherent to the text, 90.6% presented abstracts containing the objectives, methods, main results and conclusions, and 75% presented an introduction with sufficient scientific base. All studies described ethical approval and no work reported a blind controlled study. Most studies (87.5%) related the therapeutic dose administered, 90.62% reported the route of administration and 96.87% the treatment duration. The choice of administration route was not justified in any study. Most studies 96.87% reported the animal strain investigated. The sex and weight were reported in 84.37% and 93.75% of the works respectively, but only 31.25% informed the age of the animals. 59.37% of the studies informed the experimental conditions (temperature, humidity, light cycles, feed and water). A statistical analysis was conducted by all studies, but only 68.75% specified the data analyzed. 84.37% of the studies reported the number of animals in each group and 68.75%. None study reported mortality or modifications on the experimental protocol by adverse events. A coherent interpretation of the results and direct relationship between objectives and hypothesis were described in 75% of all included studies (Table S2).

In general, the animals treated with isolates and fractions of plants presented an

elevated closure rate of the wound, representing 72.72% of the studies, (30,31,33,36,37,38,39,40,42,43,46,48,49,50,51,52,53,54,55,56,57,58,59), increase tissue reepithelialization 30.3% (28,31,32,33,36,40,48,51,56), increase of the traction strength on the cicatrice tissue 75.75% (28,30,31,32,33,35,36,37,38,40,44,46, 47,48,49,50,51,52,53,54,55,56,57,58,59), greater content and organization of the extracellular matrix on fast expansion of the granulation tissue 42.42% (30,31,36,43,45,46,49,51,52,53,55,57,58,59), and stimulation of the activity of endogenous antioxidant enzymes 9.09% (29,34,35) (Table 2A and 2B).

#### **4. Discussion**

The use of plant based strategies is opening a new perspective for the treatment of skin wounds, mainly in developing countries, once it represents a simple, low cost and affordable therapy [1,7,61-63]. There are several studies indicating beneficial effects of herbal medicines in all phases of the healing process. In fact, most of the studies included in this systematic review reported that plant fractions and isolates were able to improve the skin wound healing. Apparently, these medicines were especially favorable in controlling the cutaneous inflammatory and oxidative response, and stimulating the granulation tissue formation, collagen maturation and reepithelialization.

On this review, we did not included studies testing crude plant extracts, since the chemical characterization of the extracts makes difficult to determine the herbal components responsible for the effects reported. Even including only studies with murine models, different animal strains were observed. This aspect difficult the generalizability of the results, since the biological variability directly influence the response to the treatments. In addition, among the 32 analyzed studies, there was a large methodological variation and discrepancies in the measure outcomes. An evident

example was the wide variation in wound area and time of wound closure. These considerations are important because they are directly associated to the tensile force experienced by the tissue, which profoundly affect the speed and quality of skin repair [65,66]. Our findings show that 20% of the studies that utilized fractions neglected the analysis of wound closure, an essential information to assess the ability of any intervention to stimulate the healing process. In addition, the interval between measurements of wound area and the used protocols for the calculations were variable, representing methodological flaws that compromises the study reproduction and generalizability of the findings [67,68].

Considering that the reepithelization and organization of the granulation tissue are fundamental aspects to understand how chemical substances acts to stimulates wound healing, only 60% of all studies analyzed the reepithelialization rate and 75% evaluated the molecular components of the extracellular matrix, especially collagen. These parameters indicate if the wound closure follows a normal process, in which the newly formed tissue gradually develops drastic structural changes to reconstitute the morphofunctional characteristics of the intact skin. Works which demonstrated the importance of these analyses asserts that the type and quantity of collagen fibers deposited on the tissue can be used as a marker of tissue mechanical resistance [2,3,9,63]. The connective and epithelial tissues form a structure support to promote the correct closure of the wound [62,69], reducing the chances of opportunistic infections in the wounded area [70,71]. During the granulation tissue formation there is a predominance of sulfated molecules which attract water, facilitating the cellular migration, and also serve as a support structure for the first formed collagen (type III collagen) [72]. There are enough evidences that the synthesis and differentiation of cells and matrix components are crucial for a normal wound closure [66]. It is already known

that the oxidative stress induces cell damage, lipid, protein and nucleic acids oxidation [75,76]. It is recognized that cutaneous trauma increases the tissue oxidative stress in the wounded area [75-78]. Although reactive species are able to activate cell signaling pathways and stimulate cell proliferation, differentiation and neoangiogenesis; excessive production of these molecules acts inhibiting the healing process, especially by inducing cell death and molecular damage in the extracellular matrix [36,79,80]. Thus, there is a notorious importance in analyzing the redox balance during skin repair. However, from all analyzed studies, only 15% investigated the oxidative status. This is a surprising finding, since the antioxidant effect is a pivotal mechanism indicated in several studies to support the applicability of plant extracts in the treatment of tissue damage, including skin wounds [75-78]. Another fundamental result on the cutaneous repair process is the restoration of the biomechanical properties, especially the tensile force of the newly formed tissue, which provides functional estimates on the quality of the healing process [50]. On this review, only 35% of all studies investigating plant fractions evaluated the traction resistance of the scar tissue, aspects investigated in 61.53% of the studies with plant isolates.

In our findings, we see that the majority of the studies used male animals, an aspect potentially associated to the hormonal stability, which is not observed in female animals due to the estrous cycle [81]. The use of rats as the experimental model was higher (75%), aspect potentially related to the large body area needed to perform experimental wounds (1 to 5). Thus, it is possible to construct a larger number of wounds and use a smaller number of animals in each group. In addition, in rats it is possible to collect enough fragments in order to fully analyze the healing process. Another interesting data was the age of the animals, which presented a large variation (rats, 2 to 8 weeks; mice, 5 to 12 weeks). However, 71.8% of the studies did not report this information, making it

difficult to establish a temporal basis to determine the effectivity of the herbal treatments investigated. More than half of the studies (59.37%) did not describe the popular name of the plant species. The large number of works that did not describe important variables such as age of the animals and plant species represent a concerning number, once that these characteristics are of great importance for ensure the study reproducibility and to allow the elaboration of broad reports with a critical review of the findings [28]. The orientation cited on the ARRIVE guideline describe the minimum information that all scientific publications using animals should include. This guide also brings items that help to understand on the quality of the writing and potential methodological bias that compromise the quality of the evidence [28]. The work title should refer to the readers a brief summary of the article content, providing keywords and terms that could be researched in electronic databases [82]. Only 78.12% of the studies presented a coherent title, while 90.6% presented abstracts with clear information relative to the objectives, methods, main results and conclusions. Furthermore, 75% presented introduction with enough scientific base, what can make it harder to the reader understand the relevance of the study. Another observation made through ARRIVE guide refers to the health conditions of the animals during the experiment. Thus, aspects such as information about environmental conditions (temperature, humidity), mortality, feeding, randomization, reactions indicative of systemic or local toxicity were neglected in most studies, demonstrating that the report bias is a serious limitation of these preclinical tests that compromise the reliability of the results and the quality of the evidence [83].

## **5. Conclusions**

The current evidence indicates that fractions and isolated molecules from plant extracts stimulates the healing process in cutaneous wounds. Apparently, the main

effects of these herbal medicines are associated with the stimulation of collagen synthesis, expansion of the granulation tissue, reepithelialization, modulation of the inflammatory response and oxidative stress during tissue repair. Together, these effects promote increase the speed of wound closure and the biomechanical resistance of newly formed tissue. However, the serious methodological flaws and report bias observed in most included studies makes fragile the current evidence. Thus, the relevance of fractions and isolated molecules from plant extracts in the treatment of skin wound cannot be accurately determined. Considering these limitations, seem to be impossible use these evidences to construct a rational basis that supports clinical studies. Therefore, there is an urgent need to improve research reports in experimental studies with herbal medicines in murine models of skin wound healing. This task requires a collective effort of authors, journal editors, reviewers, and from financial organisms, to ensure the reproducibility, reliability, and generalizability of the evidence, fundamental elements to determine to what extent herbal medicines are promising in the treatment of skin wounds.

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### **Conflict of interests**

The authors declare that there is no conflict of interest regarding the publication of this paper.

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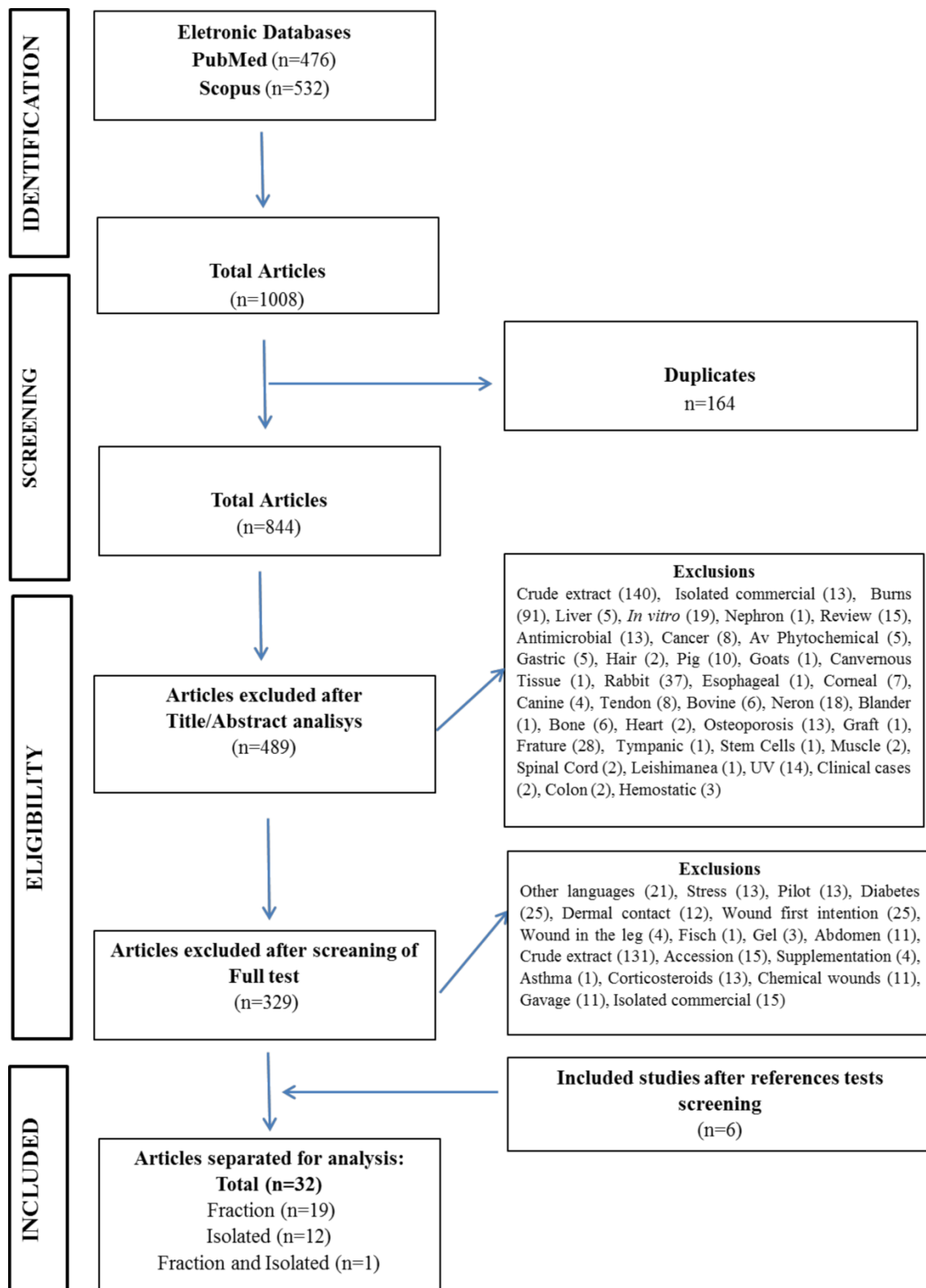
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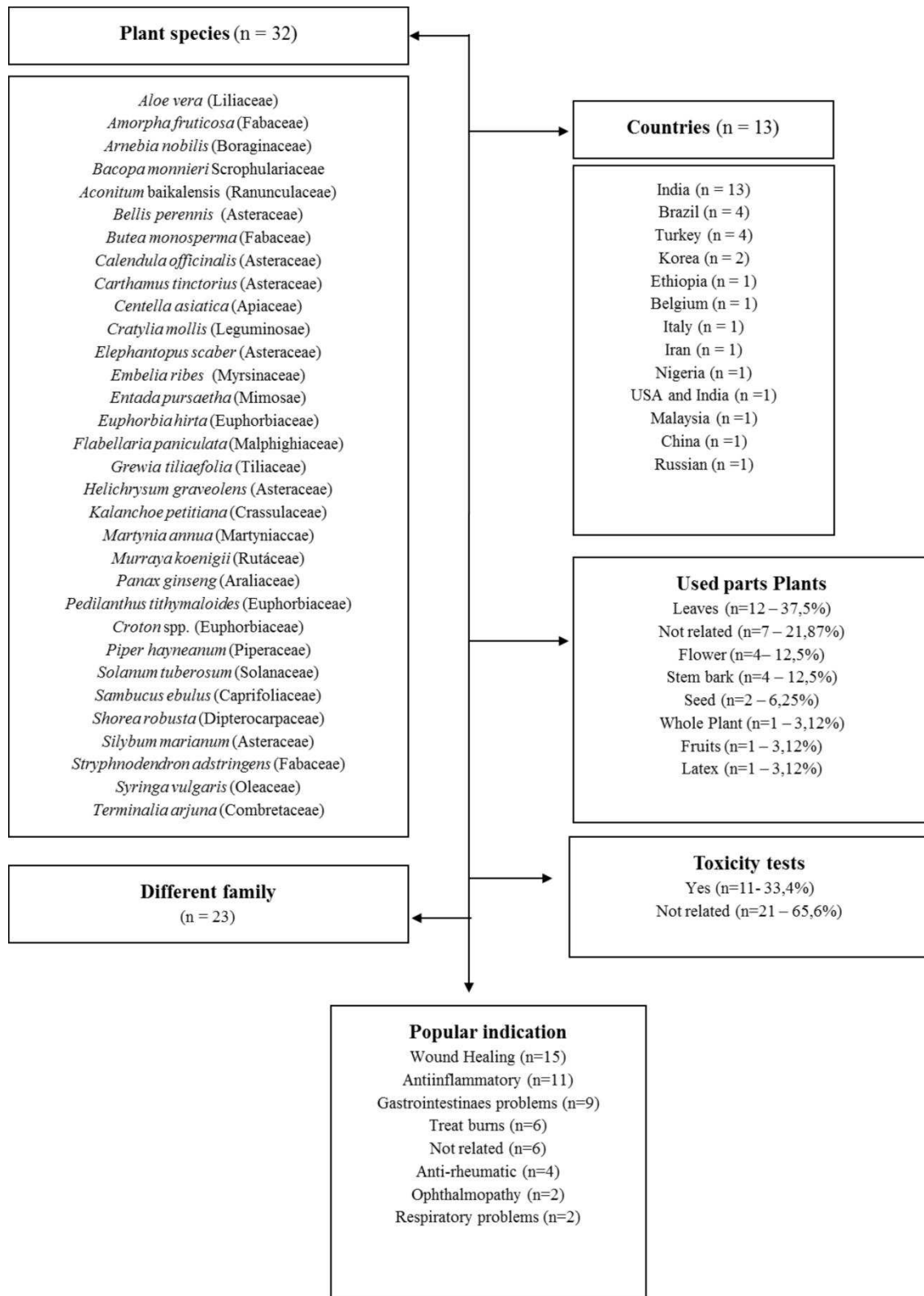
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**Figure 1:** Flowchart of the strategy applied to recover preclinical studies according to the PRISMA statement.



