HISTOPATHOLOGICAL CHANGES OF EXPERIMENTAL BURNS IN RABBITS TREATED WITH BERBERIN NANO GEL

Nguyen Ngoc Tuan[1], Le Quoc Chieu[1], Ngo Ngoc Ha[1], Nguyen Thuy Linh[2]

[1]Le Huu Trac National Burn Hospital

[2]Department of Pathology, Military Medical University

ABSTRACT

Berberin, isolated from Coscinium fenestratum and some plants in the family Ranunculaceae, has been shown to have healing effects on wounds, burns. Study to determine the effect of treatment of superficial burns in rabbits on the pathophysiology of burns of berberine nano gel produced by the National Burn Hospital.

The study was conducted on 45 rabbits, creating circular burns with a diameter of 4cm on both sides of the rabbit's back skin; rabbits were divided into 3 groups (15 rabbits, 30 burns/group), study group (A): Treated site by Berberin nano gel; control group (B): Treatment with SSD; Standard group (C): Treatment with Natri Chloride 0.9%. Follow-up histopathology at the rabbit burn site until the end of the study. Results: Group A burns had a lower number of inflammatory cells and a higher number of fibroblasts and blood vessels than group B and C burns. Conclusion: Berberin nano gel for treatment of superficial burns has been shown to have anti-inflammatory effects and stimulate wound healing.

Keywords: *Berberine nano gel, rabbit, superficial burns*

1. INTRODUCTION

Skin functions as a barrier to the outside body environment to maintain fluid homeostasis and body temperature, while providing sensory information along with metabolic and immune support. Damage of this barrier after burn injuries causes disorder of the innate immune system and increases susceptibility to bacterial infections. Burn patients are at high risk of infections, especially drug-resistant infections, which often lead to significantly longer hospital stays, slow wound healing, higher costs for treatment, and higher mortality. Therefore, infection prevention and management is a primary concern in the treatment of burn patients [1].

Using common topical drugs such as Betadin-iodine organic, Silver Sulfadiazine... for a long period would lead

[1]Chiu trách nhiệm: Nguyễn Ngọc Tuấn, Bệnh viện Bỏng quốc gia Lê Hữu Trác
Email: ngoctuan64@gmail.com

Ngày nhận bài: 05/1/2023; Ngày phản biện: 10/1/2023; Ngày duyệt bài: 15/1/2023
https://doi.org/10.54804/yhthvb.6.2022.184

to the appearance of drug resistance. On the other hand, according to the natural survival rules, the bacteria in the burn also adapt to survive. Therefore, research on a new effective antibacterial drug preparation is necessary and frequent. One trend is the modernization of herbal remedies [1, 2].

Berberine is extracted from "*Vang Dang*" tree and some plants of the family Ranunculaceae, which has many therapeutic effects. Berberine is famous and is considered a plant antibiotic with advantages such as a large raw material source, sustainable effect, low toxicity...

Berberine (mainly in solution form) is the main drug for the treatment of wounds, burns, and has been shown to be effective against bacterial infections. However, the berberine solution for external skin treatment has disadvantages such as low concentration (0.1%) due to hard water solubility, short duration of action due to evaporation, risk of resistance due to low concentration used for a long time. We have researched to prepare Berberine nano gel to overcome these disadvantages of Berberine. The gel containing Berberine nanoparticle size has improved concentration, prolonging the duration of action compared to normal Berberine; Stimulating wound healing, safe. We evaluated the therapeutic effects of Berberine nano gel in experimental burns. Within the scope of the paper, we evaluated the histopathological changes in experimental wounds treated with Berberine nano gel.

2. METHODS AND MATERIALS

• Research materials

Berberin nano gel 1.2% prepared by Hospital of Burns, meets basic standards.

SSD cream; natri chorid 0.9%. Specialized instruments for skin tissue biopsy; Normal microscope; tools and chemicals to process histological specimens.

• Research animals

Healthy domestic rabbits, regardless of breed, meeting research standards, weighing 1.8kg - 2.2kg. The number of rabbits is 45. Before the study, selected rabbits that are healthy, agile, smooth, free from skin and gastrointestinal diseases. The researched rabbits are reared under general laboratory conditions for 3 days before the study.

