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HEALING OF WOUNDS IN DIABETIC RATS TREATED WITH ROSA RUBIGINOSA OIL

CICATRIZAÇÃO DE FERIDAS DE RATOS DIABÉTICOS TRATADOS COM ÓLEO DE *ROSA RUBIGINOSA*

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ABSTRACT

Purpose: This experimental study aimed to determine if *Rosa rubiginosa* oil (Rro) influences the healing of skin wounds of diabetic rats. **Methods:** Four groups of Wistar rats were used (6 rats/group). Group 1: skin wounds were topically treated with normal saline drops (NS). Group 2 had skin wounds treated with Rro (30%) for 10 days. Diabetes was induced with Streptozotocin (STZ) in groups 3 and 4. In Group 3 the wounds were treated with NS. Group 4 wounds were treated with Rro. After anesthesia, skin excisional wounds (0.8 cm diameter) were performed in all rats. On the 10th day, blood glucose and wound tissue pro-inflammatory cytokines were measured. Histometry of wounds was studied. **Results:** Previous glycemia levels of 3 and 4 group rats were >250 mg%. Diabetic and non-diabetic rats treated with Rro had wound tissues TNF-α, IL-1β, and IL-6 expression significantly lower than in rats receiving NS. The histological score of Rro-treated diabetic rats (22±2.5) was significantly higher than in diabetic rats treated with NS (17±1.8). **Conclusion:** The topical treatment of skin wounds in diabetic rats with Rosa rubiginosa oil positively influenced the healing.

Keywords: Wound healing. Diabetes induction. *Rosa rubiginosa*. Oil. Rats.

RESUMO

Objetivo: Este estudo experimental teve como objetivo examinar se o óleo de *Rosa rubiginosa* (Rro) influencia na cicatrização de feridas cutâneas de ratos diabéticos. **Métodos:** Foram utilizados quatro grupos de ratos Wistar (6 ratos/grupo). Grupo 1: as feridas cutâneas foram tratadas topicamente com gotas de solução salina normal (SN). O grupo 2 teve feridas cutâneas tratadas com Rro (30%) por 10 dias. O diabetes foi induzido com Estreptozotocina (STZ) nos grupos 3 e 4. No Grupo 3 as feridas foram tratadas com SN. As feridas do grupo 4 foram tratadas com Rro. Após a anestesia, feridas excisionais na pele (0,8 cm de diâmetro) foram realizadas em todos os ratos. No 10º dia, glicemia e citocinas pró-inflamatórias do tecido das feridas foram dosados. A histometria das feridas foi estudada. **Resultados:** Os níveis de glicemia inicial dos ratos dos grupos 3 e 4 foram >250 mg%. Ratos diabéticos e não diabéticos tratados com Rro apresentaram expressão de TNF-α, IL-1β e IL-6 nos tecidos das feridas significativamente menor do que em ratos que receberam SN. O escore histológico de ratos diabéticos tratados com Rro (22±2,5) foi significativamente maior do que em ratos diabéticos tratados com SN (17±1,8). **Conclusão:** O tratamento tópico de feridas cutâneas em ratos diabéticos com óleo de *Rosa rubiginosa* influenciou positivamente na cicatrização.

Palavras-chave: Cicatrização de feridas. Indução de diabetes. *Rosa rubiginosa*. Óleo. Ratos.

INTRODUCTION

The healing process of wounds is very complex, and it depends on the interaction with several regulated factors, which have the objective to restore the barrier function of damaged skin. Many events occur in the superficial wounds, but there can be problems especially with some diseases such as diabetes. When healing occurs with defects, it results in a chronic scar that has negative repercussions for the patients and the Health System. In patients with diabetes[1], chronic wounds have a high cost of treatment per year. It must be considered that the incidence of diabetes has grown annually, in addition to the prevalence of other chronic diseases that can affect wound healing[2]. The oxygenation of healing tissues is very important, among other reasons, the fact that there is a need for oxygen to interact with various cytokines, with the viability of proliferating cells, neutrophils, and macrophages as well. A wound requires at least

an oxygen tension of 20 mmHg to heal[3]. In wounds with impaired healing in diabetics, the O_2 tension is approximately 5 mmHg[4].