**Figure 2:** Summary of the studies describing the plants species, families, used parts of each specie, toxicity tests and popular indications.

**Table 1A.** Description of the main characteristics of the studies with fractions obtained from plant extracts.

Reference	Country	Animal strain	Animal number	Sex	Age	Weight	Experimental groups	Animal number per group	Numbers of animals per box	Treatment control group	Plant species	Native/Exotic	Used parts	Fractions	Dose	Popular indication	Wound area	Measurement interval	Wound area calculation	Treatment time
Bastos, et al., 2011 (28)	Brazil	Wistar rats	12	♂	4mo	300-320g	4	3	1	Miconazole and non-ionic cream	Piper hayneanum (Piperaceae)	?	L	[CHCl <sub>3</sub> -EtOAc 1: 1 (hastes, A) and CHCl <sub>3</sub> -MeOH 1: 1 (raizes, B),	?	Anti-inflammatory, infectious skin diseases and healing, wounds, hematoma, and ecchymosis	6 mm <sup>2</sup>	daily	?	15 days
Shukla, et al., 1999 (29)	India	Sprague Dawley rats	?	♂	?	200-220g	3	?	1	Saline Solution	Centella asiatica (Apiceae)	?	?	Asiaticoside	20 µL of 0.2%	Healing activity	8 mm <sup>2</sup>	7/7 days	?	14 days
Muralidhar, et al., 2011 (30)	India	Wistar rats	42	?	?	150-200g	7	6	?	Ointment base	Butea monosperma (Fabaceae)	N	Sb	PETFR: petroleum ether fraction BENFR: benzene fraction, CHLFR: chloroform fraction, ACEFR: acetone fraction	200 mg/ kg-	Antitumour, antiulcer, antifungal and anti diarrheal activities	500 mm <sup>2</sup>	4/4 days	?	16 days
Süntar, et al., 2013 (31)	Turkey	Sprague-Dawley rats	?	♂	?	160-180g	?	6	?	Ointment base	Helichrysum graveolens (Asteraceae)	N	F	Hg-hexane; Hg-CH <sub>2</sub> Cl <sub>2</sub> ; Hg-EtOAc, Hg-BuOH; Hg-R-H <sub>2</sub> O; Hg-Fr.B1; Hg-Fr.B2, Hg-Fr.B3, Hg-Fr.A, Hg-Fr.B; Hg-Fr.C	0.5 g	Antimicrobial, antioxidant, anti-inflammatory, sedative, antidiabetic and cytotoxic activities	5 mm <sup>2</sup>	daily	Reduction in wounded area, using AutoCAD program	12 days
Mekonnen, et al., 2013 (32)	Ethiopia	Swiss Mice/ Wistar rats	24	?	8-10 wk / 3-5 mo	30-40g / 180-200g	4	6	1	Sodium carboxyl methyl cellulose xerogel and nitrofurazone	Kalanchoe petitiiana (Crassulaceae)	N	L	Methanolic and chloroform fractions were 16%, 8.76%, 7.5%, and 5.6%, respectively.	?	wound healing , hemorrhoids, antibacterial activities	312 mm <sup>2</sup>	daily	% wound contraction = wound area on day 0 - wound area on day n / wound area on day 0 x 100	10 days
Pieters, et al., 1995 (33)	Belgium	Wistar rats	?	♀	?	250-300g	20	2	1	Not treated	Croton spp. (Euphorbiaceae)	E	Lx	Polyphenolic: PEG ointment, PEG 400 10%	0,5 ml / 2x day	wound healing	3 cm	daily	?	18 days
Korkina, et al., 2007 (34)	Italy	Wistar rats	40	♂	?	350-400g	5	10	?	Saline Solution	Syringa vulgaris (Oleaceae)	N	F	Two phenylpropanoid glycosides: verbascoside and teupolioside	100 µL (0,2 mg/ml)	wound healing, anti-inflammatory, anti-rheumatic, anti-pyretic, anti-fungal activities	2.25 cm <sup>2</sup>	4/4 days	The recorded wound were measured by planimetry using special computer program.	8 days
Bigoniya, et al., 2013 (35)	India	Wistar rats	30	♂	?	175 ± 10g	5	6	?	Vehicle (not related)	Euphorbia hirta (Euphorbiaceae)	N	Wp	flavonoid fraction (EHTF)	?	antimicrobial, antifungal, antiviral anti-inflammatory, anti-arthritic and antioxidant activities	500 mm <sup>2</sup>	4/4 days	?	20 days
Lodhi, et al., 2011 (36)	India	Wistar rats	30	♂♀	?	150-200g	5	6	1	Not treated	Martynia annua (Martyniaceae)	N	L	M. annua fraction - MAF-A, MAF-B and MAF-C	?	?	500 mm <sup>2</sup>	2/2 days	% Wound contraction = Healed area /Total wound area X 100	20 days

**Table 1A. (Continued)**

Reference	Country	Animal strain	Animal number	Sex	Age	Weight	Experimental groups	Animal number per group	Numbers of animals per box	Treatment control group	Plant species	Native/Exotic	Used parts	Fractions	Dose	Popular indication	Wound area	Measurement interval	Wound area calculation	Treatment time
Tabandeh, et al., 2013 (37)	Iran	Wistar rats	60	♂	?	200 ±50 g	4	15	?	Saline Solution	Silybum marianum (Asteraceae)	E	?	flavonoid silibinin (SB)	10% and 20% SB powder	hepatoprotective and liver regenerating activities	1 cm	daily	?	30 days
Sonmez, et al., 2015 (38)	Turkey	Wistar rats	24	♂	?	180–260g	3	8	?	Saline Solution	Solanum tuberosum (Solanaceae)	?	?	polysaccharide haemostat (APH)	3mg of wheat meal in group 2 and 3mg of APH in powder form	?	2x2x2 cm	3,7,14 days	Percentage of contraction=[100 – (total wound area on the 14th day/total wound area on the 3rd day)X 100].	14 days
Karakaş, et al., 2012 (39)	Turkey	Wistar rats	12	♂	?	200-250g	2	12 and 8	?	Not treated	Bellis perennis (Asteraceae)	E	F	n-butanol fraction	?	Activities in sore throat, headache, eczema, skin boils, gastritis	4 mm <sup>2</sup>	1,5,10, 30 days	Percentage of wound area = Wound area in day X / Wound area in the first day X 100; Percentage of wound healing = 100 – Percentage of wound area	30 days
Choi, et al., 2001 (40)	Korea	Hairless mice	10	♂	?	?	2	10	?	Vehicle (not related)	Aloe vera (Liliaceae)	?	?	glycoprotein fraction named G1G1M1DI2	10 mg/g ointment Gentamicin 0.1% , every day	wound healing, thermal injury healing, anti-inflammation and immunomodulation activities	154 mm <sup>2</sup>	daily	?	8 days
Parente, et al., 2011 (41)	Brazil	Wistar rats	36	♀	60 days	160-190g	2	18 and 6	1	Distilled water	Calendula officinalis (Asteraceae)	E	F	DCF: dichloromethane fraction at 1%; HCF: hexanic fraction at 1%.	?	anti-inflammatory, first-degree burns, skin rashes activities	1 cm	4,7,14 days	?	14 days
Olugbuyiro, et al., 2010 (42)	Nigeria	Wistar rats	16	♂	?	250-300g	2	4	1	Gentamicin and Saline Solution	Flabellaria paniculata (Malpighiaceae)	N	L	Chloroform fraction and Aqueous fraction	100 mg/ml	Activities in skin infections, wounds and sores; dysentery	2x2 cm	7,12,14 , 18 days	?	18 days
Süntar, et al., 2010 (43)	Turkey	Sprague–Dawley rats / Swiss mice	?	♂	?	160-180g / 20-25g	9	6	?	not treated	Sambucus ebulus (Caprifoliaceae)	N	L	polyamide column fractions from the methanolic extract (Fr A, B, C, D , E)	0,5g	hemorrhoids, rheumatic pain, treat burns, infectious wounds, edema, eczema, urticarial, inflammations	5 mm <sup>2</sup>	2/2 days	Wound contraction was calculated as percentage of the reduction in wounded area	12 days
Kim, et al., 2013 (44)	Korea	Hairless mice	10	♀	2 mo	?	2	5	?	Natrigel solution	Panax ginseng (Araliaceae)	?	L	ginsenoside Rd	10 mL	Strengthen Immune system, Atherosclerosis activities	?	3/3 days	?	9 days

**Table 1A. (Continued)**

Reference	Country	Animal strain	Animal number	Sex	Age	Weight	Experimental groups	Animal number per group	Numbers of animals per box	Treatment control group	Plant species	Native/Exotic	Used parts	Fractions	Dose	Popular indication	Wound area	Measurement interval	Wound area calculation	Treatment time
Chaudhari, et al., 2006 (45)	India	Wistar rats	30	♂♀	?	180-250g	5	6	?	Soft paraffin (85%), cetosteryl alcohol (5%), hard paraffin (5%) and wool fat (5%)	Terminalia arjuna (Combretaceae)	N	Sb	Fraction I hydroalcohol ; Fraction II phytoconstituents extraction of tannins; Fraction III consisted of saponins ..	0.5 g	Diuretic, cooling, aphrodisiac, expectorant, antidiysenteric, urinary astringent, antioxidant, antibacterial activities	4 cm <sup>2</sup>	2/2 days	?	16 days
Swamy, et al., 2006 (46)	India	Wistar rats	24	♂♀	?	150-200g	4	6	?	Framycetin ointment	Embelia ribes (Myrsinaceae)	?	L	embelin	4 mg/ml of 0.2% sodium alginate gel	anti-inflammatory to relive rheumatism and fever activities	500 mm <sup>2</sup>	4/4 days	?	16 days
Hernandes, et al., 2010 (47)	Brazil	Wistar rats	15	♂	?	180-200g	3	5	1	Ointment base	Stryphnodendron adstringens (Fabaceae)	?	Sb	EtOAc fraction	?	Antioxidant, cicatrizant, antiinflammatory activities	7 mm <sup>2</sup>	4,7,10 days	?	10 days
Sidhu, et al., 1999 (48)	USA and India	Sprague–Dawley rats	?	♂	?	250-300g	4	?	1	vehicle PBS	Arnebia nobilis (Boraginaceae)	N	?	Arnebin-1 (5,8-dihydroxy-2-(19-b,b-dimethylaryoxy-49-methylpent-3-enyl)-1,4-naphthoquinone	?	?	8 mm <sup>2</sup>	daily	?	11 days
Paramesha, et al., 2015 (49)	India	Wistar rats	18	?	?	150-200g	3	6	?	sodium alginate	Carthamus tinctorius (Asteraceae)	N	L	ehydrobietylamine of C. tinctorius L., var. Annigeri-2	(50 g) to get 0.2 % (w/w) ointment gel.	laxative, appetizer and diuretic also useful in urorrhoea and ophthalmopathy activities	?	4/4 days	?	16 days
Nagappan, et al., 2012 (50)	Malaysia	Sprague Dawley rats	84	♀	?	200-250g	7	12	1	Not treated	Murraya koenigii (Rutaceae)	N	L	carbazole alkaloids mahanine (1) (0.40%) (C <sub>23</sub> H <sub>25</sub> NO <sub>2</sub> ), mahanimbicine (2) (0.24%) (C <sub>23</sub> H <sub>25</sub> NO) and mahanimbine (3) (0.66%)	Mahanine (1) (0.40%) (2) (0.24%) (3) (0.66%) (w/w)	stimulants, tonics, treat influenza, fever, bronchial asthma activities	8 mm <sup>2</sup>	daily	(%) of Wound contraction = Ø of wound area - Ø of unhealed w.a / Diameter of w.a.(wound area) × 100%	18 days
Qu, et al., 2013 (51)	China	Sprague-Dawley rats	54	♂	?	200-220g	9	6	1	Vaseline	Amorpha fruticosa (Fabaceae)	N	Fr	6a,12a-dehydroamorphin, D-3-O-methyl-chiro-inositol, Kaempferol-3-glucoside, 7-rhamnoside, 7,2',4',5'-tetra-ethoxyflavone, dehydrosermundone, tephrosin, 7,4'-dimethoxyisoflavone.	?	?	500 mm <sup>2</sup>	2/2 days	percent wound contraction = (original wound area - unhealed area)/original wound area × 100%.	22 days

Ad: Adults; wk: week; mo: month; d: days; ♂: Male; ♀: Female; N: Native; E: Exotic; L: Leaves; F: Flowers; Sb: Stem Bark; S: Seed; Wp: Whole plant; Fr: Fruits; Lx: Latex; ?: not related.

