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Use of Human Umbilical Cord Serum to Treat Animal Skin Burns

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Abstract

The objective of the study was to test the hypothesis that human umbilical cord blood crude serum applied topically may promote an early healing for animal models with burn injury. Human umbilical cord serum HUCS was collected and screened for transmitted diseases such as hepatitis B, hepatitis C and HIV. The HUCS was subjected to microbial testing to demonstrate the presence or absence of viable contaminating microorganisms. Mice (no=45) and rabbits (n=16) were scalded by boiling water then treated with HUCS in comparison to untreated animals. Another group of rabbits (n=24) were subjected to hot water (70°C) and 1N NaOH and treated with HUCS in comparison to cetrimide 0.5% cream treated group and control group (without treatment). Topical application of HUCS promoted the healing process; complete healing was seen after 10 days in the mice group and 7 days in the rabbit group. Cetrimide group applied to the second rabbit group showed slow healing and needed 10 days for hair regrowth. Control group in both mice and the two rabbits' group, showed very slow response and the burn area diameter remained the same for over 10 days, and no hair regrowth was observed after 10 days. In conclusion the results of the current study indicated that HUCS is a promising therapy for healing of burns caused by boiling water alone or by alkali with hot water. More clinical trials are needed to explore the long-term effects after UCS use and incorporation of HUCS in suitable dosage form.

Keywords: HUCS, Burn injury, Cetrimide, Animal models.

استعمال مصل الحبل السري البشري لعلاج حروق جلد الحيوان

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الخلاصة

الغاية من البحث هو لإثبات فرضية ان المصل المستخلص من دم الحبل السري للإنسان قد يعجل الشفاء عند مسحه على جلد الحيوان المحروق. تم جمع مصل الحبل السري HUCS بعد التأكد من خلوه من الامراض الانتقالية كالتهاب الكبد الفيروسي نوع (ب) و(ج) ومرض نقص المناعة المكتسب واخضع للتجارب الميكروبية المختبرية للتأكد من خلوه من البكتيريا والعفن المرضية. تم حرق جلد الفئران (عدد ٤٥) والارانب (عدد ١٦) بالماء المغلي وعلاج البعض بمصل الحبل السري ومقارنتهم بالذي لم يتلق علاج. ومجموعة أخرى من الارانب تم حرقها بواسطة ماء حار (٧٠° س) والصودا الكاوية (1N NaOH) وثم علاجها بمصل الحبل السري او بالسترامايد (Cetrimide) بالمقارنة مع مجموعة لم تخضع للعلاج. ان مصل الحبل السري عجل الشفاء والتئام كامل في مجموعة الفئران (خلال ١٠ أيام) والارانب (خلال ٧ أيام)، اما مجموعة الارانب التي تم علاجها بالسترامايد تحسنت ولكن ابطأ من المجموعة التي عولجت بالمصل واحتاجت ١٠ أيام لنمو الوبر من جديد. الحيوانات التي لم تتلق أي علاج سواء كانت الفئران والارانب التي تعرضت لنوعي الحرق، أظهرت تحسن بطيء جدا وبقي مساحة الحرق كبيرة، ولم ينم وبرها حتى بعد مرور ١٠ايام. نستنتج ان مصل الحبل السري وسيلة فعالة لتعجيل الشفاء للحروق الناتجة عن الماء المغلي او الماء الحار والصودا الكاوية. ويلزم دراسة سريرية مستفيضة لمعرفة الاثار الناتجة بعد استخدام المصل ودراسة طرق تحضيره كمستحضر صيدلاني.

الكلمات المفتاحية: مصل الحبل السري، حروق جلد الحيوان، السترامايد.

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Introduction

Burn is a thermal injury caused by contact to a physical, chemical or electrical source [(1)] and degree of the burn depends on the temperature of the skin, temperature of heat source and the duration of exposure [(2)].

Burn injury represents a cellular stress in the skin. Burns are associated with inflammation which exacerbate pain and impair wound healing, and infection due to damage of the external epidermal layer which disrupts the innate immune system and increases susceptibility to bacterial infection [(3)]. One of the most important complications of burn healing is infection, especially burnt in- patients which suffer from multi-drug resistance [(4)].

