The Use of Lectin Gel in the Treatment of Thermal Burns in Rats Immunocompromised

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Abstract—This study aimed at evaluating the use of lectin gel in the treatment of second-degree burns in rats immunocompromised. Thirty-two male rats were randomly divided into two groups (G1 = treatment with hydrogel containing 100 μg / ml Cramoll 1, 4 and G2 = Control, hydrogel without lectin). Thermal lesions were produced in the animals of both groups, positioning a massive aluminum bar 10 mm in diameter (51 g), preheated to 99°C ± 2°C/10 min in the dorsal proximal region for 15 sec. After 7, 14, 21, and 28 days, animals were euthanized. The percentage of tissue shrinkage in the group treated with lectin at 28 days was 81.0 ± 2.2%. There was no sign of infection, bleeding or secretion. There were no significant differences in biochemical and hematological parameters analyzed. Histological evaluation of G1 revealed: on the 7th day moderate inflammatory infiltrate and mild fibrosis, on the 14th day intense autolysis, neoepithelialization, mild fibroblast proliferation and intense fibrosis, on the 21st day re-epithelialization, non-modeled and dense collagen, moderate fibrosis and on the 28th day complete tissue epithelialization. These results extend the potential of therapeutic applications for Cramoll 1, 4 in the treatment of thermal burns in immunocompromised animals.

Keywords—lectin, burn, immunocompromised.

1. INTRODUCTION

Lectins are proteins or glycoproteins of plant, animal or bacterial origin that bind to cell surfaces through specific carbohydrate containing receptor sites [1]. These proteins vary remarkably in their specificity, not only in terms of the recognition of monosaccharides, but also in terms of differential binding to complex carbohydrates [2]. Legume lectins are central to the study of the molecular basis and specificity of protein–carbohydrate interactions [3] and they also have medical implications for the understanding of cell–cell recognition, adhesion, tumor spread, bacterial and viral infection, and inflammation [4].

Similarly, Cramoll a specific glucose / mannose lectin, which has multiple forms designated: Cramoll 1, Cramoll 2, Cramoll 3, Cramoll 4 [5], extracted from seeds of Cratylamollis Mart., a plant native to Northeast Brazil, has shown high clinical potential due to its immunomodulatory profile in the production of IFN-γ and nitric oxide [6], mitogenic activity in human lymphocytes [7] and antitumor activity [8]. In a study conducted by Oliveira et al. [9], the Cramoll 1, 4 is able to induce IL-6, IL-17A, IL-22 and IL-23 cytokines in vitro was demonstrated to be better than Concavalin A, besides immunologic memory generation, being a potential biotechnological tool in Th17 pathway studies.

History reveals that concern for wound healing has always existed since, for some patients a small wound is enough to give rise to a chronic lesion at risk of presenting serious complications. Burns are traumatic wounds caused in most cases, by thermal agents, chemical, electrical and radioactive. The extent and
severities vary with the type of agent, time of exposure, depth and location body [10,11]. It is estimated around two million burn accidents per year in Brazil [12]. Burns are considered injuries that cause severe trauma, since they can lead patients to death or cause emotional and social disorders. According to information from the Brazilian Ministry of Health, in the period between 2013 and 2014, more than 15,000 cases of burn hospitalization were reported in children aged 0 to 10 years [13].

In addition to second-degree burns cause the destruction of the skin’s mechanical components, which are natural defense barrier, the impairment of humoral and cellular immune defense becomes an aggravating factor, directly related to patients’ clinical conditions that favor the acquisition of infections [14]. In turn, the immune response to burns is a complex phenomenon influenced by a number of factors such as the extent and burn severity, depth, age, presence or absence of infection, type of treatment, etc. [10]. Several local and systemic factors can delay or prevent healing, such as: inadequate nutritional support, oxygenation deficit in tissue necrosis, dry environment, immunosuppression, etc. [15]. Any change in the repair process leads to pathological scarring, which can be broadly grouped into: deficient formation of scar tissue, excessive formation (keloid and hypertrophic scar) and formation of contractures [16].

Despite being observed the benefits of promoting a moist environment in the healing of wounds in the clinical practice, until the early 60's there were few studies directed to this study line. However, the publication of Winter in 1962, which demonstrated the increased rate of epithelialization of wounds in a wet environment with consequent minimization of crust formation, encouraged the research, production and marketing of wet dressings. In 1982 the hydrocolloids-based coverage are released in the United States and Europe, becoming widely used in partial thickness wounds. These covers were not available in the market from the 90's, and their high cost was an initial barrier to diffusion [17].