**Table 1B.** Description of the main characteristics of the studies with isolated molecules from plant extracts.

References	Country	Animal strain	Animal number	Sex	Age	Weight	Experimental groups	Animal number per group	Numbers of animals per box	Treatment control group	Plant specie	Native/Exotic	Used parts	Isolated	Dose	Popular indication	Wound area	Measurement interval	Wound area calculation	Treatment time
Ghosh, et al., 2012 (52)	India	Wistar rats / Swiss albino mice	36	♂	?	150-180 g / 20-25 g	6	6	?	Ointment base	Pedilanthus tithymaloides (Euphorbiaceae)	N	L	2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromen-4-one and 1, 2-tetradecanediol, 1-(hydrogen sulfate)	50 mg	antiviral, antibacterial, antihemorrhagic, antitumor, abortive, anti-inflammatory activities	500 mm <sup>2</sup>	3/3 days	(%) wound contraction $\frac{1}{4}$ (initial X final wound area) X100.	21 days
Mukherjee, et al., 2013 (53)	India	Swiss albino mice / Wistar rats	?	?	?	18-20 g / 150-180 g	10	?	?	ointment base and povidone-iodine	Shorea robusta (Dipterocarpaceae)	N	L	Compound I - Bioactive bergenin and Compound II - triterpene ursolic acid	0.025 g of isolated compound 1 and 2 mixed with 10 g ointment base	wounds and burn healing	6 cm	3/3 days	Two days interval / (Wound area on day 0 x Wound area on day n) / Wound area on day 0X100.	?
Melo, et al., 2011 (54)	Brazil	Swiss mice	30	♀	12 wk	45.0 ± 2.0 g	4	?	1	NaCl	Cratylia mollis (Leguminosae)	N	S	Cramoll 1,4 lectin	100 g/mL	?	0.5 cm	2,7, 12 days	$A = \pi \times R \times r$	12 days
Pieters, et al., 1995 (33)	Belgium	Wistar rats	40	♀	?	250-300g	20	2	1	Not treated	Croton spp. (Euphorbiaceae)	E	Lx	3',4'-O-dimethylcedrusin, Taspine hydrochloride	0,5 ml / 2x day	wound healing activities	3 cm	daily	?	18 days
Ahamed, et al., 2008 (55)	India	Wistar rats	24	♂♀	?	240-250 g	4	6	6	Tween 80	Grewia tiliifolia (Tiliaceae)	N	Sb	gulonic acid $\gamma$ -lactone (GAGL)	50 mg / 1x day	burns, skin diseases, inflammation, diarrhea and pruritis, chronic wounds, gastric ulcers	?	4/4 days	?	16 days
Zyuz'kov, et al., 2012 (56)	Russian	?/Mice	136	♂	2 mo	22-24 g	6	?	?	Water	Aconitum baikalensis (Ranunculaceae)	?	?	songorine, napelline, hyaconitine, 12-epinapelline N-oxide and mesaconitine	30 mL	?	10x10 mm	daily	?	16 days
Singh, et al., 2005 (57)	India	Wistar rats	20	♂♀	?	150-200 g	5	6	?	Tragacanth	Elephantopus scaber (Asteraceae)	N	L	deoxyelephantopin	50 mg	dysuria, diarrhea, dysentery, and in stomach pain; eczema and ulcers; wound healing	500 mm <sup>2</sup>	4/4 days	?	14 days
Sharath, et al., 2010 (58)	India	Wistar rats	?	♂♀	?	200-250 g	2	5	?	Nitrofurazone	Bacopa monnieri (Scrophulariaceae)	N	?	Bacoside-A	200 mg	Laxative, ulcers, anemia, leucoderma, Scabies	500 mm <sup>2</sup>	4/4 days	?	16 days
Vidya, et al., 2012 (59)	India	Wistar rats	30	?	?	160-200 g	4	6	?	Nitrofurazone	Entada pursaetha (Mimosaceae)	N	S	Entadamide, phaseoloidin, and entagenic acid	?	cancer, dropsy, eye diseases, wounds, snake bite, respiratory problems, antibacterial	500 mm <sup>2</sup>	4/4 days	?	16 days

Ad: Adults; wk: week; mo: month; d: days; ♂: Male; ♀: Female; N: Native; E: Exotic; L: Leaves; F: Flowers; Sb: Stem Bark; S: Seed; Wp: Whole plant; Fr: Fruits; Lx: Latex; ?: not related.

**Table 2A:** Main Parameters analyzed in the studies demonstrating the action of fractions from plants in the treatment of skin wounds in murine models.

**PARAMETERS OF WOUND HEALING IN MODELS MURINES – FRACTIONS**

Reference	Wound closure analysis	Reepithelialization analysis	Oxidative Estress	Granulation Tissue Fill	Tensile strengths
Bastos, et al., 2011	?	Fraction A and B- Moderated 9 day Fraction A and B- 100% 15 day	?	After 15 days in the treated rats, the wound healing process by stimulating different biological events such as network of fibrin, epithelialization, granulation tissue, neovascularization, and wound contraction	?
Shukla, et al., 1999	?	?	<b>Increase:</b> superoxide dismutase (35%), catalase (67%), glutathione peroxidase (49%), <b>Reduced:</b> glutathione (17%).	?	?
Muralidhar, et al., 2011	Petroleum ether fraction - (86.83±0.87%) 16 day; Benzene fraction - (86.67±0.67%) 16 day; Chloroform fraction - (88.0±0.57%) 16 day Acetone fraction - (96.0±0.37%) 16 day Control - (85.17±0.79%) 16 day	<b>Epithelialization in days</b> Petroleum ether fraction 21.17±0.48%, Benzene fraction 21.67±0.42%, Chloroform fraction 21.83±0.48% Acetone fraction 16.67±0.42% Control 22.0±0.37%	?	<b>Hydroxyproline content (µg/mg)</b> Petroleum ether fraction - 21.57±0.21 Benzene fraction - 20.96±0.08 Chloroform fraction - 21.84±0.08 Acetone fraction - 23.50±0.17 Control - 21.48±0.17	Petroleum ether fraction - 155.83±2.26 g Benzene fraction - 151.0±2.59 g Chloroform fraction - 163.33±1.33 g Acetone fraction - 212.83±2.02 g Control - 147.33±1.23 g
Süntar, et al., 2013	<b>Wound area(mm2) ± S.E.M. (Contraction%) in 12 days</b> Hg–MeOH - 0.96 ± 0.30 (65.71%) Hg–Hexane1 - 2.37±0.11(15.36%) Hg–CH2Cl2 1 - 2.35±0.29 (16.07%) Hg–EtOAc1 - 1.47±0.32 (47.50%) Hg–BuOH - 1.74±0.48 (37.86%) Hg–R–H2O - 2.63±0.17(6.07%) Hg–Fr.A - 2.20±0.39 (20.29%) Hg–Fr.B - 1.65±0.09 (40.22%) Hg–Fr.C - 1.83±0.14 (33.69%) Control - 2.76±0.30 (6.44%)	Tissues treated with Hg–MeOH, Hg–EtOAc, Hg–Fr.B demonstrated a good wound recovery with faster re-epithelialization compared to the other groups tested.	?	<b>Hydroxyproline content (µg/mg)</b> Hg–MeOH – 26.3 ±1.0 Hg–Hexane1 – 18.5 ±2.1 Hg–CH2Cl2 – 19.7 ±1.9 Hg–EtOAc1 – 31.2 ±0.9 Hg–BuOH – 15.6 ±1.8 Hg–R–H2O – 13.3 ±1.8 Hg–Fr.A – 15.4 ±1.2 Hg–Fr.B – 25.5 ±1.2 Hg–Fr.C – 16.3 ±1.9 Control – 8.9 ±2.1	Hg–MeOH – 30,11% Hg–Hexane1 – 17,5% Hg–CH2Cl2 – 15,2% Hg–EtOAc1 – 28,5% Hg–BuOH – 25,8% Hg–R–H2O – 11,6% Hg–Fr.A – 13,9% Hg–Fr.B – 25,2% Hg–Fr.C – 21,3% Control – 5,8%
Mekonnen, et al. 2013	<b>Wound contraction in 12 days</b> Chloroform: Xerogel - (77.517±1.88), 5% - (79.91 ±71.30), 10% - (82.63±1.74) Methanol: Simple ointment - (86.21±1.5), 5% - (90.86±0.21), 10% (92.09±2.00) Control - (96.63±0.32)	<b>Epithelialization in days</b> Chloroform: Xerogel - (17.83±0.30), 5% - (17.16±0.60), 10% - (16.83±0.65) Methanol: Simple ointment - (17.33±0.33), 5% - (15.66±0.21), 10% - (15.33±0.66) Positive control (14.00±0.44)	?	<b>Hydroxyproline content (µg/mg)</b> Chloroform: Xerogel - (3.01±0.46 5%), 5% - (5.83±0.79), 10% - (7.08±2.08) Methanol: Simple ointment - (3.29±0.66), 5% - (11.01±0.53), 10% - (15.33±0.66) Control (12.57±2.59)	Chloroform: Xerogel - 190.83±15.62 g (14.26%), 5% - 238.33±22.86 g (24.89%), 10% - 265.00±33.04 g (38.86%) Methanol: Simple ointment - 201.50±10.05 g (20.65%), 5% - 322.00±23.63 g (59.80%), 10% - 336.83±28.39 g (67.16%) Control - 402.33±30.26 g

**Table 2A. (Continued)**

Reference	Wound closure analysis	Reepithelialization analysis	Oxidative Estress	Granulation Tissue Fill	Tensile strengths
Pieters, et al., 1995	PEG ointment – (70%) 15 days PEG 400 10% - (80%) 15 days Polyphenolicfraction from Dragon's blood in H2O – (90%) 15 days Control - (60%) 15 days	PEG ointment – ++ (15 days) PEG 400 10% - ++ (15 days) Polyphenolicfraction from Dragon's blood in H2O – ++ (15 days) Control - + (15 day)	?	<b>Crust presence</b> PEG ointment – after 4 days PEG 400 10% – after 5 days Polyphenolicfraction from Dragon's blood in H2O – after 1 days Control – after 3 days	?
Korkina, et al., 2007	Both verbascoside 56% (46,29 ± 12,21%) 8 days Both verbascoside 97% (124,29 ± 31,23%) 8 days Teupolioside 70% (78,39 ± 21,75%) 8 days Teupolioside 97% (98,45 ± 24,26%) 8 days Control - (150,16 ± 65,46%) 8 days	?	<b>Lipid peroxidation</b> Both verbascoside 56% (7,4 ± 0,6%) Both verbascoside 97% (5,8 ± 0,4%) Teupolioside 70% (12,0 ± 0,7%) Teupolioside 97% (9,4 ± 0,6%) Control - (10,3 ± 1,0) <b>Glutathione (GST)</b> Both verbascoside 56% (3,0 ± 1,3%) Both verbascoside 97% (5,1 ± 1,3%) Teupolioside 70% (3,4 ± 1,3%) Teupolioside 97% (5,9 ± 1,2%) Control - (3,6 ± 1,3%) <b>Superoxide dismutases</b> Both verbascoside 56% (2,5 ± 0,1%) Both verbascoside 97% (2,2 ± 0,1%) Teupolioside 70% (3,1 ± 0,3%) Teupolioside 97% (1,0 ± 0,1%) Control - (4,5 ± 0,5%)	?	?
Bigoniya, et al., 2013	EHTF 200 (71,01±4,25%) 16 days EHTF 400 (69,98±3,34%) 16 days EHTF 600 (6,02±0,79%) 16 days Control (71,65±3,21%) 16 days	EHTF 200 (19,66±2,85%) EHTF 400 (19,50±2,63%) EHTF 600 (17,50±1,56%) Control (21,50±1,22%)	<b>Vehicle control:</b> Catalase (0,46±0,02%);SOD (1,15±0,12%) and Total protein (2,60±0,06%). <b>EHTF 200:</b> Catalase (0,45±0,03%), SOD (1,16±0,06%) and Total protein (2,69±0,07%). <b>EHTF 400:</b> catalase (0,52±0,09%),SOD (2,63±0,15%) and Total protein (3,34±0,05%). <b>EHTF 600:</b> Catalase (0,75±0,19%), SOD (5,06±0,09%) and Total protein (4,02±0,03%).	<b>Hydroxyproline content</b> EHTF 200 (15,89±1,28%) EHTF 400 (17,89±2,26%) EHTF 600 (24,14±2,23%) Control (16,09±1,35%)	?
Lodhi, et al., 2011	MAF A (100,00%) 20 days MAF B (100,00%) 20 days MAF C (100,00%) 18 days Control (90,37±2,07%) 20 days.	MAF A and B (20 days); MAF C (18 days); Control (24 days).	?	<b>Hydroxyproline content:</b> MAF A (37,11±1,25%); MAF B (32,86±0,85%) MAF C (42,01±0,82%) Control (21,74 ±1,85%) <b>Protein content</b> MAF A (56,30±0,55%); MAF B (52,50±1,70%) MAF C (83,60±0,72%).Control (47,30±1,72%);	MAF A (603,00±12,01%) MAF B (635,00±9,68%) MAF C (850,00±11,89%) Control (423,00±10,96%)

**Table 2A. . (Continued)**

Reference	Wound closure analysis	Reepithelialization analysis	Oxidative Estress	Granulation Tissue Fill	Tensile strengths
Tabandeh, et al., 2013	Silibinin 10% - 100% (18 day) Silibinin 20% - 100% (22 day) Control - 100% (26 day)	?	?	<b>Content N-acetyl glucoseamine and n-acetyl galactoseamine:</b> Silibinin 10 and 20% ↑ compared with the control groups at days 10, 20 and 30. <b>Hydroxyproline and collagen content:</b> Silibinin 10 and 20% ↑ compared with the control groups at days 10, 20 and 30	?
Sonmez, et al., 2015	Absorbable Polysaccharide Haemostat (APH) – (94.74 ± 0.02% ) 14 days Control - 87.33 ± 0.02% 14 days	?	?	<b>Type 1 Collagen</b> APH - 4.25 Control - 3.25 <b>Fibroblast density</b> APH - 2.87 Control - 1.75	?
Karakaş, et al., 2012	HOT - (80%) 30 days HOTBp – (100%) 30 days Control - (80%) 30 days	?	?	HOT - ↑ fibroblastic and lymphocytes: 5 days HOTBp – ↑ fibroblastic and lymphocytes: 5 days Control - ↑ fibroblastic and lymphocytes: 5 days HOT - ↑ Collagen fibrils: 10 days HOTBp – ↑ Collagen fibrils: 10 days Control - ↑ Collagen fibrils: 10 days	?
Choi, et al., 2001	G1G1M1DI2 – (98,9%) 8 days Control – (69,5%) 8 days	<b>Epithelialization in 8 days</b> G1G1M1DI2 - 98,9% Control - 69,5%	?	<b>EGF receptor</b> G1G1M1DL2 0,5% - (113%) G1G1M1DL2 50% - (220±8%) Control: 100%. <b>Fibronectin</b> G1G1M1DL2 0,5% - (294±34%) G1G1M1DL2 50% - (408±80%) Control: 100% <b>Fibronectin receptor</b> G1G1M1DL2 0,5% - (159±11%) G1G1M1DL2 50% - (220±19%) Control: 100%	?
Parente, et al., 2011	?	?	?	Number of blood vessels HCF 1 (0/4) DCF 2 (0/13) Control 2 (0/13) Days 4 and 7 presence of fibrin both groups	?