Details: Eating foods according to food standards for research animals, freely drinking water (boil to cool). Rabbits are kept in separate cages to avoid possible cross-contamination by inhalation and contact. Room temperature 23 ± 30^0C, humidity: 50 - 60%, alternating day (light) night time: 12/12 hours (OECD 402, 2017, pp 3 - 4) [3].

• Experimental model

Burns are made by the method of Pocidalo J.J. and Hladovec J (described by Nguyen Thi Ty 1989) [4], the method of Danielle dos Santos Tavares Pereira [5].

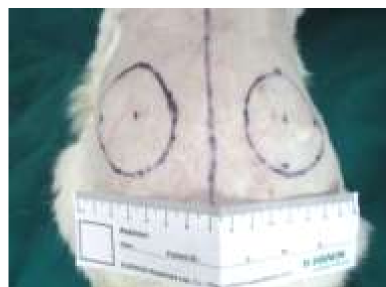
The rabbit is cut off the back hair, then fixed in the prone position on the table. Anesthetize rabbits with Ketamine, dose 50mg/kg. The tool that causes burns is a cylindrical stainless steel tube that has a round bottom with a diameter of 4cm and a height of 20cm (with a handle).

Pour boiling water into the scalding tube up to 10cm (equivalent to 1L of water), and still place the scald tube in the kettle of boiling water for 5 minutes. Then quickly place the bottom of the tube into the

prepared skin, place a 1kg weight on top of the tube.

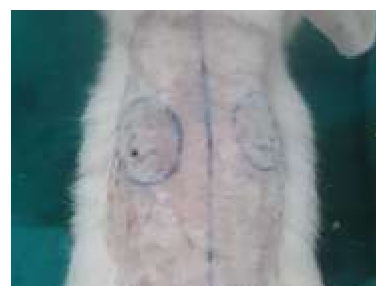
Note that the rabbit is leaning to one side, keep the rabbit's skin taut by hand, place the tube perpendicular to the rabbit's skin. Hold without pressing to keep the handle of the tube balance, not moving and in contact with the entire surface of the skin. The contact time between the bottom of the tube and the rabbit's skin is 15 seconds.

Tools to make burns



Design of burn area

Causing thermal burns with a metal tube filled with boiling water on rabbits



Rabbit skin after causing burn injury

Picture 2.1. Method of causing thermal burn wounds on experimental rabbits

Each rabbit is made 2 symmetrical burns on the back. All burns have a similar depth (partial thickness burn) (picture 2.1.) and a similar dimension ($3.14 \times 2^2 = 12.56$ cm^2)

Rabbits wake up after a few minutes of wounding and eat after a few hours. Rabbits are numbered in order, given a registration form to monitor their progress, and use medicine on the burn until the burn is healed. After causing burns, rabbits are randomly divided into 3 groups (15 rabbits each, 30 burns).

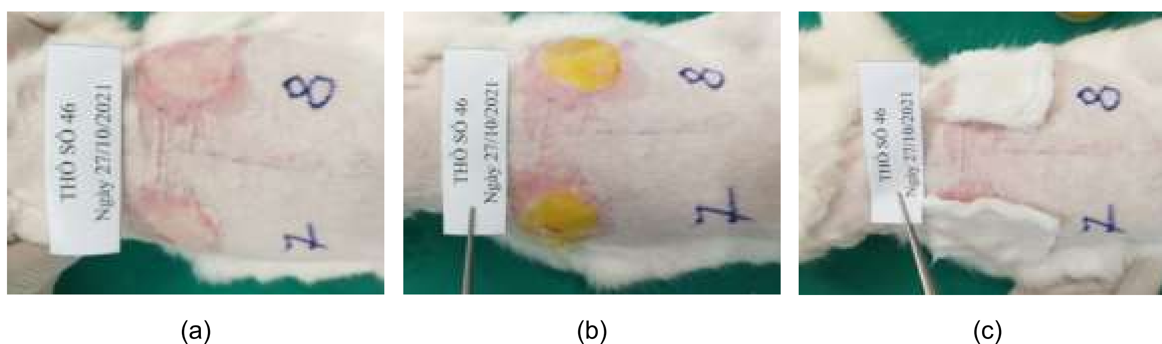
Group 1 (A): Group to be treated by nano ber gel on burns. Change the bandage following the procedure; apply Berberine nano gel impregnated with gauze (approximately 4 - 6g of gel/150cm^2 of gauze) on the burn; Apply 4 - 6 layers of sterile dried gauze, cover the burn, and fix it with Urgo tape (picture 2.2.). Change the bandage every day

