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AVALIAÇÃO DA EFICÁCIA DO HIDROGEL DE CRAMOLL-1,4 IRRADIADO NO REPARO TECIDUAL DE QUEIMADURAS TÉRMICAS DE SEGUNDO GRAU

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TITULARES

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RESUMO

Esta pesquisa visou avaliar a eficácia do hidrogel contendo lectina Cramoll 1,4 irradiado no tratamento de queimaduras térmicas de segundo grau em *in vivo*. Inicialmente foi realizada a extração e purificação da Cramoll-1,4 e desenvolvida uma formulação em hidrogel utilizando-se Carbopol como veículo contendo Cramoll 1,4 irradiada com raios gama de Co60 em uma dose de 15 kGy h^{-1}. A formulação proposta na concentração de 100 µg manteve a atividade hemaglutinante *in vitro*. Posteriormente foi estabelecido um modelo experimental para a obtenção de queimaduras térmicas de segundo grau, de modo que a lesão resultante tivesse tamanho e profundidade semelhante em todos os animais. Em todos os procedimentos os animais foram devidamente anestesiados. Os procedimentos foram conduzidos no Núcleo de Cirurgia Experimental da Universidade Federal de Pernambuco, utilizando *Rattus norvegicus*, albinos, da linhagem Wistar, machos, entre 8 a 10 semanas, pesando 250 ± 50, sadios e imunoderpimidos. Os resultados obtidos neste estudo revelou que o protocolo experimental empregado na indução de queimaduras térmicas de segundo grau originou lesões semelhantes tanto sob o aspecto clínico quanto histológico. Em paralelo, a aplicação tópica regular do hidrogel contendo Cramoll-1,4 na concentração de 100 µg irradiado utilizado no tratamento de queimaduras cutâneas de segundo grau, acelerou os processos de granulação, reepitelização e retração da lesão térmica em ratos sadios. Já os animais imunodeprimidos também tratados com hidrogel contendo Cramoll revelaram um atraso no processo de reparação da lesão, quando comparados ao grupo controle, apresentando reepitelização completa do tecido, autólise e neoformação vascular ausente, proliferação fibroblástica discreta, presença de malha de colágeno denso modelado e fibrose moderada. Os resultados permitem concluir que o hidrogel contendo Cramoll 1,4 irradiado promove o reparo de queimaduras térmicas de segundo grau em ratos sadios e imunodeprimidos, apresentando grande potencial terapêutico.

Palavras-chave: Cramoll 1,4, Queimadura, Hidrogel, Cicatrização.

ABSTRACT

This research aimed at evaluating the efficacy of hydrogel containing 1.4 Cramoll lectin spent in the treatment of second-degree thermal burns in vivo. It was initially carried out the extraction and purification of Cramoll-1,4 and developed a formulation in hydrogel using Carbopol as vehicle, which was irradiated with gamma rays in a Co60 kGy at 15 h^{-1}. The formulation proposed in the concentration of 100 µg retained the hemagglutinating activity in vitro. It was subsequently established an experimental model for obtaining second degree thermal burns, so that the resulting lesion had similar size and depth in all animals. In all procedures the animals were under anesthesia. The procedures were conducted at the Center for Experimental Surgery, Federal University of Pernambuco, using *Rattus norvegicus*, albino Wistar male rats, between 8 and 10 weeks, weighing 250 ± 50 g, healthy and immunocompromised. The results of this study revealed that the experimental protocol used in the induction of second-degree thermal burns originated similar lesions both from the clinical and histological appearance. In parallel, the regular topical application hydrogel irradiated from the Cramoll 1,4 at 100 µg for the treatment of second-degree skin burns accelerated the processes of granulation and re-epithelization and wound contraction in healthy rats. The immunosuppressed animals also treated with hydrogel irradiated from Cramoll revealed a delay in the process of lesion repair, compared to the control group, with complete tissue re-epithelialization, autolysis and absent neovascularization, mild fibroblastic proliferation, presence of modeled dense collagen mesh and moderate fibrosis. The results indicate that the hydrogel irradiated from Cramoll 1.4 promotes the repair of second-degree thermal burns in healthy and immunosuppressed mice, with great therapeutic potential.

Keywords: Cramoll 1.4, Burn, Hydrogel, healing

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1. INTRODUÇÃO

A utilização de animais como modelos experimentais em diferentes áreas da pesquisa biológica foi incentivada por CLAUDE BERNARDE, que por volta de 1865, descreveu em seu trabalho intitulado "Introdução ao Estudo da Medicina Experimental" o uso de animais como modelo de estudo e transposição para a fisiologia humana. Modelos experimentais em mamíferos são essenciais no estudo sobre queimaduras. Existem relatos na literatura da utilização de coelhos (BASHKARAN *et al*., 2011), suinos (SINGER *et al.*, 2011), cachorros (HU, CHE & TIAN, 2009), ratos (CAMPELO *et al.*, 2011) e camundongos (ASAI *et al.*, 2010) utilizados como modelos no estudo de queimaduras.

A história revela que a preocupação com a cicatrização de feridas sempre existiu e que vários extratos vegetais foram utilizados visando à cura destas lesões (ANDRADE *et al*., 1992). No estado de Pernambuco, uma lectina tem sido purificada a partir de sementes de feijão camaratu (*Cratylia mollis*), planta leguminosa comum da região semi-árida do nordeste, pertencente à família *Phaseoleae*, subfamília *Dioclinae*, a qual abrange o gênero *Canavalia* (CORREIA & COELHO, 1995). Esta lectina, denominada Cramoll, é fortemente inibida por metil α-D-manosídeo e conforme, portanto com a classe de lectinas ligantes de glicose/manose, similar às isoladas da *Canavalia ensiformis* (*Concanavalina A*, Con A) e *Lens culinaris* (lectina de lentilha) (LIMA *et al*.,1997).

Formas moleculares múltiplas de *C. mollis* têm sido estudadas para avaliar as suas diversas funções na natureza, para análises estruturais e para as mais diversas aplicações biotecnológicas. A cramoll mostra forte ligação a tecidos neoplásicos malignos humanos, particularmente aqueles derivados de glândulas mamárias, útero e cérebro e inibiu o crescimento acelerado do carcinoma de células epidermóides (Hep-2) (BELTRÃO *et al*., 1998). Experimentos envolvendo atividade antiinflamatória utilizando a cramoll livre e encapsulada em lipossomas para atividade antitumoral (ANDRADE *et al*., 2004) e atividade mitogênica de lifócitos (MACIEL *et al*., 2004) revelam um alto desempenho fisiológico desta lectina.

As queimaduras são lesões tissulares de origem térmica por exposição às chamas, líquidos e superfícies quentes, frio extremo, algumas substâncias químicas, radiações, atritos ou fricção (JORGE & DANTAS, 2003). Mesmo com a melhora no prognóstico (BARRET & HERNDON, 2003) e com o progresso no emprego de substitutos biológicos

da pele (RAMOS-E-SILVA & RIBEIRO DE CASTRO, 2002), as queimaduras ainda representam importante causa de mortalidade (SHERIDAN *et al.*, 2000).

A cicatrização através do meio úmido tem as seguintes vantagens quando comparadas ao meio seco: prevenir a desidratação do tecido que leva à morte celular; acelerar a angiogênese; estimular a epitelização e a formação do tecido de granulação; facilitar a remoção de tecido necrótico e fibrina; servir como barreira protetora contra microrganismo; promover a diminuição da dor; evitar a perda excessiva de líquidos; e evitar traumas na troca do curativo (HUTCHINSON & MCGUCKIN, 1990; JOHNSON, 1992; SANTOS, 1993; BORISKIN, 1994; HULTÉN, 1994; MORGAN, 1994; BROUGHTON *et al*., 2006).

Assim, a escolha de um agente tópico ou do tipo de cobertura a ser usada no tratamento de queimaduras deve ser realizada com base na avaliação das características da lesão e em evidências relatadas na literatura específica. Estes produtos devem apresentar características tais como: atividade antimicrobiana ou bacteriostática, ausência de toxicidade e hipersensibilidade, aderência, redução do tempo de cicatrização e custo/benefício. Todavia, muitos dos métodos aplicados nos curativos de lesões causadas por queimaduras são controversos (FRANCO & GONÇALVES, 2008).

Essa pesquisa foi desenvolvida devido à crescente demanda por curativos mais eficazes. De forma a suprir essa necessidade, os curativos úmidos de contendo moléculas naturais e sintéticas tem se mostrado efeito significativo no mecanismo de cicatrização. O modelo experimental proposto trata-se de uma inovação no tratamento de queimaduras utilizado lectina (Cramoll 1,4) o que representa uma nova oportunidade de desenvolvimento sustentável para a população do semi-árido nordestino. A biotecnologia tornou-se uma ferramenta de destaque no mercado mundial contribuindo para o desenvolvimento de novos medicamentos. Por outro lado, relaciona-se diretamente com a inovação de processos, produtos e formas de uso que abrem novas oportunidades de negócios promovendo a prestação de serviços de referência em saúde.

2. REVISÃO DA LITERATURA

2.1. O MECANISMO DE CICATRIZAÇÃO DA PELE

A pele é o maior e um dos mais complexos órgãos do corpo, representando aproximadamente 15 % do peso corporal (SAMPAIO & RIVITI, 2001). A pele encontra-se constantemente exposta a agressões físicas e mecânicas, que podem ter consequências físicas permanentes ou não. Suas principais funções são: impermeabilização, proteção, sensorial, termorregulação, excreção, metabolismo, etc (HESS, 2002; KEDE & SABATOVICH, 2004).

A camada mais externa da pele é formada pelo tecido epitelial, do tipo pavimentoso estratificado queratinizado, que constitui a epiderme. A epiderme é constituída essencialmente por quatro tipos celulares: queratinócitos, malanócitos, células de Langerhans, células de Merkel (GARTNER & HIATT, 2003). Morfologicamente a epiderme é dividida em camadas (estratos) da mais profunda à superfície: Camada germinativa ou basal; Camada espinhosa ou malpighiano; Camada granulosa; Camada lúcida (presente nas regiões palmo-plantares) e Camada córnea (CUCÉ & NETO, 200; GARTNER & HIATT, 2003). As características de cada camada do tecido epitelial refletem as propriedades mitóticas, sintéticas e o grau de diferenciação dos queratinócitos (BALASUBRAMANIAN & ECKERT, 2007).

Por ser um tecido avascular, todos os nutrientes utilizados pela epiderme derivam do tecido conjuntivo subjacente a epiderme, denominado derme, constituído por diversos tipos celulares separados por abundante material intercelular, fibras de colágeno e elastina, vasos sanguíneos e linfáticos, terminações nervosas e estruturas derivadas da epiderme como os folículos pilosos e as glândulas sudoríparas e sebáceas (BARANOSKI & AYELLO, 2004). A derme é formada por duas camadas: papilar e reticular (FIGURA 1). Logo abaixo a derme encontrasse o tecido subcutâneo ou hipoderme, camada de tecido adiposo que promove o suporte e união da derme com os órgãos subjacentes, além de atuar como reserva energética, proteção contra choques mecânicos e isolante térmico (GUIRRO & GUIRRO, 1995; KITCHEN & YOUNG, 1998).

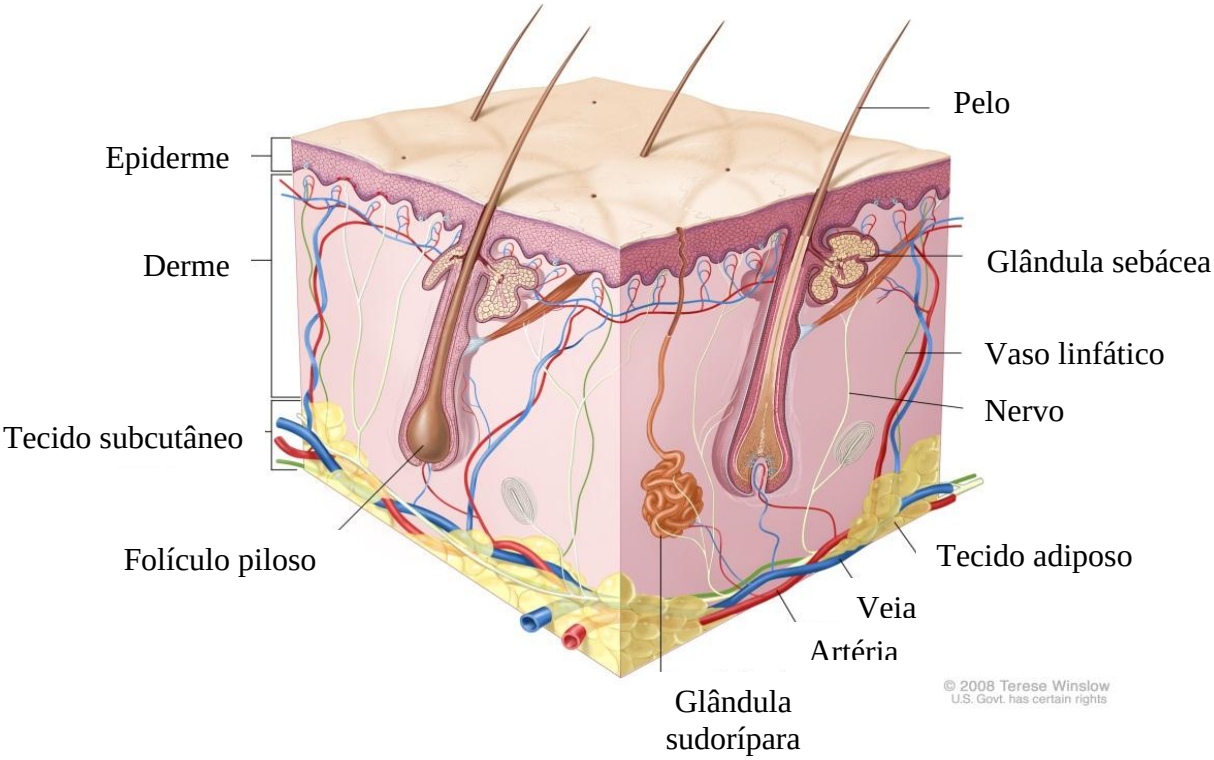


FIGURA 1. Tecido cutâneo íntegro (Epiderme, Derme e Tecido subcutâneo). Fonte: Adaptado de http://www.uchospitals.edu/online-library/content=CDR258035.

O reparo tecidual existe para garantir a restauração da integridade estrutural e funcional da pele, visando manter a homeostase. A reparação pode ocorrer por duas vias distintas: *i*) regenerativa em que o reparo tissular ocorre pela substituição na área lesionada por células idênticas ao do tecido integro; *ii*) fibroplasia, em que o reparo ocorre pela substituição do tecido lesionado por tecido conjuntivo, com perda funcional e posterior formação de cicatriz (CONTRAN, KUMAR & COLLINS, 2000). Porém, o mecanismo de cicatrização, sem a formação de cicatriz, ou seja, por regeneração, com substituição de tecido lesionado por tecido neoformado, só é observado em seres humanos durante o desenvolvimento fetal (TURAN *et al.*, 2004).

Quando há lesão da pele, seja por agente físico, químico ou biológico o organismo reage reparando-o através de um processo de cicatrização (MARTIN, 1997). O mecanismo de cicatrização tecidual consiste em uma sequência de eventos bioquímicos e fisiológicos dinâmicos com a função de impedir a infecção e restabelecer a integridade dos tecidos

lesionados, reestabelecendo assim a funcionalidade da pele (SINGER & CLARK, 1999). Todavia, a cicatrização é um processo complexo e multifatorial, que compreende basicamente 4 fases que se sobrepõem: *i*) coagulação; *ii*) inflamação; *iii*) proliferação e *iv*) remodelagem (STEED, 2003).

2.1.1. COAGULAÇÃO

As lesões do tecido cutâneo geralmente causam a ruptura de vasos sanguíneos que desencadeiam uma resposta vasculomotora que leva a vasoconstricção transitória com oclusão dos vasos injuriados. O efeito vasoconstritor é mediado pela descarga adrenérgica e pela desgranulação de mastócitos, propiciando a homeostasia (STADELMANN, DIGENIS & TOBIN, 1998). Passados alguns minutos, ocorre a vasodilatação local e exsudação de componentes plasmáticos não celulares e mediadores pró-inflamatórios através dos espaços entre as células endoteliais venosas (BLACKFORD & BLACKFORD, 1995). As plaquetas são ativadas pela exposição de colágeno subendotelial iniciando a cascata intrínseca da coagulação.

O agregado plaquetário (coágulo) que juntamente com a fibrina e a fibronectina tampona provisoriamente a lesão endotelial induz a liberação de citocinas, fator de crescimento derivado de plaqueta (PDGF), fatores de transformação de crescimento alfa e beta (TGF-α e TGF-β), fator de crescimento de fibroblasto (FGF), fator de crescimento epidérmico (EGF) fator de angiogênese derivado de plaquetas (PDAF), fator plaquetário 4 (PF-4), substâncias vasoativas e proteínas da via clássica do complemento, como C3a e C5a que contribuem significante no processo de inflamação, reepitelização, fibroplasia e angiogênese (WITTE & BARBUL, 1997; STADELMANN, DIGENIS & TOBIN, 1998; BEANES *et al.*, 2003; SHAI & MAIBACH, 2005).

A interação destes fatores é vital para a cicatrização da pele, pois promove a matriz extracelular provisória que constitui o suporte para a migração centrípeta de fibroblastos e a quimiotaxia de neutrófilos, monócitos, macrófagos e linfócitos. Qualquer alteração que interfira nesta interação inicial implicará no maior tempo de cicatrização.

2.1.2. INFLAMAÇÃO

A fase inflamatória é caracterizada pela presença de neutrófilos e macrófagos, os quais são atraídos por fatores quimiotáticos liberados durante a fase de coagulação. O edema, eritema, a dor e o calor são manifestações clínicas geralmente associadas à inflamação.

Os neutrófilos são o tipo celular predominante no local da lesão sendo responsáveis pela fagocitose de microrganismos, fragmentos celulares e produtos bacterianos (BALBINO, PEREIRA & CURY, 2005). A atividade microbicida é dependente da ativação do sistema NADPH oxidase, ou seja, da geração de espécies reativas de oxigênio e mobilização de cátions no fagossomo e a ação de proteinases, tais como elastases, catepsina G e proteinase 3) (BORREGAARD *et al.*, 1993; HAMPTON, KETTLE & WINTERBOURN, 1998; EMING *et al.*, 2007, 2009). Em baixas concentrações as espécies reativas de oxigênio atuam como agente antimicrobiano e como mensageiro celular participando na ativação dos fatores de transcrição e na liberação de citocinas pró-inflamatórias (RHEE, 1999).

A produção de citocinas pró-inflamatórias como IL-1 e IL-6, fator de necrose tumoral alfa (TNF-α) ocorre imediatamente após o dano tissular cuja liberação induz a ativação de células endoteliais e a expressão de moléculas de adesão importantes no recrutamento e acúmulo de fagócitos no sítio de inflamação (MOLLINEDO, BORREGAARD & BOXER, 1999). Todavia, os neutrófilos têm meia vida curta, mas havendo processo infeccioso no local da lesão a presença dos neutrófilos pode prolongar e/ou comprometer a cicatrização (EMING *et al.*, 2007). Porém, na ausência de infecção, os neutrófilos não são essenciais para a qualidade e taxa de cicatrização (SIMPSON & ROSS, 1972).