**Table 2A. . (Continued)**

Reference	Wound closure analysis	Reepithelialization analysis	Oxidative Estress	Granulation Tissue Fill	Tensile strengths
Olugbuyiro, et al., 2010	<b>Flabellaria paniculata</b> Chloroform fraction: 0.0 (100%) 14 days Aqueous fraction: 25.0 ± 3.0% (71.4%) 14 days Control: 87.5 ± 7.5%	<b>Flabellaria paniculata on non-infected rat wounds</b> Chloroform fraction:(14.0 ± 0.0%) Aqueous fraction: (21.5 ± 0.5%) Control: ( 24.5 ± 0.5%)	?	?	?
Süntar, et al., 2010	mm2 (%) Fr. A- 1.60 ± 1.53 (44.4%) Fr. B- 1.59 ± 0.11 (44.8%) Fr. C- 0.99 ± 0.31 (65.6%) Fr. D- 0.77 ± 0.03 (73.3%) Fr. E- 1.98 ± 0.63 (31.3%) Control- 2.88 ± 0.72 (17.5%)	?	?	?	mm2 (%) Fr. A- 21.52 ± 1.15 (13.9%) Fr. B- 24.97 ± 3.18 (32.3%) Fr. C- 25.63 ± 1.43 (35.8%) Fr. D- 26.61 ± 2.05 (40.9%) Fr. E- 22.95 ± 2.73 (21.6%) Control- 18.88 ± 2.67 (16.3%)
Kim, et al., 2013	The ginsenoside Rd-treated wounds were significantly smaller than the wounds treated with control matrigel day 6 and 9	?	?	Ginsenoside Rd↑ proliferation and migration fibroblasts. Ginsenoside Rd at 0.1–10 mM↑collagen type I protein and ↓ MMP-1 protein in fibroblasts.	?
Chaudhari, et al., 2006	?	Fraction I - 9 days Fractions II - 23 days Fraction III- 20 days	?	Fraction I increase in hexosamine fractions II and III did not reveal increase in the hexosamine content of granulation tissue.	Fraction I 719.33g ± 0.88 Fractions II 572.33g ± 2.46 Fractions III 590.33g ± 1.87
Swamy, et al., 2006	Embelin - (98.50% ± 1.64) 16 day Control - (85.33% ± 3.66) 16 day	<b>Epithelialization in days</b> Embelin - 18.17 ± 1.47 Control - 20.33± 2.66	?	Granulation tissue showed complete healing with more of fibroblasts, collagen and increased number of blood vessels.	Embelin - 528.00g ± 15.85 Control - 374.67g ± 5564
Hernandes, et al., 2010	The 1% ethyl-acetate fraction from barbatimão did not influence wound contraction	No difference in the length of newly formed epithelium was found between the treated and control wounds	?	?	?

**Table 2B:** Main Parameters analyzed in the studies demonstrating the action of fractions from plants in the treatment of skin wounds in murine models.

**PARAMETERS OF WOUND HEALING IN MODELS MURINES - ISOLATES**

Reference	Wound closure analysis	Reepithelialization analysis	Oxidative Estress	Granulation Tissue Fill	Tensile strengths
Sidhu, et al., 1999	Arnebin-1 reduced wound width wounds in compared with control.	Arnebin-1 - 7 days Control - only epithelial migration over the dermis	?	The organization of the granulation tissue was more advanced in arnebin-1-treated wounds with thick bundles of well-aligned collagen compared with controls	?
Paramesha, et al., 2015	Dehydroabietylamine - (97.78% ± 2.15) 16 day Control - (82.92% ± 1.83) 16 day	<b>Epithelialization in days</b> Dehydroabietylamine - 17.67 ± 2.62 Control - 23.17 ± 1.14	?	<b>Hydroxyproline content (µg/100 g)</b> Dehydroabietylamine - 2106,50 ± 2,62 Control -1369,67 ± 10,54	Dehydroabietylamine - 425.67g ± 10.03 Control - 277.00g ± 9.39
Nagappan, et al., 2012	Mahanine and mahanimbicine - (88.5% ± 2.03 to 93% ± 2.04) 16 day Control - (82.7% ± 2.13) 16 day	Mahanine and mahanimbicine - 18 day Control - 18 day	?	<b>Collagen deposition</b> Mahanine and mahanimbicine (65.63% ± 0.87 to 67.76% ± 0.85) 21 day (81.56% ± 1.04 to 88.54% ± 1.34) 28 day Control - (61.84% ± 0.94) 21 day (78.06% ± 1.22) 28 day	?
Qu, et al., 2013	Compound I to compound VII (96.8%±1.9 to 87.0%±2.6) 16 day Control - (87.2%±3.1) 16 day	Compound I and compound V - 18 day Control and others group - 22 days	?	<b>Hydroxyproline content (mg/g tissue)</b> Compound I to compound VII 58.4±3.7 to 80.3±4.4 Control - 60.2±4.1	Compound I to compound VII 431.5g±8.3 to 547.3g±7.9 Control - 436.5g±7.6
Ghosh, et al., 2012	Compound I to compound II - (100%) 18 days Control - (100%) 22 day	Compound I - 17.16 ± 0.4 day Compound II - 17.25 ± 0.25 day Control - 22.00 ± 0.1 days	?	Compounds I and II - fibrous connective tissue with well-collagenation Control - fibrosis and more aggregation of macrophages with less collagen fibers	Compound I - 565.10g ± 3.1 Compound II - 561.12g ± 3.9 Control - 372.13g ± 3.23
Mukherjee, et al., 2013	Compound I (2,5%) - (89.91%±0.55)18 day Compound II (2,5%)- (97.89%±0.77) 18 days Control - (75.44%±0.37) 18 day	Compound I (2,5%) - 17.16 ± 0.4 day Compound II (2,5%) - 16.01±0.33 day Control - 21.00 ±0.11 days	?	<b>Hydroxyproline content (mg/g tissue)</b> Compound I (2,5%) - 158.23±0.44 Compound II (2,5%) - 198.16±0.33 Control - 151.9±2.69	Compound I (2,5%) - 538.00g ± 1.89 Compound II (2,5%) - 535.12g ± 3.59 Control - 322.39g ± 2.66
Melo, et al., 2011	Cramoll 1,4 - (100%) 10 day Control - (100%) 12 day	?	?	<b>Crust presence:</b> Cramoll 1,4 - 13.1 ± 7.02 Control - 5.4 ± 3.3 <b>Collagen presence:</b> Cramoll 1,4 - (higher collagen deposition and annex sprouts) 12 day, Control - (matrix poor in collagen fibers) 12 day	?
Pieters, et al., 1995	3',4-0-dimethylcedrusin - (85%) 15 day Taspine - (75%) 15 day Control - (60%) 15 day	3',4-0-dimethylcedrusin - ++ (15 day) Taspine - + (15 day) Control - + (15 day)	?	<b>Crust presence</b> 3',4-0-dimethylcedrusin - after 5 days Taspine - after 5 days Control - after 3 days	?

**Table 2B.** (Continued)

Reference	Wound closure analysis	Reepithelialization analysis	Oxidative Estress	Granulation Tissue Fill	Tensile strengths
Ahamed, et al., 2009	Gulonic acid $\gamma$ -lactone - ( 94.02% $\pm$ 0.20) 16 day  Control - (79.53% $\pm$ 0.97) 16 day	<b>Epithelialization in days</b> Gulonic acid $\gamma$ -lactone - 18.62 $\pm$ 0.21  Control - 22.59 $\pm$ 0.15	?	<b>Hydroxyproline content (<math>\mu</math>g/100 g)</b> Gulonic acid $\gamma$ -lactone - 1 780.48 $\pm$ 50.73 Control - 1 346.15 $\pm$ 14.54 <b>Fibroblast count / high power field <math>\times</math> 400</b> Gulonic acid $\gamma$ -lactone - 53.26 $\pm$ 2.37 Control - 97.53 $\pm$ 4.26 <b>Blood vessel count / high power field <math>\times</math> 400</b> Gulonic acid $\gamma$ -lactone - 21.94 $\pm$ 1.15 Control -11.63 $\pm$ 1.11	Gulonic acid $\gamma$ -lactone - 561.12g $\pm$ 5.18 Control - 327.63g $\pm$ 6.37
Zyuz'kov, et al., 2012	Songorine - 100% (9-16 day) Napelline - 100% (9-16 day) Hypaconitine -100% (9-16 day) 12-epinapelline N-oxide - 89.93% $\pm$ 5.53 (9-16 day) Mesaconitine - 97.8% $\pm$ 2.2 (9-16 day) Control - 89.72% $\pm$ 4.72 (9-16 day)	Songorine - Napelline - Hypaconitine Newly formed epithelium by the wound edges represented a cell layer of varying thickness without vertical anisomorphism - (5 day)	?	<b>Leukocytic infiltration</b> Songorine - Reduction (3 day) Napelline - Reduction (3 day) Hypaconitine - Reduction (3 day) 12-epinapelline N-oxide - ? / Mesaconitine - ? / Control - ? <b>Counts of fibroblasts</b> Songorine - Increased (3 day) Napelline - Increased (3 day) Hypaconitine - Increased (3 day) 12-epinapelline N-oxide - ? / Mesaconitine - ? / Control - ?	?
Singh, et al., 2005	Deoxyelephantopin- 98.8% $\pm$ 0.35 (16 day) Control - 85.8% $\pm$ 0.69 (16 day)	<b>Epithelialization in days</b> Deoxyelephantopin- 14.0 $\pm$ 0.26 Control - 20.0 $\pm$ 0.86	?	Deoxyelephantopin - $\downarrow$ macrophages and $\uparrow$ collagen formation Control - $\uparrow$ macrophages and $\downarrow$ collagen formation	Deoxyelephantopin- 412.0g $\pm$ 11.37 Control - 298.6g $\pm$ 8.48
Sharath, et al., 2010	Bacoside-A - 98.18% $\pm$ 0.05 (16 day) Control - 85.22% $\pm$ 0.02 (16 day)	<b>Epithelialization in days</b> Bacoside-A - 18.30 $\pm$ 0.01 Control - 20.20 $\pm$ 0.04	?	Bacoside-A - $\uparrow$ Blood vessels and $\uparrow$ collagen formation Control - $\uparrow$ Inflammatory cells $\downarrow$ blood vessels $\downarrow$ collagen formation	Bacoside-A - 538.47g $\pm$ 0.14 Control - 380.48g $\pm$ 0.11
Vidya, et al., 2012	Entadamide - 92.22% $\pm$ 0.05 (16 day) Phaseoloidin - 88.50 $\pm$ 0.10 (16 day) Entagenic acid - 96.08% $\pm$ 0.04 (16 day) Control - 83.31% $\pm$ 1.06 (16 day)	<b>Epithelialization in days</b> Entadamide - 19.92 $\pm$ 0.01 Phaseoloidin - 21.16 $\pm$ 0.02 Entagenic acid - 18.08 $\pm$ 0.01 Control - 24.00 $\pm$ 0	?	<b>Hydroxyproline content (<math>\mu</math>g/100 g)</b> Entadamide - 1891.17 $\pm$ 2.75 Phaseoloidin - 1690.33 $\pm$ 2.80 Entagenic acid - 2001.33 $\pm$ 3.53 Control - 1369.67 $\pm$ 10.54	Entadamide - 463.33g $\pm$ 4.48 Phaseoloidin - 450.17g $\pm$ 7.55 Entagenic acid - 549.83g $\pm$ 2.21 Control - 260.83g $\pm$ 14.05

## **Artigo 2: Ação de extratos vegetais no reparo de feridas cutâneas em ratos diabéticos**

### **RESUMO**

**Objetivo:** Investigar o efeito da aplicação tópica da pomada à base de *Strycnos pseudoquina* nas concentrações 5 e 10% na cicatrização de feridas cutâneas em ratos diabéticos. **Material e Método:** Amostras de *S. pseudoquina* foram coletadas no Município de Rio Verde, Goiás, Brasil e em seguida submetido a prospecção fitoquímica. O extrato foi emulsificado em lanolina nas concentrações 5% e 10%. Foram utilizados trinta ratos Wistar que após a indução do diabetes com estreptozotocina, foram divididos em 5 grupos de 6 animais: Sal: feridas tratadas com 0,9% de solução salina; VH (veículo da pomada): feridas tratadas com 0,6 g de creme de lanolina; SS (Sulfadiazina de Prata): feridas tratadas com 0,6 g de creme de Sulfadiazina de Prata (0,01%); ES5: feridas tratadas com pomada a base de *S. pseudoquina* (5%); ES10: feridas tratadas com pomada a base de *S. pseudoquina* (10%). Três feridas circulares de 12 mm de diâmetro foram realizadas no dorso dos animais e fragmentos das feridas foram retirados para análises histológicas e bioquímicas a cada 7 dias durante 21 dias. **Resultados:** Os grupos que receberam o extrato de *S. pseudoquina* nas concentrações 5 e 10% apresentaram maior taxa de fechamento das feridas, maior quantidade de células, vasos sanguíneos e aumento do colágeno III e I. Os marcadores de estresse oxidativo foram menores nos grupos ES5 e ES10, e os níveis de enzimas antioxidantes foram maiores nestes mesmos grupos. **Conclusão:** Os resultados deste trabalho demonstraram que a aplicação tópica de pomada à base de *S. pseudoquina* promove um reparo cutâneo rápido e eficaz em ratos diabéticos.

