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Histomorphometry of tissue repair after the use of formulations based on extracts of *Piptadenia gonoacantha* (Mart.) J. F. Macbr in mice

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Abstract: *Piptadenia gonoacantha* is a tree species with several biological properties. The objective of this study was to evaluate the action of two pharmaceutical formulations, ointment, and balm, containing *Piptadenia gonoacantha* extract, on the healing of wounds made in 45-day-old male mice. The animals were randomly grouped and 3 treatments with 6 repetitions: an ointment containing the extract; balm containing the extract and silver sulfadiazine was used as the positive control. Tissue samples were collected on the 4th and 8th days. Treatment began immediately after the injury, and the treatments were applied once a day throughout the experiment. Treatment with the balm formulations on day 4 and the ointment on day 8 had better wound area regression rates compared to the control group, 45.4% and 81.6%, respectively. The balm and ointment treatments provided a reduction in vascularity indicating a reduction in the inflammatory process, initiating the proliferative phase in the scar tissue. These data were supported by the analysis of collagen synthesis. The results pointed out the efficacy of balm and ointment formulations concerning sulfadiazine. Therefore, the balm formulation is indicated for the beginning of the treatment, while the ointment formulation is indicated for the continuation and end of treatment.
Keywords: collagen, skin wounds, wound healing, medicinal plants, animal studies.

1. Introduction

Piptadenia gonoacantha (Mart.) J. F. Macbr. is a tree species from of the Fabaceae family, often found in the Atlantic Forest in southern and southeastern Brazil, popularly known as Pau-Jacaré (Hernández et al., 2010). *In vitro,* bioassays of *Piptadenia gonoacantha* extracts have reported antibacterial action (Carvalho et al., 2014a; Franco et al., 2021), which has stimulated new studies with the plant species and revealed other activities such as anti-inflammatory and antinociceptive (Carvalho et al., 2014b; Carvalho et al., 2010).

The therapeutic actions of plants are due to the presence of secondary metabolites, natural products that act in the plant defense system against pathogens and environmental stresses (Gobbo-Neto, Lopes, 2007; Li et al., 2020). Among the various secondary metabolites, flavonoids stand out among the most widespread and abundant groups. These compounds are essential for the growth and reproduction of plant species, besides acting against several oxidative processes (Bouderias et al., 2020). Due to these properties, research on flavonoids points to a wide range of medicinal properties (Verri et al., 2012).

Research aimed at developing new therapeutics based on plants with medicinal properties to treat injuries and healing properties has been widely developed (Souza et al., 2016; Ramalho et al., 2018). Tissue injuries are a major challenge for global health since healing is a complex process because wound repair is a process that involves cellular and extracellular mechanisms, which aim to attain cell proliferation and vascularization of the injured tissue. The increase in cell number is caused by the recruitment of inflammatory cells that secrete cytokines and attract other cells to the lesion site (Makino et al., 2014; Sarandy et al., 2015). Mediators are also released to stimulate neovascularization and improve nutrition and tissue oxygenation (Xie et al., 2013; Wong et al., 2013; Sarandy et al., 2015). This dynamic repair process is divided into three phases: inflammation, proliferation, and maturation. In addition, bacteria or fungi quickly colonize the injuries and wounds, making healing difficult and delayed. To control infections of body tissues and blood circulation by microorganisms, the use of topical antimicrobials is recommended, however, some antibiotics can have cytotoxicity, which also impairs wound healing, and antibiotic resistance is also a complicating factor (Siafaka et al., 2016).

Therefore, according to Okur et. al (2020), the use of formulations containing plant extracts may be beneficial in this process. In this sense, some natural products already stand out as tissue healing and re-epithelializing such as Calendula (*Calendula officinalis*), Barbatimon (*Stryphnodendron astringens*), Babosa (*Aloe vera*), Copaíba (*Copaiferalangs dorffi*) and Cabbage (*Brassica oleracea var. capitata*) evidencing a significant improvement in the evolution of tissue lesions (Sarandy et al., 2015; Ramalho et al., 2018). This study aimed to characterize the phytochemical constituents of the hydroalcoholic extract of *Piptadenia gonoacantha*, and to evaluate by histomorphometry the healing activity of the balm and ointment formulations containing the extract.

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2. Materials e Methods

2.1. Plant Species Acquisition

Leaflets of the species *Piptadenia gonoacantha* were collected in the municipality of Viçosa, MG, Brazil, latitude 20º 45' 14" S, longitude 42º 52' 55" W and altitude of 648 m. The collection was performed in September 2017, before the flowering, which occurs from January to August, in adult trees of this species. The identification and authentication of the material were performed by comparison with species deposited in the Horto Botanical Garden of the Universidade Federal de Viçosa, where the voucher specimen was deposited under the number 35530.