Os macrófagos, derivados de monócitos, complementam a atividade fagocitária exercida pelos neutrófilos, além disso, são responsáveis pela ativação dos linfócitos T auxiliares pela liberação de interleucina 1 beta (IL-1β) e TNF-α e quimiotaxia de fibroblastos pela liberação de PDGF e TGF-β com subsequente síntese e degradação de colágeno (DIEGELMANN & EVANS, 2004; BALBINO, PEREIRA & CURY, 2005). Os macrófagos também são considerados reservatórios de fatores de crescimento PDGF, TGF-α, TGF-β, FGF que promovem migração, proliferação celular e síntese de matriz extracelular (FALANGA & SABOLINSKI, 1999).

Os linfócitos recolhem neutrófilos inativos, induzem a angiogênese e liberam citocinas como interferon (IFN) e IL, que são mitogênicos e quimiotáticos para fibroblastos (STADELMANN, DIGENIS, & TOBIN, 1998; AGAIBY & DYSON, 1999). Sugere-se que os linfócitos T podem regular a atividade fibroblástica exuberante a qual poderia, caso esta regulação não existisse, ocorrer tardiamente na reparação cicatricial.

Esses processos que envolvem fatores de crescimento e citocinas regulam a produção e organização da matriz extracelular e proliferação de células musculares lisas e endoteliais, angiogênese e formação do tecido de granulação (BEANES *et al.*, 2003; WERNER & GROSE, 2003). O descontrole na fase inflamatória está relacionado com a incidência de câncer e de doenças autoimunes (KARIN, 2005). Um importante fator de transcrição envolvido no reparo tecidual é a proteína ativadora 1 cuja disfunção de uma ou mais subunidades desta proteína reflete numa cicatrização deficiente devido a alterações na fase de reepitelização (LI *et al.*, 2003) e no prolongamento da fase inflamatória (FLORIN *et al.*, 2006).

2.1.3. PROLIFERAÇÃO

A proliferação compreende os processos de granulação, reepitelização e contração. O tecido de granulação é produzido três a cinco dias após a lesão como um passo intermediário entre o desenvolvimento da malha formada por ácido hialurônico, fibronectina, glicosaminoglicano, proteoglicanos e colágeno (CONTRAN, KUMMAR & ROBBINS, 2000). Macroscopicamente o tecido de granulação é de um vermelho intenso, com superfície extremamente irregular, assumindo um aspecto granuloso o que determina o nome desse tecido (CHANDRASOMA & TAYLOR, 1993).

A proliferação e migração de fibroblastos são moduladas pelos fatores de crescimento, fator de crescimento de fibroblastos (FGF-α e FGF-β), TGF, EGF e PDGF, sintetizados por macrófagos, linfócitos e plaquetas (BIGLIOLO, 2004). O fibroblasto, uma vez no local da lesão, atua na construção de uma nova matriz sintetizando ativamente o colágeno, especialmente do tipo III, e dando suporte a outras células igualmente importantes no reparo tecidual (KARUKONDA *et al.*, 2000; SHAI & MAIBACH, 2005; LI, CHEN & KIRSNER, 2007).

Concomitante a síntese de colágeno, alguns fibroblastos induzidos pelo TGF-α

secretado pelos macrófagos se diferenciam em miofibroblastos. Os miofibroblastos formam conexões especializadas que promovem, após dois ou três dias, a contração das paredes marginais da lesão sendo estimulado por substâncias como TGF-β, 5-hidroxitriptofano, prostaglandina F1-α, angiotensina, epinefrina, bradicinina e vasopressina (KURUKONDA *et al.*, 2000). Por sua capacidade contrátil, similar a das células musculares lisas, os miofibroblastos, causam uma retração da ferida que pode atingir de 50 a 70 % do tamanho inicial (MONTENEGRO & FRANCO, 1999). Contudo a contração de uma ferida raramente é capaz de levar ao seu fechamento definitivo, o qual se deve principalmente à formação do tecido de granulação e a reepitelização.

Simultaneamente a fibroplasia a angiogênese local é induzida pela baixa tensão de oxigênio tissular e por vários fatores de crescimento e citocinas tais como FGF-α, FGF-β, EGF, TGF-α, PDGF e VEGF (fator do crescimento de endotélio vascular), os quais incita o brotamento capilar oriundo dos vasos circunvizinhos (ARNOLD & WEST, 1991; BIGLIOLO, 2004; LI, CHEN & KIRSNER, 2007). A rede neovascular expande-se para o centro da lesão, dando à cavidade lesionada uma aparência rosada e textura semelhante ao tecido de granulação. A neovascularização é essencial nesta fase porque permite a troca de gases e a nutrição das células metabolicamente ativa (ECHERSLEY & DUDLEY, 1988).

A reepitelização é o processo de restauração da epiderme após a lesão, envolvendo vários processos incluindo a migração e a proliferação de queratinócitos da epiderme adjacente para a área da lesão, a diferenciação do novo epitélio em epiderme estratificada, e a restauração da membrana basal que conecta a epiderme a derme subjacente (LI, CHEN & KIRSNER, 2007).

Ao final da fase de proliferação a lesão tissular está totalmente preenchida por tecido de granulação, a neovascularização restabelece a circulação local e a rede linfática passa por uma regeneração (MANDELBAUM, DI SANTIS & MANDELBAUM, 2003). O tecido de granulação, antes altamente vascularizado e repleto de células, agora apresenta características de uma massa fibrótica avascular e acelular, denominada tecido cicatricial.

2.1.4. REMODELAGEM

A fase de remodelagem dos componentes da matriz como ácido hialurônico, proteoglicanos e colágeno é o período no qual qualquer interferência na organização da matriz pode alterar as características que definem a resistência do tecido cicatricial (BALBINO, PEREIRA & CURY, 2005). O tecido cicatricial tem aproximadamente 80 % da força de tensão da pele normal, não é volumoso e é plano (FAZIO, 2000).

Nessa fase ocorre o equilíbrio entre a síntese e a degradação de colágeno anteriormente depositado. O colágeno tipo III é gradualmente substituído pelo colágeno do tipo I, que é o colágeno fibrilar maduro, com consequente remodelagem das fibras de acordo com as tensões aplicadas ao tecido (KITCHEN & YOUNG, 1998; BIGLIOLO, 2004). Os principais fatores de crescimento e citocinas envolvidas nesta fase são produzidas por fibroblastos (TNF, IL-1β, PDGF, TGF-β) e queratinócitos (EGF e TGF-β) (KARUKONDA *et al.*, 2000; DIEGELMANN & EVANS, 2004). Observa-se então a apoptose dos fibroblastos e das células endoteliais (TODD, DONOFF & CHIANG 1991). Estas alterações fazem com que o tecido de granulação agora acelular e avascular mude sua estrutura constituindo um tecido conjuntivo típico (MONTENEGRO & FRANCO, 1999) com a formação de uma cicatriz acrescida de fibras colágenas de cor pálida devido à regeneração deficitária dos melanócitos (JOHNSTON, 1990).

A resistência de uma cicatriz conjuntiva fibrosa aumenta consideravelmente durante a remodelagem da matriz e das fibras de colágeno, de modo que sejam formados feixes maiores com maior número de ligações covalentes transversais entre as fibrilas (RINGLER, 2000). Ganhos na resistência da lesão progridem assintomaticamente, porém o colágeno do tecido cicatricial, mesmo após um ano de maturação, não será igual ao colágeno encontrado na pele integra (BIGLIOLO, 2004).

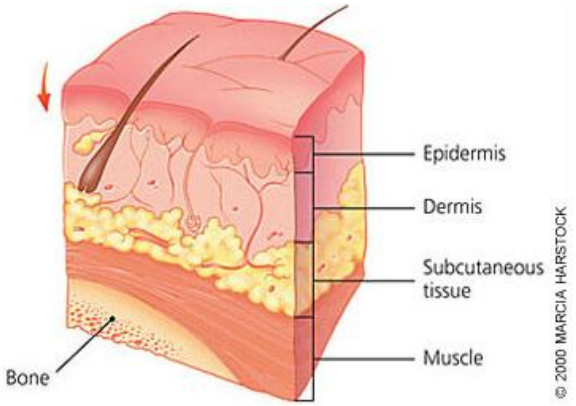
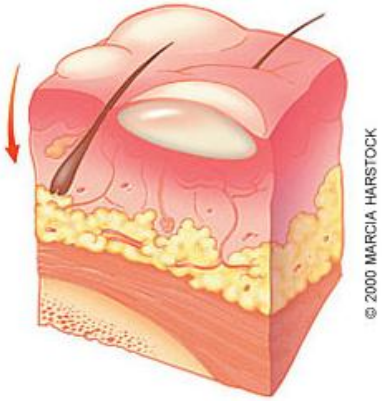
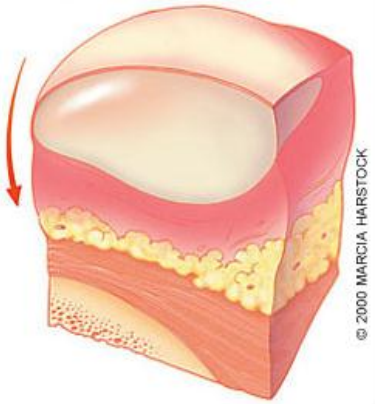
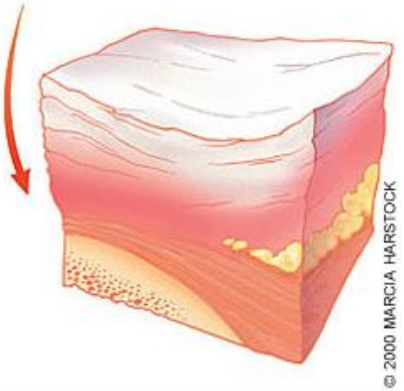
O tecido epitelial é extremamente dinâmico, e por esta razão, alterações patológicas causadas por distintos fatores locais e sistêmicos interferem negativamente no mecanismo de cicatrização. Por exemplo: o estado imunológico, a idade, as características da lesão (tamanho, local, tipo), a presença de corpos estranhos e infecções mantêm ativa a reação inflamatória; a carência de vitamina C dificulta a síntese de colágeno; condições metabólicas (diabetes mellitus), as irradiações que interferem no processo de mitose além de constituir um fator anticicatricial**,** hormônios, quimioterápicos, desnutrição etc (KOOPMAN, 1995; BIGLIOLO, 2004; BROUGHTON, JANIS & ATTINGER, 2006).

2.2. QUEIMADURAS

As queimaduras são feridas traumáticas causadas, na maioria das vezes, por agentes térmicos, químicos, elétricos e radioativos. O grau e a gravidade variam de acordo com o tipo de agente, tempo de exposição, profundidade e localização corpórea (ROSI *et al.*, 2010; MACIEL, PINTO & VEIGA JUNIOR, 2002). Podem ocorrer nos tecidos de revestimento do corpo humano, determinando destruição parcial ou total da pele e seus anexos, como também, podem atingir camadas mais profundas, representadas por tecido celular subcutâneo, músculos, tendões e ossos ([a,b]SERRA *et al*., 2004). Todavia, a pele humana pode tolerar sem prejuízo temperaturas de até 44°C. Acima deste valor, são produzidas diferentes lesões (BOLGIANI & SERRA, 2010).

A classificação tradicional das queimaduras em termos de profundidade em 1º, 2º, e 3º graus, vem sendo substituída gradualmente pelas designações de superficial, espessura parcial superficial, espessura parcial profunda e de espessura total, respectivamente (GRAY & COOPER, 2005). Queimaduras superficiais envolvem apenas a epiderme. A pele está seca e intacta, mas muito vermelha e dolorosa ao toque. Queimaduras superficiais com perda parcial da pele envolvem a epiderme e a camada mais superficial da derme. A pele normalmente forma imediatamente uma flictena, e emite exsudado hemoseroso. As queimaduras cutâneas de espessura profunda envolvem a perda parcial da epiderme e da derme. Já as queimaduras de espessura profunda envolvem a perda da epiderme, derme, camada subcutânea e/ou estruturas mais profundas. O aspecto da pele pode ser branco-cera, área cinzenta ou aparência espessa translúcida amarela/negra (TABELA 1).

TABELA 1. Classificação das queimaduras com base na profundidade da lesão e descrição do tempo estimado para a sua cicatrização. Fonte: Adaptado de MORGAN, BLEDSOE & BARKER, 2000.

Queimadura Superficial	**Queimadura Espessura Parcial Superficial**	**Queimadura Espessura Parcial Profunda**	**Queimadura Espessura Total**
			
3 a 6 dias	**7 a 20 dias**	**Mais de 21 dias**	**Indeterminado**
Tempo estimado para a cicatrização			

As queimaduras estão entre as maiores causas de lesão cutânea ocupando o segundo lugar entre os acidentes que mais comumente ocorrem no mundo. No Brasil, as queimaduras estão entre as principais caudas externas de morte registradas, perdendo apenas para outras causas violentas que incluem acidentes de transporte e homicídios (VALE, 2005).

As queimaduras são consideradas lesões que causam traumas graves, pois, podem levar o paciente à morte ou acarretar distúrbios de ordem emocional e social. Estudos mostram que a maioria dos acidentes por queimadura ocorre em ambiente domiciliar ou no trabalho (ROSSI *et al.*, 1998; GIMENES *et al.*, 2009; GONÇALVES *et al.*, 2011). De acordo com MILLER & GLOVER (1999), as causas das queimaduras podem ser acidentais, não acidentais ou tentativa de suicídio. Existem também situações patológicas (epilepsia, álcool ou depressão) que podem predispor os indivíduos a um maior risco de adquirir uma queimadura.

O resfriamento da lesão cutânea por queimadura é utilizado na tentativa de diminuir o dano tissular, a morbidade e a mortalidade, sendo a água da torneira a abordagem inicial mais recomendada na literatura (DAVIES, 1982; LAWRENCE, 1987; NOVAES, 2003; VALE, 2005). A princípio a queimadura é um ambiente estéril, porém o tecido necrótico rapidamente se torna colonizado por bactérias endógenas e exógenas, produtoras de proteases, que levam à liquefação e separação da escara, dando lugar ao tecido de granulação responsável pela cicatrização da ferida, que se caracteriza por alta capacidade de retração e fibrose nas queimaduras de terceiro grau (SUCENA, 1982).

Além de causar a destruição dos componentes mecânicos da pele que constituem uma barreira natural de defesa, as queimaduras comprometem a defesa imunológica humoral e celular tornando-se um fator agravante que favorecem a aquisição de infecções (MARTINEZ-HERNANDEZ, 1999). Por sua vez, a resposta imune às queimaduras é um evento complexo influenciado por uma série de fatores tais como a extensão e gravidade da queimadura, profundidade, idade, presença ou ausência de infecção, tipo de tratamento, etc (STOECKEL, 2006).

Em lesões cutâneas por queimadura ocorre à cicatrização por segunda intenção, que é um processo lento, com alto risco de infecção, produzindo retração cicatricial, cicatrizes extensas e alto custo de tratamento (COELHO *et al.*, 1999). Após a lesão, logo abaixo da área de necrose, é possível verificar a superfície da ferida viável (zona da lesão) que apresenta um conteúdo aumentado de fibrina, produzida pela ativação da cascata de coagulação, e de

fibronectina, sintetizada pelas células dérmicas (FIGURA 2). Segundo BAUM & ARPEY (2005) a fibrina quando em excesso, impede a migração dos fibroblastos e deposição de matriz.

Em relação ao tratamento, têm se observado grandes avanços no atendimento às vitimas de queimaduras, resultando em melhores resultados clínicos e aumentendo a sobrevida desses pacientes (LATARJET, 2002). Contudo, o reparo tecidual em lesões por queimaduras predispõe à formação de cicatrizes hipertróficas e retratéis, caracterizada pelo aumento da vascularização, de fibroblastos, miofibroblastos e colágeno, causando deformidades, as quais geram efeitos marcantes no convívio social e familiar do paciente em razão de suas implicações estéticas e específicas (CALDAS, 2004). Como consequência, na tentativa de minimizar estas sequelas torna se cada vez maior o número de cirurgias reparadoras em hospitais de atendimento especializado (THOMBS *et al.*, 2007).

Diversos fatores locais e sistêmicos podem atrasar ou impedir a cicatrização, como: suporte nutricional inadequado, déficit na oxigenação tecidual, necrose, ambiente seco, imunossupressão, etc (HESS, 2002). Qualquer alteração no processo de reparo leva à cicatrização patológica, que pode ser agrupada de forma geral em: formação deficiente de tecido cicatricial, formação excessiva (cicatriz hipertrófica e quelóide) e a formação de contraturas (ROBBINS *et al.*, 2005).

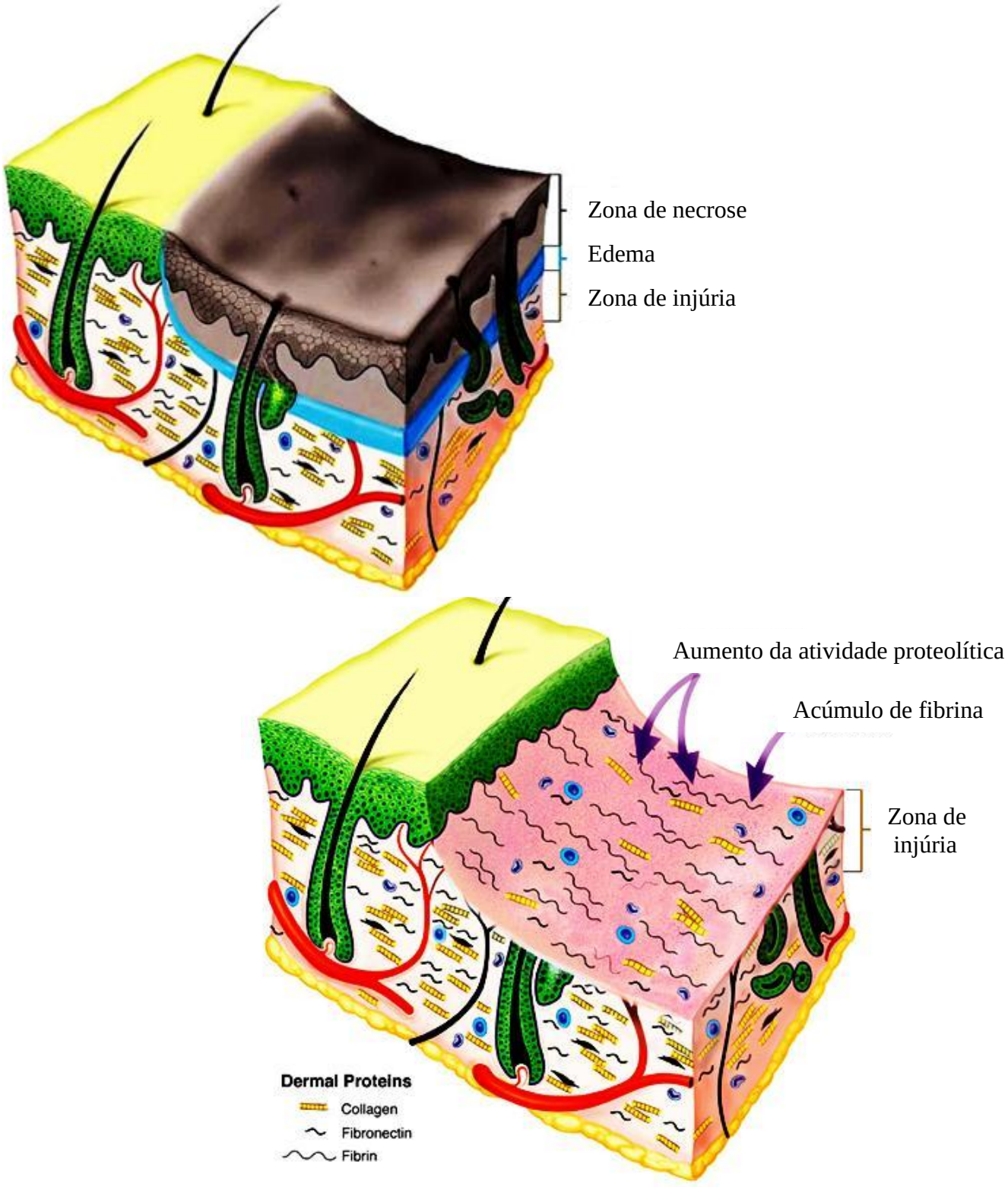


FIGURA 2. Aspecto do tecido cutâneo após indução de queimadura de segundo grau. Abaixo da zone de necrose ocorre o aumento da deposição de fibronectina. Fonte: Adaptado de http://www.plasticsurg.com/burn/Partial_Burn/part16.htm.