**Palavras-chave:** “Quina do Cerrado”; Cicatrização; Enzimas antioxidantes.

## 1. Introdução

A cicatrização de feridas cutâneas é um processo complexo e organizado caracterizado por modificações teciduais que incluem aumento da vascularização e da proliferação celular, bem como, síntese da matriz extracelular (Xie et al. 2013; Bueno et al. 2016). Estes fenômenos são responsáveis por acelerar o fechamento da ferida e promover a restauração do tecido lesado (Rosa et al. 2014). O processo de reparo cutâneo pode ser dividido em 4 fases que se sobrepõem. A primeira etapa é conhecida como hemostasia, caracterizada por constrição vascular e a formação de fibrina (Versteeg et al. 2013). Quase simultaneamente é iniciada a segunda fase conhecida como inflamatória, que é marcada pelo recrutamento de leucócitos para o local da lesão (Hsu & Mustoe, 2010, Park et al. 2014; Xing et al. 2015). As células recrutadas na fase inflamatória são responsáveis pela liberação de citocinas e fatores de crescimento que irão mediar processos de migração, proliferação e diferenciação celular, caracterizando a terceira fase, conhecida como proliferativa (Park et al. 2014; Bahramsoltani et al. 2014; Ram et al. 2015). A quarta e última etapa conhecida como fase de remodelação é marcada pela reorganização da matriz extracelular, na qual o tecido de granulação rico em vasos e fibras colágenas do tipo III, é substituído por fibras colágenas do tipo I, tornando o tecido mais forte e resistente à trações (Sarandy et al. 2015).

Entre as citocinas e fatores de crescimento liberados durante a fase inflamatória podemos destacar mediadores pró-inflamatórios como: Interleucina-1 (IL-1), TNF- $\alpha$ , e IFN- $\gamma$ , cuja principal finalidade é estimular a diapedese e proliferação celular (Hou et al. 2015). Em geral entre os mediadores pró-inflamatórios existe um predomínio na liberação de lipoxinas, Interleucina-10 (IL-10) e fator de crescimento transformador  $\beta$  (TGF- $\beta$ ) (Norris et al. 2014, Kapellos & Iqbal, 2016). Alguns mediadores são considerados universais, ou seja, existem pré-formados no organismo e são produzidos

por vários tipos de células, entre estes está o TGF- $\beta$  (Hsu & Mustoe, 2010; Siebert et al. 2011, Lamel & Kirsner, 2013), que é responsável pela quimiotaxia celular e consequentemente por estimular a produção de vasos e fibras da matriz acelerando o processo de remodelação tecidual (Guo & Diprieto, 2010, Johnson & Wilgus, 2012). Os eventos teciduais estimulados pelo TGF- $\beta$  garantem ao tecido nutrição adequada e energia suficiente para que as células possam sintetizar uma nova matriz rica em colágeno III e vasos sanguíneos que servirá de arcabouço para posterior síntese de colágeno tipo I (Row et al. 2016, Yang et al. 2016). Estas modificações são responsáveis pelo fechamento rápido e eficaz da ferida, conferindo ao tecido características semelhantes ao tecido ileso (Chen et al. 2016).

Durante o processo de reparo já é conhecido que existe a produção de muitas espécies reativas de oxigênio (ERO's), o que pode levar a um fenômeno conhecido como estresse oxidativo tecidual, sobretudo na fase inflamatória, onde ocorre a migração de fagócitos que realizam a explosão respiratória (Servetto et al. 2010, Dunnill et al. 2015). No reparo cutâneo, a produção de espécies radicalares modula a expressão de genes envolvidos na morte e na sobrevivência celular, ativando vias que vão induzir a expressão de proteínas responsáveis pela metabolização destas ERO's dentro da célula (Verri et al. 2006; Martins et al. 2016). Neste contexto, destacamos a ação das enzimas antioxidantes superóxido dismutase (SOD) e catalase (CAT) (Luchese et al. 2009, Forbes-Hernández et al. 2014). Durante o processo de estresse oxidativo ocorre a formação de radical superóxido ( $O_2^-$ ) que é transformado em peróxido de hidrogênio ( $H_2O_2$ ) pela ação da SOD (Wallace, 2005; Pieczenik & Neustadt, 2007; Moura et al. 2010) e através da ação de CAT transforma  $H_2O_2$  em  $H_2O$  e  $O_2$  (Salvi et al. 2007; Dhouib et al. 2015).

Indivíduos diabéticos apresentam o processo de reparo cutâneo comprometido,

principalmente devido às alterações vasculares e celulares tornando o tratamento de feridas um desafio para os sistemas de saúde e também para sociedade em geral (Hou et al. 2015). A prevalência dessas lesões em indivíduos diabéticos elevam os gastos, piora a qualidade de vida desses pacientes e prolonga o tempo de tratamento, constituindo-se assim um importante problema de saúde pública (Ferrari et al. 2010; Brito et al. 2014). A principal alteração estrutural observada no tecido cicatricial do indivíduo diabético é a redução da angiogênese com a conseqüente redução da proliferação celular, do suprimento de oxigênio e nutrientes para as células, resultando em disposição anormal das fibras colágenas e retardo no processo de fechamento das feridas (Kant et al. 2015; Lee et al. 2015).

Atualmente o uso de plantas medicinais para o tratamento de feridas cutâneas no diabetes vem crescendo consideravelmente, uma vez que a fitoterapia representa uma forma eficaz para o tratamento das mais variadas disfunções teciduais (Almeida et al. 2014; Kant et al. 2015). Em países como África (Agyare et al. 2016), Índia (Joshi et al. 2016), Austrália (Adams et al. 2016) e Brasil (Alerico et al. 2015), este tipo de terapia faz parte da medicina popular e vêm se mostrando uma terapia acessível e eficaz (Teló et al. 2014; Gonçalves et al. 2014). A realização da caracterização fitoquímica do extrato destes vegetais, é importante para se detectar bioativos e sua ação (Taleb et al. 2016). Compostos como flavonoides, outros polifenóis e alcaloides são conhecidos por promover o processo de cicatrização devido à sua propriedade anti-inflamatória, anti-microbiana (Kchaou et al. 2016; Taleb et al. 2016), antioxidante (Tsala et al. 2014; Udegbunam et al. 2014) e imuno-estimuladora (Thi et al. 2002), que parecem ser responsáveis pela contração da ferida e um aumento da taxa de epitelização.

O gênero *Strychnos* inclui mais de 200 espécies distribuídas em regiões tropicais do globo (Philippe et al. 2004). Muitas destas espécies são conhecidas pelas suas

propriedades medicinais (Thongphasuk et al. 2003). Neste contexto, pode-se destacar na região do Cerrado Brasileiro a espécie *Strychnos pseudoquina* A. St. Hil conhecidas popularmente como "Quina do Cerrado" e a indicação popular desta planta, refere-se principalmente ao tratamento da malária (Andrade-Neto et al. 2003) e tratamentos gastrointestinais (Silva et al, 2005; Bonamin et al, 2011).

Considerando a importância de explorar os efeitos curativos desta espécie, este estudo teve como objetivo avaliar o efeito do extrato das entrecascas de *S. pseudoquina* nas concentrações de 5% e 10% sobre características morfológicas e bioquímicas durante o reparo de feridas cutâneas de segunda intenção em ratos diabéticos.

## **2. Material e Métodos**

### **2.1. Obtenção do material vegetal e preparação dos extratos**

Amostras de entrecascas *S. pseudoquina* foram coletadas em seu ambiente natural no município de Rio Verde, Goiás, Brasil, secadas em estufa de ventilação a 40°C e, pulverizadas em moinhos de faca. Exsiccatas da espécie foram depositadas em herbário da EPAMIG-BH (número PAMG 57079). Uma parte da amostra (1.000 g) foi submetida a processo de desengorduramento por percolação, empregando-se acetato de etila como solvente extrator. A droga vegetal foi extraída exaustivamente com etanol, sendo o extrato posteriormente concentrado em evaporador rotatório para remoção do solvente. Ao final, o extrato foi submetido à liofilização, obtendo-se 132 g.

### **2.2. Prospecção fitoquímica**

Triagem fitoquímica para a presença de metabólitos secundários foi realizada utilizando análise TLC (cromatofolha de sílica gel GF<sub>254</sub>, Sigma-Aldrich®) com diferentes sistemas de fases móveis e reveladores específicos para visualização de compostos fenólicos, flavonoides, cumarinas, antraquinonas, saponinas, triterpenos/esteroides e alcaloides), de acordo com os procedimentos descritos por

Harbone (2002).

### **2.3. Quantificação de flavonóides totais**

O conteúdo de flavonoides foi determinado pelo método de cloreto de alumínio usando rutina como composto de referência. Uma amostra de 10 mg do extrato foi ressuspendida em 1,0 mL de metanol por sonicação. Em seguida, o conteúdo foi transferido para balão volumétrico de 25 mL, sendo posteriormente adicionados 8 mL de metanol; 0,6 mL de ácido acético glacial e 2,5 mL de solução metanólica de cloreto de alumínio hexaidratado a 8% (m/v) e o volume completado até 25ml com metanol. A absorção a 420 nm foi lida após 20 min. em espectrofotômetro Shimadzu UV-100 a 420 nm, utilizando cubeta de quartzo. Amostra do branco foi preparada utilizando o mesmo procedimento com exceção da adição do extrato vegetal que foi substituído por metanol. A curva de calibração foi construída sob as mesmas condições utilizando-se a rutina como padrão (2,0 a 50,0 µg/mL), apresentado na equação da reta obtida  $y=0,019x+0,0555$  com coeficiente de ajuste  $R^2$ . O experimento foi realizado em triplicata. O teor de flavonoides no extrato vegetal foi expresso como mg equivalente de rutina (RE) por g de extrato seco.

### **2.4. Preparo da formulação**

A pomada à base de extrato de *S. pseudoquina* foi preparada em uma cápsula de porcelana com auxílio de um pistilo onde o extrato seco liofilizado foi incorporado em lanolina nas concentrações de 5% (ES5) e 10% (ES10). Creme de Sulfadiazina de Prata (SS) (1%) (Rexin Farmacêutica Pvt. Ltda) foi utilizado como controle positivo (Shivhare et al. 2010; Morsi et al. 2014).

### **2.5. Animais**

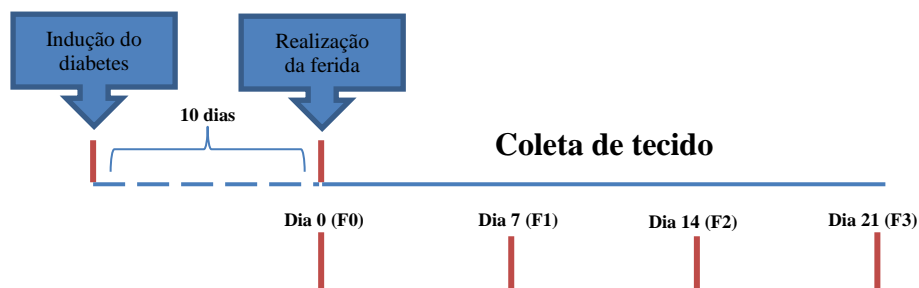
Trinta ratos Wistar (*Rattus norvegicus*), machos, com 5 semanas de idade,

pesando  $198,25 \pm 26,11$  g, foram obtidos a partir do Biotério Central da Universidade Federal de Viçosa, Brasil. Os animais foram alimentados com ração comercial e água ad libitum. Todos os animais foram mantidos em gaiolas individuais, limpas diariamente, em condições controladas de temperatura ( $22 \pm 2$  °C) e umidade (60-70%), com ciclo de luz/escuro (12/12h). Todos os procedimentos foram aprovados pelo comitê de ética de uso de animais – UFV (registro nº 730/2014).

## **2.6. Indução do diabetes mellitus e desenho experimental**

Os animais foram anestesiados por injeção intramuscular de cetamina (50 mg/kg) e xilazina (20 mg/kg) e em seguida, o diabetes foi induzido com uma única injeção de estreptozotocina (60 mg/kg) (Sigma, St. Louis, EUA). Dez dias após a indução, os níveis de glicemia foram monitorados e animais com níveis acima de 220 mg/dl foram considerados diabéticos. Após a confirmação do diabetes os animais foram anestesiados por injeção intramuscular de cetamina (50 mg/kg) e xilazina (20 mg/kg) e tiveram a região dorsolateral depilados e a área foi desengordurada com éter (Merck<sup>®</sup>, Rio de Janeiro, Brasil). Em seguida houve a aplicação de etanol (70%) e iodopovidona (10%) para a anti-sepsia (Johnson Diversey<sup>®</sup>, Rio de Janeiro, Brasil). Três feridas circulares, de 12 mm de diâmetro, foram feitas por incisão cirúrgica na pele e tecido subcutâneo com um bisturi, até a exposição da fáscia muscular dorsal (Gonçalves et al. 2010). O experimento foi conduzido em 5 tratamentos com 6 repetições cada: Sal (controle): ratos diabéticos tratados com solução salina a 0,9%, VH (veículo da pomada): ratos diabéticos tratados com 0,6 g de creme de lanolina, grupo SS (Sulfadiazina de prata – controle positivo): ratos diabéticos tratados com 0,6 g de sulfadiazina de prata (1%), ES5: ratos diabéticos tratados com pomada a base de extrato de *S. pseudoquina* (5%); ES10: ratos diabéticos tratados com pomada a base de extrato de *S. pseudoquina* (10%). Em todos os grupos, as feridas foram limpas diariamente com solução salina 0,9% antes

de cada tratamento. As feridas foram tratadas diariamente durante os 21 dias. Após este período, os animais foram sacrificados por punção cardíaca. O desenho experimental está demonstrado na figura 1.



**Figura 1:** Desenho experimental: indução do diabetes, 10 dias depois, realização das feridas e acompanhamento do processo cicatricial durante 21 dias. A cada 7 dias foram retirados tecidos de feridas diferentes. Tecido ileso (F0), tecido cicatricial 7 dias (F1), tecido cicatricial 14 dias (F2), tecido cicatricial 21 dias (F3).