Burns can result in fibro proliferative scarring, skin contractures, or chronic wounds that take weeks or months to heal. Burn injuries are highly individualized owing to wound-specific differences such as burn depth and surface area, in addition to patient-specific factors including genetics, immune competency, and age [(5)]. Other extrinsic complications such as microbial infection can complicate wound healing, resulting in prolonged inflammation and delayed re-epithelialization, with high risk of hypertrophic scarring and infection which can lead to the development of a pronounced immune response, accompanied by sepsis or septic shock, which results in hypotension and impaired perfusion of end organs, including the skin – all processes that delay wound healing [(3,6,7)].

Burns are classified into three main grades; first grade (epidermal layer), second grade superficial (superficial dermal), deep second grade (mid dermal) and the third grade (deep dermal and full dermal thickness), the third grade burns are painless, with dry appearance, with no bleeding, this type of burn is hard to heal [(1,8)].

Management of burn include antibacterial administration (topical and oral), and moisture retention dressing and /or graft replacement in third grade burns [(9)]. Sometimes antibiotics alone fail to eradicate the microorganisms causing infection, therefore the use of unconventional treatment is needed [(10)].

Human umbilical cord serum HUCS normally contains two arteries and a single vein, all embedded in Wharton's jelly, which is a collagenous matrix within the umbilical cord that protects the umbilical vessels and contains an abundance of hyaluronic acid (HA)[(11)] .

The possibility of HUCS topical application to wounds, including burns, can provide a cheap and accessible wound care for many of the neediest patients in the world [(12,13)]. Like peripheral blood serum, HUCS contains hyaluronic acid [(14)] and many growth factors and stem cells [(15)]. Therefore, it can support healing, by providing the skin basic elements for regeneration that are lacking in the conventional medications [(16)]. Human umbilical cord blood serum was proposed to improve morbidity and mortality [(13)].

Since 2003 the number of studies using stem cell from different origin (adipose tissue, bone marrow, and umbilical cord). have increased, either applied topically as a collagen-chitosan scaffold or as Biodegradable Poly (β-amino esters) nanoparticles or via intradermal injection [(17-20)]. But the number of studies using umbilical cord blood for skin injuries remains minor; Liu *et al.* injected UCB intravenously into burnt mice, while Dai *et al.* isolated mononuclear cells from mesenchymal stem cells of human umbilical cord blood and transplanted into injured nude mice by tail vein injection [(21,22)].

There are several advantages which the cord blood serum can offer than other blood-derived preparations; mainly, a large volume of serum can be gained from the umbilical vein at one time, to overcome the need for sampling of the peripheral blood from each patient and avoid repeated blood collection from patients. Furthermore, avoid the difficulty of obtaining blood from patients with infectious diseases or with abnormal materials in blood dyscrasia, also it is considered as medical waste [(23,24)].

Burn healing in third grade burns by human umbilical cord serum (HUCS) was investigated in this study.

In the current study, acute burn injury was inflicted, and the subcutaneous layer (full thickness) was compromised by boiling water or by hot water (70°C) with 1N NaOH in which, infection is the major associated complication.

Materials and Methods

Umbilical cord collection

Fresh human umbilical cord blood was collected from donor pregnant women at the time of delivery from Al-Karkh Hospital (after receiving their consent) and screened for transmitted diseases such as hepatitis B, hepatitis C and HIV. The cords and placenta were collected immediately after delivery and the blood was drained by gravity from the placenta and strained by hands and collected in sterile containers. The blood was left to clot, and the serum was isolated by centrifuging at 2000 rpm for 10 min and stored at -20°C in vials each containing 1ml [(25)].

Sterility test

The HUCS was subjected to microbial testing. All sampling was carried out under aseptic conditions in a laminar flow cabinet, and HUCS was streaked on a Nutrient agar plate (N/A) then incubated for 24 hours at 37° C to demonstrate the presence or absence of extraneous viable contaminating microorganisms.

Müeller-Hinton agar (MHA) is more commonly used for the routine susceptibility testing of non-fastidious [26].Well Diffusion assay was done in MHA using a sterile cork borer to punch holes on an agar plate; using the swab with the test organism to streak the MHA plate for a lawn of growth. The selected microorganisms included the common skin and mucosal contaminants *Staphylococcus epidermidis* (ATCC 700562) and *Staphylococcus aureus* (ATCC 6538), respectively; the pathogenic Gram-negative *Pseudomonas aeruginosa* (ATCC 15442); and *Candida albicans* (ATCC 10231), a yeast commonly isolated from umbilical cord blood. After the streaking is complete, the plate was allowed to dry for 5 minutes. The HUCS was placed in a well to study the antimicrobial effect. Then the inoculated Müeller-Hinton agar plates were placed in an incubator for 24 hours at 37° C.