2.3. CURATIVOS ÚMIDOS

Apesar de ser constatado na prática clínica os benefícios da promoção de um ambiente úmido no processo cicatricial de feridas, até o início da década de 60 eram poucas as pesquisas voltada a esta linha de estudo. Porém, a publicação do trabalho de WINTER, em 1962, que demonstrou o aumento da taxa de epitelização de feridas em um ambiente úmido com consequente minimização da formação de crostas, incentivou a pesquisa, produção e comercialização de curativos úmidos. Em 1982 as coberturas à base de hidrocolóides são lançadas nos Estados Unidos e Europa, passando a ser largamente utilizadas em feridas de espessura parcial. Tais coberturas só foram disponibilizadas no mercado brasileiro a partir da década de 90, e seu custo elevado foi uma barreira inicial para sua difusão (MANDELBAUM, DI SANTIS & MANDELBAUM, 2003).

Segundo MILLER & GLOVER (1999), o tratamento de lesões por queimadura visa entre outros fatores: 1) manter o ambiente da ferida limpo e úmido; 2) promover o conforto do paciente e 3) proporcionar proteção contra infecção e outros traumatismos. Nesse sentido, várias condutas terapêuticas têm sido empregadas com o objetivo de conseguir um resultado aceitável no tratamento destas lesões, tais como: os filmes, espumas hidrogéis, sprays, enxertos precoces, curativos biológicos com pele artificial e de animais e homoenxertos (HUTCHINSON & MCGUCKIN, 1990; JOHNSON, 1992; BORISKIN, 1994; SANTOS, 1999; PURNA & BABU, 2000).

2.3.1. HIDROGEL

Os hidrogéis podem ser descritos como polímeros reticulados formando uma rede tridimensional em seus (macrorradicais), a partir de resinas sintéticas que podem intumescer em meio aquoso e reter uma grande quantidade de água na sua estrutura (PEPPAS *et al.*, 2000; OLIVEIRA *et al.*, 2005). As interações responsáveis pela absorção de água incluem processos relacionados à presença de grupos hidrofílicos no polímero, e a processos de difusão capilar entre áreas com diferentes pressões osmóticas (ROSIACK, 1991).

Segundo EISENBUD *et al.*, (2003), o hidrogel encontra-se entre os produtos mais

utilizados no tratamento de queimadura devido ao custo/benefício e fácil aplicação. De acordo BLANES (2004), a aplicação do hidrogel promove uma sensação refrescante reduzindo a dor e evitando a desidratação das terminações nervosas. Dentre os hidrogéis comerciados atualmente no Brasil encontra-se: Hydrosorb, Duordem gel, Nugel, Intrasite-gel, Dermagran, Hydrosorb plus, Hypligel, Purilon e Elasto-gel (NEVES, 2010).

Uma diversidade de polímeros hidrofílicos vem sendo utilizada na formação de hidrogéis visando sua aplicação biotecnológica particularmente no tratamento de ferimentos e como suporte para liberação de fármacos (MARTINEZ-RUVALCABA, CHORNET & RODRIGUE, 2007). A utilização de hidrogéis a base de carbopol tem sido amplamente empregado por apresentar baixa propriedade irritante, sem efeito sobre a atividade biológica da droga o que o torna um excelente veículo, pois devido ao seu peso molecular extremamente elevado, não penetram na pele nem afetam a atividade da droga (GUMMA, 1971; MACHIDA *et al.*, 1980; SANGHAVI, PURI & KAMATH, 1989; RUIZ *et al.*, 1994).

Ao contrário do processo térmico, pouquíssima energia da radiação é consumida em aumentar a energia térmica das moléculas que a absorvem. Além disso, a energia necessária para esterilização pela radiação é de cerca de 50 vezes menor do que a requerida para esterilização pelo calor. Por isso esta é conhecida como - esterilização a frio (CENA, 2006). Além disso, o uso da radiação ionizante na esterilização de hidrogéis permite obter um produto puro, não contaminado com resíduos de materias tóxicos. Logo a aplicação da radiação ionizante é segura para o homem e para o meio ambiente (ROSIACK *et al.*, 1995).

Algumas das vantagens da irradiação com raios gama sobre outros métodos é o seu alto teor de energia e grande poder de penetração e letalidade. Seu poder de penetração é instantâneo, uniforme e profundo, permitindo o tratamento de produtos de tamanho, forma e densidades variáveis (URBAIN, 1989; EHLERMANN, 1990; FRANCO & LANDGRAF, 1996; HOBBS & ROBERTS, 1998). O Co60 é o isótopo radioativo mais utilizado comercialmente em todo mundo por sua disponibilidade, custo, por apresentar-se na forma metálica e ser insolúvel em água, proporcionando com isso maior segurança ambiental (EHLERMANN, 1990; CHMIELEWSKI & HAJI-SAEID, 2005).

2.4. LECTINAS

Lectinas são proteínas capazes de reconhecer sítios específicos em moléculas e ligar-se reversivelmente a carboidratos, sem alterar a estrutura covalente das ligações glicosídicas dos sítios (ETZLER, 1998). Devido a esta habilidade, as lectinas ou hemaglutininas, apresentam alto grau de especificidade em suas reações com grupos sangüíneos do sistema ABO e MN (SHARON & LIS, 1993). Esta interação ocorre através de ligações de hidrogênio e interações hidrofóbicas em uma porção limitada da molécula protéica denominada de Domínio de Reconhecimento a Carboidrato (KENNEDY *et al.*, 1995; NISHIMURA *et al.*, 2006;).

As lectinas apresentam uma distribuição ubíqua na natureza, sendo encontradas tanto em organismos procariotos como em eucariotos (WANG & NG, 2003). É fato que a maioria das lectinas conhecidas foram isoladas de sementes, folhas, cascas, frutos, raízes, bulbos e tubérculos (RUDIGER *et al.*, 2000). Sendo essas proteínas extraídas principalmente de sementes de leguminosas (BHATTACHARYYA *et al.*, 1990; GEGG *et al.*, 1992; YAMAGUCHI *et al.*, 1993; SHARON & LIS 1995). MARTIN-CABREJAS *et al.* (1995) encontraram quantidades consideráveis de inibidores de tripsina/quimotripsina e α-amilase e elevada atividade de lectinas em cinco cultivares de feijões (*Phaseolus vulgaris*) frescos e estocados por cinco anos. Entre as lectinas de plantas mais estudadas e caracterizadas estão incluídos: *Phaseolus vulgaris* (PHA), *Canavalia ensiformis* (com A), *Phosphocarpuis tetragonolobus* (WBL), *Triticum vulgare* (WGA) e *Lycopersicon esculentum* (lectina do tomate) (KOMPELLA & LEE, 2001).

Cada molécula de lectina contém dois ou mais sítios de ligação para carboidratos; di ou polivalentes. As lectinas podem interagir com os açúcares da superfície das células podendo originar uma ligação cruzada levando a precipitação (de polissacarídeos, glicoproteínas, peptidoglicanos, ácido teicóico, glicofosfolipídios, etc.), fenômeno este denominado aglutinação celular (CORREIA & COELHO, 1995; MO *et al.*, 2000; ZENTENO *et al.*, 2000). A capacidade de aglutinar células (FIGURA 3) distingue lectinas de outras macromoléculas ligantes de açúcares como as glicosidases e glicotransferases (GOLDSTEIN *et al.*, 1980).

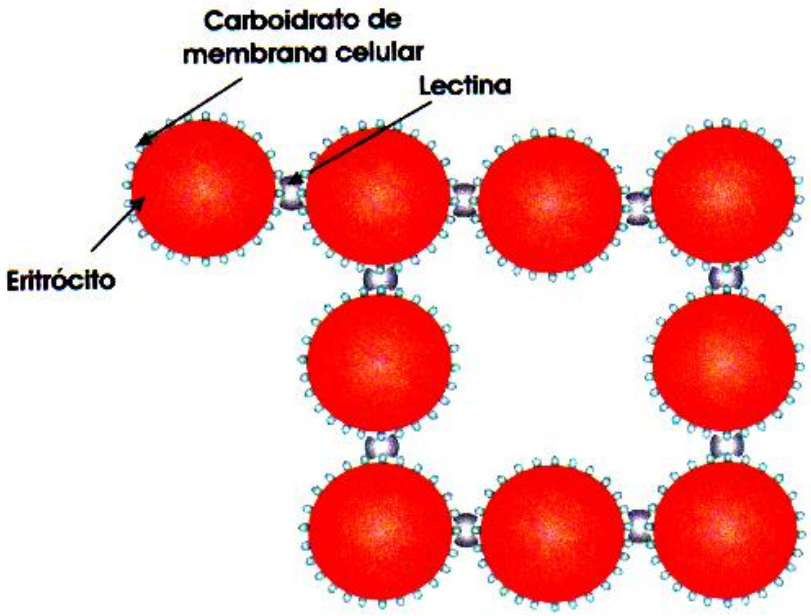


FIGURA 3. Rede de hemaglutinação mediada por lectinas. Fonte: PIMENTEL, 2006.

As lectinas são consideradas moléculas que reconhecem e decifram as informações contidas nos oligossacarídeos da superfície celular (RINI, 1995). A especificidade das lectinas é definida pelo monossacarídeo ou oligossacarídeo que inibe as reações de precipitação ou aglutinação induzidas por lectinas (KOMPELLA & LEE, 2001). Esta interação fraca entre a lectina e o carboidrato aumenta tanto a afinidade como a especificidade através de subsítios e subunidades (FIGURA 4). Esta ligação ao carboidrato é diretamente responsável pela atividade biológica (PEUMANS & VAN DAMME, 1995). Por existirem plantas que possuem duas ou mais lectinas que diferem na especificidade, SHARON & LIS (2003) denominaram estas lectinas de isolectinas.

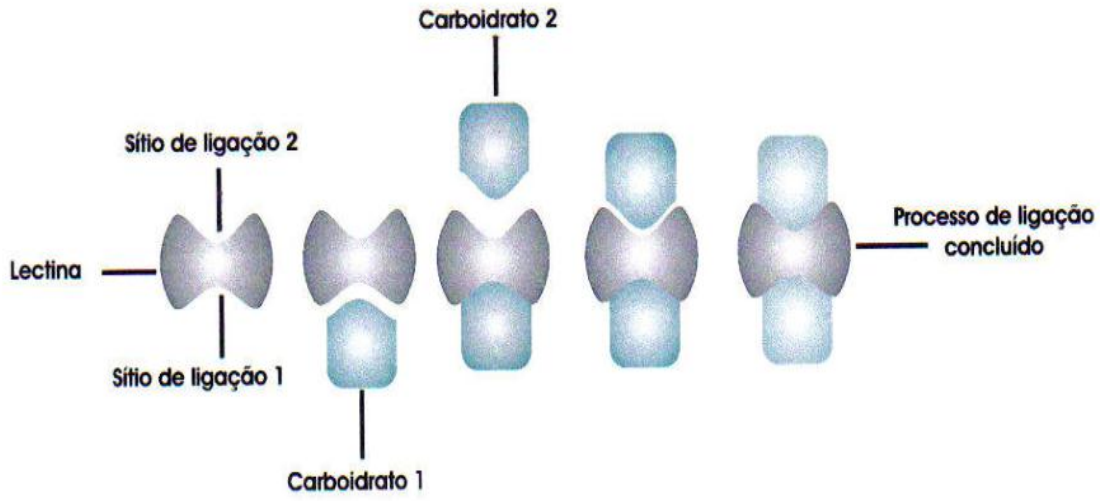


FIGURA 4. Ligação específica lectina e carboidrato. Fonte: PIMENTEL, 2006.

Isolectinas são definidas como um grupo de proteínas intimamente relacionadas, resultantes da expressão de diferentes genes, com estruturas semelhantes em uma mesma espécie, e apresentam formas moleculares com mobilidade eletroforética diferente. O termo isoforma foi proposto para lectinas pertencentes à mesma espécie, cuja heterogeneidade de origem genética não foi bem definida (PAIVA & COELHO, 1992). Apesar de muitas plantas possuírem uma lectina com especificidade para um único carboidrato, são conhecidas plantas que contém duas ou mais lectinas com especificidade para açúcares diferentes, por exemplo: *Ulex europaeus*, *Bandeiraea simplicifolia*, *Dioclea lehmani* e *Sambucus nigra* (VAN DAMME *et al.*, 1998; PEREZ, 1998).

A caracterização físico-química de lectinas é importante na elucidação de seu comportamento em diferentes sistemas biológicos (SOUZA *et al.*, 2001). A estabilidade e integridade estrutural de proteínas oligoméricas são determinadas por suas interações inter e intracadeias. Lectinas de leguminosas são similares nas suas estruturas primária, secundária e terciária (SRINIVAS *et al.*, 2001) e por esta razão tornam-se um excelente modelo para estudos de desdobramento de proteínas diméricas e tetraméricas, e da oligomerização na estabilidade e integridade estrutural. Além disso, estas glicoproteínas têm sido consideradas uma importante ferramenta em pesquisas biomédicas (RUDIGER, 1998).

De forma geral as lectinas isoladas a partir de vegetais são comumente subdivididas em (TABELA 2): **merolectinas** - lectinas com apenas um domínio de ligação a carboidratos, que são incapazes de precipitar glicoconjugados ou aglutinar células; **hololectinas** - lectinas que possuem no mínimo dois ou mais domínios homólogos de ligação a carboidratos; **quimerolectinas** - proteínas com um ou mais domínios de ligação a carboidratos e um domínio não relacionado que possui uma atividade biológica distinta e independente; e **superlectinas** - incluem as lectinas que possuem dois domínios de ligação a carboidratos estrutural e funcionalmente distintos (VAN DAMME, 1998).

Esta classificação abrange significativamente inúmeras proteínas vegetais, porém existem algumas exceções, a exemplo da lectina, presente na semente de *Parkia platycephala*, homóloga a família das hidrolases (FIGURA 5). Tal lectina possui um domínio com um sítio enzimático específico para a quitina, e dentro deste mesmo domínio, um outro sítio de reconhecimento a carboidrato (CAVADA *et al*., 2006).

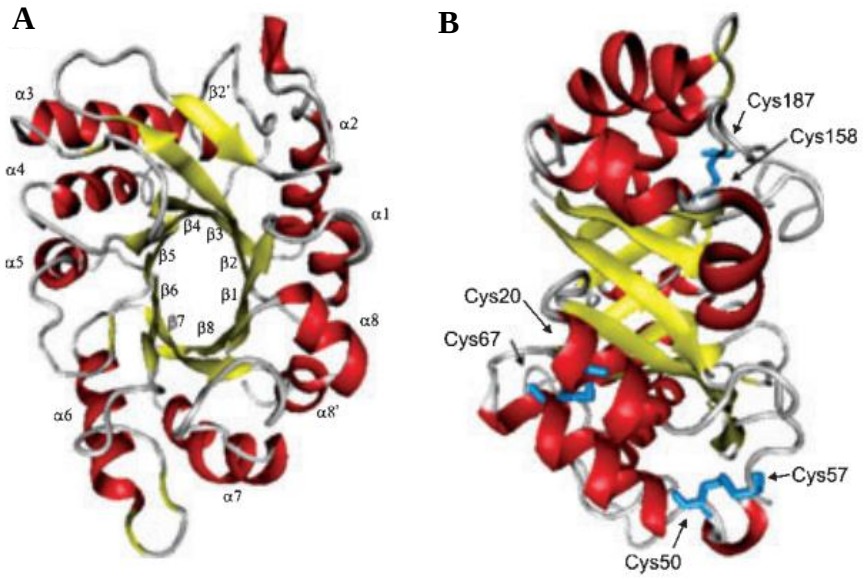
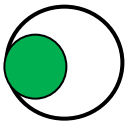
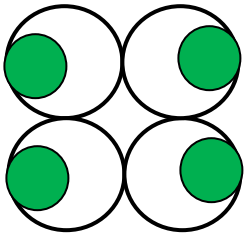
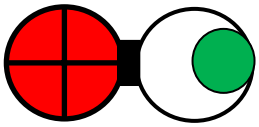
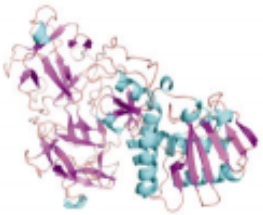
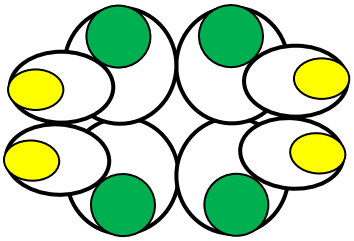
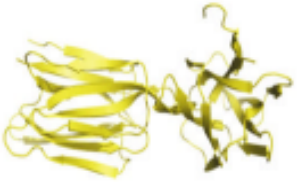


FIGURA 5. Estrutura cristalina da lectina de *Parkia platycephala*. (A) e (B) mostram duas visões da dobra barril. Pontes dissulfeto estão destacadas em azul. Em (B), o sítio ativo da fenda (loops) estão localizados na face direita do modelo. Fonte: CAVADA *et al.*, 2006.

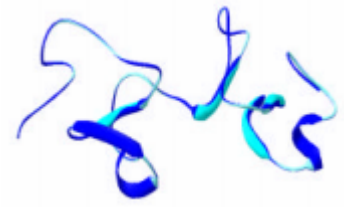
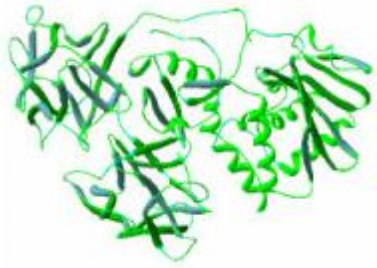
TABELA 2. Classificação das lectinas quanto aos aspectos estruturais. Fonte: Adaptado de MORENO, 2008.

Representação Esquemática	Exemplo
Merolectina Proteína monomérica com um único sítio ativo. 	 Lectina de *Hevea brasiliensis* ANDERSEN *et al.*, 1993
Hololectina Proteína tetramérica com quatro sítios ativos homólogos. 	 Lectina de *Arachis Hypogaea* RAVISHANKAR *et al.*, 2001
Quimerolectina Proteína com um sítio de ligação a carboidrato e um outro domínio que possui uma função não lectínica. 	 Lectina de *Ricinus communis* RUTEMBER *et al.*, 1991
Superlectina Proteína com dois domínios diferentes com afinidade por carboidratos distintos. 	 Lectina de *Musa acuminata* MEAGHER *et al.*, 2005

Atualmente é possível classificar as lectinas não somente quanto aos aspectos estruturais vistos anteriormente, mas em famílias evolutivamente relacionadas, das quais podemos citar, (TABELA 3): *i*) lectinas de monocotiledôneas do tipo manose; *ii*) lectinas específicas a quitina e homólogas a heveína; *iii*) lectinas homólogas a jacalina; *iv*) lectinas homólogas ao tipo RIP-2 (Ricina); *v*) lectinas de leguminosas; *vi*) lectinas da família das Amarantaceae; e *vii*) lectinas de floema de Curcubitaceae (MORENO, 2008).