On the other hand, the healing mechanism involves an extremely complex series of events that has aroused the interest of many researchers engaged in the search for new therapeutic technologies that can solve or minimize the flaws in the process of tissue repair, in particular the tissues damaged by thermal burns. On the other hand, according to Thomas and colleagues [18], the use of hydrocolloids in burns is related to better wound healing rates, greater comfort for the patient due to the mobility of the dressing, pain relief, less frequency in the dressing change and should be considered as a treatment for partial thickness burns. In this context, this study aimed at evaluating the effect of topical gel use containing 1 and 4 isoforms of the lectin from C. mollis in the healing of second-degree thermal injuries deep in experimentally immunosuppressed rats.

II. EXPERIMENTAL PROCEDURES

2.1. Animals

Male wistar rats, Rattus norvegicus, albinus, (n = 16 / group), 8 - 10 weeks old and 250 ± 300 g were raised at the animal facilities of Laboratório de Experimentação Animal – UFPE. Each animal was maintained in individual cage, under controlled environmental conditions (12 h light / dark cycle, temperature 23 ± 2 °C and humidity 55 ± 10 %) with water and commercial food ad libitum (Labina®). All rats were treated and sacrificed in accordance with the Ethical Committee of Universidade Federal de Pernambuco for Experiments with Laboratory Animals (23076.015015/2009-31).

2.2. Lectin extraction and purification

C. mollis seed extract (10% w/v prepared in 0.15M NaCl) was fractionated using ammonium sulphate (40–60% w/v) and the fraction obtained was submitted to affinity chromatography in Sephadex G-75. Cramoll 1,4 preparation was bioselectively eluted with 0.3 M d-glucose in 0.15M NaCl, dialyzed against 0.15M NaCl during 24 h and lyophilized [5].

2.2.1. Lectin hydrogel (Cramoll 1,4)

Carbopol® was used as vehicle suspended in boric acid buffer (pH 6.0) at 25 °C. After extraction and purification, Cramoll 1,4 solutions were added in sufficient quantity to achieve the final concentration of 100 μg Cramoll 1,4 per ml of hydrogel. Irradiation was performed at room temperature using Co60 at 15 kGy h⁻¹ [19].

2.3. Immunosuppression induction

Methotrexate (MTX) was administered to each animal using a low-dose (0.8 mg / kg / week). MTX was administered, according to [20], intramuscularly in 0.15 M NaCl weekly at 7 days before surgery, on surgery day and 7 days after surgery.

2.4. Experimental protocol and groups

Animals were divided into two groups (n = 30 / group) and were anesthetized for the surgical procedure using 2 % xilazinechloridrate (10 mg / kg) and 10 % ketamine chloride (115 mg / kg) in subcutaneous injections [20]. Each animal was placed in a prone position and prepared for aseptic surgery using 1 % polyvinylpyrrolidone-iodine. A standard Burns were symmetrically caused on depealated areas through contact with an aluminum bar (diameter = 10 mm), preheated for
100 °C for 15 s (Figure 1). After burn injury and animal awakening, once the procedure completion, analgesia was processed by means of intramuscular dypirone application (0.01 mg kg⁻¹) to prevent pain. Injuries were observed during 35 consecutive days followed by the application of 100 µl hydrogel on the burn as follows: Group-1 immunocompromised animals topically treated with hydrogel containing 100 µg / ml Cramoll 1.4; Group-2 (control) immunocompromised animals topically treated with hydrogel without isolectin.

Fig.1: Appearance of the deep second-degree thermal lesion induced in experimentally immunosuppressed male Wistar rats. 10-mm burn in diameter made aiming at evaluating the healing effect of the lectin hydrogel (Cramoll 1.4).

2.5. Clinical Evaluation

Clinical characteristics of the experimental lesions were observed every day, considering the following aspects: edema, hyperemia, exudation and the firmness of wound surface, presence or absence of granulation tissue, presence or absence of scar tissue and crust. Wounds were considered closed if moist granulation tissue was no longer apparent and wounds seemed covered with new epithelium.

All the rats were examined weekly under anesthesia for observation of wound contracture. The wound retraction was evaluated in 7, 14, 21 and 28 days after burn induction. Wound contraction was expressed as reduction in percentage of original wound size. % wound contraction on day-X = [(area on day 0 - open area on day X) / area on day 0] x 100 [21].