## 2.7. Cálculo da área da ferida e a taxa de contração da ferida

A área e a taxa de contração da ferida foram avaliadas a cada 7 dias utilizando imagens digitalizadas com as dimensões de  $320 \times 240$  pixels (24 bits / pixel) obtidas usando uma câmera digital (W320 Sony, Tóquio, Japão). A área das feridas foi calculada por planimetria computadorizada usando o programa Image-Pro Plus 4.5 (Media Cybernetcs, Silver Spring, EUA). O Índice de Contração da Ferida (ICF) foi calculado utilizando a seguinte equação: 
$$\frac{(A_o) - (A_I)}{(A_o)} \times 100$$
 onde (Ao) = área inicial da ferida (AI) = área medida em um determinado dia (Gonçalves et al. 2014). A ferida mais cranial (F1) teve tecido retirado no dia 7, e a ferida do meio (F2) o tecido foi retirado no dia 14. A terceira ferida (F3) foi utilizada como referencial para mensurar o ICF uma vez que esta lesão teve tecido retirado para análises histológicas e bioquímicas somente ao final do experimento (21 dias).

## 2.8. Coleta de tecido para análise histológica

Fragmentos de tecido para análise histológica foram obtidas de diferentes feridas a cada 7 dias, utilizando o mesmo procedimento anestésico descrito anteriormente. Cada fragmento continha tecido do centro da ferida e parte do tecido ileso sem tratamento, presente na região adjacente à borda da ferida. Os fragmentos foram fixados em solução de Karnovsky, embebidos em parafina e seccionados (4  $\mu\text{m}$ ) (Leica Multicut 2045<sup>®</sup>, Reichert- Jung produtos, Jena, Alemanha). Utilizou-se 1 a cada 20 secções para evitar a repetição da análise da mesma área histológica. Os cortes foram corados com hematoxilina e eosina (H&E) para análise dos fibroblastos e vasos sanguíneos, Sirius Red (Sirius Red F3B, Mobay Chemical Co., União, NJ, EUA) para diferenciar as fibras de colágeno sob microscopia de polarização (Sigma, St. Louis, Mo, EUA) (Gonçalves et al. 2012) e para a diferenciação de fibras elásticas foi utilizada a coloração de Resorcina fucsina (Junqueira & Carneiro, 2005). O azul de toluidina foi utilizado para evidencição de mastócitos (Churukian & Schenk, 1981). Usando uma lente objetiva de 40x, 10 campos histológicos foram analisados totalizando uma área de  $1,53 \times 10^6 \mu\text{m}^2$  em microscópio de luz CX40<sup>®</sup> (Olympus, Tokyo, Japão). Cada secção histológica apresentou uma área de  $0,153\text{mm}^2$ . Esta área foi calculada a partir do número de campo=20/40, em que r representa o raio:  $A=\pi r^2$ .

## 2.9. Estereologia da pele

Os cortes foram analisados por meio de exame de imagem. As imagens foram capturadas usando um microscópio de campo claro (BX-60<sup>®</sup> Olympus, Tóquio, Japão) ligado a uma câmara digital (3<sup>®</sup>-QColor Olympus). Foi examinada uma área de tecido total de  $1,53 \times 10^6 \mu\text{m}^2$ . Para cada corte foram obtidos, de forma randomizada, dez campos histológicos em cada secção da pele usando uma lente objetiva de 20. Uma grade contendo 300 pontos foi sobreposta em cada imagem. Parâmetros estereológicos

de densidade volumétrica ( $V_v$ ) foram calculados por contagem dos pontos sobre fibroblastos, vasos sanguíneos, fibras colágenas tipo III, tipo I e fibras elásticas, usando a relação:  $V_v = \frac{PP}{PT} \times 100$  onde PP é o número de pontos que ocorre sobre a estrutura de interesse, e PT é o número total de pontos da grade (Novaes et al. 2015). Fibras de colágeno foram analisadas de acordo com a propriedade diferencial da birrefringência, onde fibras colágenas grossas (tipo I) aparecem em tons de cores brilhantes que vão do vermelho ao amarelo. As fibras reticulares finas (colágeno tipo III) aparecem em verde brilhante (Gonçalves et al. 2014) sob polarização.

### **3.10. A análise do status oxidativo**

Uma parte dos fragmentos de tecido retirados de cada ferida foram rapidamente congelados em nitrogênio líquido e armazenados em freezer  $-80^{\circ}\text{C}$ . Amostras destes fragmentos foram homogeneizadas em solução tampão fosfato-salino (PBS) e centrifugadas a  $-5^{\circ}\text{C}$ . O sobrenadante foi usado para análise de proteínas carboniladas e substâncias reativas do ácido tiobarbitúrico, além da análise de proteínas antioxidantes: superóxido dismutase (SOD) e catalase (CAT). A atividade da SOD foi realizada em leitor de Elisa 570 nm e baseou-se na capacidade desta enzima em catalisar a reação do superóxido ( $\text{O}_2^-$ ) em peróxido de hidrogênio ( $\text{H}_2\text{O}_2$ ) (Siddiqui et al. 2005) e a atividade de CAT utilizando o método Aebi (1984), medindo a taxa de decomposição de peróxido de hidrogênio. A mensuração de malondealdeído (MDA) realizada pela técnica da substância reativa do ácido tiobarbitúrico (TBARS) foi realizada de acordo com o protocolo descrito por Gutteridge e Halliwell (1990) e as proteínas de carboniladas foram analisadas utilizando protocolo descrito por Jana et al. (2014). Os dados bioquímicos foram normalizados em relação aos níveis de proteína total no sobrenadante, quantificada de acordo com o método de Bradford (1976).

### **3.11. Análise da expressão de TGF- $\beta$**

Amostras de tecido cicatricial, coletadas nos dias 7 (F1) e 14 (F2), foram congeladas a  $-80^{\circ}\text{C}$  e homogeneizadas em PBS, tampão pH: 7.4 contendo 0,05% de Tween e centrifugado a 3.500G por 30 minutos. Os níveis de TGF- $\beta$  no sobrenadante foram analisados utilizando-se kits de imunoenensaio pelo método de ELISA (Boster Biological Technology Ltd, China), seguindo o protocolo do fabricante. Placas de poliestireno de alta afinidade (Corning, New York, USA) foram sensibilizadas com 100  $\mu\text{L}$ /poço de anticorpos monoclonais específicos para o componente a ser dosado (anticorpo de captura), diluídos em tampão carbonato-bicarbonato 0,1M (pH 9,6) por 12 horas à  $4^{\circ}\text{C}$ . Em seguida, as placas foram bloqueadas com solução de PBS acrescido com 10% de soro fetal bovino inativado (Sigma) durante 1 hora em temperatura ambiente. Nos poços foram adicionados, em duplicata, compostos recombinantes (curva-padrão) e as amostras do homogenato de tecido cicatricial. As placas foram incubadas em temperatura ambiente durante 2 horas e em seguida lavadas cinco vezes com PBS-Tween e adicionados os anticorpos secundários específicos para cada componente de interesse conjugados com biotina (anticorpo de detecção) associada à avidina-peroxidase. A reação foi revelada com tetrametilbenzidina (TMB) e bloqueada após 20 minutos com ácido sulfúrico 2M, sendo a leitura realizada em leitor de microplacas em 450 nm (Power Wave X- BioTek Instruments, Inc. Winooski, Vermont, USA).

### **3.12. Análise estatística**

Os resultados foram representados como média e desvio padrão (média  $\pm$  DP). A distribuição dos dados foi avaliada por meio do teste D'Agostino-Pearson. Os dados com distribuição paramétrica foram comparados por meio de análise de variância (one-way ANOVA) seguido pelo teste Student-Newmann-Keuls. Os dados não paramétricos

foram comparados por meio do teste Kruskal-Wallis. A confiabilidade dos testes foi estabelecida em 95%. A significância estatística foi estabelecida em  $p < 0,05$ .

## 4. Resultados

### 4.1. Análise fitoquímica

A prospecção fitoquímica do extrato de *S. pseudoquina* revelou a presença de constituintes fenólicos, flavonoides e alcaloides. O teor de flavonoides totais encontrado foi de 0,59 mg/g equivalente de rutina (RE).

### 4.2. Área da ferida e índice de contração

Os animais dos grupos ES5% e ES10% apresentaram uma área da ferida menor nos dias 7, 14 e 21 quando comparado aos demais grupos (Tabela 1). Não houve diferenças no índice de contração de feridas nos grupos analisados (Figura 2).

**Tabela 1:** Área (mm<sup>2</sup>) e taxa de contração da ferida (mm<sup>2</sup>/dia) em animais diabéticos tratados com pomada a base de extrato de *S. pseudoquina*

		Sal	VH	SS	ES (5%)	ES (10%)
<b>F0</b>	<b>Área</b>	160,3±23,6	164,5±13,6	165,3±12,5	160±12,1	166,5±26,5
	<b>ICF</b>	00,00±00,00	00,00±00,00	00,00±00,00	00,0±00,0	00,0±00,0
<b>F1</b>	<b>Área</b>	115,4±15,9	113,1±13,9	109,6±12,1	95,5±11,3*	76,8±16,06*
	<b>ICF</b>	31,9±17,4	39,7±11,5	37,7±13,1	32,4±11,7	50,5±12,6
<b>F2</b>	<b>Área</b>	30,0±11,3	25,2±12,9	35,3±10,4	15,9±8,8*	16,8±8,1*
	<b>ICF</b>	84,7±9,3	88,3±7,9	81,3±9,1	89,1±6,05	89,9±5,5
<b>F3</b>	<b>Área</b>	10,4±3,4	9,1±4,1	8,18±3,6	2,8±1,7*	3,2±1,5*
	<b>ICF</b>	94,3±5,3	94,0±5,3	94,9±2,6	98,0±1,2	96,7±2,4

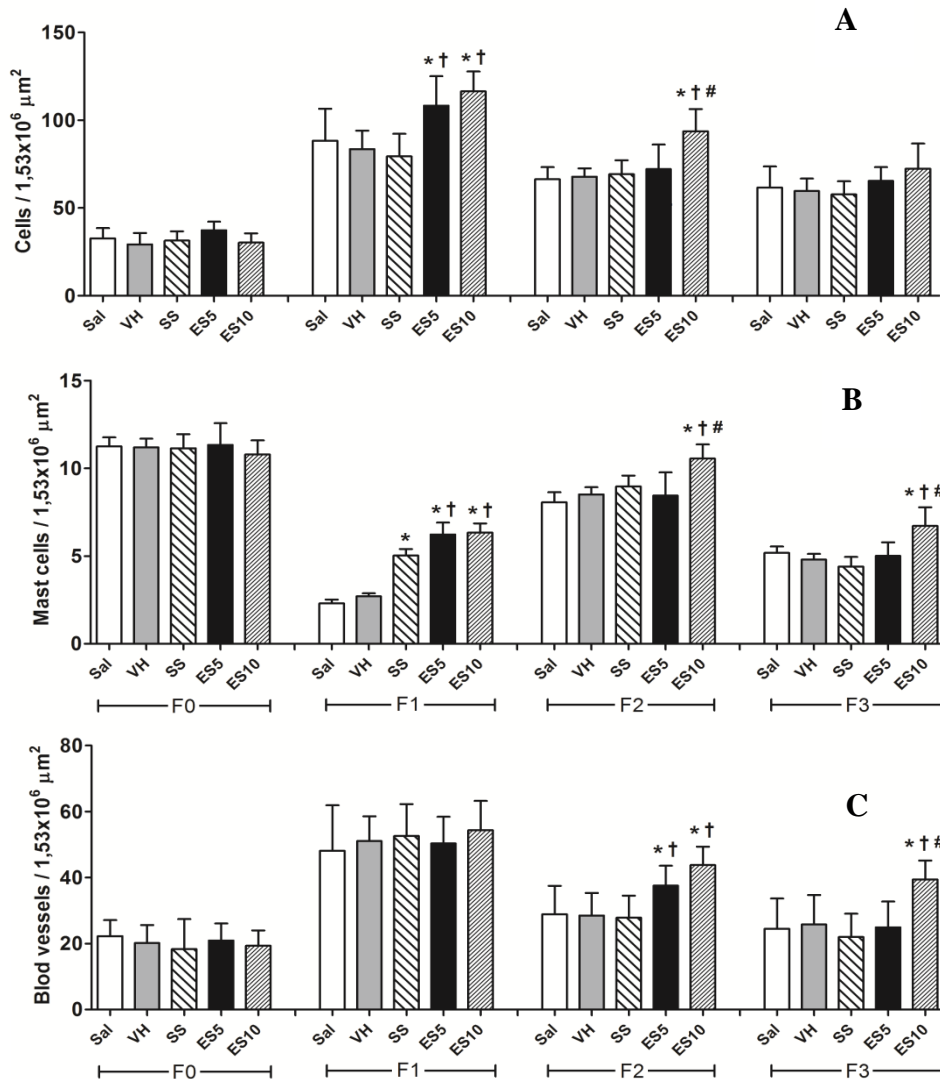
Área da ferida e Índice de Contração da Ferida (ICF) durante 21 dias de tratamento. Sal: Salina a 0,9%; VH: veículo da pomada; SS: sulfadiazina de prata (1%), ES5: Pomada a base de *S. pseudoquina* (5%); ES10: Pomada a base de *S. pseudoquina* (10%). Os tratamentos foram aplicados diretamente nas feridas durante 21 dias. F0= tecido intacto; F1, F2, F3= tecido cicatricial após 7, 14 e 21 dias respectivamente. \* indica diferença estatística vs Sal e VH.



**Figura 2:** Representação das feridas de segunda intenção demonstrando a evolução do reparo cutâneo em função do tempo de tratamento. Sal: Salina a 0,9%; VH: veículo da pomada; SS: sulfadiazina de prata (1%), ES5: Pomada a base de S. pseudoquina (5%); ES10: Pomada a base de S. pseudoquina (10%). F1, F2, F3= tecido cicatricial em 7, 14 e 21 dias respectivamente.

