THE RESEARCH ON THE TREATMENT EFFECTS OF NANO BERBERIN GEL ON SUPERFICIAL BURN THICKNESS

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ABSTRACT

Objectives: *To evaluate the analgesic effect of nano Berberin gel in the treatment of superficial burns.*

Subjective and Method: *Study on 32 pediatric burn patients, treated at the Pediatric Burn Department in Le Huu Trac National Burn Hospital from 1 January 2022 to 30 April 2022. The method of study is a Prospective study, a randomized controlled trial. with comparison before-after. In the same patient, the burn was divided into 2 areas of equal area and depth. Study area: burns treated with a gel containing nano Berberine and comparison area: burns treated with 1% SSD cream.*

Results: *1. Having an anti-inflammatory effect: Reduction of the degree and duration of inflammation and reduced significantly the exudation on the study burn wound compared to the control area.*

2. Nano Berberin gel dries wet necrosis (obvious after 2 - 3 days of using the drug).

3. Gel has the effect of preventing infection. A significant reduction in the number of positive cultures and the number of bacteria after 1 - 2 weeks of treatment, (78.33 ± 46.69 vs 105.16 ± 91.28 p < 0.05). The number of infected wounds after 1 week also decreased significantly in the control area, (25% vs 61.5% in zone A; 33.3% vs 58.3% in zone B) p<0.05. 4. The time for 50% epithelialization, the treatment time in the study area is also shorter than in the control area, (6.9 ± 3.6 vs 8.1 ± 4.2; p < 0.05).

Conclusion: *Initial remarkable recognition of the treatment effect of Nano Berberin Gel on the pediatric superficial burn.*

Keywords: *Nano Berberine gel, superficial burn, pediatric*

INTRODUCTION

Berberine is extracted from Cosinium Myrtaceae, Berberine has been a traditional medicine to treat diarrhea... Currently, Berberine has been proven to have many pharmacological effects and is widely used in many diseases. The outstanding advantage of berberine is as a plant antibiotic and its anti-inflammatory, anti-cancer, and hypoglycemic effects [1, 2]. It affects many types of bacteria, fungi, and viruses. Berberine is also used to treat infections caused by bacteria, streptococcus, and burn wounds. It has many effects such as safety, pain relief,

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inflammation reduction, and stimulation of healing wounds [3, 4, 5]. Nano Berberin gel is the gold topical agent to treat the pediatric superficial burn.

1. THE OBJECTS AND METHOD

1.1. Objects

The conduction of 32 pediatric burn patients, treated at the Pediatric Burn Department in Le Huu Trac National Burn Hospital from the 1st day of January 2022 to 30 April 2022.

The criteria for enrollment

- Child burn patients in the Pediatric Burn Department - Le Huu Trac National Burn Hospital.

- Child patients below the age of 18 years old.

- Admit during 72 hours after burn injury.

The patient's family consented to the study.

The exclusion criteria:

Patient with chronic disease or severe systemic disease.

1.2. Research material

Comparative drugs: SSD 1% cream

Research drug: Berberin nano gel, prepared by Le Huu Trac National Burn Hospital.

1.3. Research Methods

Prospective study, with comparison before-after. In the same patient, the burn was divided into 2 areas of equal area and depth. Study area: burns treated with a gel containing nano Berberine and comparison area: Burns treated with 1% SSD cream.

Calculation of burn area method: Combination of the 9th method of Pulaski E.J; Tennison C.W (1949) and Wallace A (1951); the method of calculating the burn area by the patient's palm (equivalent to 1 - 1.25%) of Blokhin N.N and Glumov I. I (1953); the number 1 - 3 - 6 - 9 - 18 of Le The Trung method (1965) [3, 7].

The Depth diagnosis method: using the 5-level depth classification of Le The Trung (1965) [7]. Diagnosis of burn depth is based on:

Clinical features: burn wound clinical... pain sensation test at the burn wound. Necrotic incision procedure to release compression and diagnose deep burn injury [3].

The course of burn wound: with superficial burns, the burn is capable of self-healing thanks to epithelialization. Deep burns: after rotten necrosis, the granulation tissue formed after necrosis comes off, requiring an autologous skin graft [3].