O espectro das funções biológicas das lectinas não está totalmente esclarecido, pois estas proteínas apresentam ampla ocorrência, diversidade estrutural e especificidade glicídica. Além disso, uma lectina particular pode assumir diferentes funções dependendo de onde e quando é expressa (RUDIGER *et al.*, 2000). Algumas das funções atribuídas a esta classe de proteínas, são: renovação de glicoproteínas do soro (VIJAYAN & CHANDRA, 1999); defesa contra patógenos (CHANG & ZHU, 2002); proteínas de estocagem (NAKAMURA *et al*., 2004); adsorção viral (BOTOS & WLODAWER, 2005); resposta imunológica (CHEN et al., 2005); transporte de carboidratos (KAMIYA *et al*., 2005); mediação da interação célula-célula e patógeno-hospedeiro (SAOUROS *et al*., 2005). Porém uma das grandes importâncias fisiológicas da lectinas está associada a sua utilização dessas proteínas como reagentes policlonais para investigar as bases moleculares no controle da ativação e proliferação de linfócitos; para identificar e fracionar células do sistema imune e como drogas (SINGH *et al*., 2005).

TABELA 3. Classificação das lectinas quanto às famílias evolutivas. Adaptado de MORENO, 2008.

Classificação	Referências
Lectinas de monocotiledôneas do tipo manose  Lectina de *Scilla campanulata*	WRIGHT *et al.*, 2000
Lectinas especiíficas a quitina e homólogas a heveína  Lectina de *Phytolacca americana*	FUJII *et al.*, 2004
Lectinas homólogas a jacalina  Lectina de *Parkia platicephala*	GALLEGO DEL SOL *et al.*, 2005
Lectinas homólogas ao tipo RIP-2  Lectina de *Sambucus ebulus*	PASCAL *et al.*, 2001

2.4.1. CRAMOLL

A *Cratylia mollis* (FIGURA 6), facilmente encontrada na região do semi-árido nordestino, pertence à família Leguminosae, subfamília Papilionoideae, tribo Phaseoleae, subtribo Diocleinae que compreende 13 gêneros, dos quais desatacam-se os gêneros: *Canavalia*, *Cratylia*, *Calopogonium*, *Dioclea*, *Galactia* e *Herpyza* (POLHILL *et al.*, 1981).

FIGURA 6. *Cratylia mollis*. Arbusto (à esquerda) e sementes (à direita).

A partir das sementes secas e trituradas do feijão camaratu coletado no município de Ibimirim/PE, localizado a aproximadamente 346 Km da capital recife (FIGURA 7), é purificada pela fração 40-60% de ´precipitação de sulfato de amônio, as isoformas 1 e 4 associadas, denominada Cramoll-1,4 (PAIVA & COELHO, 1992; CORREIA & COELHO, 1995). A mistura de Cramoll 1 (em maior concentração nas sementes) e sua isoforma, Cramoll 4, podem ser separadas por cromatografia de troca iônica (CORREIA & COELHO, 1995). Posteriormente as isoformas Cramoll 2 e Cramoll 3 foram isoladas por PAIVA & COELHO (1992). A classificação das lectinas foi realizada de acordo com a migração eletroforética em gel para proteínas básicas nativas; Cramoll 1, proteína mais básica, apresenta a maior migração, seguida de Cramoll 2; Cramoll 3 é a menos básica das três e Cramoll 4 (PAIVA & COELHO, 1992; CORREIA & COELHO, 1995).

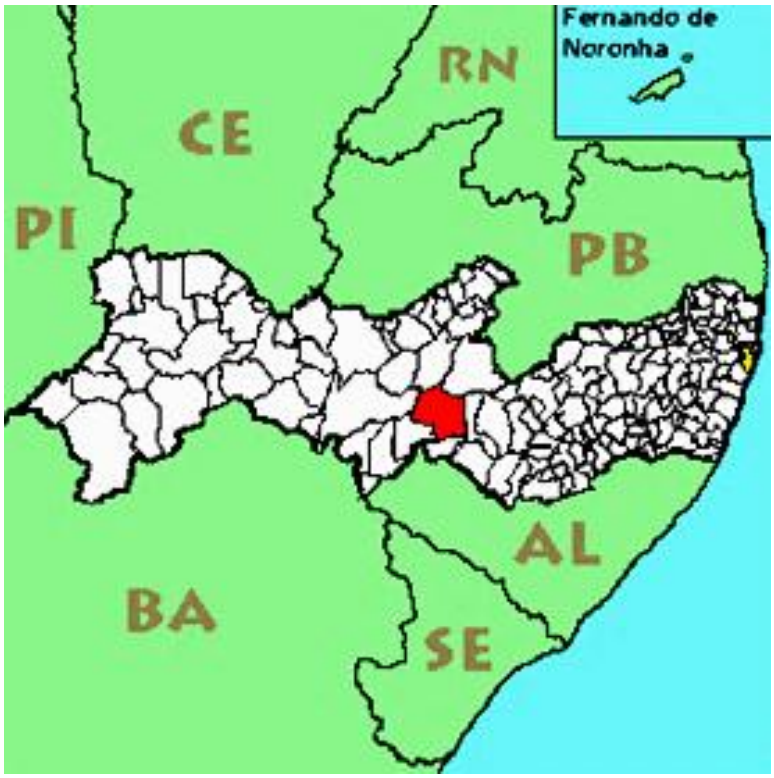


FIGURA 7. Mapa do Estado de Pernambuco, destacando o município de Ibimirim (em vermelho). Fonte: http://www.cidades.com.br/cidade/ibimirim/002561.html

A Cramoll-1,4 apresenta-se estável até 80° C, com ponto isoelétrico em torno de 8,6 e caráter básico (FIGURA 8). Possui uma banda principal de 31 kDa e dois fragmentos da banda principal de 16 e 14 kDa (CORREIA & COELHO, 1995). O melhor potencial eletroquímico para *C. mollis* livre ou imobilizada foi obtido utilizando-se 1,0 mg/ml, a 5 e 10° C, 87 e 102 mV, respectivamente. O desenvolvimento de técnicas para definir a interface de parâmetros elétricos poderá dar informações sobre a adsorbância de grupos carregados na superfície da membrana celular, revelando interações em sistemas biológicos (SOUZA *et al.*, 2003).

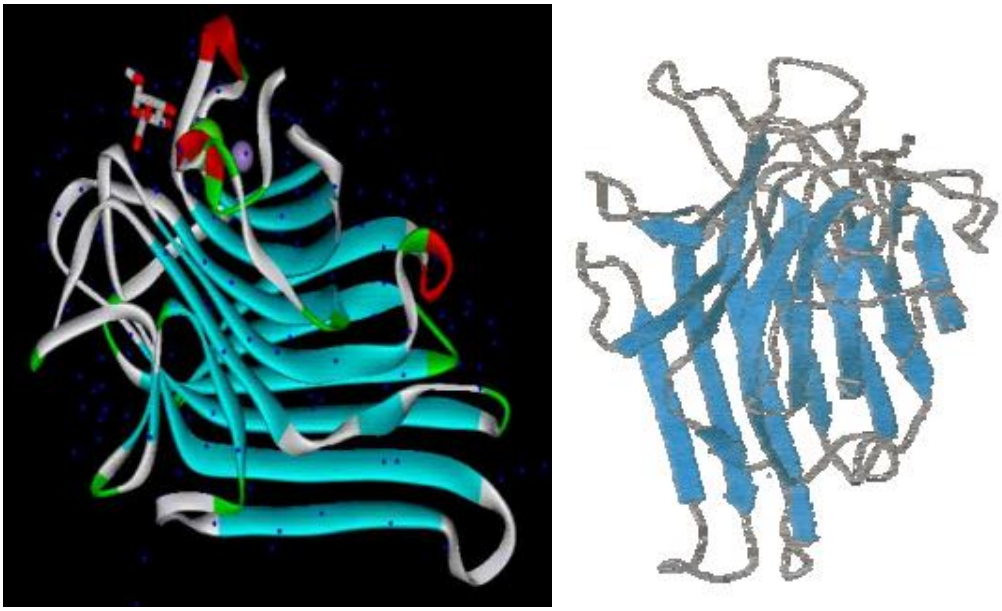


FIGURA 8. Estrutura terciária da lectina Cramoll 1,4 (à esquerda) e modelo da isoforma Cramoll 1 (à direita). Fonte: SOUZA *et al.*, 2003; http://webenligne.cermav.cnrs.fr/lectines/.

Várias atividades biológicas têm sido atribuídas as diferentes isoformas isoladas de *C. mollis*, das quais podemos citar: atividade mitogênica de linfócitos (MACIEL *et al*., 2004), adjuvante na terapia do câncer (BELTRÃO *et al.*, 1998; ANDRADE *et al*., 2004), atividade imunoestimulatória (OUDRHIRI *et al.*, 1985; GHOSHA *et al.*, 1999; MELO *et al*., 2010a; MELO *et al*., 2010b), atividade anti-inflamatória e anti-helmíntica (FERNANDES, 2010; MELO et al., 2011c) e na reparação de feridas em ratos sadios e imunodeprimidos (YASUOKA *et al*., 2003; ALBUQUERQUE *et al*., 2004; MELO *et al.*, 2011b).

3. JUSTIFICATIVA

O avanço tecnológico tem possibilitado o surgimento de novos tratamentos que aceleram o mecanismo de cicatrização das feridas e contribuem no restabelecimento da qualidade de vida do paciente. Por outro lado, o preço destes curativos algumas vezes é elevado o que inviabiliza sua utilização pela população de baixa renda. Assim, a avaliação da eficácia da lectina de feijão camaratu (Cramoll 1,4) no reparo de lesões apresenta grande potencial terapêutico, o que torna relevante estudar o efeito da aplicação tópica contínua da formulação de hidrogel irradiado de Cramoll 1,4 em um novo modelo experimental voltado para a cicatrização de queimaduras cutâneas em ratos sadios e imunodeprimidos. Este projeto justifica-se ainda pelo caráter multidisciplinar que envolve todas as etapas da experimentação proposta, a qual fornece informações importantes quanto ao aspecto clínico, histopatológico, bioquímico e hematológico das queimaduras de segundo grau.

4. OBJETIVOS

4.1. GERAL

Avaliar o processo de reparo de queimaduras cutâneas experimentais em ratos normais e imunodeprimidos submetidos ao tratamento contínuo com hidrogel associado à lectina Cramoll 1,4 irradiado.

4.1.1. ESPECÍFICOS

- Desenvolver um modelo experimental de queimaduras térmicas de segundo grau em ratos Wistar;
- Realizar o tratamento das lesões cutâneas experimentais utilizando hidrogel da lectina Cramoll 1,4 irradiado;
- Acompanhar a evolução do processo de reparo tecidual em ratos sadios, avaliando os sinais clínicos, bioquímicos, hematológicos, microbiológico e histológico;
- Induzir e acompanhar a imunodepressão nos animais experimentais;
- Acompanhar a evolução do processo de reparo tecidual em ratos imunodeprimidos, avaliando os sinais clínicos, bioquímicos, hematológicos, microbiológico e histológico.

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6. ARTIGO I

Aceito para publicação no Journal of Biomedicine and Biotechnology

DEVELOPMENT OF ANIMAL MODEL FOR STUDYING DEEP SECOND-DEGREE THERMAL BURNS

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Abstract

Thermal lesions were produced in 12 male Wistar rats, positioning a massive aluminum bar 10 mm in diameter (51 g), preheated to 99° C ± 2° C/10 min. on the back of each animal for 15 sec . After 7, 14, 21 and 28 days, animals were euthanized. The edema intensity was mild, with no bubble and formation of a thick and dry crust from the 3rd day. The percentage of tissue shrinkage at 28 days was 66.67 ± 1.66 %. There was no sign of infection, bleeding or secretion. With 28 days reepithelialization was incomplete, with fibroblastic proliferation and moderate fibrosis and presence of modeled dense collagen fibers. It is concluded that the model established is applicable in obtaining deep second-degree thermal burns in order to evaluate the healing action of therapeutic agents of topical use.

Keywords: thermal burn, experimental model, rats.

1. Introduction

Burns are tissue lesions from thermal origin for exposure to flames, hot surfaces and liquids, extreme cold, chemicals, radiation or friction [1]. Even with improved prognosis [2] and progress in the use of biological skin substitutes [3], Burns are an important cause of mortality [4].

Burns are classified depending on the lesion severity into superficial or first-degree, when lesion is restricted to the epidermis or skin causing redness; partial thickness or second-degree that can be superficial when reaching the epidermis and superficial dermis, showing hypersensitivity and pain, or deep when it extends to the deepest layer of the dermis and may have reduced sensitivity with red and/or white coloration of the tissue; and full-thickness or third-degree when lesion involves the subcutaneous layer, with no sensitivity and white coloring [5].

The use of animals as experimental models in different areas of biological research was encouraged by Claude Bernard [6], who around 1865, described in his paper entitled "Introduction to the Study of Experimental Medicine" the use of animals as a model for study and transposition into human physiology. Experimental models are essential in mammals when studying on burns. There are literature reports on the use of rabbits [7], pigs [8], dogs [9], rats [10] and mice [11] as models in the study of burns.

The healing of skin lesions induce the burn injured tissue inflammation, edema and hypertrophic and unsightly scars [12]. Thus, the choice of a topical agent or the type of coverage to be used in treating burns should be conducted based on the assessment of lesion characteristics and evidence reported in the specific literature. These products must have features such as: antimicrobial or bacteriostatic activity, absence of toxicity and hypersensitivity, compliance, reduced healing time and cost/benefit [13]. However, many of the methods used in healing injuries caused by burns are controversial [14].

In this context, the objective of this study was to establish an experimental protocol for induction of deep second-degree thermal lesions in Wistar male rats to obtain clinical and histopathologic data that will facilitate understanding of results concerning the evolution of the healing action of topical therapeutic agents.

2. Materials and Methods

2.1. Animals

The experiment was conducted at the Department of Experimental Surgery, Federal University of Pernambuco, using albino Wistar male rats (*Rattus norvegicus)* weighing 250 ± 50 g, kept in individual cages of polypropylene measuring 30x20x19cm and controlled lighting conditions (12 h light /dark phtoperiod), temperature (24 ± 2 ° C), receiving water and food (Labina®) ad libitum. The experimental procedure was approved by the ethics committee on animal experimentation of the Federal University of Pernambuco (Case No 23076.015015/2009-31).

2.2. Thermal Burn Experimental Model

Initially, 12 animals were weighed and intramuscularly pre-anesthetized with atropine sulfate (0.04 mg /kg) and 10 minutes after subjected to anesthetic combination of 10% ketamine (90 mg /kg) and 2 % xylazine (10 mg /kg) intramuscularly [15, 16]. With the animal properly anesthetized trichotomy of back was performed and antisepsis with 1 % polyvinylpyrrolidone-iodine. Thermal injuries were made with a solid aluminum bar 10 mm in diameter (Figure 1A), previously heated in boiling water and so that the temperature reached 100° C measured with a thermometer. The bar is maintained in contact with the animal skin on the dorsal proximal region for 15 sec (Figure 1B). The pressure exerted on the animal skin corresponded to the mass of 51g of aluminum bar used in the burn induction. Immediately after the procedure, analgesia with dipyrone sodium (40 mg /kg) was performed intramuscularly, being maintained for three consecutive days sodium dipyrone at 200 mg /kg orally administered in the drinking water supplied to animals.

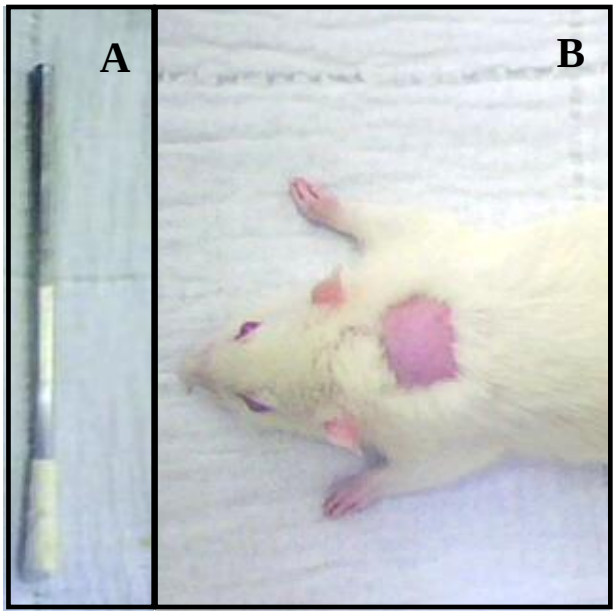

Figure 1. Experimental model of second-degree thermal burn in male Wistar rats. **A** - Solid aluminum bar of 10 mm in diameter and 51 g used in the induction of thermal burns by direct heat transference. **B** - Proximal dorsal region chosen for burn induction.

2.3. Clinical Evaluation

The clinical course of skin lesions by burns was evaluated for 28 consecutive days based on the following aspects: blistering, swelling, redness, crust, bleeding, secretion, granulation tissue and scar tissue.

The wound retraction was evaluated using a caliper 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 [17].

2.4. 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.

2.5. Histological Analysis

At the pre-established times for biopsy (7, 14, 21 and 28 days after burn induction), three animals randomly selected underwent anesthesia combination of 10 % ketamine (90 mg /kg) and 2 % xylazine (10 mg /kg), intramuscularly [15, 16] for tissue samples collection. Euthanasia was performed by excessive doses of sodium pentobarbital intraperitoneally (100 mg /kg) [18].

Tissue samples were immediately fixed by immersion in 4% formaldehyde (v /v) prepared in PBS (0.01 M, pH 7.2), followed by routine histological processing (paraffin embedding, microtomy with 4 µm cuts and Masson's trichrome staining. Histological study was performed by comparative descriptive analysis of the experimental groups in binocular optical microscope (Zeiss - Axiostar model) where were evaluated the evolution of skin healing after thermal trauma.

The histological analysis was performed by 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 leukocytes (PNM), 2) granular tissue: characterized by the presence of fibroblasts, myofibroblasts and neovascularization, 3) fibrosis: characterized by the density of collagen fibers identified by the intensity of blue color observed under optical microscopy due to staining by Masson's trichrome. A score was made for all parameters evaluated : - = absent, + = mild presence, + + = moderate presence; + + + = strong presence.

2.6. Statistical Analysis

Data were analyzed using non-parametric tests. To detect differences between groups, the Kruskal-Wallis was used. All results were expressed as mean values for group ± standard deviation and analyzed considering $p < 0.05$ as statistically significant.

3. Results and Discussion

3.1. Study Design

This experimental model was established to standardize thermal burn injuries in order to obtain injuries with the same size and depth degree. The choice of Wistar rats is due to these animals show a great ease of handling, accommodation and resistance to surgical aggressions and infectious processes, with low mortality [19, 20]. However, the choice of male rats is due to variations in hormonal cycles in females that could intervene in the process of tissue repair [21]. Infected wounds heal more slowly, re-epithelisation is more prolonged and there is also the risk of systemic infection [22].

Shaving the back of the animals was performed by manual traction of hair (Figure 2A) thus preventing secondary skin lesions that often occurs by the use of laminated devices [23]. The option to induce only one burning in the dorsal-proximal aimed at preventing the animal itself could reach the burn so that altering the outcome of the clinical evaluation of lesions. The use of individual aluminum bar for each animal in the experimental group is important in reducing the interval between the induction of a burn and another within the same group, thus avoiding large variations in the assessment of healing time. The size of lesions showed uniform average distribution of 10 ± 1 mm in diameter (Figure 2B). Similar studies by Heredero and colleagues [20] and Meyer and Silva [24] revealed that it is not possible to perform a perfectly uniform burn in all experimental rats.