2.2. Extraction process

The extracts of *Piptadenia gonoacantha* were obtained from its leaflets, which were dried in a circulating air oven at 40 ± 2ºC for 96 hours and ground in a knife mill. The leaf powder was used in a 1:5 ratio (100g:500mL of ethanol/water solution 80% v/v with 0.3% citric acid), resulting in a concentration of 20% dry extract (w/v). The extract was then macerated for 72h at 25°C in a shaking incubator. Afterward, it was vacuum filtered (Marconi MA 058®), and the filtrate was collected and stored in an amber flask. The retained residue (cake) in the filter was taken again to extraction by maceration 2 times. The filtrates were gathered at the end of the process in an amber flask and protected from light until the rotary evaporation process that occurred under negative pressure at 60°C was completed and then they were freeze-dried at 10^{-1} mbar at -60ºC. The lyophilized was used for the preparation of the formulations (Balm and Ointment) and in the healing tests.

2.3. Chemical characterization by RP-UPLC-MS/MS of the extracts

The chromatographic separation was performed using a C18 column (250x4.6mm), a pre-column at room temperature (21±5°C) and isocratic elution mode with water, 0.1% (w/w) ortho-phosphoric acid (solvent A) and acetonitrile (solvent B) for 60min at conditions of 80% A and 20% B at a flow rate of 0.8mL·min–1 with a UV/Vis detector at a wavelength of 210nm. Liquid chromatography analyses were coupled to mass spectrometry (HPLC-MS/MS), where they were prepared at the concentration of 2000 ppm (mass of plant/volume of solvent), and diluted up to 500 ppm. The results obtained were expressed to LODi (Instrument Detection Limit) and LOQi (Instrument Quantification Limit).

Standard compounds of gallic acid, chlorogenic acid, catechin, vanillic acid, caffeic acid, 6-hydroxycoumarin, p-coumaric acid, ferulic acid, rutin, 4-hydroxycoumarin, rosmarinic acid, quercitrin, myricetin, physetin, resveratrol, trans-cinnamic acid, quercetin, luteolin, apigenin, kaempferol, 3,6-dihydroxyflavone, chrysin, and galangin were used for the analysis.

2.4. Preparation of the formulations

The freeze-dried extract of *Piptadenia gonoacantha* was suspended in ethanol at 80 % (v/v), in a 1:1 ratio. Subsequently, the solution obtained (50% - m/v) was incorporated into the balm and ointment bases in the proportion of 20% to the freeze-dried extract, according to specifications of the Brazilian pharmacopeia National Form / Brazil (Anvisa, 2012; Corrêa, 2012).

The following were used to prepare the ointment base: cetostearyl alcohol 90%, cetylstearyl sulfate sodium salt 10%, almond oil, ethoxylated lanolin, liquid vaseline, solid vaseline, beeswax, preservatives, and purified water. The preparation technique is performed by heating the components until the waxes melt and cooling with agitation (Anvisa, 2012).

For the preparation of the balm base, two distinct phases were used, where the components of the oil phase (butylhydroxytoluene, propylparaben, sunflower oil, copaiba oil, ethoxylated hydrogenated castor oil, cetostearyl alcohol 90% and cetylstearyl sulfate sodium salt 10%) were heated to 70±5°C in a water bath, separately from the components of the aqueous phase (pro-vitamin b5, methylparaben, caprylic acid capric triglycerides, and demineralized water), also heated to 70±5°C in a water bath. The aqueous phase components were poured over the oil phase components with constant stirring until cooling.

2.5. Animal experiment

2.5.1. Evaluation of the healing activity of Piptadenia gonoacantha balm and ointment formulations

Eighteen male mice (Balb C), 45 days old, from the Biotério Central da Universidade Federal de Viçosa-MG (UFV) were used. The animals remained in individual polypropylene cages, sanitized daily at a controlled temperature of 23±2°C and a 12-hour light/dark cycle. Throughout the experimental period, the animals were fed with feed and water ad libitum. After the experiment, the animals were euthanized following the recommendations of the ethics committee on animal experimentation under the number (CEUA/UFV, nº 597/2017), and the carcasses were collected by the biosecurity service from UFV.

2.5.2. Surgical Incision and Animal Treatment

The animals were anesthetized using xylazine hydrochloride (8.0 $mg.kg^{-1}$) with ketamine hydrochloride (140.0 $mg.kg^{-1}$), intraperitoneally. Subsequently, trichotomization and antisepsis with 2% chlorhexidine were performed. Using a circular scalpel (6 mm), a surgical incision and removal of the skin from the dorsal region were performed (Time "0"), according to the methodology proposed by Carvalho et al., 2013. The wounds were kept open, without any local manipulation, only with the application of the treatments.

Three treatments with 6 repetitions were performed: (Pom) treatment with an ointment containing *Piptadenia gonoacantha* extract; (Bal) treatment with balsam containing *Piptadenia gonoacantha* extract and (SULF) treatment with silver sulfadiazine 1%,

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used as a positive control. Treatment started immediately after the injury, with the application of the treatments once a day. Later, samples were collected from the wounds on days 4 and 8 after the beginning of the treatment.

2.5.3. Clinical and histomorphometry evaluation of wounds

Wound assessment was performed daily, visually, observing possible changes in signs of inflammation, erythematous halo, epithelialization time, and healing time. The wound area was assessed using a handheld pachymeter with measurements taken in the greatest length and greatest width directions on days 1, 4, and 8 after the incisions.