To restore the skin after a surgical wound, some immunological mechanisms are activated for the phagocytosis of bacteria, foreign bodies and other materials. An increase in metabolic demand occurs, with the participation of inflammatory cells, cytokines, chemokines, and other important molecules. Several of these processes take place simultaneously, during the inflammatory, proliferative, and remodeling phases[5].

After a rapid vasoconstriction, the vascular permeability and vasodilation take place. So, the blood leukocytes migrate to the wound. A great apport of cytokines such as TNF-α, IL-1, IL-2, IL-6, etc is expressed in the wound, and at this moment monocytes are converted to macrophages[6]. Macrophages play an important function in wound healing. At the begining they promote inflammation, phagocytosis of bacteria, debris, and apoptotic cells. So, they secrete important growing factors that regulate the proliferation of keratinocytes, fibroblasts, and endothelial cells for neovascularisation and wound closure. In diabetic and nondiabetic patients, macrophages increase secretion of pro-inflammatory cytokines[4]. The endothelial cells enter a rapid growth phase, angiogenesis occurs within the granulation tissue, and a wound transition into remodeling and maturation phase take place. At this remodeling phase the collagen type I predominates (80%), compared to type III collagen (20%). After that, the maturation of collagen take place. It is characterized by a large amount of intra and intermolecular collagen cross-linking, restoring the tensile strength of uninjured skin for about 70% [5,6].

Diabetes is difficult to control, and the skin wound in diabetes is difficult to heal. This is due to poor tissue blood supply, low phagocytic capacity of neutrophils and low inflammatory response[7,8]. Some treatments have been proposed for healing of diabetic wounds[9,10]. Therapies by using fibroblast growth factors have shown good results. Nevertheless, they are too expensive and are only used in controlled experimental studies[11]. So, the development of new dressings based on bioactive materials and herbals must be urgently developed for the treatment of wounds in diabetic patients[12,13].

The properties and composition of *Rosa rubiginosa* oil extracted from its seeds have been studied. The *Rosa rubiginosa* belongs to the genus Rosa, from the *Rosaceae*

family[14]. Original from the Mediterranean region, it has been cultivated in some regions of Brazil[15,16].

Some health benefits attributed to the *Rosa rubiginosa* oil, can be highlighted: anticarcinogenesis, antiatherosclerosis, inhibition of free radicals, changing in the metabolism of adipose tissue, antibacterial, immunomodulation and antidiabetic activity[17]. The seeds oil is rich in minerals, saturated fatty acids such as caprylic, capric and myristoleic acids. At the same time, antioxidant activity have been studied in a great amount of phospholipids found in its oil. Some of them are: quercetrin, pectin, mineral salts, ascorbic acid, t-retinoic acid, amino acids, anthocyanins, benzaldehyde, eugenol, saponin and tannin[18,19]. A significant percentage of flavonoids, proanthocyanidins and carotenoids have been found in *Rosa rubiginosa* oil, as well as polyphenols. The use of *Rosa rubiginosa* seed oil on open wounds of patients has shown good results[20].

The aim of the present study, is to examine whether *Rosa rubiginosa oil* influences the healing of dorsal skin wounds in diabetic rats.

METHODS

Rosa rubiginosa oil

Rosa rubiginosa oil was purchased from Guarani Cosméticos, Gramado-RS, Brazil. It was manipulated at 30% using polyethylene glycol from Merck (Ref. 202398), São Paulo, Brazil and was stored at room temperature until use.

Animals

Adult Wistar rats (Rattus norvegicus) weighing 280±23g, were from the Animal Vivarium of the CCS-UFRN, Brazil. All animals were housed in laboratory cages, in a refrigerated environment (22◦C), with a 12h light:12h dark cycle. They had ad libitum access to water and laboratory rats' food (Prevence®). The protocol was approved by the Institutional Revision Board for the Use of Animals in Research (Protocol No. 01/2021).