Group 2 (B): Group to be treated burns by Silvirin (control drug)

Group 3 (C): Group to be treated burns by Physiological Saline solution 0.9%.

Monitoring indicators

- Histopathological examination at the wound: Biopsy of the burn tissue by a specialized biopsy tool (biopsy punch) (5mm diameter, 1mm depth, produced by SFM watchers - Germany). Stable the specimen with Boin solution, then transfer the specimens to an alcohol solution with increasing concentrations of 700, 800, 900, 1000, and xylene 1, xylene 2 for 3 hours to push the alcohol out of the specimen. Mold specimens in molten paraffin at 560C into individual blocks and number them sequentially. Blocks were cut by a microtome, each slice was 5 μm thick. Each block was cut into 5 slides, placed the slices on a clean slide, and glued with albumin water. The slides are placed in incubator 370 for 12 hours. Remove the paraffin with xylene 1, xylene 2, then use alcohol 1, and alcohol 2 to remove xylene.



(a) (b) (c)

Picture 2.2. Images of injury immediately after causing burns (a); Berberine nano gel was applied (b) and then covered with dry gauze, closed bandage (c)

Specimens are stained by Hematoxylin-eosin (HE) then observed by microscope with magnification of 40-400 times to evaluate the change of cells and tissue structure. Count inflammatory cells (neutrophils, macrophages, lymphocytes), fibroblasts, neovascularization per unit area (122400m^2) under a microscope with microvessels eyepiece. Each specimen is counted for 4 area units and taken average value for each specimen.

A histological examination was conducted at the Department of Pathology, Military Medical Academy. Time: before applying the research drug (day 1 after the burn), then 1 week to recover.

• **Data analysis:** The collected data are calculated as the mean, compared between groups using the T-test and chi-square, SPSS 20.0 software. The difference is significant when $p < 0.05$.

3. RESULTS

3.1. General observation

a) At time T0: Immediately after causing burns, before applying the researched drug: the lesions are round and grayish-white with a pink border. After that, the necrosis gradually becomes clear, the progression is different between the 3 groups.

b) At time T1 (after 5 - 7 days):

Group A: The border is clear between necrosis and healthy skin. Necrosis is clearer, tends to dry with a light and dark

brown partially or fully. Dry necrosis is together with reducing exudative edema. Epithelialization from the margin together with traction narrows the area.

Group B: The burn is more obvious. There is a wet necrotic burn that dissolves (3 burns) or tends to become drier, grayish-white, or light brown, gradually turning completely dry (day 7: 22/30 burns). Inflammation and exudation are more frequent than that of group A. Marginal epithelialization causes sloughing of necrotic scales not as clear as in area A.

Group C: Wet necrosis is more obvious, edema is still strong. Burns tend to be drier, grayish-white, light brown, or dry mostly or fully, with 15/30 burns on day 7. The edema is stronger than in groups A and B, and more fluid than in A and B. Epithelialization from the margin leading to necrotic sloughing is not as obvious as in zone A.

c) At time T2 (after 2 weeks): Area A: dry burn, dry necrosis, flakes from the margin to the center, exposing the healed dermis or epithelium. Epithelization from the margin is extensive. The swelling is gone, the lesion area is significantly narrowed. Area B and Area C: Similar to area A, the difference is mainly narrowing in area.

d) At time T3 (after 3 weeks to 4 weeks): The lesion narrows to a few cm^2 to heal. The burn is dry, not inflamed. The healed burn has a smooth, flat base that grows hair over time. The main difference is the treated time.