Animals

Mice weighing 18-23g (10-12-wk. old) and albino rabbits weighing 2-2.5kg (6 m. old) from the laboratory animal house of Al-Razi Center in accordance with the Institutional Animal Care and Use Committee. The animals were kept in the animal house at a temperature of about 25°C, in which the sun was the light source, and they were provided with standard diet and drinking water *ad libitum*. The animals were held tight and their back hair was shaved. Thermal burns were induced on the animals by two methods, first; wet thermal source (scalds), this was performed on the mice and rabbits, the shaved area was subjected to boiling water (100ºC) for 5 sec., the burn diameter for mice was about 1cm in diameter, while the first rabbit group burns were about 7-8 cm in diameter [27]. While the rabbit second group which were subjected to both scalding by water 70°C for 5sec., and chemical burning by applying 2-3 drops of (1 N NaOH) on 4 circles points on each rabbit's back of about 1 cm^2; all the animals showed 3rd degree burns. This procedure was sufficient to induce burns of third grade [24]. Mice were chosen due larger population possibility [27] .Rabbits were chosen because of larger size and will not be affected by the burn area size [28].

Methods

In the initial experiments HUCS was taken out of the freezer on the day of experiment and thawed to room temperature and applied few drops to sterile gauze. The mice and rabbits' wet thermal burns were dressed daily with HUCS impregnated sterile gauze. Digital photographs were taken on day 3, 7, 10 and 20 for macroscopic evaluation to measure the wound surface and wound contracture and compared with control (animals without treatment). While, the HUCS was swabbed on rabbits back (burnt by wet thermal and chemical burns daily).

The treatment method was divided into three groups:

- Group A this group received HUCS treatment.
- Group B which received (Cetrimide 0.5% cream) Celavex® SDI a mild antiseptic daily.
- Group C which didn't receive any treatment as a control.

Statistical analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effect of difference groups in study parameters. Least significant difference –LSD test or T-test was used to significant compare between means (0.05 and 0.01 probability) in this study [29].

Results and Discussion

Microbiological assessment showed after incubation under testing conditions, no growth was seen on nutrient agar streaked with the HUCS (Figure 1a, 1b) which means that HUCS did not support microbial growth, and no inhibition zone was seen (Figure 1c, 1d) against the tested microbes. However, satisfactory results, only indicated that, no contaminating microorganism had been found in the sample examined under the conditions of the test, also cord serum had bacteriostatic effects due to antimicrobial peptides [30,31].

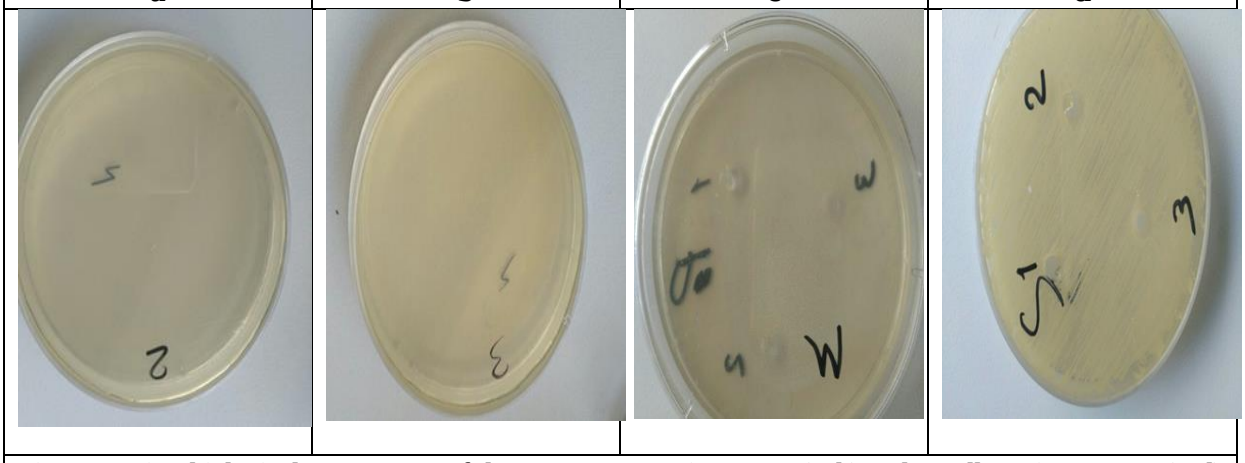


Figure 1.Microbiological assessement of the HUCS on Nutrient Agar (a, b) and Müeller-Hinton Agar (c, d)