2.5.4. Collection and histological processing of the material

On the 4th day of treatment, three animals from each group were euthanized, and a surgical incision using a circular scalpel (6 mm) was made to remove the tissue fragment destined for histopathological analysis. The same procedure was performed on the 8th day of treatment. The skin fragments were collected from each animal and fixed in 4% paraformaldehyde for 24 h and stored in bottles containing 70% ethanol until the histological slides were made. Procedures for paraffin embedding were performed following the steps: dehydration using increasing concentrations of ethanol (70% to 100%) and transferred to xylene for diaphanization. Five-micrometer sections were obtained on a rotating microtome, which was then prepared on slides stained with Picrosirius and others stained with hematoxylin-eosin (HE).

To perform the fibroblast cells, count and vascularization in the scar tissue on slides stained with HE, a digital camera coupled to the optical microscope was used. Ten fields per slide were photographed with a 20X objective lens, with a total tissue area submitted to stereological analysis of 7.2x106µm^2. For this analysis, a grid area composed of 300 points in the test area, used as a standard (TA) of 1.2x105µm^2, was superimposed on each image (Vieira, 2015). The analysis of collagen fibers present in the connective tissue was performed with specialized software. Type I collagen fibers were visualized in red and yellow and type III collagen fibers were visualized in green, following the methodology described by Vieira, (2015).

3. Results

The phytochemical analysis in UPLC-MS/MS of the hydroalcoholic extract of *P. gonoacantha* allowed the identification of the following compounds: rutin, ferulic acid, p-coumaric acid, quercitrin and kaempferol (Table 1).

Compounds	UPLC-MS/MS Results (ng mL^{-1})	Pharmacological Activity
Rutin	33.4	Antioxidant (Al-Rejaie et al., 2013; Paula et al., 2014), antibacterial, anti-hepatotoxic, anti-hemorrhoidal, anti-allergic, anti-inflammatory, anti-tumor, antiplatelet properties, antispasmodic, antiviral, anti-ulcerogenic, anti-diarrheal, vasodilator, cytoprotective, antihypertensive and antimutagenic (Caillet et al., 2005; Calabrò et al., 2005; Domitrović et al., 2012; Janbaz, Saeed, Gilani, 2002; Mahmoud, 2012; Oliveira, 2008; Yang, Guo, Yuan, 2008).
Ferulic Acid	11.6	Antioxidant (Soares, 2002), melanogenesis, angiogenesis and accelerates wound healing (Zduńska et al., 2018).
p-Cumaric Acid	10.5	Antimelanogenic antioxidant inflammatory (Boo, 2019; Lee et al., 2019), antibacterial (Lou et al., 2012).
kaempferol	7.6	Anti-inflammatory (Santangelo et al., 2007; Yoon, S. Baek, 2005), hypoglycemic, antioxidant activity (Barreto-Silva et al., 2013; Da Silva et al., 2000; Pepato et al., 2002) and Antitumor (Alonso-Castro et al., 2013; Cid-Ortega and Monroy-Rivera, 2018).
Quercitrin	7.4	Antioxidant, anti-carcinogenic, anti-inflammatory, and antiviral activities (Neto, 2008; Yao et al., 2016).
Apigenin	2.2	Antiulcerogenic e anti-inflammatory (Chen et al., 2006c; Duarte et al., 2011b; Liu, Bao, Yan, 2002b; Lopez-Jornet et al., 2014b).
6-Hydroxycoumarin	1.9	Wound healing (Afshar et al. 2020), antioxidant e Anti-inflammatory (Hadjipavlou-Litina et al., 2007).

Table 1 – Metabolites evaluated by liquid chromatography-mass spectrometry (UPLC-MS/MS) of the hydroalcoholic extract of *P. gonoacantha.*

The follow-up of the animals showed that the ones treated with ointment (Pom), balsam (Bal), or sulfadiazine (Sulf) did not develop signs of infection at the wound site, as well as no change in body weight throughout the experimental period. The animals developed a crust at the wound site, as shown in Figure 1.

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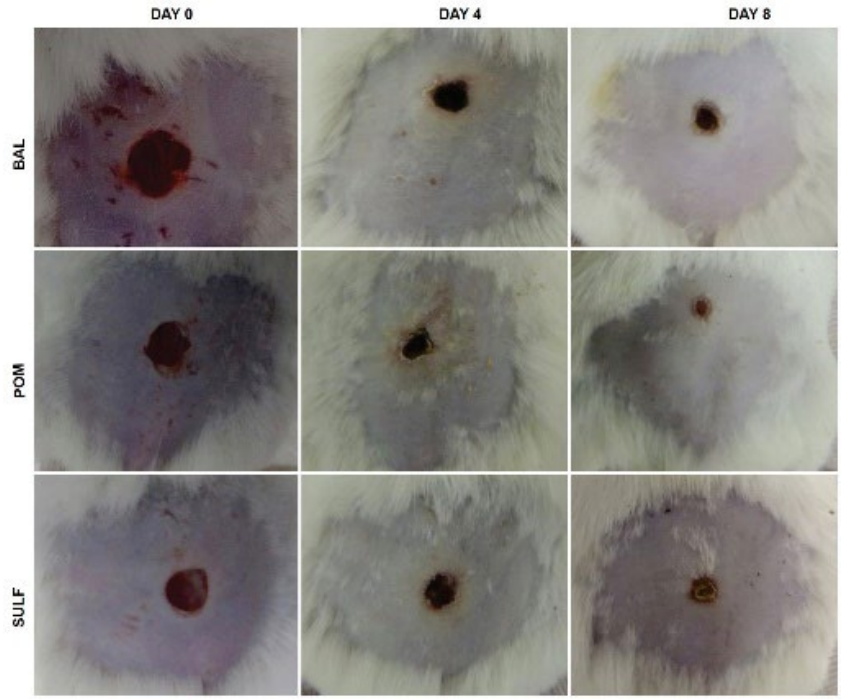