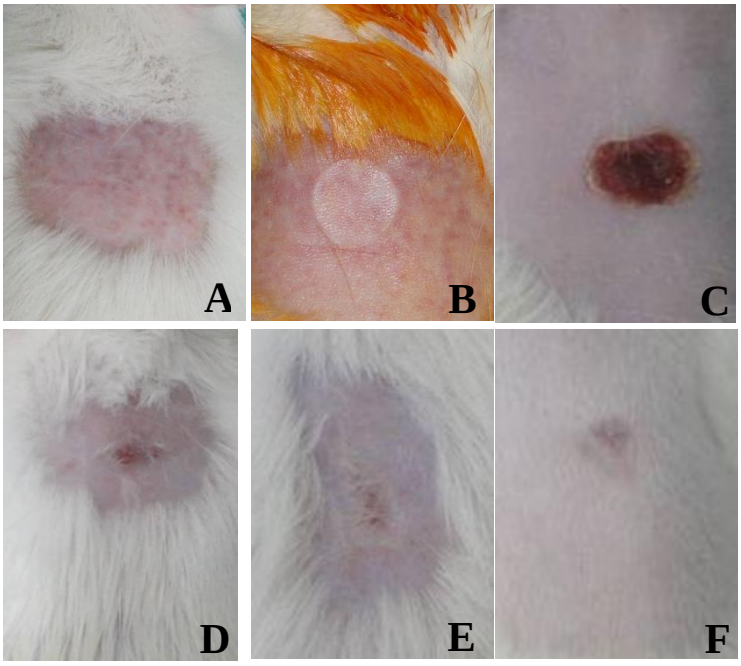

Figure 2. Clinical evolution observed in the experimental model of deep second-degree thermal burns in male Wistar rats: **A** - Animal's skin after shaving. **B** - Thermal lesion obtained with 10 mm diameter bar, with presence of mild edema. **C** - Injured tissue on day 7 after burn induction, presence of thin and dry crust with homogeneous staining and discreet detachment on the edges. **D** - Damaged tissue on day 14 after burn induction, presence of granulation tissue in the center of the lesion with a second discreet crust and formation of scar tissue at the edge. **E** - Injured tissue on day 21 after the burn induction: discreet presence of granulation tissue with the presence of scar tissue. **F** - Injured tissue at day 28 after burn induction: tissue with incomplete healing.

According to Vale [25], the burn depth depends on the intensity of the thermal agent, generator or heat transmitter and time of contact with the tissue, which is the determinant of the aesthetic and functional result of the burn. Medeiros et al [26], caused thermal burns by using 5 cm^2 aluminum plate heated to 130° C, which were pressed into the skin of the back for 5 seconds. However, this method can generate lesions with different depths depending on the pressure during the procedure. In our study, the pressure was equivalent to the mass of the aluminum bar (51 g) there being no interference by researcher, thus ensuring the reproducibility of thermal injuries.

The standardization of procedures, systematization and organization of knowledge about the interrelationships of models it is necessary to provide more reliable knowledge advance [27]. The most common method for obtaining second-degree thermal burns uses hot water as heat transfer agent. Khorasani et al. [28] induced second-degree thermal burns on the back of rats using submersion in hot water (90° C) for 6 seconds. In this experiment, 10 % body surface of the animal was injured producing lesions of variable size. According to Meyer and Silva [23], burns when reaching 26 % to 30 % of total body surface area of these mice cause mortality rates of 40 % after three days, 52.5 % after 7 days, 57.5 % after 15 days and 62.5 % after 25 days.

3.2. Macroscopic Evaluation

Results of this study revealed thermal burns white in color, painful, with no bubbles, mild edema until the 3rd day after injury (Figure 2B). Similar definition is reported by [29, 30] that describes the deep second-degree burns and injuries that have pale color with pain in lower intensity compared to superficial second-degree burn. In our evaluation was observed variation of the degree of hyperemia in the first three days of experiment that changed from slight to absent (Table 1). The formation of a thick and dry crust was observed from day 3 after burn induction. Signs of the scar tissue formation at the edge of the lesion were observed from day 14 (Figure 2D).

Table 1. Clinical parameters evaluated in the experimental model of deep second degree thermal burns in male Wistar rats.

Time	Animal	Clinical signs of the experimental model			
		Edema	Hyperemia	Crust	Scar tissue
7th day	1	+	+	*	-
	2	+	-	*	-
	3	+	-	*	-
14th day	1	-	+	-	+
	2	+	-	-	+
	3	-	-	-	+
21st day	1	-	-	-	+++
	2	-	-	-	+++
	3	-	-	-	+++
28th day	1	-	-	-	++
	2	-	-	-	+
	3	-	-	-	+

The intensity of clinical signs was scored as: - = absent; * = present, + = mild, + + = moderate, + + + = strong.

The burn healing occurs by second intention, which is a slow process with high risk of infection, producing scar retraction, which depending on the area of injury can cause extensive scarring and consequently high cost in treatment [31]. The contraction of skin lesions occurs centripetally from the injury edges being caused by the action of myofibroblasts present at the site. In turn, myofibroblasts may promote lesion retraction from 50 to 70 % of original size [32]. The percentage of lesion contraction at the end of the experiment was 66.67 ± 1.66 %. Values obtained in this study are similar to those published by Zohdi and colleagues [33], who observed 72.75 ± 1.8 % of reduction in control rats treated with hydrogel without drug (placebo) at 28 days of study.

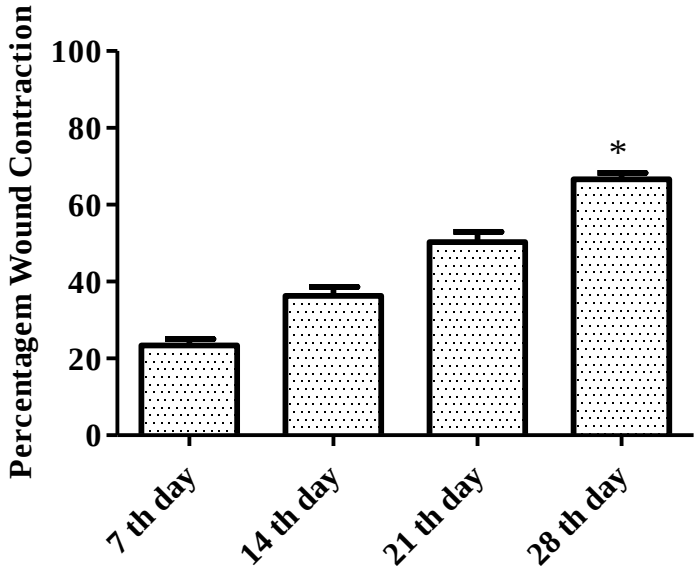

Figure 3. Contraction area percentage of deep second-degree thermal burns in the experimental model in male Wistar rats: n = 3. Values are mean ± SEM. * p <0.05.

According to Mandelbaum and colleagues [34], the mechanism of tissue repair is the integration of dynamic cellular and molecular processes involving biochemical and physiological phenomena aiming at ensuring tissue restoration. For this reason, only the clinical evaluation of a burn injury does not provide information on the evolution degree of tissue healing, being of fundamental importance the histopathologic evaluation of these lesions.

Microscopic Evaluation

The histopathological findings confirmed the acquisition of deep second-degree burns based on the observation of total autolysis of both the dermis and epidermis, without reaching the hypodermis. These data are consistent with reports of several authors who characterize it as deep second-degree burn injuries that cause partial or total destruction of nerve endings, hair follicles and sweat glands [25, 35, 36].

Thermal injury was observed on the 7th day and extensive inflammatory exudate featuring an intense inflammatory reaction. Orgaes and colleagues [22] describe in their study

the occurrence in the control group, treated with saline solution, an acute inflammatory process on the 6th day of evaluation. By day 14 the inflammatory response was classified as moderate with presence of macrophages, progressing to discreet at day 21. By day 28 was not observed signs of inflammatory response in the animals evaluated (Table 2).

Table 2. Histopathological analysis on the degree of inflammatory intensity, presence of granulation tissue and fibrosis in the skin after deep second-degree thermal burn. Samples were obtained on days 7, 14, 21 and 28 day after burn wound induction

Time	Animal	Inflammatory response	Granulation Tissue	Fibrosis
7th day	1	+++	+	-
	2	+++	+	-
	3	+++	+	-
14th day	1	+	++	+
	2	++	+++	+
	3	++	+++	+
21st day	1	+	+	+
	2	+	+	++
	3	+	++	++
28th day	1	-	-	++
	2	-	-	++
	3	-	-	++

Intensity of the evaluated parameters was scored as: - = absent, + = mild presence, + + = moderate presence; + + + = strong presence.

Tissue still presented a complete destruction of the dermis and epidermis and maintenance of the hypodermis (Figure 4A) on the 7th day after lesion induction. Histopathology section of the burned skin of control animals on 5th day showed denuded epidermis, diffuse infiltration of plasma cells, lymphocytes and polymorphs [37]. After 14 days the histopathological evaluation revealed moderate autolysis of the tissue, with discrete neovascularization and fibroblast proliferation, with loose collagen fibers, not modeled with mild fibrosis and crust absence (Figure 4B). Yaman and colleagues [38] confirm the presence

of crust formed by remnants of necrotic tissue and infiltration of mononuclear cells on the 4th day of experimentation in the control group. The crust detachment was only observed by these authors on the 14th day of study.

By day 21 we observed the absence of autolysis, discrete neovascularization and intense fibroblastic proliferation, with dense collagen, not modeled and moderate fibrosis (Figure 4C). By the end of the experiment at 28 days, histological observations showed incomplete re-epithelialization of the injured tissue with autolysis and absent neovascularization, showing moderate fibroblastic proliferation and fibrosis with the presence of modeled dense collagen fibers (Figure 4D).

Wound healing includes number of stages like clotting, inflammation, granulation, fibrosis, arrangement of collagen with spasm of wound and epithelization. The time required for complete healing of deep second-degree burns, without the application of specific therapeutic agents, can be three to six weeks or more, and these burns will leave a scar tissue that may hypertrophy and contract itself [29, 30].

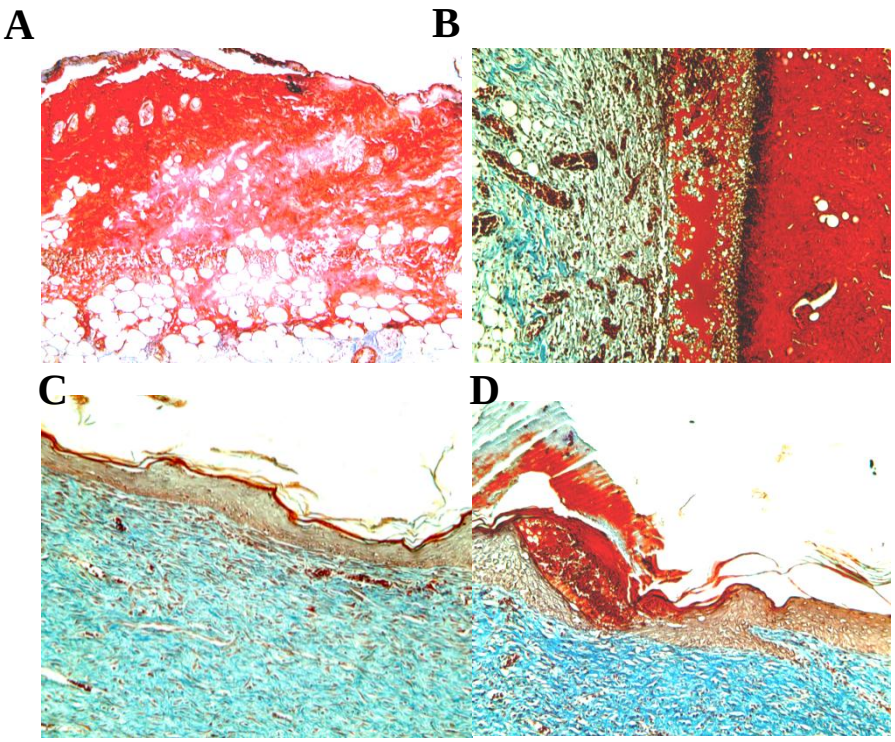

Figure 4. Histopathological aspects of deep second-degree thermal burns. Masson's trichrome staining. 100x Magnification. **A** - Animal showing thin crust and epithelial tissue with complete destruction of dermis and epidermis and hypodermis maintenance at the 7th day after the thermal lesion induction. **B** - Animal at day 14, with crust and tissue reepithelialization, showing collagen, not modeled and slight fibrosis. **C** - Animal at day 21, tissue repithelialization showing intense fibroblastic proliferation with the presence of dense collagen, not modeled and moderate fibrosis. **D** - Animal at day 28, with incomplete tissue repithelialization, moderate fibroblastic proliferation, presence of modeled dense collagen mesh and moderate fibrosis.

4. Conclusion

In this new model of second-degree thermal burns, injuries are easy to create and easily reproducible. There are similarities with the human second-degree burns in clinical and pathologic aspects. Thus, the animal model presented in this study is applicable in evaluating the use of therapeutic agents in the healing evolution of deep second-degree burns.

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7. ARTIGO II

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Research Article

Topical Application Effect of the Isolectin Hydrogel (Cramoll 1,4) on Second-Degree Burns: Experimental Model

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Abstract

This study aimed at evaluating the use of hydrogel isolectin in the treatment of second-degree burns. Twenty male rats were randomly divided into two groups (G1 = treatment with hydrogel containing 100 μg / ml Cramoll 1,4; and G2 = Control, hydrogel). After 7, 14, 21, 28 and 35 days, animals were euthanized. On the 7th day G1 showed intense exudates, necrosis and edema. On the 14th day G1 showed tissue reepithelialization and moderate autolysis. On the 21st day G1 showed intense fibroblastic proliferation, presence of dense collagen and moderate fibrosis. On the 28th day G1 showed complete tissue epithelialization. On the 35th day G1 showed modeled dense collagen. The significant wound contraction was initiated from day 14 in the G1. There were no significant differences in biochemical and hematological parameters analyzed. These results extend the potential of therapeutic applications for Cramoll 1,4 in the treatment of thermal burns.

1. Introduction

Since prehistory, plants and their by-products were used to treat wounds. Cramoll 1.4 is lectin extracted from seeds of *Cratylia mollis* Mart, a plant native to north-eastern Brazil. Cramoll is specific for glucose/mannose. Four multiple forms have been purified from *C. mollis* - Cramoll 1, Cramoll 2, Cramoll 3, Cramoll 4 – and preparations containing multiple combined forms as 1 and 4, named Cramoll 1.4 [1]. Studies have demonstrated that Cramoll 1,4 is capable of: i) isolating glycoproteins from human plasma [2], ii) characterizing transformed mammary tissue [3], iii) inducing mitogenic activity in human lymphocytes [4], iv) producing IFN-y and nitric oxide [5] and v) antitumor activities [6].

Burn wounds are one of the health problems in modern societies associated with irreparable harms and side many problems for patients and their families [7]. Burns are classified by their depth and severity such as 1st, 2nd, 3rd and 4th degrees. The pathophysiologic reaction to a burn injury is complex and varies with the cause (thermal, chemical, electrical or radiation). In thermal injuries changes in the burn wound are mainly caused by heat direct effects, but superimposed on these are changes associated with the acute inflammatory process. It is these latter changes that account for the widespread and devastating effects of major burns on the entire body's homeostatic function [8]. In addition to the physiological morbidity that burns these types of injuries are associated with a huge financial burden on the public health system.

In order to ease the pain of burning and minimize the number of dressing changes, several studies have been carried out in search of formulations that help in healing. The advent of dry bandages occurred in the nineteenth century due to the germ theory authored by Louis Pasteur. In the twentieth century, with advances in knowledge about the mechanisms involved in tissue lesion healing, the theory that the wounds in a wet environment have better healing capacity was developed [9]. In order to meet this need, the wet bandages containing natural and synthetic molecules have shown significant effect on the healing mechanism. In this sense, aiming to evaluate the effects of topical application of hydrogel containing 1,4 Cramoll isolectin, this study investigated *in vivo* the clinical and histopathological features of second-degree thermal burns demonstrated experimentally in rats of Wistar strain.

2. Materials and methods

2.1. Plant material

2.1.1. *Cratylia mollis* (extraction and purification)

Cramoll 1,4 isolectin was purified from a 10 % (w/v) seed extract of *Cratylia mollis* in 0.15 M NaCl according to the protocol reported in Correia & Coelho [10]. Briefly, all seeds (camaratu bean) collected in Ibimirim City, State of Pernambuco were washed with distilled water, dried at room temperature and blended in 0.15 M NaCl. After 16 h of gently stirring at 4 °C, the extract was filtered and centrifuged for 12 000 *g*. The extract was ammonium sulfate fractionated, dialysed against 0.15 M NaCl (fraction 40 - 60 %) and affinity chromatographed on Sephadex G-75 (Sigma Chemical Company) in column (70.0 x 1.9 cm) containing 200 ml packed matrix, balanced with 0.15 M NaCl. After sample application, 0.15 M NaCl was passed through the column until *A*280 nm was less than 0.1; isolectin was eluted with 0.3 M glucose in 0.15 M NaCl. Fractions with highest *A*280 nm were pooled, exhaustively dialysed in buffer citrate phosphate and then lyophilized. The native isolectin has 8.5 - 8.6 p*I* measured by isoelectric focusing in polyacrylamide gel and 31 Kda main polypeptide.

2.2. Isolectin Hydrogel

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 Co^{60} at 15 kGy h^{-2}[11].

2.2.1. Evaluation of hemagglutinating activity of the isolectin hydrogel

The hemagglutinating activity was performed in microtiter plates according to Correia and Coelho [10]. Samples of isolectin hydrogel (50 μl) were serially diluted in 0.15 M NaCl

before adding 5 µl of a 2.5 % (v/v) rabbit erythrocytes suspension previously treated with glutaraldehyde. The title was expressed as the highest dilution showing hemagglutinating activity. Assay performed in triplicate.

2.3. Animals and experimental wounds

2.3.1. Animals

All animals received humane care and studies reported in this manuscript have been carried out in accordance with the guidelines for human treatment of animals set by the Brazilian College of Animal Experiment. The study was approved by the Committee on Animal Research at the Federal University of Pernambuco, Brazil (23076.015015/2009-31). A total of twenty male Wistar rats (*Rattus norvegicus*, albinus), 8-10-week-old and weighing approximately 250 - 300 g were used in this study. Food pellets and water were provided *ad libitum* throughout the experiment.

2.3.2. Burn injury

Animals were divided randomly into two groups of 10 (G1 and G2) and pre-anesthetized with atropine sulfate at 0.04 mg kg^{-1} intramuscularly. After ten minute an anesthetic combination was used through an intramuscular injection of xylazine 10 mg kg^{-1} and ketamine 90 mg kg^{-1} with subsequent dorsum trichotomy by direct hair tension (area measuring approximately 3 cm^2) (Figure 1A) and antisepsis with 1 % polyvinylpyrrolidone-iodine. Burns were symmetrically caused on depilated areas through contact with an aluminum bar (r = 10 mm), preheated for 100 $^{\circ}C$ for 15 s (Figure 1B). 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 (Figure 1C). Group-1 was treated with empty hydrogel containing 100 µg Cramoll 1,4. Group-2 (control) was treated with hydrogel without isolectin.