The efficacy of HUCS in the healing of skin burns was evaluated by the clinical evidence of injured area decrease in diameter, inflammation reduction, and hair regrowth after treatment. The results may be related to growth factor supplementation, and angiogenesis [(32)].The burnt area was proportional to the animals' surface area.

Results in table 1 and figure 2 showed the burn healing process when the HUCS was applied topically to mice, in 3 days the burn area started to contract and fibrin clot was formed, and after 10 days remission was complete, and the hair started to grow again. The accelerated healing process by HUCS was evident, and mice back hair grew tremendously faster after HUCS application in comparison with control group. Although experiments using mice are more convenient, but the mouse model has many disadvantages, mouse have a thinner epidermis and dermis compared to humans, the mouse hair cycle is usually three weeks, where as human hair cycles can last several years [(33)]. Also, mice wound healing process involves contraction while human healing process involves re-epithelialization and granulation [(34)].

Table 1. Comparative mice groups at different stages of treatment with control subjected to scalding

Duration of treatment with HUCS (Day)		Treated group	Untreated control group	T-Test (P-value)
0	No of animals	(n=25)	(n=20)	-
	Burn area %	23.86034 ± 0.382082	23.86034 ± 0.382082	1.035 NS (0.548)
3	No of animals		(n=17) (85%)	-
	Death %		15% Died (n=3)	-
	Burn area %	15.97429 ± 2.172889	18.81667±2.016667	4.932 NS (0.092)
7	No of animals		(n=15)	-
	Death %		11.7% Died (n=2) Overall (25%)	-
	Burn area %	6.034755 ± 0.289341	11.20269 ± 2.434742	3.834 * (0.0372)
10	No of animals		(n=10)	-
	Death %		33.3% Died (n=5) Overall (50%)	-
	Burn area %	3.125701 ± 0.52276	6.357143±0.071429	1.638 * (0.0252)
20	No of animals		(n=7)	-
	Death %		30% Died (n=3) Overall (65%)	-
	Burn area %	0.721154 ± 0.240385	3.911765±1.088235	1.085 * (0.349)

* ($P<0.05$), NS: non-significant.