Figure 1 – Macroscopic evaluation of the healing process of the exceptional wounds and treatment of the mice with balm formulations (bal), ointment (pom) based on extracts of Piptadenia gonoacantha or silver sulfadiazine (sulf).

Macroscopically, there was a higher rate of wound regression in the animals with treatment A on day 4 and with treatment B on day 8 compared to the other groups. Figure 2 shows the values of the area (mm^2) and the wound shrinkage index (%) of the surgical wounds in the animals treated with Pom, Bal, and Sulf.

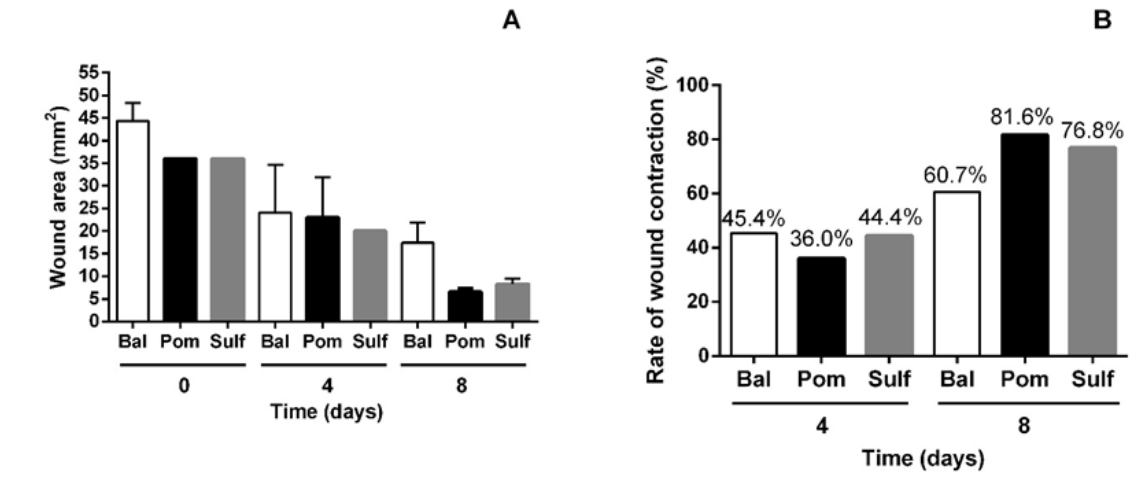

Figure 2 – Effect of treatment with balsam, ointment and control group. Wound area (A). Wound contraction rate (B) in mice was evaluated on days 4 and 8 of treatment. Pom: ointment; Bal: balsam and Sul: silver sulfadiazine.

On the 4th day of treatment, the groups treated with Sulf and Bal had wound area regression rates of 44.4% and 45.4%, respectively (Figure 2). On the 8th day of treatment, there was a higher percentage of wound regression in the Pom group (81.6%), followed by the Sulf (76.8%) and Bal (60.7%) groups. Treatment with Bal provided a reduction in the time to wound closure (day 4 of treatment) when compared to the animals treated with Pom and Sulf. However, treatment with Pom was able to reduce the wound area by 81.6%, being more effective than the other treatments on day 8, followed by Sulf (76.8%) and Bal (60.7%) (Figure 2B). The treatment with balm containing *P. gonoacantha* extract provided greater speed in the healing process in the first days of treatment. The treatment with *P. gonoacantha* ointment is more effective after the 4th day of treatment. In other words, both the Bal and Pom treatments are superior to the control treatment, Sulf. However, in different phases, Bal has greater efficacy at the beginning

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of the treatment and Pom at the continuation and end of the treatment. This reduction promotes shorter treatment time, improving patients' quality of life and reducing financial expenses.

Regarding the number of total cells, all treatments presented statistically significant results ($p<0.05$) (Figure 3A), compared to the tissue removed on the first day, called control. However, they did not differ statistically from each other in the first 4 days of treatment, yet the data showed an increase in cellularity by 8.10% and 9.73% with the Bal and Pom treatments, respectively, compared to the control-treated with Sulf.