- Method of using topical drugs: the dressing change according to the procedure, ensuring the principle of sterility. Wash the skin around the burn wound with NaCl 0.9%, then disinfect with 70^0 alcohol. Wash the burn wound with 0.9% physiological saline. Remove necrotic. Pat the burn wound with sterile gauze. Apply gauze with nano Berberin gel on the study burn wound and SSD cream on the comparison burn wound (approximately 4 - 6g of drug/150cm^2 of gauze). Apply 4 -6 layers of sterile dry gauze on top of the medical gauze, cover. Dressing changes once a day until healed [7].

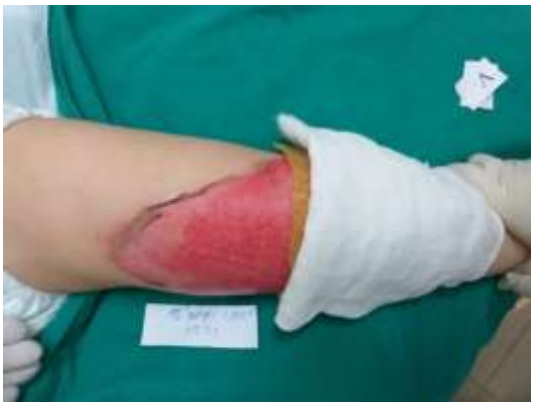

Figure 1.1. Research lesions in the left thigh area, grade II III, all blister has slipped, area of 1%; before applying for the medicine, on the 2nd day after-burn

Figure 1.2. The lesion is divided into 2 areas, Area B: below the thigh, using SSD 1% gauze

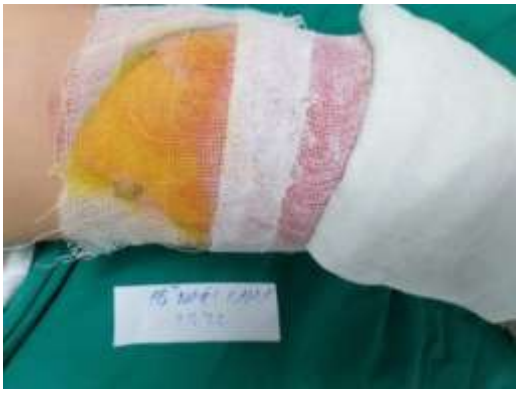

Figure 1.3. Injury to area A, upper thigh, apply gauze impregnated with Berberin nano gel

1.3.1. Clinical

- Age, gender, circumstances, agent of burns.

- The day of hospitalization after burns. The first and the end study date.

- Monitor the whole body: Pulse, temperature, blood pressure, respiratory, neuropsychological, digestive, urine output before and after application, allergic (rash, erythema, chest tightness, shortness of breath...).

- Daily monitoring of symptoms of the burn wound.

+ Burn size and depth of burn injury.

+ Burn size and depth of burn injury between the study area and the comparison area.

+ Pain sensation when applying for medicine: Assessed according to the modified CHEOPS scale (Children's Hospital of Eastern Ontario Pain Scale) scale.

+ Inflammation of the wound and the normal skin, with manifestations such as edema, red, heat, pain, maybe appeared erythema, swelling may spread to normal skin; much pain.

+ The condition of exudative, pus-filled fluid seeping out of the dressing at the burn wound. Assess discharge status according to 4 levels:

Extensive degree: fluid seeps all the dressing. Moderate: Fluid penetrates the inner gauze layer. Low level: the fluid only penetrates the innermost layer of gauze. Out of fluid: the wound is dry.

+ Allergic conditions, manifestations of rash, itching, inflammation...

+ The epithelialization: Assessed by the time of epithelialization 50% of the burn size.

- Time of hospitalization: Counting from the first time of study to the day when the burn is completely healed.

- Quality of wound healing: Evaluated with criteria such as color, softness, and elasticity, compared with surrounding normal skin.

1.3.2. Microbial monitoring

Collect pus at the burn site, culture and identify bacteria at the following stages: before the study (apply research drug), after 1 week and after 2 weeks.

The technique of taking samples, determining bacteria species, the number of bacteria on the surface of a burn wound is similar to that in experimental burns, conducted at the Microbiology laboratory, National Burn Hospital.

1.3.3. Subclinical

Paraclinical tests were conducted at the time before the study, after 1 week and after 2 weeks, at the Paraclinical Department, National Burn Hospital.

Hematological indicators: Red blood cells, hemoglobin, white blood cells, platelets, hematocrit. Biochemical indicators: protein, albumin, glucose, AST, ALT, urea and creatinine, serum electrolytes. The obtained values are compared with the normal physiological index of Vietnamese children.