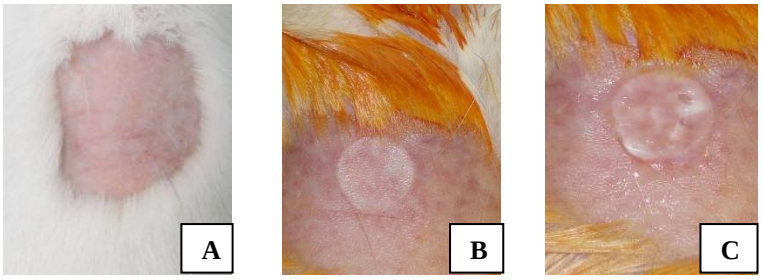

Figure 1.: Induction of second-degree thermal burns in male Wistar rats. **A** – Back trichotomy by direct hair tension, **B** - depth second-degree thermal burn with r = 10 mm; **C** - Treatment of thermal burn using 100 µl hydrogel.

2.4. Pathological observations

2.4.1. Clinical parameters

Burns surface were evaluated based on the following parameters for 35 consecutive days: edema, hyperemia, exudation and the firmness of wound surface, presence or absence of granulation tissue and scar tissue. Wounds were considered closed if moist granulation tissue was no longer apparent and wounds seemed covered with new epithelium. Body weight was determined using electronic balance (accuracy to g) on the day of burn induction as well as day 7, 14, 21, 28, and 35 after wounding.

2.4.2. Wound retraction quantification

All the rats were examined weekly under anesthesia for observation of wound contracture. The wound retraction was evaluated in 7, 14, 21, 28 and 35 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 [12].

2.4.3. Biochemical and hematological evaluations

Blood from two animals per group were collected on days 7, 14, 21, 28 and 35 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.

2.4.4. Histopathology

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.

2.5. Statistical analysis

Data were analyzed using non-parametric tests. To detect differences between groups, the Mann–Whitney U test was used. All results were expressed as mean values of groups ± standard deviation and analyzed considering $p < 0.05$ as statistically significant.

3. Results and Discussion

Overall, all animals were clinically well (showing normal behavior of species and ingestion of food and water) during the experiments. There was no bleeding during surgery. Neither the rats under treatment nor the control group showed any statistically significant changes on the body weight throughout the experiments, showing that analgesia was adequate

for the injury caused. As reported by Hellebrekers [13], the main signs of pain in laboratory animals subjected to experimental procedures are directly related to changes in behavior, being anorexia one of the most significant signs.

3.1. Hemagglutinating activity

Due to the immunostimulating and mitogenic activities attributed to lectins, the therapeutic use of these proteins in tissue repair processes has been subject of much research either related to the lectin concentration or the formulation used [14].

Lectins or hemagglutinins can be detected and characterized by their ability to agglutinate erythrocytes. In the evaluation of hydrogel containing 1,4 isolectin Cramoll was observed that Cramoll-1,4 at 50 μg / ml in both pure and gel formulation after irradiation lost their hemagglutinating activity. On the other hand, the concentration 100 μg / ml remained constant for the irradiated pure isolectin and that combined to the hydrogel excipient (Figure 2).

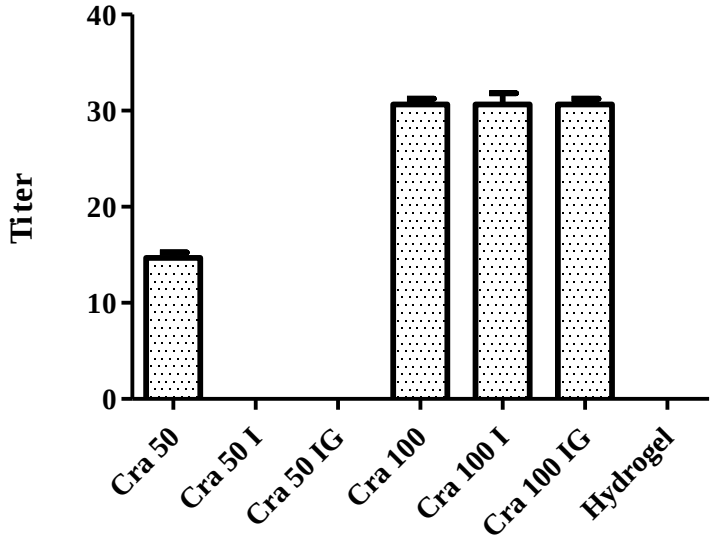

Figure 2: Evaluation of hemagglutinating activity of 1,4 Cramoll isolectin combined to the hydrogel excipient. Cra 50: Pure Cramoll 1,4 isolectin at 50 μg / ml. Cra 50 I: Pure Cramoll 1,4 isolectin at 50 μg / ml irradiated (15 kGy h^{-2}). Cra IG: Pure Cramoll 1,4 isolectin at 50 μg / ml associated with hydrogel excipient and irradiated (15 kGy h^{-2}). Cra 100: Pure Cramoll 1,4

isolectin at 100 µg / ml. Cra 100 I: Pure Cramoll 1,4 isolectin at 100 µg / ml irradiated (15 kGy h^{-2}). Cra IG 100: Pure Cramoll 1,4 isolectin at 100 µg / ml associated with hydrogel excipient and irradiated (15 kGy h^{-2}). Hydrogel irradiated without lectin. The title was expressed as the highest dilution showing hemagglutinating activity. Values are mean ± SEM.

Several aspects make the hydrogel an ideal bandage for treatment of tissue lesions, such as: hydrophillicity, biocompatibility, non-toxicity, biodegradability, easy replacement, transparency, adhesion, absorption, and prevention of body fluid losses [15, 16]. Burd [17] evaluated the use of hydrogel sheet dressings in comprehensive burn wound care, noting that use of hydrogel in burn wound care reduces patient's pain sensation. Osti [18] evaluated the use of a transparent adhesive film possessing selective permeability combined with a hydrogel (Burnshield) in burns treatment. For about 2 years, this type of therapy was used in the first aid treatment of 48 burn patients, 4 were lost during therapy and 4 were unavailable for following-up. In the reepithelialization phase complications were recorded in 8 of the 40 patients: 7 (18 %) had residual inflammation and 1 (2 %) had hypertrophic scar. During the follow-up, late complications were recorded in 2 (5 %) of the 40 patients. A gel was used in 8 patients: in 6 of the 7 patients with residual inflammation the complication was solved, while in 1, despite therapy, the residual inflammation evolved into hypertrophic scarring.

3.2. Clinical parameters

Wound cooling caused by burn is an urgency measure which proved to be beneficial in clinical and experimental practices. Hydrogels are cross-linking three-dimensional structures with high water percentage that can be transferred from the gel to the scar wound to facilitate hydration. The healing process of animals with aseptic experimental thermal burns treated topically with isolectin, had better response than the control in the clinical examination in several ways such as: (1) presence of edema in the first 24 h after induction of second-degree thermal burn, (2) thickening of the crust, which began to emerge spontaneously in 6 days of experiment, (3) discrete hyperemia observed in the range between 24 and 48 h after injury; (4) presence of scar tissue with 13 days of experiment (Figure 3). During the study period lesions showed no signs of infection. Severely burned skin ceases to perform its natural protective and

barrier role and allows a dramatic increase in water loss and can become a portal for bacterial invasion. The local treatment of second-degree burns is targeted at maintaining a wet microenvironment and stimulating the formation of a well-vascularised granulation tissue, and the re-epithelization of the lesion while counteracting the development of microorganisms, which is able to delay or prevent the biological phenomena of cicatrization and reepithelization [19].

3.3. Wound retraction quantification

The wound contraction is a parameter used for assessing wound healing. The lesion area decreased gradually with the progress of healing time in both groups. The significant wound contraction was initiated from day 14 in the G1 that showed highest rate of lesion contraction compared with G2, indicating that isolectin has inducing effect on the lesion contraction as illustrated in Figure 4. These results are consistent with studies *in vitro* and *in vivo* performed by Sezer et al [20], which demonstrated the efficacy of hydrogels in the treatment of dermal burns in rabbit model revealing that the application of fucoidan-chitosan hydrogel promotes burn wound contraction and inducing the healing.

Wound contraction, wound shrinking process, depends on the tissue's reparative abilities, type and damage extent and tissue health general state [21]. On the other hand, the wound contraction is rarely able to take to its permanent closure, which is mainly due to the presence of fibroblasts found in the granulation tissue that later differentiate into myofibroblasts [22].

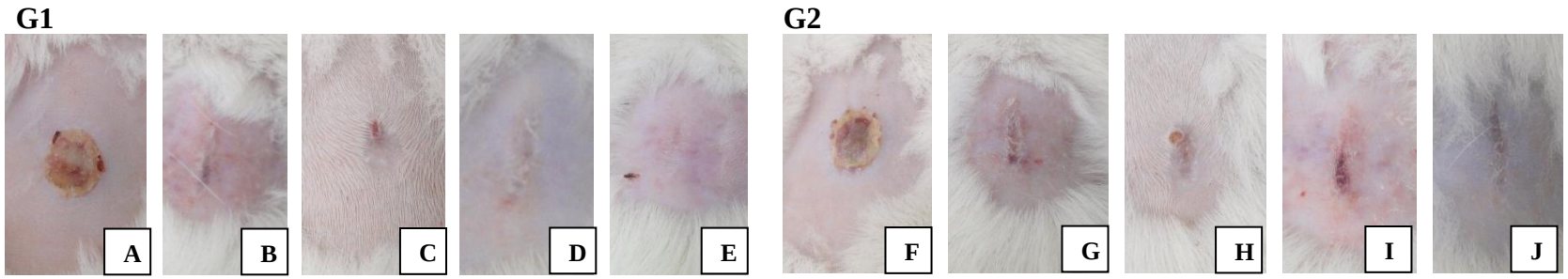

Figure 3.: Clinical evaluation of 2nd degree burn healing in Wistar male rats. **G1**: experimental group treated with hydrogel containing isolectin Cramoll 1,4 at 100 µg / ml. **A** - Thermal lesion aspect after 7 days: macroscopically shows thin and dry crust with detachment of edges. **B** - Thermal lesion aspect after 14 days of treatment: absence of crust and the presence of scar tissue. **C** - Thermal lesion aspect after 21 days of treatment: presence of scar tissue and a small detachment point of the crust. **D** - Thermal lesion aspect after 28 days of treatment: presence of scar tissue only. **E** - Thermal lesion aspect after 35 days of treatment: view of a discrete scar tissue. **G2**: control group treated by topical application of hydrogel excipient. **F** - Thermal lesion aspect in control animals after 7 days: view of thin and dry crust with detachment of edges. **G** - Thermal lesion aspect in control animals after 14 days: absence of crust and the presence of scar tissue. **H** - Thermal lesion aspect in control animals after 21 days: presence of scar tissue, with the point of detachment of the crust. **I** - Thermal lesion aspect in control animals after 28 days: presence of scar tissue and a second crust. **J** - Thermal lesion aspect in control animals after 35 days: view of scar tissue.

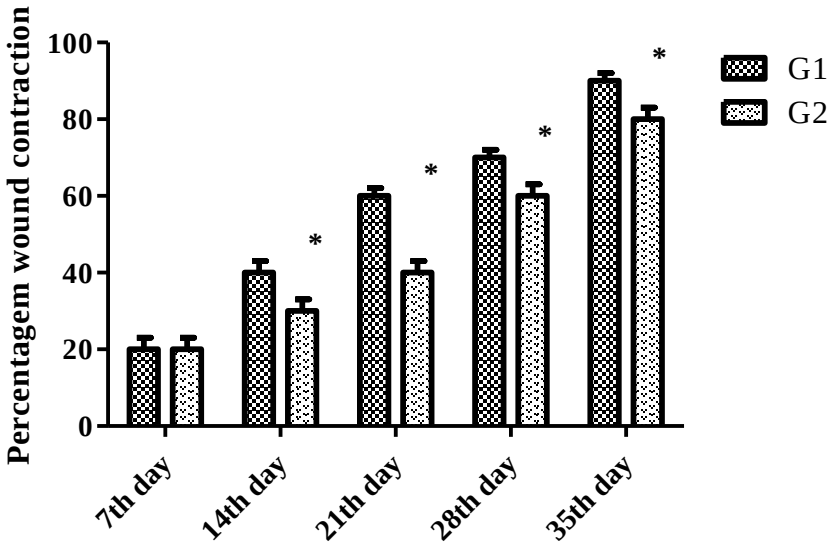

Figure 4. Effect of hydrogel topical application on the burn wound expressed as percentage of wound contraction. G1 = Treatment, G2 = Control. n = 2. Values are mean ± SEM. *p <0.05.

3.3. Biochemical and hematological evaluation

Hematological values obtained in this study showed no significant changes as function of burn induction during the period analyzed (Erythrocytes: 7.6 ± 0.48; Hemoglobin: 13.65 ± 0.5; Platelets: 846400 ± 0.71, leukocytes: 7980 ± 0.71; Basophils: 0.2 ± 0.05; Eosinophils: 1.38 ± 0.18; Lymphocytes: 82.37 ± 0.83; Monocytes: 1.9 ± 0.2) (Table 1), revealing normal values in rats [23]. 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 [24].

However, average values of biochemical parameters analyzed in this study were consistent with previously reported specific data to normal animals (Calcium: 10.04 ± 0.42; Pro-Thrombin: 9.94 ± 0.16; Fibrinogen: 457.32 ± 0.25; Alkaline Phosphatase: 212.68 ± 0.52; Glutamic Oxalic Transaminase: 180.02 ± 0.35; Glutamic Pyruvic Transaminase: 53.28 ± 0.41; Gamma-Glutamyl Transpeptidase: 5.76 ± 0.23, Creatine: 0.54 ± 0.04; urea: 46.34 ± 0.04 and Amylase: 842.06 ± 0.48) (Table 2). The biochemical evaluation revealed increased ALT levels

in response to injury by burning and alkaline phosphatase-related to inflammatory period of the healing process. On the other hand, metabolic changes are considered high risk in third-degree burns with hyperglycemia [25] and high protein catabolism [26] as the main aggravating factors to the injury.

After burn trauma, inflammatory mediators, oxygen free radicals, arachidonic acid metabolites and complement [27], released in the wounds promote a great edema. According to Beukelman et al [28], liposomal hydrogel with 3 % povidone-iodine (PVP-ILH, Repithel®) has shown clinical benefit in settings where inflammation and/or reactive oxygen species are thought to impede wound healing (e.g., burns and chronic wounds in smokers). According to Moller-Kristensen et al [29], the MBL, mannan-binding lectin, modulates not only inflammatory factors such as cytokines and chemokines, but also cell adhesion molecules, the binding growth factor protein and, MPPs in particular, metalloproteinase matrix, which are the most likely direct effectors in scabs detachment.

Considering the influence of carbohydrates in numerous cell signaling phenomena whether physiological or pathological, the use of lectins in the treatment of cutaneous lesions among other diseases, stimulates the activation and modulation events such as communication, cellular differentiation and proliferation [30, 31, 32].

3.4. Histopathology

Deep partial thickness burns are injuries that cause partial or total destruction of nerve endings, hair follicles and sweat glands. On the seventh day was observed intense fibroblastic proliferation, neovascularization, necrosis and edema. In upper layer of dermis most hair follicle walls, sebaceous follicles and sudoriparous glands disappeared and only their residual bodies could be found. Capillary vessels were fractured. The epidermis showed necrosis with infiltration of large numbers of neutrophils and few monocytes, leukocytes and plasma of the dermis (Figure 5B). These data are similar to observations reported by Nunes et al [33] that when evaluating the application of a collagen film containing acid usnic as bandage to treat second-degree thermal burns found intense inflammatory response after 7 days with presence of neutrophils distributed throughout the length of the burn.

With 14 days of experiment, G1 and G2 showed granulation tissue with presence of discrete neovascularization and neoformation of skin appendages. Angiogenesis is essential to restore the supply of nutrients and oxygen during tissue healing [34]. In group 1 was observed increased number of fibroblasts and presence of collagen begins organized in the lesion center (Figure 5C). Experiments performed by Sezer et al [35] demonstrated that fibroblast and collagen amounts in fucosphere treated groups increased at day 14 compared to that at day seven, but decreased at day 21. The reepithelialization time was lower for animals treated with isolectin hydrogel and started around the burn edge on the 14th day. Epithelialization is necessary in the repair of all type of wounds if water tight seal occur. Protection from fluid and particulate-matter contamination and maintenance of internal milieu are dependent on keratin's physical characteristic [36]. The experimentally-induced thermal injuries have been completely reepithelialized in both groups with 35 days.

Histopathology revealed the intense fibroblast proliferation at 21st day, presence of dense collagen, not modeled and moderate fibrosis (Figure 5D). Collagen deposition in the fibroplasia phase is required for the efficient arrival of fibroblasts to the burn site. Mature fibroblasts produce a delicate matrix that gives mechanical support to the new capillaries [37]. The collagen deposited at the injury site will not have the same unique organization of an intact tissue, being required a period of two months to complete restructure [38]. The decrease in epithelium thickness on the day 21 was considered by Sezer et al [39] as the result of higher healing rate, particularly on the superficial burn wound treated with chitosan film containing fucoidan.

After 28 days, both G1 and G2 showed gradual decrease in the number of fibroblasts with greater organization of the collagen matrix with reduced inflammatory infiltration (Figure 5E). Finally, 35 days after burn procedures, the injured tissue of group 1 is at the stage of maturation and remodeling, with the presence of few fibroblasts and inflammatory cells (Figure 5F). The histological analysis of liver sections in group G1 showed no cytotoxic effects resulting from topical application of isolectin hydrogel at the end of treatment after 35 days. These results are consistent with previous studies performed by our group that found the healing action of isoforms 1 and 4 of *Cratylia mollis* lectin in the repair of skin wounds in normal and immunosuppressed mice [40].

Several studies have confirmed the use of lectins in the immune system activation,

enlisting neutrophils through indirect mechanisms [41], promoting pro-inflammatory effects in polimorphonuclear cells and inducing the cytokines release [42], as well as triggering fibroblasts proliferation [43]. Previous assays accomplished by our group have shown a potential pro-inflammatory and immunomodulatory activity induced by Cramoll 1,4 lectin. The importance of glycoproteins (including lectins) as components of *Aloe vera* extract gel has been asserted for promoting wound, burn and frost-bite healing, and showing anti-inflammatory and antifungal properties [44]. Sell and Costa [45] also described improved effect of PHA lectin in the skin tissue repair process of Wistar rats compared to *Triticum vulgaris* (WGA) and *Artocarpus integrifolia* (jacalin) lectin.