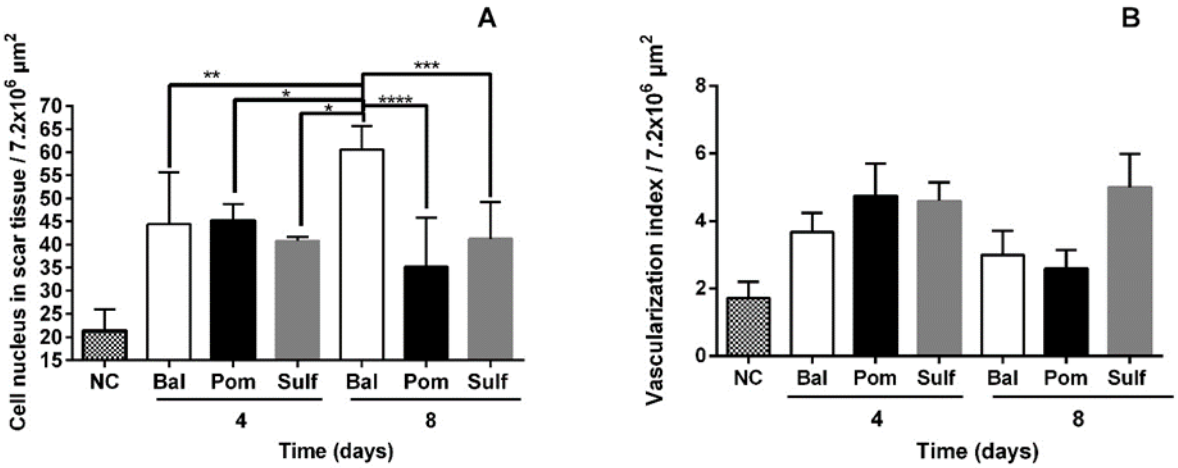

Figure 3 – Effects of the formulations on the scar tissue of animals treated with Bal, Pom or Sulf. Cellularity (A) and vascularization in scar tissue (B). Bal - balm; Pom - Pomade; Sulf - Silver sulfadiazine.

In assessing tissue cellularity on the 8th experimental day (Figure 3A; Figure 4), there was an increase in the number of cells within the group of animals treated with Bal compared to the Pom ($p<0.0001$) and Sulf ($p<0.001$) groups. The statistical difference observed demonstrated that the healing process provided by the use of Bal was favored by the increase in cell number in the scar tissue.

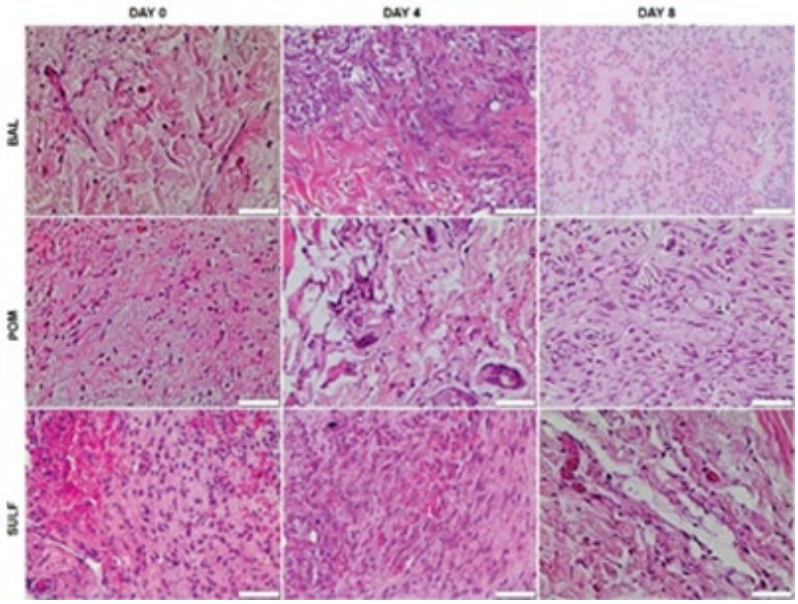

Figure 4 – Representative photomicrographs of tissue cellularity in mouse skin sections were observed under a light microscope. H&E staining, Bars = 200µm. Tissue fragments were collected on days 0, 4 and 8 of treatment with Bal, Pom or Sulf. Bal - balm; Pom - ointment; Sulf - silver sulfadiazine (Positive control).

Regarding tissue vascularization (Figure 3B, Figure 4), all treatments provided an increase in vascularization and differed statistically ($p<0.05$) from the control group. However, there was no statistically significant difference between the Bal, Pom, and Sulf treatments in the initial four days. However, on day 8, there was a reduction in vascularization in tissue treated with Bal ($p<0.01$)

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and Pom ($p<0.001$), being statistically different compared to the Sulf control group (Figure 3B, Figure 4). The reduction in vascularization observed in the tissue treated with Bal and Pom reflects a reduction in the inflammatory process in the scar tissue. This reduction is fundamental to initiating the proliferative phase, allowing the healing process to continue.

In the evaluation of type III collagen synthesis, no statistically significant difference was observed between the treatments. However, in absolute values, Bal treatment provided an increase in type III collagen fibers compared to the control group (51.1%), Sulf (68.1%) on the 4th day, and Sulf (59.5%) on the 8th day of treatment (Figure 5A).