2. RESEARCH RESULTS

2.1. Clinical development of patients with superficial burns

Table 2.1. General characteristics of patients

Evaluation criteria	Quantity	Ratio
Age: Under 1 year old	4	12,5%
1 - 5 years old	21	65,6%
6 - 10 years old	4	12,5%
Over 10 years old to 16 years old	3	9,4%
Total	32	100%
The average age	28.37 ± 21.31 months	
Gender: Male	21	65,6%
Female	11	34,4%
Total	32	100%
Average burn area	8.6% ± 4.0%	
The average depth of burn area (n = 7)	4.3% ± 1.9%	
The average length of hospital stay	19.8 ± 9.3	
Burning agent: Wet heat	28	87.5%
Dry hea	4	12.5%
Total	32	100%

The study was mainly in the group of children aged 1 - 5 years old (65.6%), mainly men (65.6%), in patients with less than 10% body area (62.5%). The study was conducted mainly on patients with wet heat burns (87.5%).

- The clinical statement during the study: Monitoring before and after applying the drug Specifically: did not see any abnormal changes. The patients do not have a fever before and after applying for the medicine. Organs: Nervous, respiratory, digestive... all show no disorder. No allergic related used drug manifestations such as edema, allergic rash in healthy skin, anaphylaxis...

Table 2.2. Location, time and the average of the study area

Available		area A	area B
Location	Front of body	4	4
	Rear body	2	1
	Upper limb	6	5
	Lower extremities	20	22
	Total	32	32
Post-burn study time (days)		2.35 ± 1.19	
Average study area		1.71 ± 0.99%	2.11 ± 1.5%

The study was mainly conducted in the lower extremities, in the first 3 days after burn.

Table 2.3. Local developments of superficial burns

Symptoms	Area A	Area B
Pain	No	No
Inflammation of the burn wound	- Less swelling than zone B; Discount from 2nd day, end on 4 - 6th days after using the drug - No inflammation or wide inflammation of the normal skin	- Superficial burns: the same. The degree of swelling is clearer than in zone A. No inflammation to the normal skin
Exudate, purulent fluid	Discharge, purulent fluid in B region is less. The epidemic decreased from the 2nd to the 4 - the 6th day. The pink background is clean, and less pseudomembranous in the B area. The significant difference between the two areas A and B after an average of 1.5 ± 0.5 days. The gauze adheres to the burn wound and medicated gel, creating a thin scab on the burn wound until it heals. - III degree necrosis: The necrotic layer dried and clears after 2 - 3 days, then the necrosis falls off (after 4 - 5 days); epithelial background.	- The exudate, purulent, pseudomembranous fluid is more than in area A. The exudate in superficial burns decreases gradually from day 2nd to 6th. The drug creates a thin, white gel on the surface of the burn wound. When it epithelializes: drug gauze adherents slightly. - III degree necrosis: the drug does not dry up the necrosis, when it falls off, it is usually later, with inflammation, and purulent fluid.
allergies	No	No

Table 2.4. The time of 50% epithelialization and the time of healing superficial

Targets		Area A	Area B	p
The time of 50% epithelialization		6.9 ± 3.6	8.1 ± 4.2	< 0,05
Treatment time	Second degree burns	5.38 ± 1.85 (n = 21)	5.4 ± 1.7 (n = 22)	< 0,05
	Third degree burns	12.23 ± 4.4	13.8 ± 4.5	< 0,05

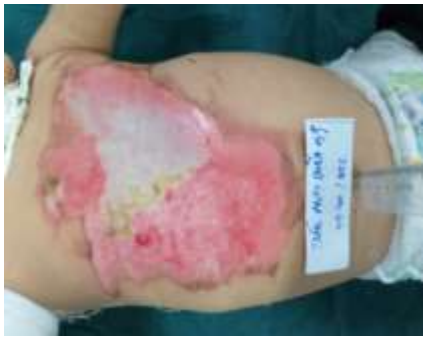

Figure 2.1. Image of a second-degree burn injury caused by boiling water before the study

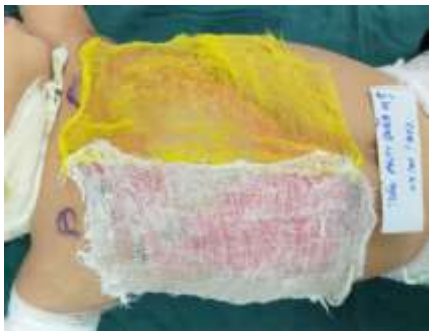

Figure 2.2. The lesion is divided into 2 areas: Area A with Berberin nano gel; B: add SSD 1%