2.6. Biochemical and hematological evaluations

Blood from three animals per group were collected on days 7, 14, 21 and 28 after burn induction for biochemical determination. Levels of creatinine, urea, glutamic pyruvic transaminase, glutamic oxalacetic transaminase, gamma glutamyl transferase, amylase, alkaline phosphatase, calcium, prothrombin and fibrinogen were determined. Hematological parameters (erythrocytes, leukocytes and platelets) were determined immediately after blood collection. Evaluations performed in triplicate. Animals in both G1 and G2 were sacrificed by injecting 30 mg kg⁻¹ thiopental sodium.

2.7. Microbiological evaluation

Microbiological evaluation was carried out using “swabs” in the injury area at the moment of surgery and respective days of biopsies. This sample was transferred to a Petri dish of 20 x 150 mm containing nutrient agar medium in a laminar flow chamber. After 24h incubation, plates inoculated in triplicate for each sample were evaluated. This routine evaluation was performed to evaluate the degree of contamination of injuries.

2.8. Histopathologic Evaluation

After collection, tissue samples were fixed in 4 % formaldehyde (v/v) prepared in PBS (0.01 M, pH 7.2) followed by histological processing through paraffin embedding, microtome with 4 µm cuts and Masson's trichrome and hematoxylin-eosin staining. Histological analysis was performed by comparative descriptive analysis of experimental groups in binocular optical microscope (Zeiss – Axiostar model) where cellular and tissue characteristics of skin were evaluated after thermal injury and subsequent healing pattern.

The histological analysis was performed by an independent pathologist who was experienced in the examination of burn wound specimens, in the following way: 1) Inflammatory response: characterized by the presence of polymorphonuclear cells (SMC), 2) granular tissue: characterized by the presence of fibroblasts, myofibroblasts and neovascularization; 3) fibrosis: characterized by densities of collagen fibers identified by blue staining intensity observed under optical microscopy, resulting from staining by Masson's trichrome. The score made for parameters was: - = absent, + = mild presence; + + = moderate presence; + + + = strong presence.

2.9. Statistical analysis

To detect differences between groups, the Kruskal-Wallis was used. The results from at least eight independent experiments performed in triplicate are displayed as mean values ± standard deviation. For comparative analysis of the quantitative variable the
Student’s-t-test was applied considering the value of \( p < 0.05 \) as statistically significant.

III. RESULTS AND DISCUSSION

3.1. Lectin Hydrogel

The hydrogel of Cramoll 1.4 showed uniform, transparent sheets of three-dimensional networks and good transparency, which allowed the monitoring of healing progression of thermal injuries. The formulation pH equal to 6 was chosen by being similar to that observed in the skin and by not altering the hemagglutinating activity of isoelectin Cramoll 1.4. In turn, the gamma irradiation was effective in the microbiological control of the gel formulation without causing changes on the hemagglutinating activity of lectin. In addition to these results, various aspects described in the literature make the gel formulation optimal display for treatment of injuries, such as biocompatibility, lack of toxicity, biodegradability, adhesion and absorption [22,23].

3.2. Clinical Evaluation

Results of this study revealed thermal burns white in color, painful, with no blistering, mild edema until 2 days after injury induction in both groups. The hyperemia degree varied from mild to absent in the first two days for group 1, being present in group 2 until the 3rd day of experimentation. The formation of a dense and dry crust was observed in 90% of the animals in the G1 (Figure 2A) and 85% in G2 (Figure 3A) from the third day after burn induction. At 14 days after injury was observed in 33.4% of the animals in G1 (Figure 2B) and 41.6% of the animals of group 2 (Figure 3B), the presence of a second dry and thin crust, smaller than the first crust located in the burn center.

The granulation tissue was observed in the lesions of group 1 at day 12 after injury being visible until day 21 (Figure 2C). In the control group was verified the presence of red color granulation tissue, located at the skin height, similar to that observed in G1 between day 12 and day 23 after injury (Figure 3C). Signs of the scar tissue formation at the lesion edge were observed from day 14. At 28 days the scar tissue was still present but to a lesser degree in group 1 (Figure 2D) compared to its respective control (Figure 3D).