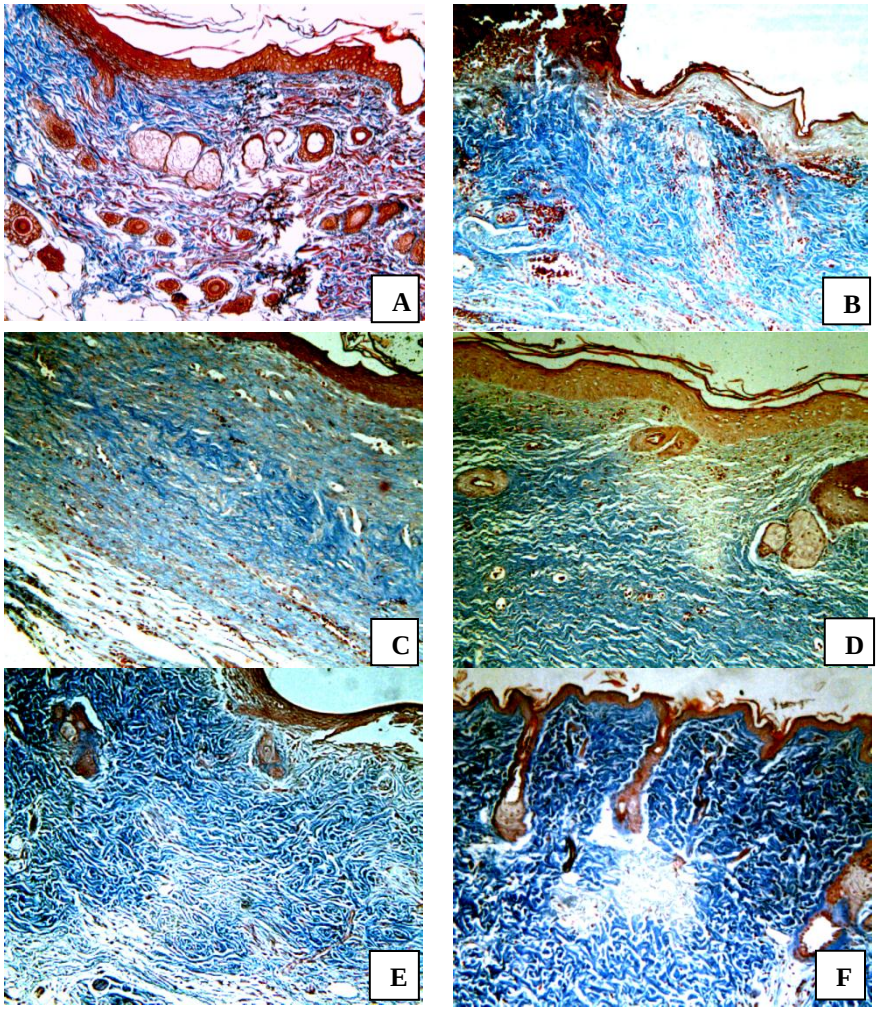

Figure 5.: Epithelial tissue of rats in group 1 subjected to second-degree thermal burns. Masson's trichrome staining. 100x Magnification. **A** - Normal epithelial tissue with all skin appendages. **B** - Animal presenting epithelial tissue with complete destruction of the dermis and epidermis showing exudates albumin/leukocyte/macrophage intense, necrosis, edema and crust at the 7th day after injury induction. **C** - Animal at the 14th day with tissue re-epithelialization, moderate autolysis, moderate exudate albumin/leukocyte/macrophage, intense neovascularization, discrete fibroblast proliferation with the presence of loose collagen and mild fibrosis. **D** - Animal at the 21st day with incomplete tissue reepithelialization, mild exudate albumin/leukocyte/macrophage, moderate neovascularization, intense fibroblastic proliferation, presence of dense collagen, not modeled and moderate fibrosis. **E** - Animal at the 28th day with complete tissue epithelialization, exudate albumin/leukocyte/macrophage discrete in the epidermis, moderate fibroblastic proliferation, presence of modeled dense collagen mesh and moderate fibrosis. **F** - Animal at the 35th day with complete reepithelialization, mild fibroblastic proliferation, presence of modeled dense collagen mesh and moderate fibrosis.

5. Conclusion

The present study has demonstrated that the regular topical application of Cramoll 1,4 hydrogel containing in treatment of second-degree burns accelerates the granulation, reepithelialization process and wound retraction. These results extend the potential of therapeutic applications of isolectin Cramoll 1,4, which can be used in combination with other byproducts in the treatment of thermal burns.

Table 1.: Effect of topical administration of hydrogel containing 100 μg per ml isolectin Cramoll 1,4 on the hematological parameters of Wistar rats. Assays performed in triplicate for each parameter. G1 = Treatment, G2 = control. Mean ± SD (n = 2).

Parameters	7th Day		14th day		21st Day		28th day		35th day	
	G1	G2	G1	G2	G1	G2	G1	G2	G1	G2
Erythrogram										
Erythrocytes mil/mm^3	6.7 ± 0.01	7.2 ± 0.14	7.1 ± 0.16	7.57 ± 0.69	7.6 ± 0.69	7.5 ± 0.42	6.32 ± 0.56	8.1 ± 0.21	6.3 ± 0.71	7.7± 0.92
Hemoglobin g/dl	13.9 ± 0.01	13 ± 0.14	14.8 ± 0.52	15.59 ± 0.41	15.6 ± 0.41	12.4 ± 0.64	13.7 ± 0.38	12.8 ± 0.49	14.1 ± 0.32	14.5 ± 0.42
Hematocrit %	38.7 ± 0.22	41.1 ± 0.49	40.6 ± 0.56	43.22 ± 0.96	43.2 ± 0.96	41 ± 0.85	38.5 ± 0.55	39.8 ± 0.92	41.9 ± 0.84	40.8 ± 0.49
Platelet Count										
Platelets mil/mm^3	844000 ± 0.71	805000 ± 0.71	656000 ± 0.71	926000 ± 071	788000 ± 0.71	820000 ± 0.71	844000 ± 0.71	789000 ± 0.71	749000 ± 0.71	892000 ± 0.71
WBC										
Leukocytes %	7200 ± 0.71	8000 ± 0.71	8100 ± 0.71	7900 ± 0.71	12000 ± 0.71	8100 ± 0.71	9300 ± 0.71	7900 ± 0.71	8000 ± 0.71	8000 ± 0.71
Neutrophils %	15.1 ± 0.07	26.8 ± 0.78	26.4 ± 0.28	31.3 ± 0.56	8.7 ± 0.63	28.8 ± 0.42	14.7 ± 0.71	27.5 ± 0.71	16.1 ± 0.2	33.1 ± 0.99
Eosinophils %	0 ± 0	0.1 ± 0.14	0.1 ± 0.07	1.6 ± 0.28	0.1 ± 0.07	2.4 ± 0.28	0.1 ± 0.14	1.3 ± 0.14	0.1 ± 0	1.5 ± 0.07
Basophils %	0.2 ± 0	0.2 ± 0.14	0.2 ± 0.07	0.2 ± 0	0.2 ± 0	0.2 ± 0.14	0.2 ± 0	0.2 ± 00	0.2 ± 0	0.1 ± 0
Typical Lymphocytes %	81.4 ± 0.64	81.5 ± 0.49	68.5 ± 0.70	86.85 ± 0.78	87.1 ± 0.84	82.7 ± 0.49	81.5 ± 0.56	79.9 ± 0.71	83.7 ± 0.46	80.9 ± 0.78
Atypical Lymphocytes %	0 ± 0	0 ± 00	0 ± 00	0 ± 00	0 ± 0	0 ± 0	0 ± 0	0 ± 00	0 ± 0	0 ± 0
Monocytes %	1.4 ± 0.07	2 ± 0.71	1.2 ± 0.07	2 ± 00	1.2 ± 0.07	1.5 ± 0.71	1.4 ± 0.07	1.5 ± 0.71	1.2 ± 0.07	2.5 ± 0.71

Table 2.: Effect of topical administration of hydrogel containing 100 μg per ml isolectin Cramoll 1,4 on the biochemical parameters of Wistar rats. Doses performed in triplicate for each parameter. G1 = Treatment, G2 = Control. Mean ± SD (n = 2).

Parameters	7th Day		14th Day		21st day		28th Day		35th day	
	G1	G2	G1	G2	G1	G2	G1	G2	G1	G2
Pro-thrombin time %	10.1 ± 0.07	9.7 ± 0.02	9.62 ± 0.11	10.1 ± 0.21	9.2 ± 0.21	10.1 ± 0.28	10.5 ± 0.71	10.1 ± 0.436	10.5 ± 0.70	9.7 ± 0.56
Fibrinogen mg/dl	460.5 ± 0.71	457.9 ± 0.07	407.1 ± 0.14	460.8 ± 0.21	412 ± 0.71	465.6 ± 0.47	380 ± 0.92	440.1 ± 0.142	407.7 ± 0.41	462.2 ± 0.34
Cálcium mg/dl	10.3 ± 0.14	9.6 ± 0.46	8.4 ± 0.98	9.6 ± 0.42	11.6 ± 0.14	9.4 ± 0.16	11.5 ± 0.71	11.5 ± 0.658	9.7 ± 0.59	10.1 ± 0.42
Alkaline Phosphatase U/l	193.6 ± 0.56	199.6 ± 0.49	212.7 ± 0.42	201.4 ± 0.57	208 ± 0.71	198.2 ± 0.31	275 ± 0.71	244.6 ± 0.601	209.7 ± 0.38	219.6 ± 0.62
Gamma glutamyl transferase U/l	5 ± 00	5.9 ± 0.14	5.7 ± 0.35	5.7 ± 0.29	5.8 ± 0.14	5.9 ± 0.02	5.3 ± 0.07	5.2 ± 0.012	5.6 ± 0.14	6.1 ± 0.72
Oxalic Transaminase glutamic U/l	142 ± 0.07	176.6 ± 0.54	136.5 ± 0.71	208.2 ± 0.33	193 ± 0.71	179.9 ± 0.04	141.5 ± 0.64	156.6 ± 0.506	177.9 ± 0.15	178.8 ± 0.31
Transaminase glutâmico pirúvica U/l	60.7 ± 0.42	50.8 ± 0.19	55.7 ± 0.04	54.5 ± 0.58	47 ± 0.42	48.7 ± 0.33	48.5 ± 0.63	51.9 ± 0.129	58.5 ± 0.71	60.5 ± 0.78
Urea mg/dl	46.3 ± 0.49	46.9 ± 0.05	43.7 ± 0.35	50.6 ± 0.91	40 ± 0.71	45.9 ± 0.06	41.5 ± 0.71	46.8 ± 0.331	37.9 ± 0.11	41.5 ± 0.68
Creatinine mg/dl	0.2 ± 0.07	0.6 ± 0.04	0.5 ± 0.07	0.6 ± 0.12	0.4 ± 0.07	0.6 ± 0.01	0.6 ± 0.04	0.50 ± 0.011	0.5 ± 0.14	0.4 ± 0.04
Amylase U/l	838 ± 0.14	846.6 ± 0.56	789 ± 0.71	866.7 ± 0.42	808.3 ± 0.87	887.5 ± 0.71	856.6 ± 0.84	799.7 ± 0.469	814.5 ± 0.71	809.8 ± 0.27

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8. ARTIGO III

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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 sem lectina). 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, neovascularization, 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.

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].

History reveals that concern for wound healing has always existed and that several plant extracts have been utilized to cure lesions. Cramoll 1,4 is a lectin extracted from seeds of *Cratylia mollis* Mart., a plant native to Northeastern Brazil. Cramoll 1.4 is a lectin extracted from seeds of *Cratylia mollis* Mart., A plant native to Northeastern Brazil. Cramoll lectin is a specific glucose/mannose having multiple four forms: Cramoll 1, Cramoll 2, Cramoll 3, Cramoll 4 [5]. Several studies have demonstrated the immunomodulatory profile of this lectin, production of IFN-γ and nitric oxide [6], mitogenic activity on human lymphocytes [7] and antitumor activity [8].

Burns are traumatic wounds caused in most cases, by thermal agents, chemical, electrical and radioactive. The extent and severity vary with the type of agent, time of exposure, depth and location body [9,10]. It is estimated around two million burn accidents per year in Brazil [11]. Burns are considered injuries that cause severe trauma, since they can lead patient to death or cause emotional and social disorders. Reference to the burn care in Pernambuco (Brasil), the Restoration Hospital reported 15 % increase in the number of visits made during the July festivities in 2011, over the same period in 2012. During this period there have been several accidents caused by fireworks, bonfires and coal [12].

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 [13]. In turn, the immune response to burns is a complex event influenced by a number of factors such as the extent and burn severity, depth, age, presence or absence of infection, type of treatment, etc. [9]. Several local and systemic

factors can delay or prevent healing, such as: inadequate nutritional support, oxygenation deficit in tissue necrosis, dry environment, immunosuppression, etc. [14]. 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 [15].

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 [16].

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 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.

2. 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 0.15 M 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 Co^{60} at 15 kGy h^{-1}[17].

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 [18], 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 % xilazine chloridrate (10 mg / kg) and 10 % ketamine chloridrate (115 mg / kg) in subcutaneous injections [19]. Each animal was placed in a prone position and prepared for aseptic surgery using 1 % polyvinylpyrrolidone-iodine. A standard Burns were symmetrically caused on depilated areas through contact with an aluminum bar (diameter = 10 mm), preheated for 100 °C for 15 s (Figure 1). After burn injury and animal a wakening, 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.

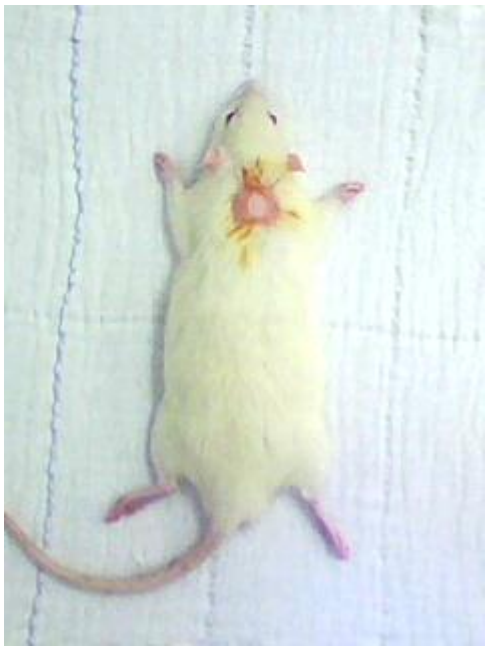

Figure 1. Appearance of the deep second-degree thermal lesion induced in experimentally immunosuppressed male Wistar rats. 10-mm burn in diameter made aiming at evaluating the healing effect of the lectin hydrogel (Cramoll 1.4).

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 [20].

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 24 h 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.

3. 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 isolectin 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 [21,22].

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 of 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 [16], 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 [23, 24]. In turn, the myofibroblasts can promote 50-70% lesion retraction from the initial size [25].

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 [26]. 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). 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 [27] and high protein catabolism [28] as the main aggravating factors to the injury. The other biochemical parameters were similar to those reported in the literature for healthy animals.

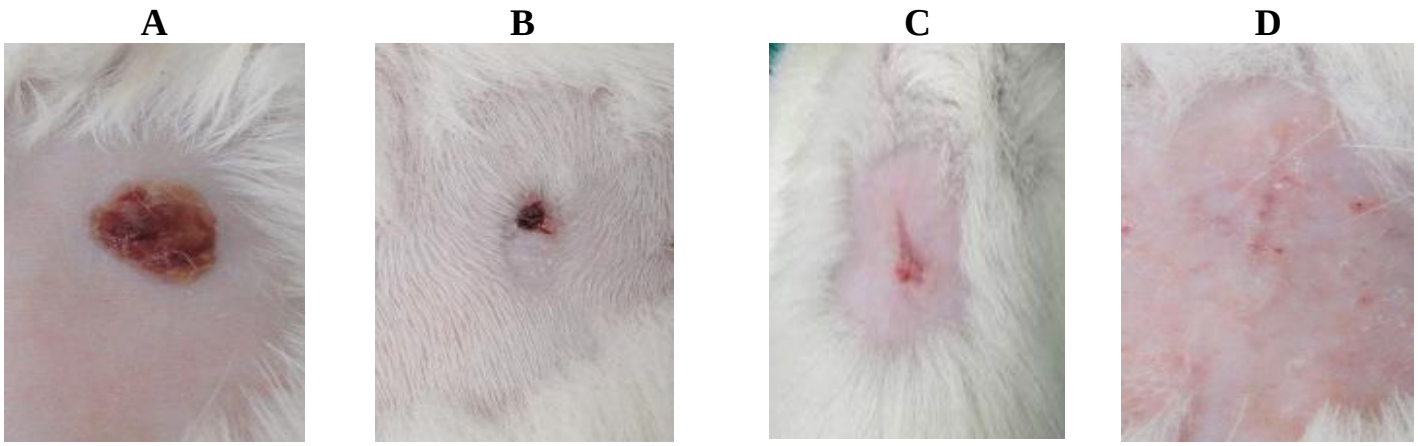

Figure 2. Healing clinical evolution of second-degree thermal burns in immunosuppressed rats experimentally treated by daily topical application of hydrogel containing 100 µl of lectin Cramoll 1.4 at 100 µg / ml. **A** - Presence of thin and dry crust with slight edges detachment. **B** - Presence of 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.

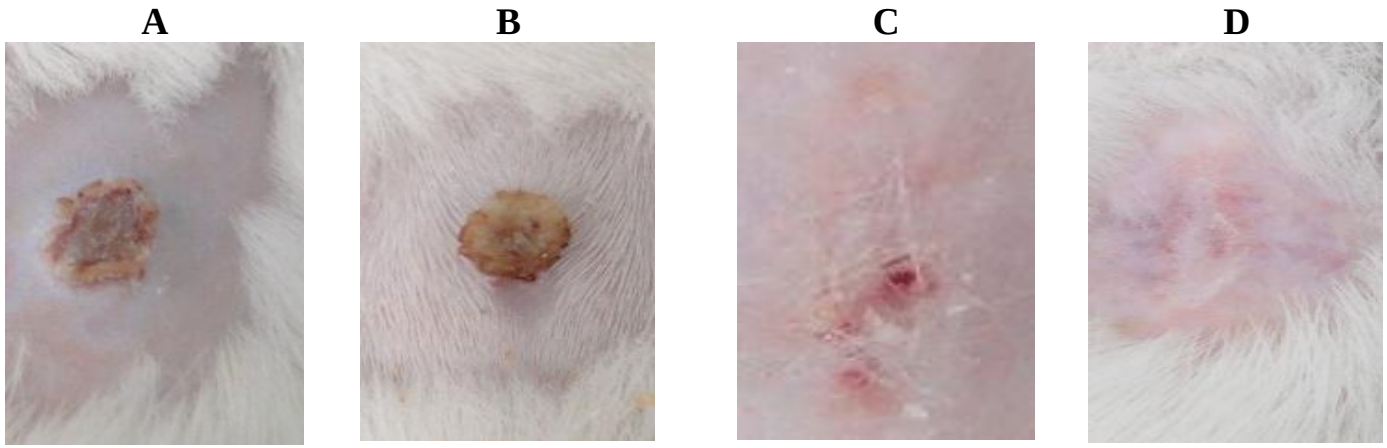

Figure 3. Healing clinical evolution of second-degree thermal burns in immunosuppressed rats experimentally treated by daily topical application of 100 µl hydrogel without lectin (control group) **A** - Presence of thin and dry crust with slight edges detachment. **B** - Presence of crust with strong 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.

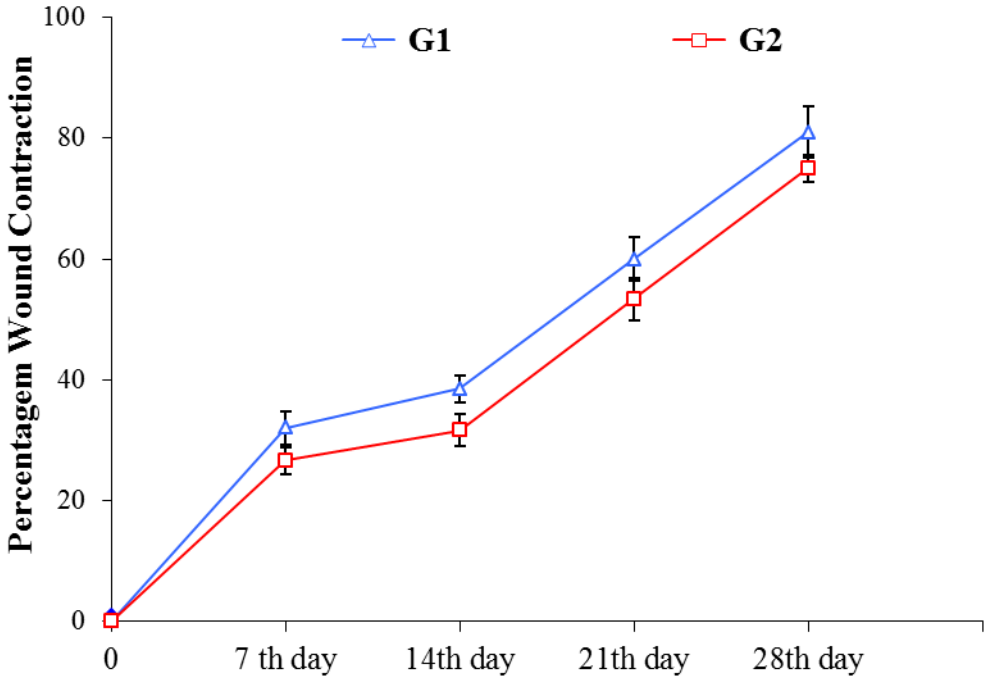

Figure 4. Contraction area percentage of deep second-degree thermal burn in the experimental model, in male Wistar rats. n = 3. Values are mean ± SEM. * p <0.05.