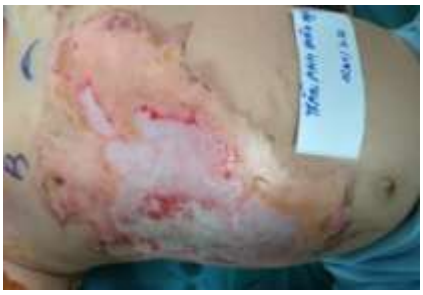

Figure 2.3. Lesions after 5 days of studying, area A is well epithelialized, significantly narrowed, cleaner, less pseudomembranous and exudative than area B

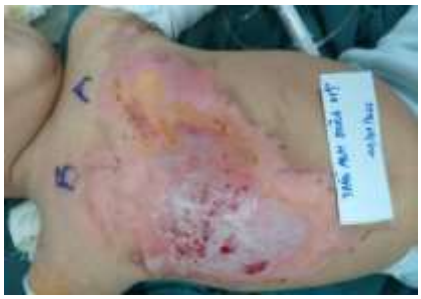

Figure 2.4. Lesions after 7 days of studying, area A is well epithelialized and clean; Area B is still deeply in III degree burn with many pseudomembranous and exudates

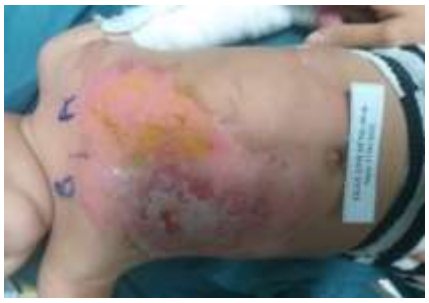

Figure 2.5. After 10 days of study, area A healed. Zone B: III-degree burn and IV, pseudomembranous, much more fluid

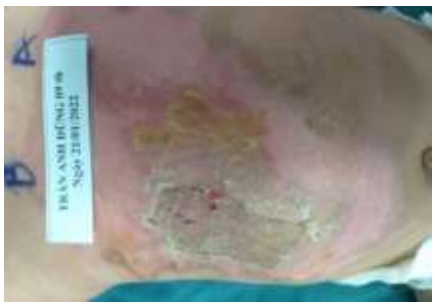

Figure 2.6. Lesions after 14 days of studying, III degrees burn in zone B healed

2.2. Microbial evolution at the superficial burn surface

Table 2.5. The proportion of positive cultures in superficial burn

Time		Area A		Area B		p
		n	%	n	%	
Before the study (T0)	Positive Bacterial culture	12	37.5%	13	40.6%	> 0,05
	Negative Bacterial culture	20	62.5%	19	59.4%	
	Total	32	100%	32	100%	
After 1 week (T1)	Positive Bacterial culture	16	61.5%	14	58.3%	> 0,05
	Negative Bacterial culture	10	38.47%	10	41.7%	
	Total	26	100%	24	100%	
After 2 weeks (T2)	Positive Bacterial culture	3	25%	5	33.3%	> 0,05
	Positive Bacterial culture	9	75%	10	66.7%	
	Total	12	100%	15	100%	
p		$p_{T0-T1, T1-T2} < 0.05$				

Comparison in each group: The number of infectious burn wounds increased after 1 week, then decreased, $p < 0.05$. Comparison between 2 groups each time: no difference, $p > 0.05$.

Table 2.6. the number of bacteria at the superficial burn surface (x5 x10^3)

Time	Number of zones A	Number of zones B	P
Before the study (T0)	167.75 ± 139.46	115.2 ± 129.1	< 0.05
After 1 week (T1)	105.16 ± 91.28	144.31 ± 104.58	< 0.05
After 2 weeks (T2)	78.33 ± 46.69 (n = 3)	92.2 ± 38.95 (n = 5)	> 0.05
	$p_{T0-T1, T1-T2} < 0.05$		

Table 2.7. Bacterial species in superficial burns over time

Type of bacteria	Occurrences in area A			Occurrences in area B		
	T0	T1	T2	T0	T1	T2
S. aureus	5	7	1	7	6	2
P.aeruginosa	3	9	1	2	10	2
S.saprophyticus	1			2		
E.coli	1	1		1		
A. baumanii	1	1				
S. horminis	1	0		2		
Proteus			1			1
Total	12	18	3	14	16	5

Note: First culture: 1 patient in group B grew 2 species of bacteria. The second culture of areas A and B both had been growing 2 species of bacteria.