Table 3.1. Wound healing time

Index	group A (n=30)	group B (n=30)	group C (n=30)	P
Average treated duration (days)	24.46 ± 4.95	28 ± 2.12	28.37 ± 4.65	p_{a-b}, $p_{a-c} < 0.05$; $p_{b-c} > 0.05$

3.2. Microscopic observation

a) At time T0:

Group A: Complete loss of epidermis, thick superficial coagulation necrosis. The dermis is coagulated with protein, congested blood capillaries. No or minimal infiltration of inflammatory cells, mainly N leukocytes. The stroma is mildly edematous, and skin appendages may be seen.

Group B, Group C: Lesions similar to area A.

b) At time T1:

Group A: Thickened coagulation necrosis at the surface; many white blood cells, the dermis is coagulated with protein, may encounter skin appendages; Capillary congestion, vascular necrosis may occur. The stroma is not inflammatory or has few inflammatory cells; observation of granulation tissue with blood vessels, fibroblasts in the wound background. The proliferation of colloidal fibers and fibroblasts predominates higher than the group using physiological saline 0.9%.

Group B: Skin with thickened superficial necrosis, leukocytes at the surface. The dermis is coagulated with protein, congested capillaries. Few inflammatory cells infiltrate. Buffer tissue edema. Granulation images were not found.

Group C: The lesion image is still superficial necrosis, to the subcutaneous fat layer. The dermis is coagulated with protein, the blood capillaries are congested. Inflammatory cell infiltration. The stoma is edematous. Granulation images were not found.

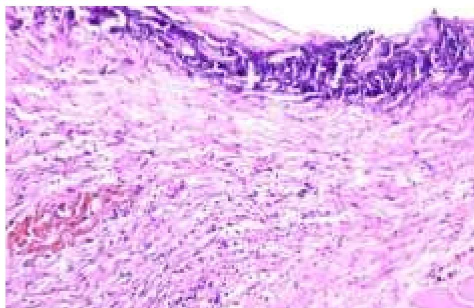


Figure 3.1. Image of the area A after 1 day of burn, the surface has a thin layer of necrosis, the underlying tissue has congested blood vessels interspersed with few N white blood cells

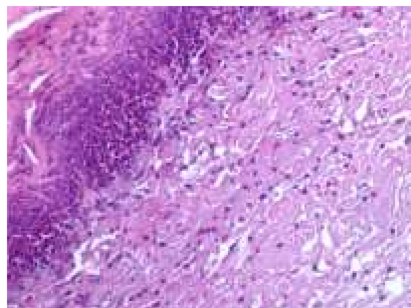


Figure 3.2. Image of area B after 1 day of burn, the surface has a thick layer of necrosis, the underlying tissue is congested with blood vessels, infiltrated with many N white blood cells

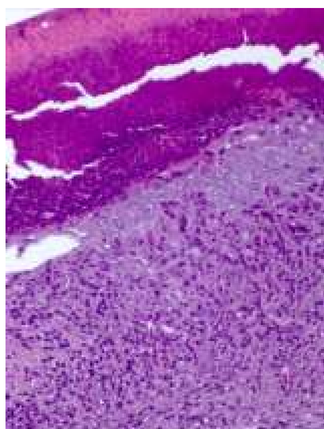


Figure 3.3.Area A after 1 week: Superficial necrotic layer, fine underlying granulation tissue, proliferative squamous epithelium crawling from the wound edge

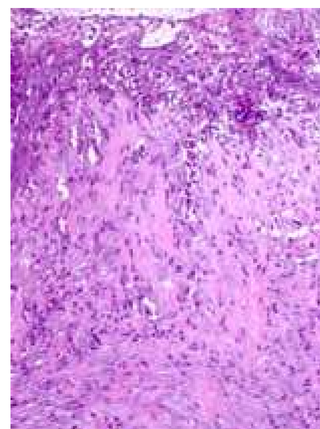


Figure 3.4. Area B after 1 week: The necrotic layer has disappeared, epithelial islands have crawled in to cover the lesion. Underneath the epithelial layer is thin of granulation tissue

c) At time T2

Group A: Skin with a thin coagulated necrotic epidermis, many white blood cells or necrotic surface, stratified squamous epithelium crawling into the wound can be seen. The dermis is coagulated with protein, some blood vessels are congested, and skin appendages are also visible. Underneath the necrotic layer, there is granulation with congested capillaries, a proliferation of fibroblasts, and inflammatory cells infiltrated.