The shrinkage percentage of the induced thermal lesion in immunosuppressed rats was observed by measuring the total burn area with the aid of a caliper on days 7, 14, 21 and 28 after injury induction. Lesion areas gradually decreased in both groups overtime. However, when groups were compared among each other, averages of the contraction percentages were similar (Figure 4). The contraction of skin lesions is centripetally from the lesion edges. According to Mandelbaum et al [17], the tissue contraction in a healing process by second intention, such as those in burns, can induce a reduction rate of up to 62% of the total surface area of the initial injury. However, the contraction is only possible due to the myofibroblasts movement that generates a tensile strength to the smooth muscle cells [24, 25]. In turn, the myofibroblasts can promote 50-70% lesion retraction from the initial size [26].

3.3. Hematological and biochemical evaluation

Rats, like other mammals, have to maintain strict control of the internal environment thus ensuring homeostasis. It is known that rats can produce changes in these parameters as a result of pathological processes or external factors such as sex, ancestry, age, diet, handling and environment [27,28]. When analyzing the hematological data in group 1, treated with hydrogel containing Cramoll 1.4, there is a change on the increase in the number of leukocytes (mononuclear and polymorphonuclear) in the 7th, 14th and 21th days of treatment, which was higher than the control group (Table 1). The prevalence of polymorphonuclear cells induced by Cramoll 1.4 is important to remove cellular debris and microorganisms in the wound, favoring healing [29]. Moreover, the number of monocytes showed high in both groups.

The biochemical evaluation revealed increased ALT levels in response to injury by burning and alkaline phosphatase-related to inflammatory period of the healing process animals (Table 2). On the other hand, metabolic changes are considered high risk in third-degree burns with hyperglycemia [30] and high protein catabolism [31] as the main aggravating factors to the injury. The other biochemical parameters were similar to those reported in the literature for healthy animals.
Fig. 2: Healing clinical evolution of second-degree thermal burns in immunosuppressed rats experimentally treated by daily topical application of hydrogel containing 100 µl of lectin Cramoll 1.4 at 100 µg/ml. A - Presence of thin and dry crust with slight edges detachment. B - Presence of a small crust in the lesion center. C - Presence of granulation tissue, red color, skin height, located in the lesion center. D - Mild presence of scar tissue at the burn induction site.

Fig. 3: Healing clinical evolution of second-degree thermal burns in immunosuppressed rats experimentally treated by daily topical application of 100 µl hydrogel without lectin (control group). A - Presence of thin and dry crust with slight edges detachment. B - Presence of crust with strong edges detachment; C - Presence of granulation tissue, red color, skin height, located in the lesion center. D - Mild presence of scar tissue at the burn induction site.

Fig. 4: Contraction area percentage of deep second-degree thermal burn in the experimental model, in male Wistar rats. n = 3. Values are mean ± SEM. * p < 0.05.
3.4. Microbiological Evaluation

The lesions of both groups were not contaminated at any time during the experimental evaluation. For this reason, it was not observed the presence of secretion and exudates in the lesion area during the daily clinical evaluation. Infected wounds heal more slowly, re-epithelialization is longer and there is also the risk of systemic infection [32]. Severe burn trauma is generally associated with bacterial infections, which causes a more persistent inflammatory response with an ongoing hypermetabolic and catabolic state. This complex biological response, mediated by chemokines and cytokines, can be more severe when excessive interactions between the mediators take place [33].

3.5. Histological analysis

The assessment of histological sections of animals treated with 100 µl lectin hydrogel (Cramoll 1.4) revealed the presence of points with necrosis, hemorrhage, fibrin, and extensive inflammatory exudate characterizing an acute inflammatory reaction assessed by the presence of polymorphonuclear cells (Figure 5A) at 7 days of treatment. In the control group (G2) are visualized signs of bleeding in the dermis, similar to the G1, presence of fibrin and discrete inflammatory infiltrate (albumin/leukocyte/macrophage) (Figure 6A). Fibrosis was classified as mild to the 7th day of treatment in both groups (Table 3).

The collagen deposition in fibroplasia phase, necessary for the efficient arrival of fibroblasts at the lesion site was classified as mild in the control group and intense in the group treated with hydrogel containing 1.4 Cramoll. Methotrexate has been shown to have an effect on both circulating and cutaneous lymphocytes [34]. In vitro, keratinocytes were a thousand times more resistant to their cytotoxic effects than lymphoid cells, [35] confirming their immunosuppressive properties. For this reason, the use of methotrexate to induce immunosuppression in male Wistar rats causes negative effects on the healing process.