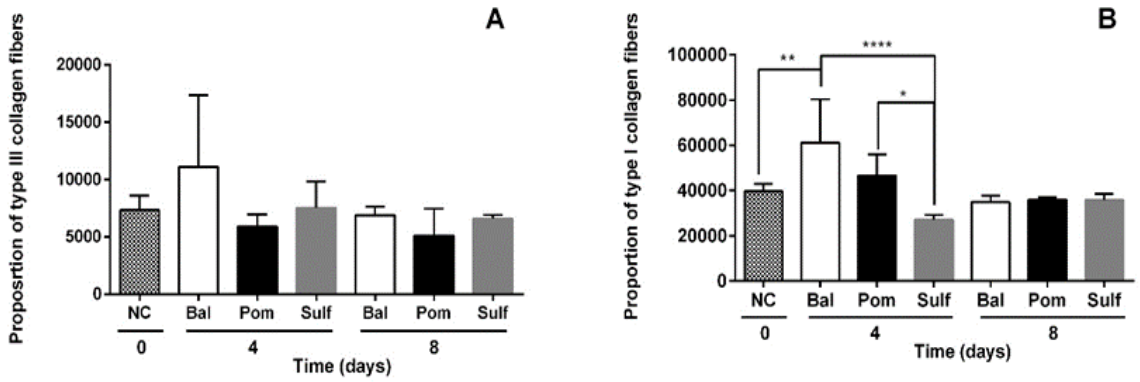

Figure 5 - Proportion of collagen fibers type I (A) and type III (B) in the scar tissue of mice evaluated on the 4th and 8th day of treatment with formulations Bal, Pom or Sulf. Bal - bal; Pom - ointment; Sulf - silver sulfadiazine (Positive control). *= $p<0.05$, **=$p<0.01$ and $p<0.0001$.

Concerning the quantification of collagen fibers on the 4th and 8th day of treatment, the Bal treatment showed itself to be more effective than the other groups in inducing type III collagen formation, showing greater inflammatory response and angiogenesis, with a shorter healing time.

When we analyzed the synthesis of collagen type I, fundamental to the healing process, there was an expressive increase in the treatments with Bal and Pom concerning the control (Time 0) and Sulf groups (Figure 5B; Figure 6). The Bal treatment provided an increase in type I collagen fibers, statistically significant concerning the Sulf treatment ($p<0.0001$) and the control (Time 0) ($p<0.001$). It is possible to infer that the bioactive compounds of *P. gonoacantha* extract, present in the Bal and Pom formulations, promoted the proliferation and maturation of fibroblasts, as well as the synthesis of type I collagen.

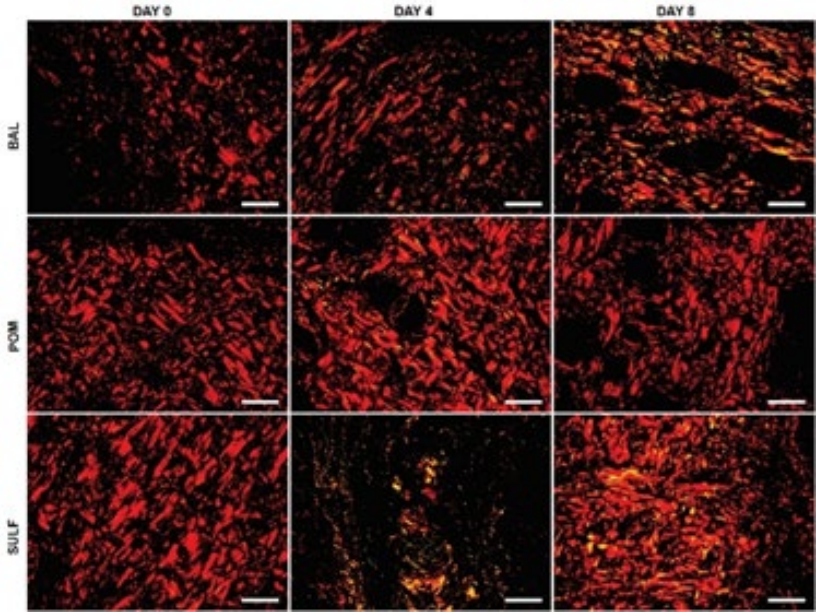

Figure 6 – Photomicrographs of collagen synthesis in the healing process of animals. Tissue fragments were collected on days 0, 4 and 8 of treatment with formulations Bal, Pom or Sulf. Picrosirius staining, Bars = 200 µm. Bal - balm; Pom - ointment; Sulf - silver sulfadiazine (Positive control).

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Treatment with the balm formulations on day 4 and the ointment on day 8 showed the highest wound area regression rates, 45.4%, and 81.6%, respectively, compared to the control group, Sulf. The Bal and Pom treatments provided a reduction in vascularization indicating a reduction in the inflammatory process, initiating the proliferative phase in the scar tissue. These data were supported by the analysis of collagen synthesis, where the Bal and Pom treatments promoted greater stimulation of fibroblasts and synthesis of type I collagen fibers.

4. Discussion

Concerning the chemical characterization, preliminary studies with *P. gonoacantha* extracts have demonstrated the presence of phenolic compounds, flavonoids, and coumarins (Carvalho et al., 2010). The results indicate the presence of secondary metabolites related to biological activities, beneficial in the treatment of various pathologies. To this class of polyphenols, several activities have been attributed, among them antitumor, antioxidant, antiviral, and anti-inflammatory (Cazarolli et al., 2008; Simões, 2016; Veitch, Grayer, 2011).