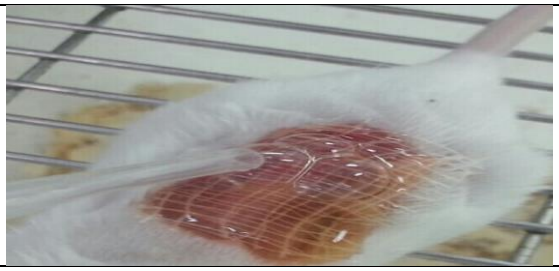
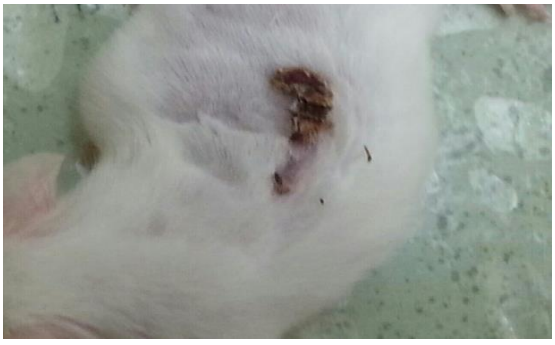
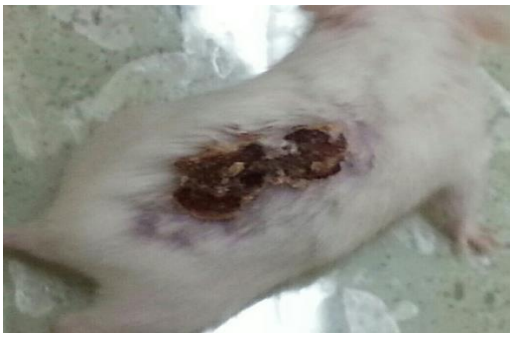
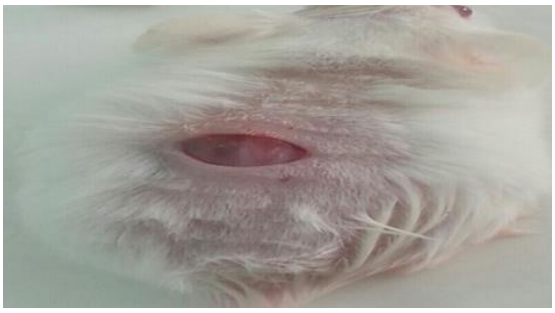
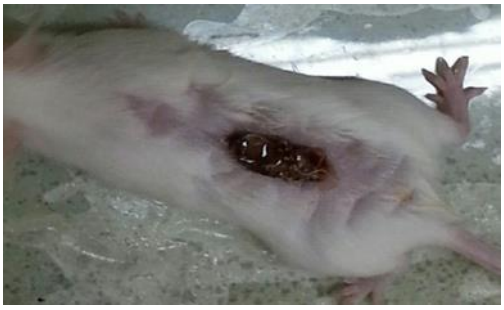
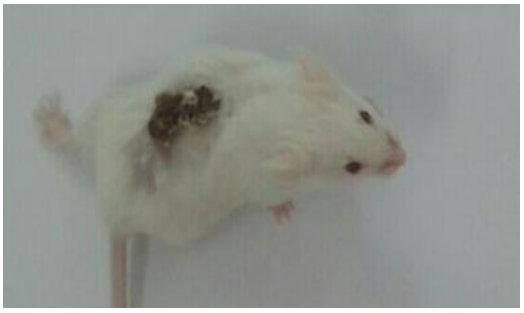
Day after treatment	Treatment group	Control group
0		
3		
7		
10		
20		

Figure 2. The difference in healing process between the mice treated with HUCS in comparison with control without treatment

Rabbits were used as a second model; the results were almost the same as mice. The rabbits were subjected to boiling water (Figure 3, and Table 2), the burn area of the group treated with HUCS showed accelerated healing and wound coverage with complete remission in 3 weeks in comparison with the untreated group.

Table 2. Comparative burn area (%) in rabbit groups subjected to scalding by boiling water

Duration of treatment with HUCS (Day)	Treated group Burn area %	Untreated Control group Burn area %	T-Test (P-value)
0	22.91667±2.172889	22.91667±2.172889	3.784 NS (0.688)
3	11.15177± 0.037422	19.21856±0.01221	1.0953 ** (0.0001)
7	7.414966± 0.748299	17.84059±0.697734	3.029 ** (0.0001)
10	3.846154± 0.675676	11.86331± 0.636694	3.261 ** (0.0001)
20	0.500992± 0.054563	7.450054± 0.713212	1.369 ** (0.0001)