Peters et al. [36] observed that CD18 present in the neutrophil surface during migration emits a chemical signal that induces infiltrations of macrophages to secrete TGF-β 1. Therefore the lack of CD18 in one or another cell leads to an extremely reduced release of TGF-β 1 due to defective adhesion and to subsequent extravasation of the phagocyte in the injury area. Ronty et al. [37] additionally affirmed that this deficient release of TGF-β 1 promotes a delay in the arrival in fibroblasts to the injury site with consequential deficit collagen staple fiber deposition.

By day 14 the inflammatory response was classified as mild in G2 (Figure 6B), progressing to moderate to 21 days after the induction of thermal injury (Figure 6C). On the other hand, the intensity of the inflammatory response evolved from acute to chronic in the group 1 assessed by fibroblastic proliferation, 14 days after injury induction (Figure 5B). After 21 days of experimentation the group showed moderate inflammatory infiltrate (Figure 5C). The inflammatory reaction may impair the healing process by promoting swelling, excessive amount of exudate, which favors dehiscence, bacterial growth and consequently inhibition of fibroblast proliferation and collagen deposition [38].

Due to the large molecular diversity of lectins, they have distinct roles in modulating the physiological response participating in the activation of immune cells [39], enlisting neutrophils through indirect mechanisms [40], promoting pro-inflammatory effects in PMN and inducing the release of cytokines [41] as well as triggering the proliferation of fibroblasts [42]. Recent assays also demonstrated higher proliferative induction promoted by this lectin, in addition to IL-2, IL-6, nitric oxide and NK cell activation, in preimmunized mice with Cramoll 1.4 [43]. The IL-6 is a mediator in various stages of inflammation [44]. Among the several pro-inflammatory effects attributed to it, those closely related to the repair process are: mitotic induction of keratinocytes in a later stepand their effects on neutrophil chemoattractantsat the earliest stage [45].

At 28 days thermal injuries treated with hydrogel containing Cramoll 1.4 demonstrated excellent repair in relation to collagen deposition and early development of skin appendages compared with their respective control (Figure 5D). The control group also showed collagen deposition and re-epithelialization (Figure 6D). The decrease in collagen deposition in the phase of tissue remodeling in the control group can be explained by the deficient arrival of fibroblasts in the injury area until the 7th day of experimentation.

The scar tissue is characterized by a dense fibrous tissue, which resistance is given by the amount of collagen deposited and fibers disposal, which has only 15% of the tensile strength of the original tissue after 21 days. Thus, the process of tissue remodeling can last for months or years, with the new tissue structure being slowly modeling [46]. Although scar formation is a beneficial process to the body, the excess deposition of some proteins such as collagen can cause aesthetic and functional complications, resulting in hypertrophic scars and keloids [47]. Burned patients have a prevalence of hypertrophic scars of about 67%, which leads to high medical costs due to size of the wound surface area [48]. The histological evaluation of liver sections of animals from Group 1 showed no pathological changes resulting from daily topical application for 28 consecutive days of...
100 µl hydrogel containing 100 g Cramoll 1.4 / ml (Figure 7).

Another study related the occurrence of gradual healing process induced by a hydrogel containing Cramoll 1.4 on experimental second-degree burns in rats [49]. On the 7th day of treatment, treated group showed higher epidermis, exudates, and necrosis. With more 7 days, tissue reepithelialization and moderate autolysis were observed. With more two weeks, tissue epithelialization was completed; and in the 35th day was observed a modeled dense collage. Recently, it has been demonstrated that Cramoll 1, 4 is able to induce IL-6, IL-17A, IL-22 and IL-23 cytokines better in vitro than Concavalin A, in addition to promoting the generation of immunological memory potential biotechnological tool in the Th17 pathway studies. In fact, the healing potential of cutaneous wounds and thermal burns has been related to the immunomodulatory profile of Cramoll, described in other studies, including proinflammatory action in polymorphonuclear cells, induction of cytokine release, and fibroblast proliferation [50].

**Table 1:** Effect of topical application of hydrogel containing 100 µg of lectin Cramoll 1.4 (G1) and hydrogel without lectin (G2) in the treatment of deep second-degree burns on the hematological parameters in immunosuppressed male Wistar rats.