Group B: Skin has a thin or thick coagulated necrotic epidermis, many white blood cells, or no necrosis on the surface. The dermis is coagulated with protein, some blood vessels are congested, and skin appendages are also visible.

Underneath the necrotic layer, there is granulation with congested blood capillaries, infiltrated by inflammatory cells.

Group C: Similar to group B, Edema, congestion decreased but still had thick/thin coagulation necrosis; dermal edema strongly congested, infiltrated many inflammatory cells, granulation regenerates irregular structure.

d) At time T3

Group A: Epithelialization with the surface appearance is covered by the epidermis, with some epithelial islands growing down to the dermis. The dermis has thick fibrous connective tissue by the proliferation of fibroblasts and collagenous fibers; Some blood vessels have thick walls, little/no inflammatory cells infiltrate. Rarely, the epidermis has thin coagulation necrosis, protein clots in the dermis, some congested blood vessels, and skin appendages are seen. Underneath the necrotic layer, there is granulation tissue with congested blood capillaries, infiltrated by inflammatory cells.

Group B: Seeing thick/thin coagulation necrosis, many surface leukocytes, dermis with protein coagulation, some congested blood vessels, and skin appendages. Underneath the necrotic layer, there is granulation tissue with congested blood capillaries, infiltrated by inflammatory cells. An image of the epidermis covering the wound surface can be seen

Group C: Similar to group B, Edema, congestion decreased, there were images of thick/thin coagulation necrosis; The dermis is strongly edematous and congested, infiltrates many inflammatory cells, and the tissue regenerates irregularly.

Table 3.2. Number of inflammatory cells in the wound

Time	Number of inflammatory cells/unit area			p
	Region A	Region B	Region C	
T0	4.2 ± 5.4	4.1 ± 3.7	4.8 ± 5.2	> 0.05
T1	15.5 ± 7.6	18.5 ± 10.8	20.6 ± 12.8	$P_{A-B, C} < 0.05$
T2	17.8 ± 6.5	20.7 ± 7.9	30.3 ± 14.1	$P_{A-C} < 0.05$
T3	11.2 ± 5.7	15.4 ± 6.3	25.8 ± 16.4	$P_{A-B, C} < 0.05$
p	$P_{0-1,2,3} < 0.05$	$P_{0-1,2,3} < 0.05$	$P_{0-1,2,3} < 0.05$	

The number of cells increased during treatment, peaked after 2 weeks, then tended to decrease. At the time of T1, T2, and T3, the A region has a lower number of inflammatory cells than the B and C . regions.

Table 3.3. Number of blood vessels in the wound

Time	Number of blood vessels /unit area			p
	Region A	Region B	Region C	
T0	1.5 ± 0.9	1.3 ± 2.5	1.7 ± 1.6	> 0,05
T1	4.6 ± 1.2	2.2 ± 3.0	2.1 ± 2.7	$P_{A-B,C} < 0.05$
T2	5.5 ± 3.4	4.0 ± 2.6	4.5 ± 1.7	$P_{A-B, C} > 0.05$
T3	6.5 ± 6.3	5.0 ± 3.7	5.3 ± 3.2	$P_{A-B, C} > 0.05$
p	$p_{0-1,2,3} < 0.05$	$p_{0-1,2,3} < 0.05$	$P_{0-1,2,3} < 0.05$	

The number of new vessels in all 3 regions increased over time, but the difference was statistically significant after 1 week ($p > 0.05$).