Table 2.8. Species of bacteria at the superficial burn surface

Type of bacteria	Frequency of appearance		Total (%)
	Area A	Area B	
S.aureus	13	15	28 (41.2%)
P. aeruginosa	13	14	27 (39.7%)
E.coli	2	1	3 (4.4%)
S. horminis	1	2	3 (4.4%)
S.saprophyticus	1	2	3 (4.4%)
A. baumanii	2		2 (2.9%)
Proteus	1	1	2(2.9%)
Total	33	35	68

2.3. Subclinical developments in patients with superficial burns wound

Table 2.9. Changes in some subclinical parameters of patients

Index	Research time		
	T0	T1	T2
Hematology			
White blood cells	13.7 ± 3.7	12.5 ± 0.71	8.6 ± 4.2
Red blood cells	4.4 ± 0.3	3.99 ± 0.68	4.37 ± 0.02
Hemoglobin	125.9 ± 18.6	111 ± 12,6	119.9 ± 10.23
Hematocrit	35.1 ± 3.2	32.9 ± 2.57	36.4 ± 2.9
Platelet	315.5 ± 76.8	408.4 ± 188.6	368 ± 127.6
Blood chemistry			
Urea	3.04 ± 0.85	2,1 ± 0.5	2.5 ± 0.1
Glucose	5.8 ± 1.7	5.6 ± 0.6	4.95 ± 0.35
Creatinin	52.3 ± 17.9	56.5 ± 30.4	32.7 ± 0.57
protein	66.05 ± 8.1	64.35 ± 0.9	61.8 ± 16.1
Albumin	40.1 ± 3.48	31.3 ± 4.5	35.8 ± 1.48
ALT	26.2 ± 6.38	27.2 ± 5.3	31 ± 3.6
AST	35.3 ± 25.03	24.4 ± 12.7	35.2 ± 30.8
Na	134.8 ± 2.58	136.7 ± 2.1	138 ± 2.85
K	4.5 ± 0.1	4.2 ± 0.42	4.55 ± 0.78
Cl	103 ± 2.4	103.4 ± 1.8	102.7 ± 1.73

WBCs in the first-week increase clearly, then return to normal levels. Red blood cells decreased slightly in the 2nd test (still at a normal level) then recovered in the 3rd test. The Hb and hematocrit levels tended to decrease at the 2nd test, gradually recovered after 2 weeks (the time points were still within the same time). Platelet count is normal.

The biochemical indicators were within normal limits, except for the albumin test the 2nd time, which decreased, then recovered to normal after 2 weeks. 4 cases with Albumin decreased below 30g/L in 2 patients with deep burns injuries. Burn size (TBSA 16%, Full thickness burn 10%) and TBSA 7% Full thickness burn (3%), 2 patients with TBSA upper 10% burns. If not counting this number of patients, Albumin index the mean will be 34.99 ± 3.25 (within the normal value).

3. DISCUSSION

3.1. Anti-inflammatory effect of berberine nano gel

Clinically, we did not see any cases of inflammation spreading to normal skin. In patients with deep burns, the duration and degree of swelling in zone A are less than in region B

Corresponding to swelling inflammation is the state of exudative, purulent, and pseudomembranous Wounds. Area A burn wounds are cleaner, and exudate and pus are significantly less. This effect was evident in most of the studying patients after 1 day of using the study drug. This is a remarkable advantage of Berberin nano gels.

Regarding the anti-inflammatory mechanism, Berberine nano gels may be created thin dry scales in superficial burns and dry up necrosis of deep burns.

In superficial burns, when the burn wound dries, the gauze adheres to the medicated gel, creating thin scabs on the burn wound until it heals.

In a burn wound with the III degree burn necrosis, the drug dries up and clears the necrotic layer after 2 - 3 days of using the drug, then the necrosis falls off (after 4 - 5 days of using the drugs) the whole plaque, epithelial lesion background. While in zone B, the drug does not dry up the necrosis, when it falls off, it is usually later, dissolving accompanied by inflammation and purulent fluid. Progressive wet necrosis with purulent inflammation and extensive disintegration.