*Mean ±SD, n = 4.*

| Parameters          | 7th Day   | 14th day | 21st Day | 28th day |
|---------------------|-----------|----------|----------|----------|
| **Erythrogram**     |           |          |          |          |
| Erythrocytes mil/mm3| 7.04 ± 0.99 | 8.2 ± 0.65 | 6.59 ± 0.67 | 6.85 ± 0.47 | 7.05 ± 0.47 | 7.38 ± 0.97 | 7.7 ± 0.10 | 6.85 ± 0.01 |
| Hemoglobin g/dl     | 15.24 ± 0.19 | 16.94 ± 0.17 | 14.25 ± 0.01 | 14.4 ± 0.01 | 13.66 ± 0.45 | 14.48 ± 0.74 | 14.4 ± 0.01 | 14.4 ± 0.33 |
| Hematocrit %        | 41.1 ± 0.59 | 46.3 ± 0.99 | 58.0 ± 0.01 | 39.9 ± 0.87 | 39.3 ± 0.45 | 40.8 ± 0.24 | 41.2 ± 0.01 | 39.9 ± 0.09 |
| **Platelets Count** |           |          |          |          |
| Platelets mil/mm3   | 827000 ± 0.93 | 692000 ± 0.01 | 813000 ± 0.01 | 793000 ± 0.91 | 958000 ± 0.33 | 859000 ± 0.81 | 783000 ± 0.04 | 765000 ± 0.31 |
| **WBC**             |           |          |          |          |
| Leukocytes %        | *10300 ± 0.48 | 6200 ± 0.78 | *11400 ± 0.91 | 7700 ± 0.01 | *10600 ± 0.15 | 8200 ± 0.01 | 6000 ± 0.43 | 5600 ± 0.11 |
| Neutrophils %       | 11.2 ± 0.39 | 12.4 ± 0.01 | 12.9 ± 0.28 | 13.6 ± 0.75 | 8.7 ± 0.67 | 5.9 ± 0.42 | 9.2 ± 0.01 | 9.0 ± 0.99 |
| Eosinophils %       | 0.1 ± 0.11 | 2.0 ± 0.32 | 0.1 ± 0.07 | 0.1 ± 0.44 | 0.1 ± 0.01 | 0.0 ± 0.00 | 0.1 ± 0.01 | 0.1 ± 0.01 |
| Basophils %         | 0.3 ± 0.02 | 0.2 ± 0.17 | 0.0 ± 0.00 | 0.4 ± 0.21 | 1.2 ± 0.99 | 0.3 ± 0.10 | 0.4 ± 0.01 | 0.4 ± 0.01 |
| Typical Lymphocytes %| 87.2 ± 0.39 | 84.0 ± 0.07 | 75.5 ± 0.90 | 84.0 ± 0.15 | 88.4 ± 0.10 | 82.2 ± 0.09 | 88.1 ± 0.31 | 88.3 ± 0.20 |
| Atypical Lymphocytes %| 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| Monocytes %         | 1.2 ± 0.10 | 1.4 ± 0.01 | 2.0 ± 0.01 | 1.3 ± 0.00 | 1.6 ± 0.32 | 1.6 ± 0.31 | 2.2 ± 0.99 | 2.1 ± 0.41 |

**Table 2:** Effect of topical application of hydrogel containing 100 µg of lectin Cramoll 1.4 (G1) and hydrogel without lectin (G2) in the treatment of deep second-degree burns on the hematological parameters immunosuppressed male Wistar rats.

*Mean ±SD, n = 4.*

| Parameters       | 7th Day   | 14th Day | 21st Day | 28th Day |
|------------------|-----------|----------|----------|----------|
| **Pro-thrombin time** |           |          |          |          |
| 10.5 ± 0.01      | 10 ± 0.02 | 10.6 ± 0.01 | 10 ± 0.01 | 9.2 ± 0.21 | 10 ± 0.10 | 10.5 ± 0.71 | 10 ± 0.02 |
| **Fibrinogen mg/dl** | 430.5 ± 0.01 | 437 ± 0.01 | 413 ± 0.99 | 400 ± 0.01 | 412 ± 0.71 | 436 ± 0.71 | 468 ± 0.99 | 451 ± 0.10 |
### Table 3:  Histopathological evaluation of the degree of inflammatory intensity, presence of granulation tissue and fibrosis in the skin after deep second degree thermal injury. Samples were obtained 7 day, 14 day, 21 day and 28 day after induction of the burn wound in immunocompromised male Wistar rats. G1 = Treatment, G2 = Control.