Table 3.4. Number of fibroblasts in the wound

Time	Number of fibroblasts /unit area			p
	Region A	Region B	Region C	
T0	1.1 ± 2.4	1.8 ± 4.2	1.6 ± 3.2	> 0.05
T1	10.9 ± 4.8	3.0 ± 5.2	3.3 ± 2.5	$P_{A-B, C} < 0.05$
T2	13.2 ± 6.9	8.6 ± 8.4	7.8 ± 6.3	$P_{A-B, C} < 0.05$
T3	14.8 ± 11.0	11.1 ± 8.8	10.7 ± 4.7	> 0.05
p	$P_{1-2,3} < 0.05$	$P_{1-2,3} < 0.05$	$P_{1-2,3} < 0.05$	

The number of fibroblasts in all three regions increased with time, the difference was statistically significant at the time of T1 and T2 ($p > 0.05$).

4. DISCUSSION

The depth of burn injury determines the treatment modality. In practice, the depth of burn injury is assessed primarily clinically. Histological wound biopsy is an accurate diagnostic tool to assess burn depth [6,7]. However, it has never become a useful tool for clinical practice, because of the cost, time-consuming and scarring of the biopsy site [8]. In addition, dynamic changes in the burn site complicate both the time of biopsy and the assessment of microscopic changes, which depend on many factors [6, 7].

On macroscopic observation, Berberine nano gel has anti-inflammatory effects and stimulates the healing of burn wounds. A sizable difference is the earlier drying of wet necrosis of Berberine nano gel compared to SSD drugs and physiological saline. The combined result was that the burn healing time (wound closure) of the study group was faster than the other two groups (table 3.1). This result also corresponds to histopathology during the study.

4.1. Anti-inflammatory effect

Inflammation is important for successful burn wound healing, and inflammatory mediators (cytokines, kinins, lipids, etc.) provide immune signals to recruit leukocytes and macrophages to initiate the proliferative phase. Burn re-epithelialization during the proliferative phase through activation of keratinocytes and fibroblasts, or migration from undifferentiated hair follicles and other epidermal analogues, is mediated by cytokines during the proliferative phase. inflammatory segment. Thus, inflammation is necessary for wound healing, but abnormal inflammation also disrupts wound healing, associated with hypertrophic scarring and delayed wound healing [9, 10].

Significant edema due to several triggers including vasodilation, extravascular osmotic activity, and increased microvascular permeability is often associated with inflammation. Excessive or prolonged swelling and inflammation aggravate pain and impair

wound healing. Inflammation is the body's normal response to inflammatory factors, helping to initiate and stimulate responses for the next stages of the wound-healing process. However, if the inflammation is too much, it will affect the wound-healing process later [11-13].

Histological results also showed that the Berberine nano gel treatment group exhibited less inflammation than the 1% SSD group. On the first day after treatment, all three areas still had many inflammatory cells. By day 7 after the study, the number of inflammatory cells in the area treated with Berberine nano gel was significantly less than in the area treated with 1% SSD ($p < 0.05$). The number of inflammatory cells also decreased gradually over the study time points, along with an increase in the number of neovascular and fibroblasts (table 3.2).

Studies have also documented berberine to have anti-inflammatory effects. In vitro, Berberine inhibits platelet aggregation and adhesion, inhibits arachidonic acid secretion from cell membrane phospholipids, increases 6-keto-prostaglandin F1 alpha production in platelets [14]; inhibits the production of cyclooxygenase-2 (COX-2), thereby reducing prostaglandin E2 (PGE2), which plays an important role in the inflammatory process [15]. Berberine inhibits NADPH oxidase, reducing free radical generation in macrophages [16]; inhibits lymphocyte transformation, inhibits DNA synthesis in activated lymphocytes [17]; inhibits the proinflammatory response through AMPK activation in macrophages [18].

4.2. Stimulating effect on wound healing

Burn wound healing is also a dynamic process with overlapping stages. The inflammatory phase is intended to prevent infection during wound healing, degrade necrotic tissue, and activate the signals necessary for wound healing [19].

Next, and superimposed on the inflammatory response, the proliferative phase is characterized by the activation of keratinocytes and fibroblasts by cytokines and growth factors [20]. The keratinocytes migrate through the wound to gradually close wound, aiding in the closure and restoration of the vascular network [21].

The proliferative phase is characterized by a series of responses aimed at maintaining vascular integrity, replacing damaged tissue, and covering the wound surface. During the proliferative phase, important activities are epithelialization from keratinocytes, increased synthesis of collagen and extracellular substances, and increased angiogenesis.