Phenolic compounds, such as flavonoids, have an important function in the inflammatory process by inhibiting T lymphocyte proliferation and in the production of pro-inflammatory cytokines, TNF-*α* and IL-1 (Kim et al., 2004; Theoharis, 2007; Sarandy et al., 2015). Flavonoids are responsible for therapeutic effects, such as antioxidant, anti-inflammatory, and antifungal. In addition, it can promote quick healing because of its antimicrobial properties that promote complete collagen synthesis (Nazaruk and Galicka, 2014; Lopez-Jornet et al., 2014; Sarandy et al., 2015).

As shown in Table 1, rutin is the secondary metabolite present at the highest concentration, being a natural flavonoid with antioxidant action and important tissue protection capacity (Al-Rejaie et al., 2013). Its reducing property and chemical structure favor the sequestration of free radicals, acting in the initiation and transmission of the oxidation process (Paula et al., 2014). In addition, rutin exhibits pharmacological activities such as antibacterial, anti-hepatotoxic, anti-hemorrhoidal, anti-allergic, anti-inflammatory, anti-tumor, antiplatelet, antispasmodic, antiviral, anti-ulcerogenic, anti-diarrheal, vasodilator, cytoprotective, antihypertensive, and antimutagenic properties (Caillet et al, 2005; Calabrò et al., 2005; Domitrović et al., 2012; Janbaz, Saeed, Gilani, 2002; Mahmoud, 2012; Oliveira, 2008; Yang, Guo, Yuan, 2008). Almeida and co-workers showed in a study with hydrogels containing rutin intended for cutaneous administration that, after 5 days of treatment of wounds, hydrogels containing rutin presented a higher decrease in the wound area compared to the control hydrogels (Almeida et al., 2012).

Quercitrin was also identified in the extracts, Neto (2008) demonstrated that this flavonoid, extracted from the leaves of *Bauhinia ungulata L.,* among other substances, conferred antioxidant action in the assays performed. *In vitro* and some animal models have shown that quercetin, a polyphenol derived from plants, has a wide range of biological actions including anti-carcinogenic, anti-inflammatory, and antiviral activities; as well as attenuating lipid peroxidation, platelet aggregation, and capillary permeability (Neto, 2008; Yao et al., 2016). The apigenin, present in a great number of plants (especially chamomile), has been observed to act as a natural anti-inflammatory agent (Chen et al., 2006a; Chen et al., 2006b; Duarte et al., 2011; Liu, Bao, Yan, 2002; Lopez-Jornet et al., 2014). Its efficacy in the treatment of symptoms of gastritis, gastric ulcers, and other mucosal inflammatory processes is due to the apigenin glycosides present in the plant. Some recent studies have demonstrated that apigenin could be effective in the treatment of skin inflammatory processes induced by free radicals (such as UV, X or γ radiation, or chemical compounds) (Chen et al., 2006c; Duarte et al., 2011b; Liu, Bao, Yan, 2002b; Lopez-Jornet et al., 2014b).

Nagy and co-workers (2019), while studying cannabis, (*Cannabis sativa L.*) detected the presence of 6.1 and 7.8 $mg \cdot g^{-1}$ of kaempferol and apigenin, respectively, in methanolic extracts of Cannabis leaves and inflorescence (Nagy et al., 2019). Based on these results, *C. sativa* may represent an important source of these metabolites for nutraceutical, cosmetic, and pharmaceutical uses. These metabolites were also verified in the hydroalcoholic extract of *P. gonoacantha*, corroborating our results and pointing to a new source of these compounds.

Coumarin is a plant compound with anti-inflammatory and anti-oxidant effects. Afshar et al. (2020) showed the benefits of using coumarin in accelerating wound healing in mice (Afshar et al., 2020). The topical application of coumarin had beneficial effects on different phases of wound healing in the skin of BALB/c mice.

Concerning the clinical evaluation of the animals and histopathological evaluation of the wound healing process, the results confirmed the studies of Mandelbaum (2003) who demonstrated that open wounds crusted and epithelialized slowly. Ricardo et al. (2018) used barbatimão (*Stryphnodendron astringens*), as a treatment and observed the formation of thick, dry, and irregular crusts (Ricardo et al., 2018) that occurred probably by the astringent action of the tannins present in the species. However, in our studies, the use of the formulations promoted greater tissue hydration, caused by the characteristics of the products. Several drugs with healing purposes are used to produce moisture on the site, due to the formulation constituents, greatly improving the re-epithelialization of wounds.

The rate of wound regression is important data in the process because measurements at regular intervals are essential for monitoring the healing process. Studies by Garros et al. (2006), that used passion fruit extract (*Passiflora edulis*) to evaluate the healing of skin wounds in rats, showed that the wound area gradually decreased significantly over time in the *Passiflora edulis*-treated group and the control group (Garros et al., 2006).

Corroborated by Ribeiro (2011), who evaluated the potential in the contraction of cutaneous wounds induced on the back of rats using extracts from the leaves and stem of Santa Maria's grass (*Chenopodium ambrosioides* L), showed increased retraction of the wounds after 12 days of treatment. Faleiro and collaborators (2009) demonstrated that the extract of aloe leaves (*Aloe vera*)

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facilitated the healing process of experimental wounds in rats by using herbal medicine in the form of glycolic extract since it provided greater contraction of the experimental wounds.