### 4.3. Resultados Histopatológicos

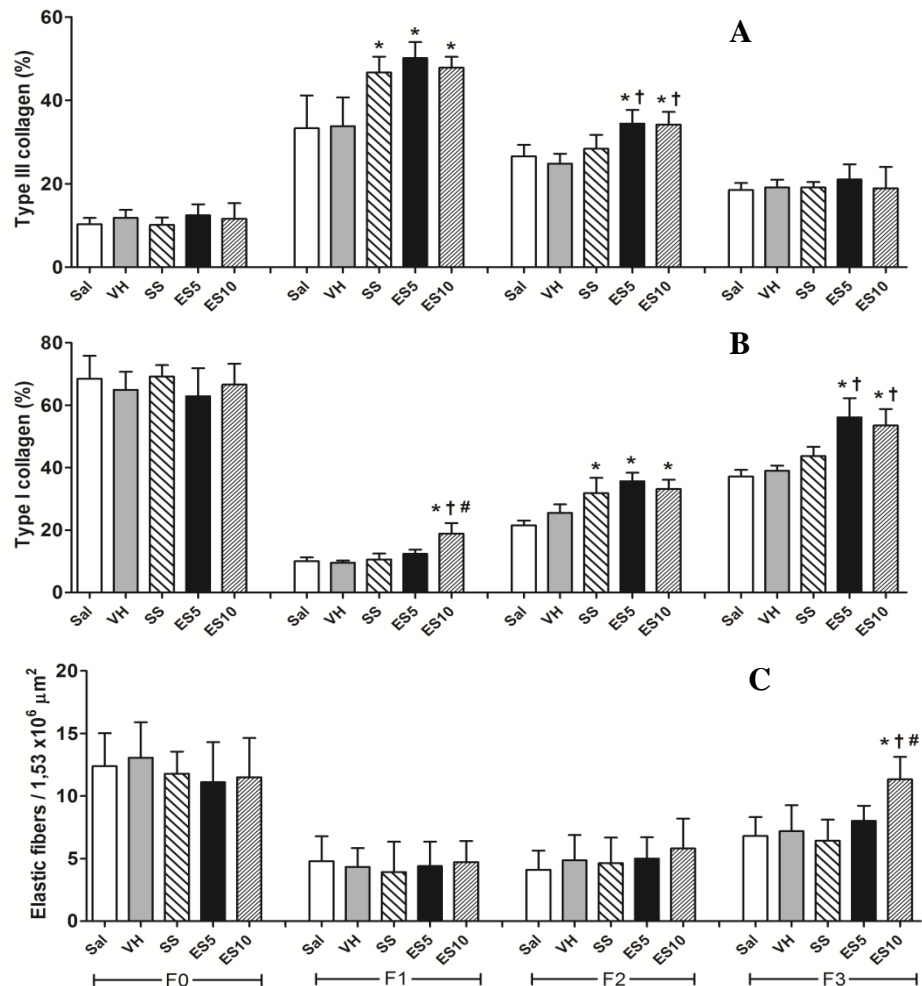
Nos grupos ES5 e ES10 houve um aumento no número de células no dia 7 (F1) em relação aos grupos controle (Sal, VH e SS). No dia 14 (F2) o grupo ES10 apresentou um aumento da quantidade de células quando comparado aos demais grupos (Sal, VH, SS e ES5) (Figura 3A). No dia 7 (F1) os níveis de mastócitos foram maiores nos grupos tratados com ES5 e ES10 em relação aos grupos controle (Sal, VH e SS), e nos dias 14 e 21 os animais tratados com extrato na maior concentração (ES10) apresentaram número maior de mastócitos quando comparado a todos os demais grupos (Figura 3B). Em relação à formação de novos vasos, no dia 14 (F2) houve um aumento da vascularização das feridas nos grupos que receberam tratamento com ES5 e ES10 em relação aos grupos controle (Sal, VH e SS) e, no dia 21 (F3) o grupo tratado com ES10 apresentou maior número de vasos quando comparado aos grupos Sal, VH, SS e ES5 (Figura 3C).



**Figura 3:** Efeito da pomada a base de S pseudoquina (5 e 10%) sobre a quantidade total de células (A), número de mastócitos (B) e numero de vasos sanguíneos (C) em feridas cutâneas de ratos diabéticos. Sal: Salina a 0,9%; VH: veículo da pomada; SS: sulfadiazina de prata (1%), ES5: Pomada a base de S. pseudoquina (5%); ES10: Pomada a base de S. pseudoquina (10%). F0= tecido ileso, F1, F2, F3= tecido cicatricial com 7, 14 e 21 dias respectivamente. Dados são relatados como média±desvio padrão (DP). \*,†,# representam diferença estatística entre os grupos ( $p \leq 0.05$ ); \* comparado com Sal e VH, \*† comparado com Sal, VH e SS, \*†# comparado com Sal, VH, SS e ES5.

Quando analisado a quantidade de fibras colágenas, observou-se que no dia 7, houve um aumento de colágeno III nos grupos tratados com SS, ES5 e ES10. No dia 14 os grupos ES5 e ES10 apresentaram um aumento destas fibras quando comparado a todos os outros grupos (Figura 4A). A análise de colágeno I mostrou que durante todo o

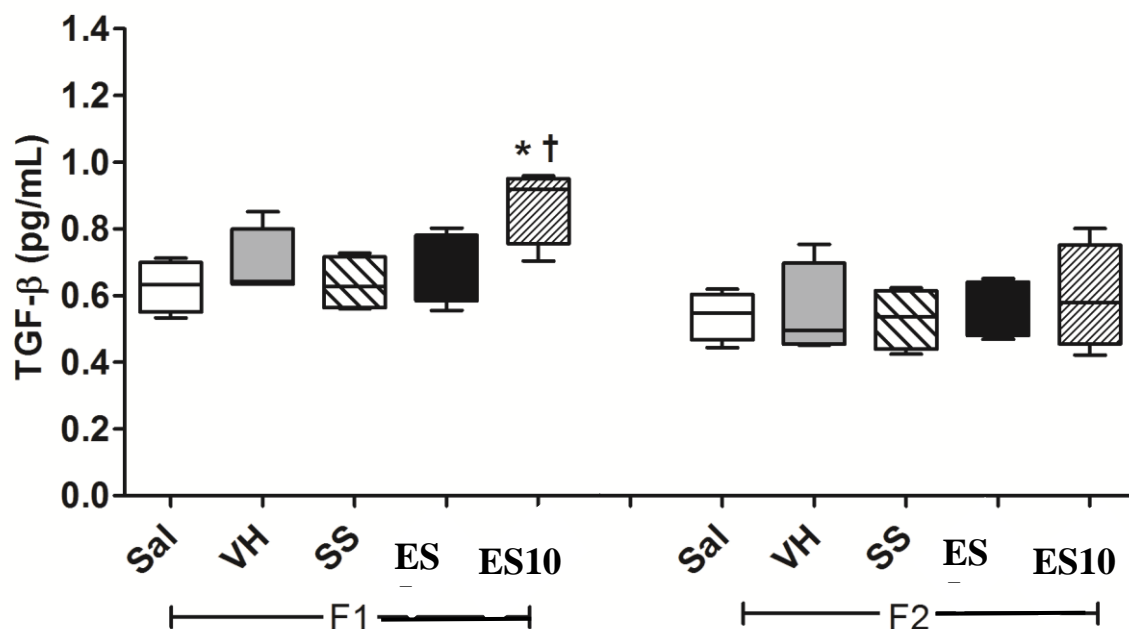
experimento houve um aumento na quantidade destas fibras no grupo tratado com a pomada a base de *S. pseudoquina* (ES5 e ES10) quando comparado aos controles (Figura 4B). Foi observado um aumento no índice de fibras elásticas no grupo tratado com pomada a base de *S. pseudoquina* 10% no dia 21 em relação aos demais grupos (Figura 4C).



**Figura 4:** Efeito da pomada a base de *S. pseudoquina* (5 e 10%) sobre a quantidade total de fibras colágenas do tipo III (A), quantidade total de fibras colágenas do tipo I (B) quantidade total de fibras elásticas (C) em feridas cutâneas de ratos diabéticos. Sal: Salina a 0,9%; VH: veículo da pomada; SS: sulfadiazina de prata (1%), ES5: Pomada a base de *S. pseudoquina* (5%); ES10: Pomada a base de *S. pseudoquina* (10%). F0= tecido ileso, F1, F2, F3= tecido cicatricial com 7, 14 e 21 dias respectivamente. Dados são relatados como média±desvio padrão (DP). \*,†,# representam diferença estatística entre os grupos ( $p \leq 0.05$ ); \* comparado com Sal e VH, \*† comparado com Sal, VH e SS, \*†# comparado com Sal, VH, SS e ES5.

#### 4.4. Análise de TGF- $\beta$

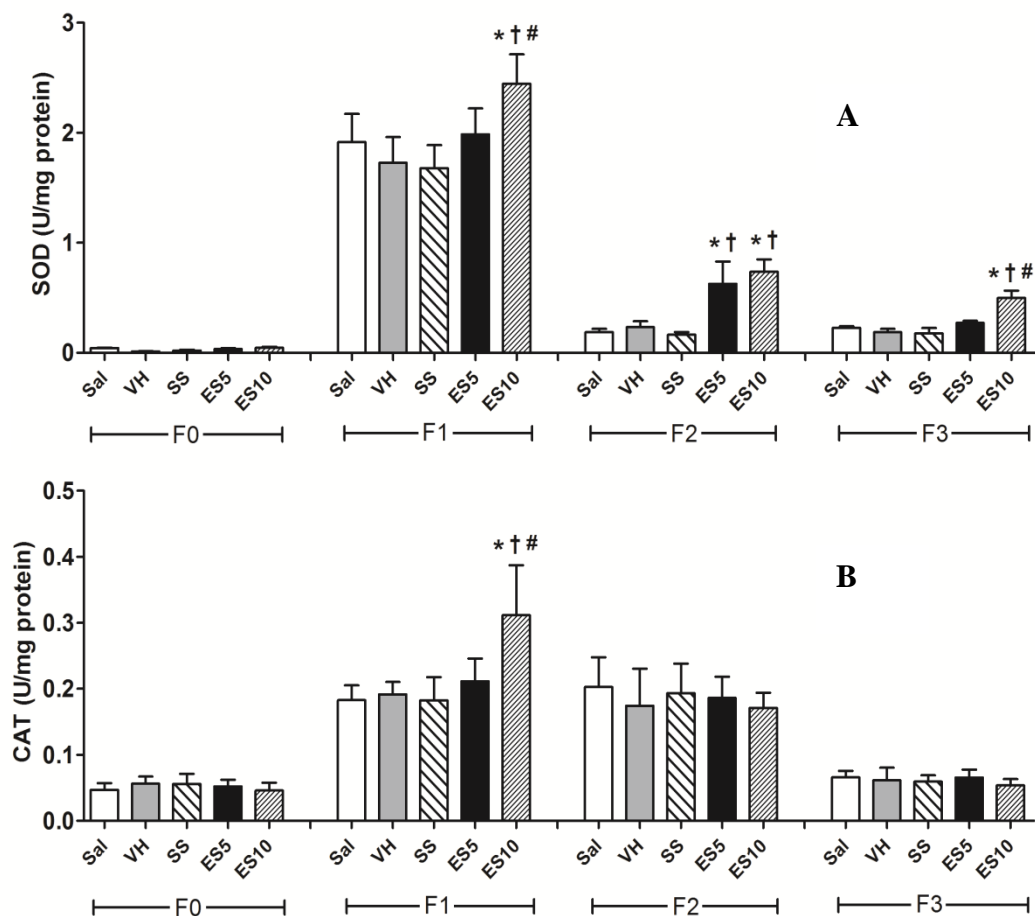
No dia 7 (F1) houve um aumento dos níveis de TGF- $\beta$  no grupo tratado com ES10 quando comparado aos grupos Sal, VH, SS e ES5 (Figura 5).



**Figura 5:** Efeito da pomada a base de *S. pseudoquina* (5 e 10%) sobre os níveis de TGF- $\beta$  em feridas cutâneas de ratos diabéticos. Sal: Salina a 0,9%; VH: veículo da pomada; SS: sulfadiazina de prata (1%), ES5: Pomada a base de *S. pseudoquina* (5%); ES10: Pomada a base de *S. pseudoquina* (10%). F1= tecido cicatricial com 7 dias e F2= tecido cicatricial com 14 dias. Dados são relatados como média $\pm$ desvio padrão (DP). \*,†,# representam diferença estatística entre os grupos ( $p\leq 0.05$ ); \* comparado com Sal e VH, \*† comparado com Sal, VH e SS.

#### 4.5. Status oxidativo do tecido cicatricial

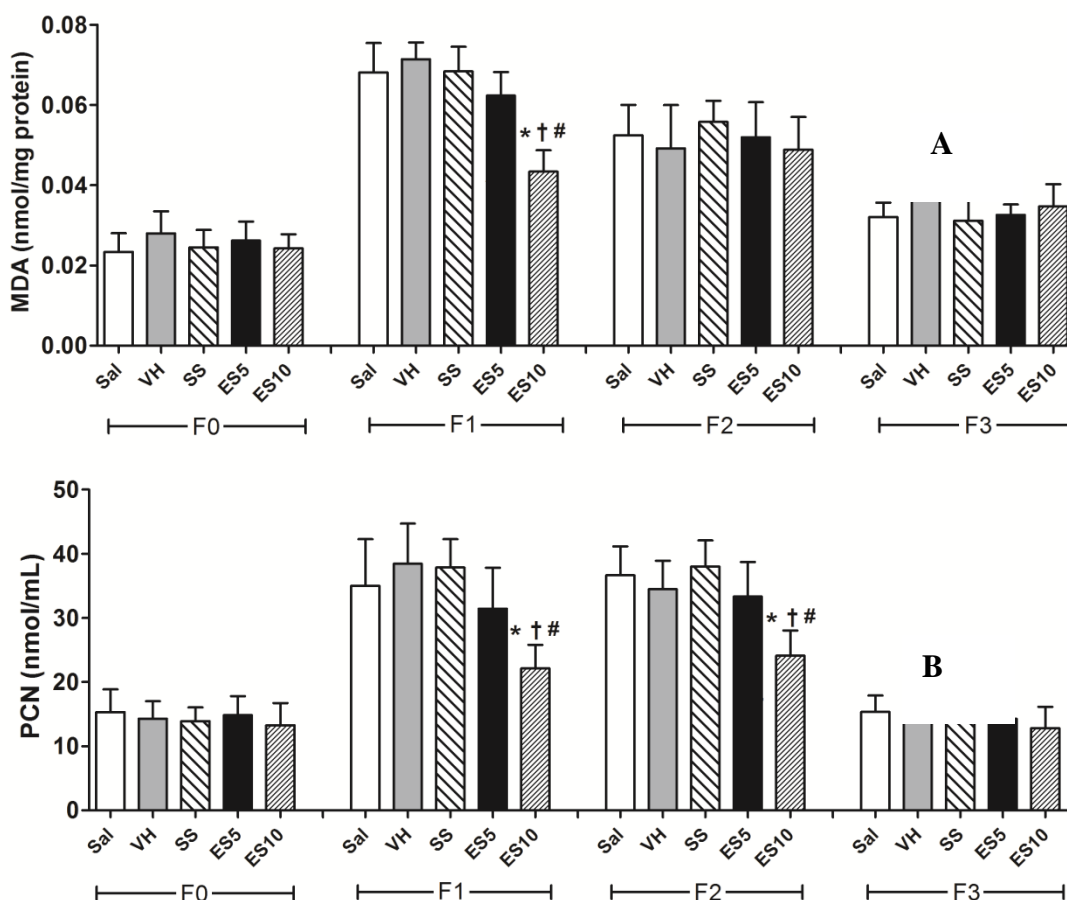
A análise das enzimas antioxidantes mostrou que os níveis de SOD foram maiores nos grupos tratados com a pomada a base de *S. pseudoquina*, principalmente na dose mais elevada (ES10), durante todo o período experimental (Figura 6A). Os níveis de CAT também se mostraram elevados no dia 7 (F1) nos grupos tratados com as maiores doses do extrato (ES10%) quando comparado aos demais (Figura 6B).