- Change in the Number of blood vessels

Neoangiogenesis or generation of new vessels is the re-establishment of microvascular integrity and function of new tissue. This process requires the migration and proliferation of endothelial cells. Under the action of collagenase and other enzymes, endothelial cells separate from the undamaged blood vessels, migrate to the injured area, and proliferate. These endothelial cells divide, and new cells form a bud-like circle in the walls of intact microvessels adjacent to the lesion. From there, they divide to form vascular tubules that connect to each other to form loops. At the same time, the vascular basement membrane is formed from the matrix components. These neovascularized tubules differentiate into arterial and venous ducts communicating with the general circulation [19, 20].

In the study, the number of neovascularization tended to increase over time. At time T1, the number of blood vessels of group A burns was significantly higher than that of the other two groups. Increasing the number of blood vessels is the material basis for granulation tissue, providing oxygen and nutrients for regeneration. This leads to faster results in group A (table 3.3.).

- Change in the number of fibroblasts

Fibroblasts are key cells of the proliferative phase, producing intercellular buffers and synthesizing several growth factors. Fibroblasts proliferate and produce intercellular buffers (collagen and other components of the matrix: fibronectin, hyaluronic acid, glycosaminoglycans...). As an early response to injury, fibroblasts from the wound margins begin to proliferate and after about 4 days begin to migrate into the lesion and switch to their primary function of protein synthesis. Fibroblasts are also differentiated into myofibroblasts involved in wound contraction [22].

In the study, the number of fibroblasts in all three regions increased over time, the difference was statistically significant at the time of T1 and T2 ($p > 0.05$) (table 3.4).

The effect of stimulating the healing of burn wounds has been recognized by many experimental studies. P.I. Jewo compared the effectiveness of MEBO - a Berberine-containing drug with SSD in superficial burn wounds in *Sprague Dawley* rats. Histologically, the MEBO-treated burn group had greater mean epidermal thickness (12 mm) compared with 10 mm in the SSD group. MEBO promotes rapid re-epithelialization [23]. Nasef M (2016) studied the therapeutic effects of Berberine on 120 adult male white rats *Sprague Dawely* causing superficial burns (compared to SSDs) [24] noted that Berberine promotes rapid re-epithelialization fast.

5. CONCLUSION

Studying the therapeutic effect on histopathology of Berberine nano gel on rabbit experimental burns (compared with cream SSD) found that Berberine nano gel had the effect of reducing inflammation and stimulating wound healing, expressing the number of inflammatory cells. decreased, the number of fibroblasts and neovascularization increased more than in the control group; the rate of epithelialization, the wound healing time is also faster, $p < 0.05$.

REFERENCES

1. Matthew P. Rowan, Leopoldo C. Cancio, Eric A. Elster, David M. Burmeister, Lloyd F. Rose, Shanmugasundaram Natesan, Rodney K. Chan, Robert J. Christy, and Kevin K. Chung, *Burn wound healing and treatment: review and advancements;* Crit Care. 2015; 19: 243.
2. D'Avignon LC, Hogan BK, Murray CK, Loo FL, Hospenthal DR, Cancio LC, et al. Contribution of bacterial and viral infections to attributable mortality in patients with severe burns: an autopsy series. *Burns.* 2010; **36**:773-9.
3. OECD guideline for the testing of chemicals, 402, 2017.
4. Nguyễn Thị Tỵ (1989*); tác dụng điều trị tại chỗ vết thương bỏng thực nghiệm của tinh dầu tràm và bước đầu ứng dụng lâm sàng*, luận án PTS khoa học Y Dược, HVQY, Hà Nội
5. Danielle dos Santos Tavares Pereira, Maria Helena Madruga Lima-Ribeiro, Nicodemos Teles de Pontes-Filho, Ana Maria dos Anjos Carneiro-Leão, and Maria Tereza dos Santos Correia, *Development of Animal Model for Studying Deep Second-Degree Thermal Burns;* J Biomed Biotechnol. 2012; 2012: 460841.