Regarding the number of total cells, the data corresponds with the results of Janning and colleagues (2011), who evaluated macro-microscopically the healing of cutaneous wounds in rats treated with topical use of the hydroalcoholic extract of Jasmine Catavento (*Tabernaemontana catharinensis*). The authors concluded that the hydroalcoholic extract has an action on vascular proliferation, endothelial proliferation, and fibroblasts. They also suggested that the extract of *T. catharinensis* could have a healing action, similar to the results obtained in our study. Barros (2016) when studying the effects of passion fruit extract (*Passiflora edulis* S.) on the healing of induced burns in mice, observed an acceleration in the healing process. Furthermore, the results showed a decrease in the number of cells involved in the inflammatory process in 7 days and an increase in fibroblasts with deposition and organization of collagen fibers in the animals in the group treated with *Passiflora edulis* S. in 14 days.

Hernandes and collaborators (2010) used an ointment composed of the semipurified fraction of barbatimão (*Stryphnodendron astringens*) bark extract on surgically treated rat skin wounds. The study reported a stimulation in the proliferation of keratinocytes by stimulating the proliferation of these cells along the margin of re-epithelialization compared to the control group on the fourth, seventh, and tenth day after injury. Zduńska and collaborators (2018) demonstrated that ferulic acid is related to the reduction in healing time.

We highlight those compounds such as Rutin (Domitrović et al., 2012), kaempferol (Santangelo et al., 2007; Yoon, S. Baek, 2005), quercitrin (Neto, 2008; Yao et al., 2016) and apigenin (Chen et al., 2006c; Duarte et al., 2011b; Liu, Bao, Yan, 2002b; Lopez-Jornet et al., 2014b) present in *P. gonoacantha* extract are recognized for their anti-inflammatory action.

Kaempferol has anti-inflammatory activity conferred by the inhibitory action of the enzymes phospholipase A2 (PLA2), 8 lipo-oxygenase, and cyclo-oxygenase and by the inhibition of nitric oxide production (Santangelo et al., 2007; Yoon, S. Baek, 2005). Ferulic acid, arising from enzymatic reactions from cinnamic acid, has actions in both the treatment and prevention of cancer and cardiovascular diseases. Research involving phenolic acids, including ferulic acid, showed that the antioxidant potential, when compared to chlorogenic, caffeic, and p-coumaric acids, presented significant results (Soares, 2002). Ferulic acid is a free radical scavenger, but also an inhibitor of enzymes that catalyze free radical generation and an enhancer of scavenger enzyme activity. It has a protective role for the main skin structures: keratinocytes, fibroblasts, collagen, and elastin, inhibiting melanogenesis, enhancing angiogenesis, and accelerating wound healing. Ferulic acid is widely applied in skin care formulations as a photoprotective agent, delayer of skin photoaging processes, and a brightening component. Nonetheless, its use is limited by its tendency to be rapidly oxidized (Zduńska et al., 2018). These actions were observed (Figures 5 and 6) and quantified in terms of collagen production in the scar tissue.

Nitz and collaborators (2006) studied the healing potential of the aqueous extract of Mastiff (*Coronopus didymus*) and Calendula (*Calendula officinalis*) reporting its efficiency in the healing process of skin wounds in rats, by promoting an increase in the number of fibroblasts and collagen fibers (Nitz et al., 2006).

Fibroblasts and endothelial cells are cells that make up the proliferative phase (Bobbo Moreski et al., 2018), being one of the main components of granulation tissue that, under the influence of growth factors and other mediators, are activated and migrate from the wound edges to its center (Balbino et al., 2005). By increasing the number of activated fibroblasts, the local production of collagen begins, with the extracellular matrix being replaced by a stronger connective tissue with greater elasticity, also promoting the formation of new blood vessels. The induction of angiogenesis occurs due to the low oxygen tension, a characteristic that occurs in the center of the wound (Bobbo Moreski et al., 2018).

Collagen is a protein found in large numbers in the connective tissue during the healing phase (Balbino et al., 2005). In this phase of repair or remodeling the healing process occurs the deposition of collagen in an organized way. These fibers are classified as types I and III according to their degree of maturation, and their quantification is essential to compare tissue repair. Type I collagen is most often present in the skin (80%), synthesized by fibroblasts in bones and tendons, and is considered mature collagen. Type III Collagen (30 to 40%) is found in blood vessels, dermis, and other soft tissues (Robinson, Steed, Franz, 2001).

5. Conclusion

The chemical characterization of *P. gonoacantha* hydroalcoholic extract, used for the development of Bal and Pom formulations, allowed the identification of the presence of rutin, ferulic acid, p-coumaric acid, quercitrin, and kaempferol. The Bal and Pom formulations showed superior efficacy in the healing process when compared to the positive control group, treated with silver sulfadiazine. The formulations promoted a reduction in the inflammatory process in the scar tissue. However, with different indications in different phases, Bal could be more useful during the beginning of the treatment and Pom during the continuation and conclusion, fundamental to the scarring process.

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