Streptozotocin-induced diabetes

Diabetes was induced with a single dose of 45 mg/kg body weight of streptozotocin (STZ, S0130, Sigma-Aldrich) in 0.1 mol/L citrate buffer (pH 4.5), injected

intraperitoneally. After two days, diabetes developed in the test groups, which was confirmed by the dosage of blood glycemia from rats. Dosing was processed using Accu-Check, Roche Diagnostics, Germany. Rats with glycemia ≥ 250 mg/dL were considered diabetic and they were included in the study.

Experimental design

Rats were allocated into four groups (6 per group). Group 1 wounds were treated with normal saline (NS) and served as controls; Group 2 received 30% of *Rosa rubiginosa* oil (RrO) and served as a sham control. Groups 3 and 4 were treated with STZ to induce diabetes. In addition to STZ treatment, Group 3 received NS topical in the wounds and served as toxic control; in Group 4, skin wounds were treated with topical RrO and served as the test group. Topical treatments started on the same day in all groups.

Skin wounds

After anesthesia with xylazine 7mg/kg and ketamine 80 mg/kg i.p., the animals' backs skin was shaved and we used 70% alcohol for antisepsis. Two full-thickness circular wounds (8 mm in diameter) were created on the back skin of the rats and employed for the treatments with NS and Rosa rubiginosa oil.

Wounds were topically treated for 10 days. After 10 days, blood glucose was once more dosed. An excisional biopsy was performed in all the wounds. Fifty percent of the wound area of each rat was collected, homogenized, centrifuged, and the extract was used for cytokine dosage of TNF-α, IL-1β, IL-6 by the ELISA technique. PeproTec kits (USA) were used, according to the manufacturer's instructions. After that, the rats were euthanized with an overdose of thiopental (100 mg/kg i.p.).

Histopathological study

Fifty percent of skin wound specimens from all rats were collected for histopathology study. The fresh tissues were uniformly washed, the samples were fixed in 10% buffered formaldehyde for 48 hours, then processed for 18 hours in a tissue processor (Leica TP 1020 equipment). The fragments were cut with a punch-type device (0.5cm diameter) and embedded in paraffin.

The histological sections (4 microns) were processed with a Leica RM 2125 RTS microtome, and put on slides. The samples were stained using hematoxylin/eosin

histochemical technique for analysis under a microscope (Olympus BX50, Japan). Quantitative assessment of collagen fibers, neovascularization, fibroblasts, macrophages, epithelialization and necrosis was performed using an adapted wound healing histological scoring system tabulated below[21]. Five microscopic fields of 6 slides per group were analyzed and the average of the scores obtained for each group was calculated. Results were expressed as mean±standard deviation.

Histological scoring system for wound healing (adapted from[21])

score	Epithelization	Collagenization	Fibroblasts, macrophages	Neovascularization	Necrosis	Granulation tissue
1	None	None	none	None	Extensive	None
2	None	None	Some	None	Focal	Immature
3	Partial	Partial	Medium	<5/HPF	None	Medium mature
4	Complete Immature	Complete Irregular	Medium	6-10/HPF	None	Moderate mature
5	Complete Mature	Complete Regular	Many	>10/HPF	None	Completely mature

HPF: High power field

Statistical analysis

A comparison was made between the groups and the results were statistically analyzed using the ANOVA test. Kruskal Wallis test and Tukey test were used as well to compare treated and control groups. Differences were considered significant at $p<0.05$.

RESULTS

Evolution of glycemia with treatments

Table 1, shows that in the control group and in the diabetes group treated with saline solution (DIAB/NS), there was no significant difference between the blood glucose levels comparing the second day of dosing and the 10th day ($p>0.05$).

Table 1 – Glycemia measurement on the 2nd and 10th day after STZ injection in rats of the study groups.

Groups	2nd DAY	10nd DAY
	Glycemia (mg/dL)	Glycemia (mg/dL)
C/NS	162.1±11.2	158.2±10.5
C/RrO	130.3±10.4	137.5±9.6
DIAB/NS	279.5±15.6	270.2±11.2
DIAB/RrO	248.7±14.1	247.8±12.6

C, control; RrO, *Rose rubiginosa* oil; DIAB, diabetes; NS, normal saline. No significant difference was observed comparing the glycemia on the 2nd and 10th days ($p>0.05$ Tukey's test).