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 [29]. 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 [30].

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. The use of methotrexate to induce immunosuppression causes negative effects on the healing process. Peters et al. [31] 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. [32] 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 [33].

Due to the large molecular diversity of lectins, they have distinct roles in modulating the physiological response participating in the activation of immune cells [34], enlisting neutrophils through indirect mechanisms [35], promoting pro-inflammatory effects in PMN and inducing the release of cytokines [36] as well as triggering the proliferation of fibroblasts

[37]. 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 [38]. The IL-6 is a mediator in various stages of inflammation [39]. Among the several pro-inflammatory effects attributed to it, those closely related to the repair process are: mitotic induction of keratinocytes in a later step and their effects on neutrophil chemoattractants at the earliest stage [40].

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 [41]. 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 [42]. 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 [43].

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).

Table 1. Effect of topical application of hydrogel containing 100 ug 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	
	G1	G2	G1	G2	G1	G2	G1	G2
Erythrogram								
Erythrocytes mil/mm^3	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	14.44 ± 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
Platelet Count								
Platelets mil/mm^3	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 ± 0.99	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

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

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 in immunosuppressed male Wistar rats. Mean ± SD, n = 4.

Parameters	7th Day		14th Day		21st day		28th Day	
	G1	G2	G1	G2	G1	G2	G1	G2
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
Cálcium mg/dl	11 ± 0.10	10 ± 0.63	8.4 ± 0.98	9 ± 0.99	11.6 ± 0.14	11 ± 0.99	11 ± 0.01	11.5 ± 0.99
Alkaline Phosphatase U/l	185.6 ± 0.07	196 ± 0.99	212.7 ± 0.42	194 ± 0.83	208 ± 0.71	190 ± 0.01	215 ± 0.34	233 ± 0.31
Gamma glutamyl transferase U/l	4 ± 0.01	5 ± 0.99	5.7 ± 0.35	5 ± 0.01	5.8 ± 0.14	5.9 ± 0.62	5.3 ± 0.01	4.5 ± 0.01
Oxalic Transaminase glutamic U/l	125 ± 0.01	130 ± 0.99	110 ± 0.05	98 ± 0.99	143 ± 0.48	144 ± 0.99	117.5 ± 0.14	111 ± 0.01
Transaminase glutâmico pirúvica U/l	68 ± 0.72	70 ± 0.01	60 ± 0.01	60 ± 0.01	68 ± 0.29	68 ± 0.10	47.5 ± 0.63	60 ± 0.99
Urea mg/dl	54 ± 0.65	59 ± 0.98	51 ± 0.31	52 ± 0.99	55 ± 0.53	55 ± 0.04	44 ± 0.09	46.8 ± 0.331
Creatinine mg/dl	0.4 ± 0.70	0.4 ± 0.31	0.5 ± 0.07	0.6 ± 0.12	0.4 ± 0.01	0.6 ± 0.53	0.5 ± 0.14	0.3 ± 0.46
Amylase U/l	755 ± 0.14	968 ± 0.09	971 ± 0.53	1153 ± 0.99	926.3 ± 0.04	1045 ± 0.99	968 ± 0.01	1120 ± 0.29

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

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.

		Inflammatory response		**Granulation tissue**		**Fibrosis**	
Time	**Animal**	**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	-	+	+	-	++	++

Intensity of the parameters evaluated was scored as: - = absent, + = mild presence, + + = moderate presence, + + + = strong presence.

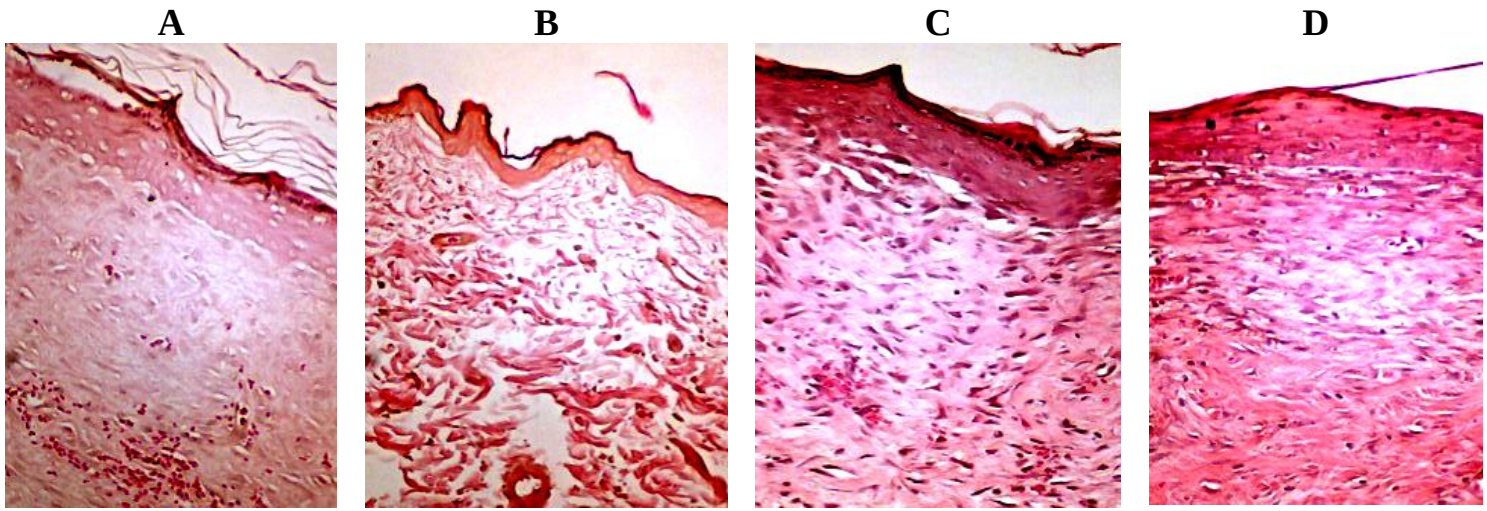

Figura 5. Epithelial tissue of rats in group 1 subjected to second-degree thermal burns. Hematoxilina - Eosina staining. 100x Magnification. **A** – Histopatological appearance of the lesion at 7 days after thermal injury presenting epithelial tissue with complete destruction of the dermis and epidermis with moderate inflammatory infiltrate and mild fibrosis. **B** - Histopatological 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** - Histopatological 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** - Histopatological 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.

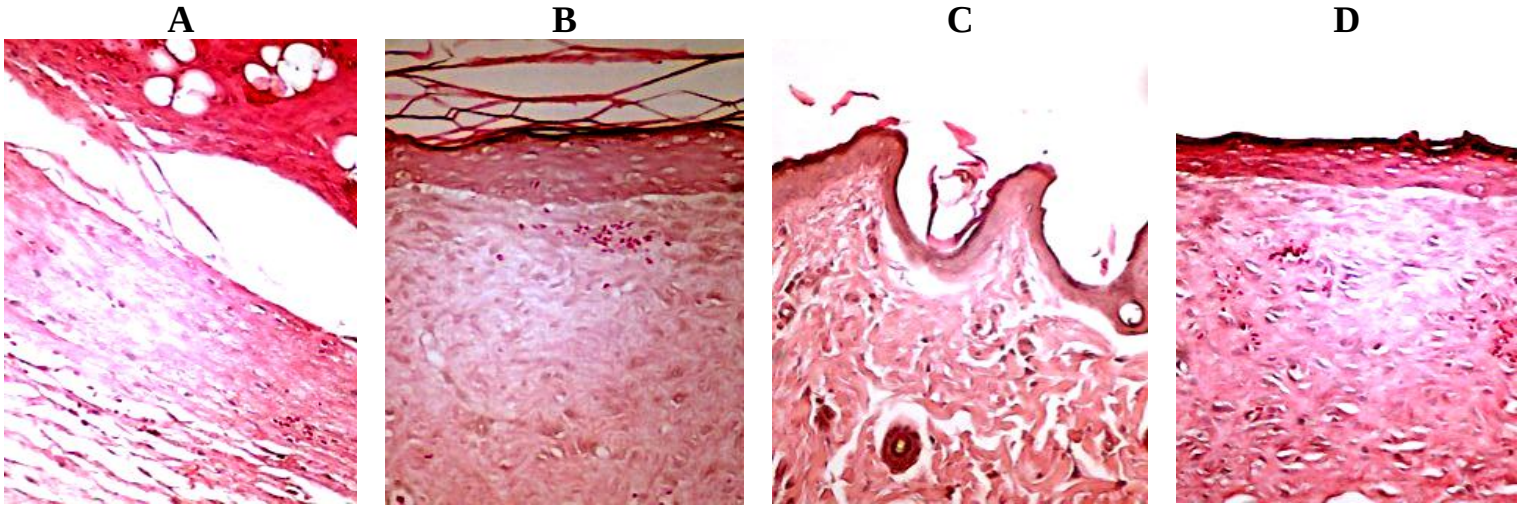

Figura 6. Epithelial tissue of rats in group 2 subjected to second-degree thermal burns. Hematoxilina - Eosina staining. 100x Magnification. **A** – Histopathological appearance of the lesion at 7 days after thermal injury presenting epithelial tissue with complete destruction of the dermis and epidermis 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.

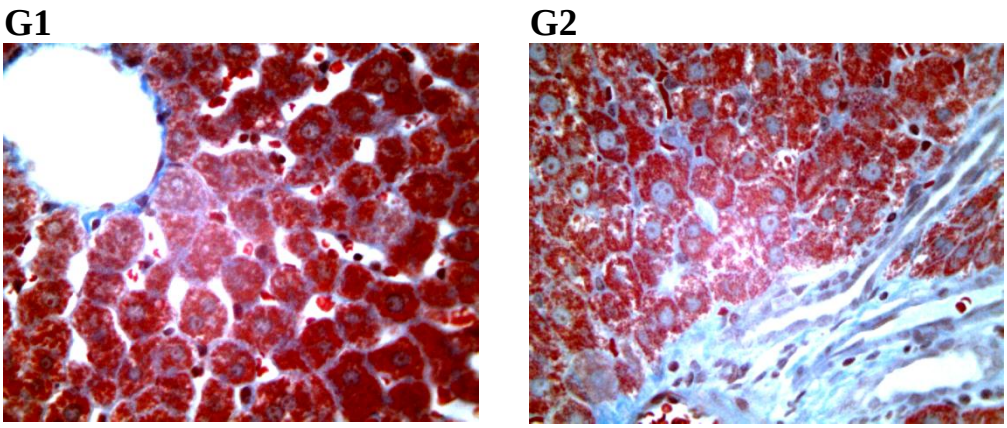

Figure 7. Evaluation of histological sections from liver of the animals in the treated group (G1) and control (G2) with 28 days of experimentation. Masson Trichrome staining. Magnification 100x.

4. Conclusion

Several studies have shown the use of lectins in the modulation of biological response. As discussed by Sell and Costa [44] 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 [45, 46, 47]. Our results showed that the lectin Cramoll 1.4 was effective in the repair of deep second degree thermal lesions induced in experimentally immunodepressed mice and may be used in the future as a biotechnological alternative in the development of therapeutic agents.

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9. CONCLUSÕES

A análise dos resultados obtidos na realização deste estudo nos permitiu obter as seguintes conclusões:

i) O modelo experimental desenvolvido para a obtenção de queimaduras cutâneas de segundo grau em ratos Wistar originou lesões uniformes e de fácil reprodução, sendo este modelo aplicável no estudo do reparo de lesões térmicas de segundo grau profunda;

ii) A formulação proposta de hidrogel de carbopol em associação com a lectina Cramoll 1,4 irradiado não alterou a atividade hemaglutinante desta lectina na concentração de 100µg / ml. Desta forma, a irradiação com raios gama pode ser empregada no controle microbiológico de formulações contendo a Cramoll-1,4 como princípio ativo;

iii) Aplicação tópica regular do hidrogel contendo Cramoll-1,4 irradiado na concentração de 100 µg para o tratamento de queimaduras cutâneas de segundo grau acelerou os processos de granulação, reepitelização e retração da ferida em ratos sadios;

iv) Já os animais imunodeprimidos, também tratados com hidrogel contendo Cramoll irradiado, apresentaram reepitelização completa do tecido, porém com proliferação fibroblástica discreta e fibrose moderada após 28 dias de tratamento;

v) Quando comparados os animais normais e imunodeprimidos tratados com hidrogel contendo Cramoll 1,4 irradiado, verificamos que os animais imunodeprimidos, apresentaram um atraso no processo de reparação da lesão, comparado com os animais sadios devido à imunossupressão.

10. PERSPECTIVA

Os resultados obtidos apontam como perspectiva a aplicação da formulação de hidrogel irradiado contendo a lectina Cramoll-1,4 como um novo biofármaco empregado no tratamento de queimaduras cutâneas de segundo grau em protocolos de reparação tecidual em humanos.

11. ANEXOS

Darwin 200 ANOS

II SicBio - Simpósio de Inovação em Ciências Biológicas
DA ORIGEM DA VIDA AO PROGRESSO DA CIÊNCIA
23 a 26 de novembro de 2009
CCB | UFPE

UNIVERSIDADE FEDERAL DE PERNAMBUCO
PROPESQ PRÓ-REITORIA PARA ASSUNTOS DE PESQUISA E PÓS-GRADUAÇÃO
CCB UFPE
PPGCB

CERTIFICADO

Conferido a **Danielle dos Santos Tavares Pereira** pela apresentação do trabalho intitulado **"Estudo da Atividade Cicatrizante de Cramoll 1,4 em Queimaduras Experimentais"** e co-autoria de **CARNEIRO-LEÃO, A.M.A.; CORREIA, M.T.S.** sob forma **de Pôster** , no II Simpósio de Inovação em Ciências Biológicas, realizado no período de 23 a 26 de novembro de 2009 no Centro de Ciências Biológicas da UFPE em Recife – PE, com carga horária total de 04 horas.

Profª. Drª. Maria Tereza dos Santos Correia
Coordenadora do PPGCB

Artur Felipe Santos Barbosa
Presidente do II Sicbio

VII CONGRESSO BRASILEIRO
Queimaduras
13 A 16 DE OUTUBRO DE 2010

Hotel Armação do Porto
Porto de Galinhas, Ipojuca, PE

REALIZAÇÃO

Sociedade Brasileira de Queimaduras

CERTIFICADO

CERTIFICAMOS QUE

LIMA-RIBEIRO, M H M; PEREIRA, D S T; SANTOS-OLIVEIRA, R; CAVALCANTI, C L B; DE PONTES FILHO, N T; CORREIA, M T S

Participaram do **VII CONGRESSO BRASILEIRO DE QUEIMADURAS**, realizado no período de 13 a 16 de outubro de 2010, no Hotel Armação, em Porto de Galinhas - PE, como **AUTORES** do Pôster: **MODELO EXPERIMENTAL DE QUEIMADURA TÉRMICA DE 2° GRAU EM RATOS WISTAR**

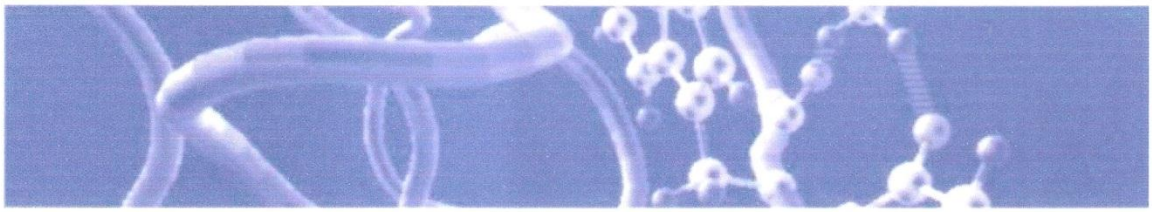
Pernambuco, 16 de outubro de 2010

TL-95

Dra. Telma Rocha
Presidente da
Comissão Organizadora

Dr. Dilmar Francisco Leonardi
Diretor Científico

Dr. Flavio Nadruz Novaes
Presidente da
Sociedade Brasileira de Queimaduras



FeSBE 2011

24 a 27 de agosto de 2011
Rio de Janeiro – RJ

CERTIFICADO

Certificamos que o resumo 10.033 – EVALUATION OF HEMATOLOGIC AND BIOCHEMICAL PARAMETERS OF RATS SUBJECTED TO SECOND-DEGREE THERMAL BURN, autoria de PEREIRA, D. S. T.; LIMA-RIBEIRO, M. H. M.; CUNHA, C. R. A.; CARNEIRO-LEÃO, A. M. A.; CORREIA, M. T. S., foi apresentado na XXVI Reunião Anual da Federação de Sociedades de Biologia Experimental - FeSBE, realizado de 24 a 27 de agosto de 2011 no Rio de Janeiro, RJ.

Comissão Organizadora

CAPES CNPq FAPESP FAPERJ Organização Pan-Americana da Saúde

Para verificar a autenticidade deste certificado, acesse www.fesbe.org.br/certificados

XLI Reunião Anual da Sociedade Brasileira de Bioquímica e Biologia Molecular - SBBq
Foz do Iguaçu, PR, Brasil - 19 a 22 de maio de 2012

Healing Activity Induced by Cramoll 1,4 Lectin in Second Degree Burns: Animal Model

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Cramoll 1,4 is a specific glucose/mannose lectin, extracted from *Cratylia mollis* Mart. seeds, a native plant from North-Eastern, Brazil and it was broadly evaluated in different biological applications. This study aimed to investigate the wound-healing efficiency of Cramoll 1,4 hydrogel in an animal model for second-degree burns. Twenty male rats were randomly divided into two groups (G1 = treatment with hydrogel containing 100 µg/ml Cramoll 1,4; and G2 = Control, treatment with hydrogel). For 35 days, it was effectuated clinical evaluation of the injury and on the 7, 14, 21, 28 and 35 days after burn induction, under anesthesia, the injuries were evaluated regarding the contraction area, lesion re-epithelialization degree and tissue excision for histopathological assessment followed by biochemical and hematological analysis by cardiac puncture, with subsequent euthanasia using thiopental. G1 showed intense exudates, necrosis and edema on the 7th day, tissue reepithelialization and moderate autolysis on the 14th day, intense fibroblastic proliferation, presence of dense collagen and moderate fibrosis on the 21th day, complete tissue epithelialization on the 28th day and modeled dense collagen on the 35th day. There were no significant differences in biochemical and hematological parameters analyzed and significant wound contraction was initiated from day 14 on the G1. The results showed that Cramoll 1,4 hydrogel accelerates the granulation and reepithelialization process and promotes higher percentage of thermal burn contraction compared with the vehicle used as control. These results extended the potential of therapeutic applications of Cramoll 1,4 in the treatment of thermal burns.

Word Keys: Burn, Cramoll 1,4, Healing.
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SUBMETIDO