**Figura 6:** Efeito da pomada a base de *S. pseudoquina* (5 e 10%) sobre a atividade das enzimas antioxidantes, Superóxido Dismutase (SOD) (A) e Catalase (CAT) (B) em feridas cutâneas de ratos diabéticos. Fragmentos de tecidos foram coletados a cada 7 dias durante 21 dias. Sal: Salina a 0,9%; VH: veículo da pomada; SS: sulfadiazina de prata (1%), ES5: Pomada a base de *S. pseudoquina* (5%); ES10: Pomada a base de *S. pseudoquina* (10%). F0= tecido ileso, F1, F2, F3= tecido cicatricial com 7, 14 e 21 dias respectivamente. Dados são relatados como média±desvio padrão (DP). \*,†,# representam diferença estatística entre os grupos ( $p \leq 0.05$ ); \* comparado com Sal e VH, \*† comparado com Sal, VH e SS, \*†# comparado com Sal, VH, SS e ES5.

A análise dos marcadores de estresse oxidativo mostrou que os níveis de MDA foram menores no dia 7 (F1) quando comparado a todos os demais grupos (Figura 7A). Em relação às proteínas carboniladas, os grupos que receberam tratamento com a pomada a base de *S. pseudoquina* na maior dose (ES10) mostraram os menores valores

nos dias 7 (F1) e 14 (F2) quando comparado aos demais grupos (Figura 7B).



**Figura 7:** Efeito da pomada a base de *S. pseudoquina* (5 e 10%) sobre os níveis de Malonaldeído (MDA) (A) e proteínas carboniladas (PCN) (B) em feridas cutâneas de ratos diabéticos. Sal: Salina a 0,9%; VH: veículo da pomada; SS: sulfadiazina de prata (1%), ES5: Pomada a base de *S. pseudoquina* (5%); ES10: Pomada a base de *S. pseudoquina* (10%). F0= tecido ileso, F1, F2, F3= tecido cicatricial com 7, 14 e 21 dias respectivamente. Dados são relatados como média±desvio padrão (DP). \*,†,# representam diferença estatística entre os grupos ( $p \leq 0.05$ ); \* comparado com Sal e VH, \*† comparado com Sal, VH e SS, \*†# comparado com Sal, VH, SS e ES5.

## 5. Discussão

Nossos resultados mostraram que a pomada a base de extrato de *S. pseudoquina* nas concentrações 5 e 10% é eficaz para o tratamento de feridas cutâneas em ratos diabéticos, uma vez que diminuiu a área da ferida, promoveu a proliferação celular e

formação de novos vasos sanguíneos aumentando consequentemente a síntese de matriz extracelular. Para comprovar esta hipótese foi utilizado como controle positivo a sulfadiazina de prata (1%), comumente empregada como medicamento cicatrizante, por possuir elevada atividade antimicrobiana e anti-inflamatória (Morsi et al. 2014). Patologias relacionadas à deficiência no reparo cutâneo como o diabetes constituem um problema clínico, econômico e social significativo em todo o mundo (O'Brien et al. 2014). Estudos têm relatado o uso de fitoterápicos cada vez mais eficientes para a reparação dos tecidos, com o objetivo de minimizar fatores que atrasam ou impedem o processo de reparo da ferida, e evidenciar o grande potencial de bioprospecção apresentado por extratos vegetais (Tofighi et al. 2014; Zhang et al. 2015, Jana et al. 2014, Silveira et al. 2016). Análise fitoquímica do extrato da entrecasca de *S. pseudoquina* revelou a presença de compostos fenólicos, flavonoides e alcalóides que são metabólitos secundários já descritos com elevado potencial terapêutico para diversas doenças (Oryan et al. 2010; Novaes et al. 2012; Nazaruk et al. 2014). A presença destes compostos em várias espécies *Strychnos* foi descrita por Silva e colaboradores (2005) e, através da investigação fitoquímica, alcalóides foram isolados sendo destacados como composto principal deste vegetal, porém, flavonóides e terpenóides também foram relatados em menor grau. Em estudos semelhantes utilizando extrato da casca de *Musa paradisiaca* relatou-se a presença de alcalóides e taninos em quantidades apreciáveis indicando que estes compostos promovem o processo de cicatrização de feridas devido a atividades antioxidantes do extrato da casca deste vegetal (Padilla-Camberos et al. 2016).

Os resultados deste estudo mostraram que os tratamentos com extratos de *S. pseudoquina* no diabetes aceleram o processo de fechamento das feridas, promovendo uma reepitelização mais rápida do tecido. Resultados semelhantes foram encontrados no

tratamento tópico de feridas utilizando óleo essencial das partes aéreas de *Rosmarinus officinalis* em ratos diabéticos que apresentou uma melhor taxa de contração da ferida em 15 dias de tratamento comparando-se com a aplicação do extrato aquoso e com grupos diabéticos não tratados (Abu-Al-Basal, 2010). Sarandy e colaboradores (2015) demonstraram que extrato de folhas de *Brassica oleracea*, fitoterápico rico em flavonoides e antocianinas, é eficaz no uso tópico de feridas cutâneas fechando totalmente as feridas em 20 dias de tratamento.

A utilização de fitoderivados tem demonstrado atividades promissoras no estímulo celular bem como na produção de componentes do tecido epitelial e conjuntivo acelerando processo de reparo das feridas cutâneas (Cheng et al. 2013; Sarandy et al. 2015). No presente estudo os animais que receberam tratamento com pomada a base de extrato de *S. pseudoquina*, principalmente na dose mais elevada, apresentaram aumento na celularidade e na vascularização tecidual, estas características são importantes para demonstrar o aumento do metabolismo do tecido durante o processo de reparo no diabetes, especialmente na fase inflamatória (Xie et al. 2013). Em geral, a fase inflamatória é prolongada no diabetes. Existem defeitos na angiogênese e diminuição da proliferação celular e, todos estes fatores contribuem para um processo cicatricial mal-sucedido (Mikaili et al. 2014). Romero-Cerecero e colaboradores, (2014) demonstraram que o uso tópico de plantas medicinais como *Ageratina pichinchensis* na cicatrização de feridas em ratos diabéticos estimulam um aumento na celularidade e vascularização do tecido lesionado. Resultados semelhantes foram encontrados na aplicação tópica de Curcumina, um pigmento presente na espécie *Curcuma longa* L. (Kant et al. 2015) e também em estudos com Asiaticoside isolados de *Centella asiatica* (Shukla et al. 1999), sendo observado um aumento da reepitelização e vascularização em ratos diabéticos. Além dos fagócitos e fibroblastos, os mastócitos também são encontrados em

abundância durante o processo de reparo tecidual e desempenham importante função na produção de fatores angiogênicos tais como VEGF e TGF- $\beta$ 1 exercendo grande influência sobre a resposta proliferativa na cicatrização de feridas cutâneas (Nishikori et al. 2014; Egozi et al. 2003). Neste estudo, observou-se um aumento do número de mastócitos nos tratamentos com extrato em diferentes doses, no entanto, o efeito dose resposta também foi observado para estas células. Resultados semelhantes foram encontrados através do uso tópico de pomada a base de extrato de *Schinus terebinthifolius* a 5%, em ratos não diabéticos, no qual houve aumento do número de mastócitos, acompanhado pelo aumento da contração da área da ferida nos dias 7 e 14 de tratamento (Estevão et al. 2015). Em nossos achados foi interessante notar que a expressão de TGF- $\beta$  acompanhou o aumento apresentado na proliferação celular para os grupos que foram tratados com a maior dose do extrato. TGF- $\beta$  é considerado um mediador universal e pode ser sintetizado por vários tipos celulares, entre eles destacam-se mastócitos e fibroblastos (Siebert et al. 2011; Sydow et al. 2016). A ação positiva do TGF- $\beta$  no processo de reparo das feridas cutâneas no diabetes já tem sido relatada, uma vez que este mediador tem importante ação anti-inflamatória e junto com outros mediadores diminui a migração celular e acelera a síntese de matriz no local da lesão (Kant et al. 2015; Lin et al. 2015).

A última fase do processo cicatricial, também conhecida como fase de remodelação, é caracterizada pelo aumento da resistência biomecânica do tecido que ocorre devido à substituição do tecido de granulação rico em colágeno III, por um tecido mais forte, rico em colágeno I (Thakur et al. 2011). Além da modificação no tipo de fibras, nesta fase as ligações entre os constituintes da matriz são do tipo covalente o que confere ao tecido maior firmeza. (Mendonça & Netto 2009; Nassar et al. 2012). Os resultados mostraram que a síntese de colágeno na matriz foi maior quando o extrato de

*S. pseudoquina* foi utilizado em ambas as concentrações e que a síntese de outros componentes da matriz também foi favorecida, como pode ser observado na análise de fibras elásticas. O estudo de extratos vegetais para promover a síntese dos constituintes da matriz tem mostrado resultados positivos, principalmente quando relacionado à síntese de colágeno (Donato-Trancoso et al. 2014; Hou et al. 2015). Recentemente Gonçalves e colaboradores (2016) demonstraram que o hormônio esteroide 5 $\alpha$ -Dihydrotestosterona, quando aplicado diretamente em feridas cutâneas promove a síntese de colágeno I e III na matriz, acelerando o processo de reparo. Semelhantemente, um curativo a base de colágeno extraído da cauda de rato (Neurotensin-loaded collagen) foi aplicado em feridas de ratos diabéticos e observou-se um aumento desta fibra no tecido cicatricial analisado (Moura et al. 2014). Aplicação tópica do extrato de partes aéreas de *Ageratina pichinchensis* também apresentou aumento de fibras colágenas na cicatrização em ratos diabéticos (Romero-Cerecero et al. 2014).

Manter o equilíbrio entre fatores oxidantes e antioxidantes é crucial para uma adequada cicatrização de feridas (Lin et al. 2015). Nossos estudos demonstraram diminuição marcante na quantidade de marcadores de estresse oxidativo e um aumento na atividade de enzimas antioxidantes como SOD e CAT, o que nos permite sugerir que o extrato de *S. pseudoquina* possui elevado potencial antioxidante que favorece o processo de reparo cutâneo. A enzima Superóxido-Dismutase (SOD) é a primeira defesa antioxidante da célula, catalisando a transformação do íon superóxido ( $O_2^-$ ), extremamente lesivo às células, em  $H_2O_2$  e sua ação é acompanhada pela enzima catalase que elimina o  $H_2O_2$  convertendo-o em água e  $O_2$  molecular (Dhouib et al. 2015). Segundo Zhang e colaboradores (2015) extratos de *Lens culinaris*, ricos em compostos fenólicos, apresentam elevada atividade antioxidante e conseqüentemente são eficazes para manter o status redox dos tecidos. Em estudos com Asiaticoside isolados de

Centella asiatica observou-se um aumento de enzimas antioxidantes SOD e CAT em tecido cicatricial o que favorece a passagem de elétrons e diminui os danos oxidativos aos tecidos (Shukla et al. 1999). Quando os tecidos estão sendo agredidos pela ação de radicais livres é comum aparecer alterações em lipídeos, proteínas e no DNA das células, levando a um quadro conhecido como estresse oxidativo (Limón-Pacheco & Gonsebatt, 2009; Guo & DiPietro, 2010; Scioli et al. 2015). Durante o reparo tecidual é comum haver a produção de muitas espécies reativas de oxigênio (ROS) devido à explosão respiratória que ocorre durante a fase de inflamação (Watson et al. 2016). Lipídios de membrana são muito sensíveis aos efeitos nocivos de ROS (Bal et al. 2012; Naziroglu et al. 2009) e estes radicais levam à remoção de um átomo de hidrogênio, causando a peroxidação lipídica em cadeia de ácidos graxos poli-insaturados. Estes por sua vez, desencadeiam um aumento da concentração de radical lipoperoxil e conseqüentemente leva à formação de malondialdeído (MDA) no tecido (Cambay et al. 2011). Os grupos carbonila nas proteínas (aldeídos e cetonas) são formados quando as proteínas são oxidadas por radicais livres provenientes do estresse oxidativo (Jana et al. 2014; Etem et al. 2014). A redução na produção de espécies reativas do ácido tiobarbitúrico (Malondialdeído) e proteínas carboniladas neste estudo evidenciam a ação positiva do tratamento com o extrato de *S. pseudoquina* 10% em ratos diabéticos. Tratamento tópico com extrato de *Albizzia lebeck*, que apresentou quantidade significativa de fenóis, taninos e flavonoides, houve um aumento de SOD bem como redução de MDA no tratamento de feridas em ratos (Joshi et al. 2013). Estudos feitos com óleo de sementes de *Joannesia princeps* reduziram os níveis de proteínas carboniladas na área da ferida no dia 14 após o ferimento, diminuindo o dano oxidativo induzido por ROS contribuindo para uma melhoria na cicatrização das feridas (Donato-Trancoso et al. 2014).

## 6. Conclusão

Nossos resultados indicam que a aplicação tópica do extrato de *S. pseudoquina* nas concentrações de 5 e 10% promove um reparo cutâneo mais rápido e eficaz. Os resultados mostraram um possível efeito dose-dependente. Estudos com a caracterização fitoquímica aprofundada deste extrato se faz necessário para avaliar e identificar dos componentes ativos e esclarecer plenamente o seu mecanismo de ação.

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