6. Papp, K. Kiraly, M. Härmä, T. Lahtinen, A. Uusaro, E. Alhava; *The progression of burn depth in experimental burns: a histological and methodological study*; Burns 30 (2004) 684-690
7. Heimbach D, Mann R, Engrav L. *Evaluation of the burn wound management decisions*. In: Herndon D, editor. Total burn care. 2nd ed. Saunders; 2002. p. 101-8.
8. Heimbach D, Engrav L, Grube B, Marvin J. *Burn depth: A review*. World J Surg 1992;16:10-5.
9. Matthew P. Rowan, Leopoldo C. Cancio, Eric A. Elster, David M. Burmeister, Lloyd F. Rose, Shanmugasundaram Natesan, Rodney K. Chan, Robert J. Christy, and Kevin K. Chung, *Burn wound healing and treatment: review and advancements*; Crit Care. 2015; 19: 243.
10. Tabas I, Glass CK. *Anti-inflammatory therapy in chronic disease: challenges and opportunities*. Science. 2013;**339**: 166-72.
11. Arturson G. *Forty years in burns research - the postburn inflammatory response.* Burns. 2000; **26**:599-604.
12. Edgar DW, Fish JS, Gomez M, Wood FM. *Local and systemic treatments for acute edema after burn injury: a systematic review of the literature*. J Burn Care Res. 2011;**32**:334-47.
13. Szpaderska AM, DiPietro LA. *Inflammation in surgical wound healing: friend or foe?* Surgery. 2005; **137**:571-3.
14. Ang ESW, Lee ST, Gan CSG (2001), *Evaluating the role of alternative therapy in burn wound management: randomized trial comparing mois exposed burn ointment with conventional metthod in the management of patients with second-degree burns*, Medscape general medicine: 3 (12) 3 -18.
15. Chilikuoa, Chin-Wen Chiab, et al (2004), *The anti-inflammatory potential of berberine in vitro and vivo*, Cancer letters, Volume 203, Issue 2, Pag. 127-137.
16. Sarna LK, Wu N, Hwang SJ, et al (2010), *Berberine inhibits NADPH oxidase mediated superoxide anion production in macrophages*; Can. J. Physiol. Pharmacol. 88(3): 369 -378.
17. Ckless K, Schlottfeldt JL, Pasqual M, et al (1995), *Inhibition of in-vitro lymphocyte transformation by the isoquinoline alkaloid berberine*. J Pharm Pharmacol; 47:1029 - 1031.
18. Jeong HW, Hsu KC, Lee JW, et al (2009), *Berberine suppresses proinflammatory responses through AMPK activation in macrophages*, Am J Physiol Endocrinol Metab296: E955-E964.
19. Reinke JM, Sorg H. *Wound repair and regeneration*. Eur Surg Res. 2012;**49**:35-43.
20. Werner S, Krieg T, Smola H. *Keratinocyte-fibroblast interactions in wound healing*. J Invest Dermatol. 2007;**127**:998-1008.
21. Pastar I, Stojadinovic O, Yin NC, Ramirez H, Nusbaum AG, Sawaya A, et al. *Epithelialization in wound healing: a comprehensive review*. Adv Wound Care. 2014;**3**:445-64.
22. Bộ môn Bỏng - Học viện Quân Y, *Liền vết thương*, Giáo trình Bỏng (dùng cho đào tạo trình độ Sau đại học), NXB Quân đội Nhân dân, 2018, trang70-90.
23. P.I. Jewo, I.O.F., O.S. Babalola, L.C. Saalu, A.S. Benebo, M.C. Izegbu, and O.A. Ashiru; *A Comparative Study of the Wound Healing Properties of Moist Exposed Burn Ointment (MEBO) and Silver Sulphadiazine.* Ann Burns Fire Disasters, 2009. **22**(2): p. 79-82.
24. Nasef M, K.H.A., Ashour M A, Nassar S L, Selim N M, El-Raey M A; *Biological Investigations of a New Natural Recipe Expected to Promote Healing of Superficial Burns;* International Journal of Pharmaceutical and Clinical Research 2016. **8**(8): p. 1230-1239