Wound tissue cytokine dosages

Significantly lower levels of TNF-α, IL-1 and IL-6 were observed in the skin wound tissues of control rats treated with Rosa rugiginosa oil (C/RrO) than in controls treated with saline (C/NS). Diabetic rats treated with normal saline(DIAB/NS) had TNF-α, IL-1 and IL-6 tissue expression significantly higher than in rats treated with Rosa rubiginosa oil (DIAB/RrO) in each group. These data are summarized in table 2.

Table 2 – Dosages of TNF, IL-1β e IL-6 in wound tissues of rats treated and untreated rats with *Rosa rubiginosa* oil in control and diabetic rats.

Groups'	TNFα (pg/ml)	IL-1β (pg/ml)	IL-6 (pg/ml)
C/NS	269±17[a]	81.2±13[a]	143.8±19[a]
C/RrO	142.4±15[a]	42.2±11[a]	107.3±13[a]
DIAB/NS	298.3±20[a]	101.3±13[a]	85.4±12[a]
DIAB/RrO	190.7±19[a]	72.3±12[a]	71.3±8[a]

C, control; RrO, Rosa rubiginosa oil; DIAB, Diabetes; NS, normal saline. Measures followed of the same letter in the same column differ significantly ($p<0.05$, Tukey test).

Histopathology

In Table 3 it is observed that the scores of wounds from rats in the control group treated with RrO (Figure 1-B) were higher (33±1.9) than the score of rats in the control group treated with NS (19±1.9). (Figure 1-A). In diabetic rats the same occurred, as well. Considering the diabetic rats, the score of rats treated with RrO (22±2.5) was significantly higher (Figure 1-D) than in diabetic rats treated with NS (17±1.8) (Figure 1-C). ($p<0.05$). The scores of skin wounds from diabetic groups were significantly lower than the control groups. These data are summarized in Table 3. Representative images related to the histopathological analysis can be seen in figure 1 (A, B, C,D).

Table 3 – This table demonstrates the values of histological scores measured using a wound healing histological scoring system.

Groups	Wound scores
C/NS	19 ± 2.3^{a}
C/RrO	$33\pm1.9^{a,c}$
DIAB/NS	$17\pm1.8^{a,b}$
DIAB/RrO	$22\pm2.5^{b,c}$

C, control; RrO, *Rosa rubiginosa* oil; DIAB, Diabetes; NS, normal saline. Measures followed by the same letter in the same column differ significantly ($p<0.05$ Tukey test).