| Time     | Animal | Inflammatory response | Granulation tissue | Fibrosis |
|----------|--------|-----------------------|--------------------|----------|
|          |        | G1 | G2 | G1 | G2 | G1 | G2 | G1 | G2 |
| 7th day  | 1      | ++ | +  | -  | -  | +  | +  | -  | -  |
|          | 2      | ++ | +  | -  | -  | +  | +  | -  | -  |
|          | 3      | ++ | +  | -  | -  | +  | +  | -  | -  |
|          | 4      | ++ | +  | -  | -  | +  | +  | -  | -  |
| 14th day | 1      | +++| +  | +  | +  | +++| +  | -  | -  |
|          | 2      | +++| +  | +  | +  | +++| +  | -  | -  |
|          | 3      | +++| ++ | +  | +  | +  | +  | +  | -  |
|          | 4      | +++| +  | +  | +  | ++ | +  | -  | -  |
| 21st day | 1      | ++ | ++ | ++ | ++ | ++ | ++ | +  | +  |
|          | 2      | ++ | ++ | ++ | ++ | ++ | ++ | +  | +  |
|          | 3      | ++ | ++ | ++ | ++ | ++ | ++ | +  | +  |
|          | 4      | ++ | ++ | ++ | ++ | ++ | ++ | +  | +  |
| 28th day | 1      | +  | +  | -  | -  | +  | +  | +  | +  |
|          | 2      | -  | -  | -  | -  | +  | +  | +  | +  |
|          | 3      | +  | +  | -  | -  | +  | +  | +  | +  |
|          | 4      | -  | +  | +  | +  | ++ | +  | +  | +  |

*Statistically different from control group (Student’s t-test, p < 0.05)*

Intensity of the parameters evaluated was scored as: - = absent, + = mild presence, ++ = moderate presence, +++ = strong presence.
Fig. 5: Epithelial tissue of rats in group 1 subjected to second-degree thermal burns. Hematoxilina - Eosina staining. 100x Magnification.
A – Histopathological appearance of the lesion at 7 days after thermal injury presenting epithelial tissue with complete destruction of the dermis and epidermis with moderate inflammatory infiltrate and mild fibrosis. B - Histopathological appearance of the lesion at 14 days after thermal injury presenting intense autolysis, neovascularization in the superficial portion of the epithelial tissue, mild fibroblastic proliferation with the presence of not modeled collagen and severe fibrosis. C - Histopathological appearance of the lesion at 21 days after thermal injury presenting tissue reepithelialization, moderate neovascularization, moderate fibroblastic proliferation, presence of dense not modeled collagen and moderate fibrosis. D - Histopathological appearance of the lesion at 28 days after thermal injury showing complete tissue epithelialization, absent autolysis, absent neovascularization, mild fibroblast proliferation, presence of dense and modeled collagen mesh and moderate fibrosis.

Fig. 6: Epithelial tissue of rats in group 2 subjected to second-degree thermal burns. Hematoxilina - Eosina staining. 100x Magnification.
A – Histopathological appearance of the lesion at 7 days after thermal injury presenting epithelial tissue with complete destruction of the dermis and epidermis and mild fibrosis. B - Histopathological appearance of the lesion at 14 days after thermal injury presenting neovascularization, not modeled collagen, and mild fibrosis. C - Histopathological appearance of the lesion at 21 days after thermal injury of tissue showing re-epithelialization, moderate fibroblast proliferation and moderate fibrosis. D - Histopathological appearance of the lesion at 28 days after thermal injury presenting incomplete tissue re-epithelialization, mild fibroblast proliferation presence of not modeled and dense collagen mesh, moderate fibrosis and vascularization present.
IV. CONCLUSIONS

Several studies have shown the use of lectins in the modulation of biological response. As discussed by Sell and Costa [51] PHA lectin has improved effect in the skin tissue repair process of Wistar rats when compared to Triticum vulgaris (WGA) and Artocarpus integrifolia (jacalin) lectins. In fact, studies have affirmed that lectin binding to glycans of the cell surface can cluster target molecules, a pivotal step for initiating cellular signaling pathways [52, 53, 54]. Our results showed that the lectin Crammoll 1.4 was effective in the repair of deep second degree thermal lesions induced in experimentally immune depressed mice and may be used in the future as a biotechnological alternative in the development of therapeutic agents.

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