This drying necrotic effect is also consistent with the clinical course of experimental burns and wounds in rabbits.

3.2. Anti-infective effect of Berberine nano gel on burns wound

In the study, we did not encounter any cellulitis cases in 2 areas, which showed swelling, redness and pain spreading to the normal skin. Clinical manifestations of exudate and purulent discharge were significantly reduced in area A when compared with area B (treated with SSD cream) in the period after 5 - 10 days post-burning and applying treatment.

On bacterial culture results: The number of infected wounds increased after 1 week, then decreased, $p < 0.05$. Comparison between 2 groups each time: no difference, $p > 0.05$. It can be said that the effect of infection burn prevention in general, the effect of Berberin nano gel on bacteria in burn wounds is equivalent to that of SSD.

S. aureus and *P. aeruginosa* are still two species of bacteria in burn wounds with the highest percentage. The other species is low prevalence (rarely, the number of samples for each species is still small) so there are not enough comparative data.

Studies have shown the antibacterial effects of Berberine, gram-positive, gram-negative, fungal and even viral [9, 10, 13]. Of which, Berberin is still effective against 2 species *P. aeruginosa* and *S. aureus*, even against methicillin-resistant *S. aureus*. In vitro studies on the inhibitory ability of Berberine against Methicillin-resistant *S. aureus* reduced minimum inhibitory concentration of 32 - 128µg/mL. Berberine reduced significantly these concentrations of ampicillin and oxacillin with methicillin-resistant *Staphylococci*, restoring the effect of β-lactam antibiotics [11, 12].

According to the research by M. Čerňákoval, The antibacterial activity of berberine varies considerably depending on the organism tested, the sensitivity decreases as follows: *S. aureus* > P. *aeruginosa S (sensitive) > E. coli > P. aeruginosa R (resistant) > E. coli R > B. subtilis > Z. ramigera > C. albicans > S. cerevisiae > A. pullulans B (black) > A. pullulans W (white) > T. viride Br (brown) > M. gypseum > A niger > F. nivale > P. chrysogenum > T. viride G* (green) [12].

Recently, there have been some studies showing that the antibacterial effect of berberine bound to NP has been markedly increased.

Mechanism of NP Berberin action against some common bacteria in burn wounds:

In a review [11], Suliman Khan synthesized that the antibacterial mechanism of Berberine, such as cell wall degradation, leakage of a cell to the outside, disruption of proton transport, the disappearance of protein bands, inhibit the synthesis of protein and DNA, and block bacterial metabolism.

3.3. Stimulating effect on clinical wound healing

Nano Berberin gel has reduced exudate, and *pseudomembranous*, reduce the risk of infection, facilitate the healing of burn wound. In third-degree burns, with necrosis, the conversion of wet necrosis to dry necrosis has reduced inflammation and swelling, secretions, and pus; thus facilitating the burn wound healing process [10].

Therefore, the results of wound healing in area A take place faster than in area B, with the time for epithelialization 50% of area A also shorter than that of area B, treatment time for third-degree burns in area A is also shorter than in area B, $p < 0.05$.

3.4. The Safety of Berberine nano gels in clinical practice

Applying Berberine nano gel medicine to the superficial burn area is painless. Conducted a study on 32 patients in the whole group of children, we did not encounter any cases of allergic reactions or irritation at the lesion site. During the whole study, we also did not see any case with systemic allergic manifestations and did not experience systemic or organ disorders related to the drug. The hematological (except leukocyte) and biochemical (except albumin) parameters were within normal limits.

This is consistent with our pre-clinical studies that have documented the safety of Berberine.

4. CONCLUSION

Effect of Berberine nano gel on 32 patients with superficial burns, compared with 1% SSD cream:

1. Gel has an anti-inflammatory effect (the degree and duration of inflammation and exudate on the burn wound are significantly reduced compared to the control area).

The gel dries wet necrosis (obvious after 2 - 3 days of using the drug), and while the area of wet necrosis develops, swelling and exudation become more abundant.

2. Gel has the effect of preventing infection, showing a significant reduction in the number of positive cultures and the number of bacteria after 1 - 2 weeks of treatment, $p < 0.05$. The number of infected wounds after 1 week also decreased significantly in the control area, $p < 0.05$.

3. The time for 50% epithelialization, the treatment time in the study area is also shorter than in the control area, $p < 0.05$.

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