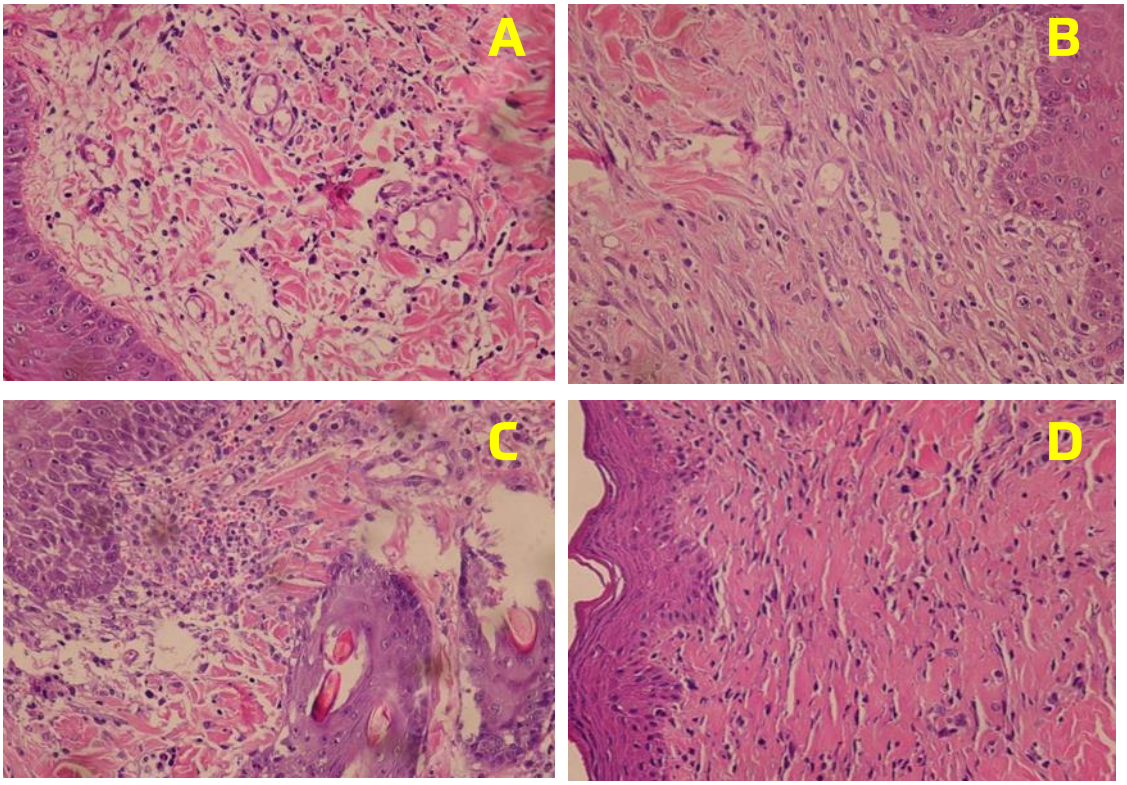

Figure 1- Representative images of the histopathological evaluation of the four groups. A, control, wound treated with normal saline; B, control, wound treated with RrO; C, diabetic rat, wound treated with normal saline; D, diabetic rat, wound treated with RrO. HE, 200x.

DISCUSSION

In the present work, an experimental model of healing of skin wounds on the back of diabetic and non-diabetic rats was used in order to study the effects of topical treatment with 30% *Rosa rubiginosa* seed oil on the healing and its evolution. In a clinical study with a control group, Moreno et al[20] described an improvement in healing time and quality of wounds among patients using 26% Rosehip oil, compared to the control group. They were patients with leg varicose ulcers, post-traumatic ulcers, contact eczema, and post-surgical wound dehiscence. All patients had positive results compared to the control group, with a difference of up to 29 days of healing between the evaluated groups (23.2 days for the therapeutic group versus 52.2 days for the control group)[20].

Macrophages are cells derived from monocytes. They positively influence the wound healing process as very active phagocytes that remove foreign bodies, bacteria and have positive effect on the granular tissue. In addition, they stimulate the appearance of fibroblasts in healing wounds, collagen and cytokine synthesis. This

inflammatory phase is characterized by fibroblast proliferation, collagen synthesis, and wound contraction[22]. These mechanisms must have been stimulated by *Rosa rubiginosa oil*, as observed in the data of the histological scores and cytokine expression.

In the present study, the histopathological examination quantitatively analyzed epithelization, neovascularization, fibroblasts, macrophages, granulation tissue, necrosis and collagen fibers. We observed that in the groups of diabetic, and non-diabetic animals, topical treatment with *Rosa rubiginosa oil* had a positive influence on the quantification of histological scores. Neovascular tissue, and macrophages carry chemical mediators, such as cytokines, enzymes, oxygen and vitamins, essential for the establishment of high-quality fibroblasts for collagen synthesis and maturation[23]. Therefore, fibroblast proliferation and collagen production are parameters to study the activity of drugs in wound healing[24,25]. Macrophage depletion during granulation tissue formation typically show a defect in re-epithelialization, granulation tissue formation, angiogenesis, wound cytokine production, and wound contraction[26].

Delayed wound cicatrization is an important complication of diabetes and, if it is left untreated, chronic wounds occur[27]. In the present study, we observed an increase in the population of macrophages and fibroblasts in the wounds of rats treated with *Rosa rubiginosa* oil[28,29]. The reduction of inflammation has been demonstrated[29]. Some studies indicate that the phenolic compounds of *Rosa rubiginosa* oil may have bacteriostatic and anti-inflammatory effects[28]. Good results have been reported with the use of *Rosa rubiginosa* seed oil in open wounds, by inducing a good granulation tissue, with no sensitization[20].

In conclusion, Rosa rubiginosa oil positively influenced the healing of cutaneous wounds in a diabetic rats after topical treatment.

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