** ($P < 0.01$), NS: non-significant, ($n = 8$)

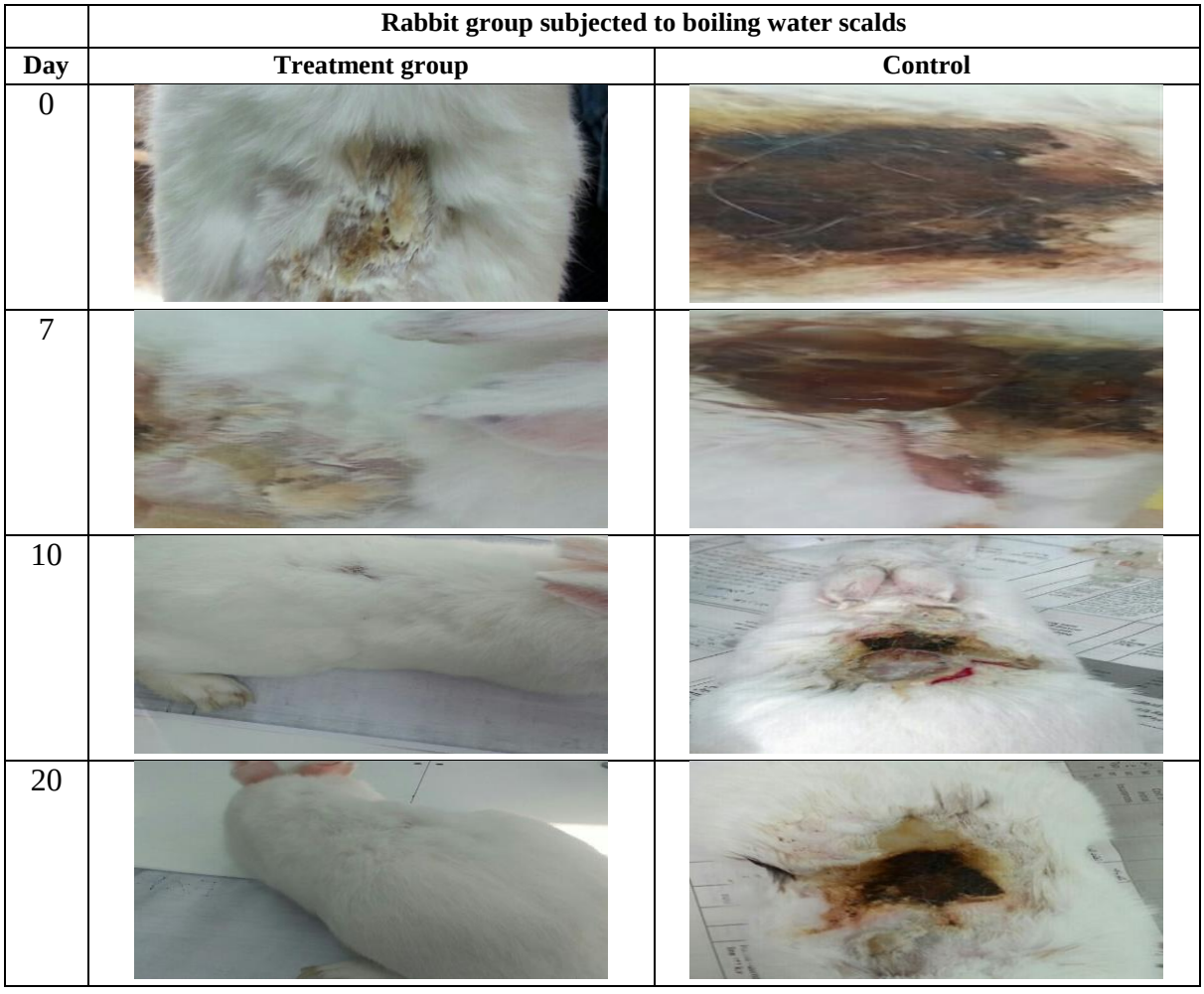


Figure 3. The difference in healing process between the scalded rabbit group treated with HUCS in comparison with control without treatment

While the second group of rabbits which were subjected to both hot water (70°C) and 1 N NaOH burns (Group A) which received the HUCS as shown in the figure 3, showed the fastest healing, decrease in diameter of the burn, reduced inflammation around the burn area, formation of fibrin clot at day 3 and hair regrowth after 7 days .

Group B which received Cetrimide showed slow healing , after 7 days the wound formed a fibrin clot and the inflammation area around the

burn decreased and needed 10 days for hair regrowth.

Group C showed very slow response and burn area diameter remained the same for over 10 days, the inflammation was at its peak at day 3, fibrin clot formed slowly, and no hair regrowth was obvious after 10 days.

Table 3. Comparative burn area (%) in rabbit groups subjected to hot water (70°C) and chemical burns within NaOH

Duration of treatment with HUCS (Day)	Group A (HUCS applied) Burn area %	Group B (Cream 0.5%) Burn area %	Group C Control Burn area %	LSD (P-value)
0	48.4308±5.039109	48.4308±5.039109	48.4308±5.039109	4.711 NS (0.794)
3	26.07895±2.453303	33.73737±2.626263	37.69737±0.197368	2.176 * (0.0377)
7	22.04199± 1.046244	26.02026±1.252463	25.15527±0.796291	2.017 * (0.046)
10	15.57353± 2.073529	20.51948±2.337662	22.22413±2.134844	4.703 * (0.0359)
20	8.465608± 1.058201	12.41176±3.588235	14.53595±1.464052	3.066 ** (0.0027)

* (P<0.05), ** (P<0.01), NS: non-significant, LSD Least Significant difference (n = 8**)**.

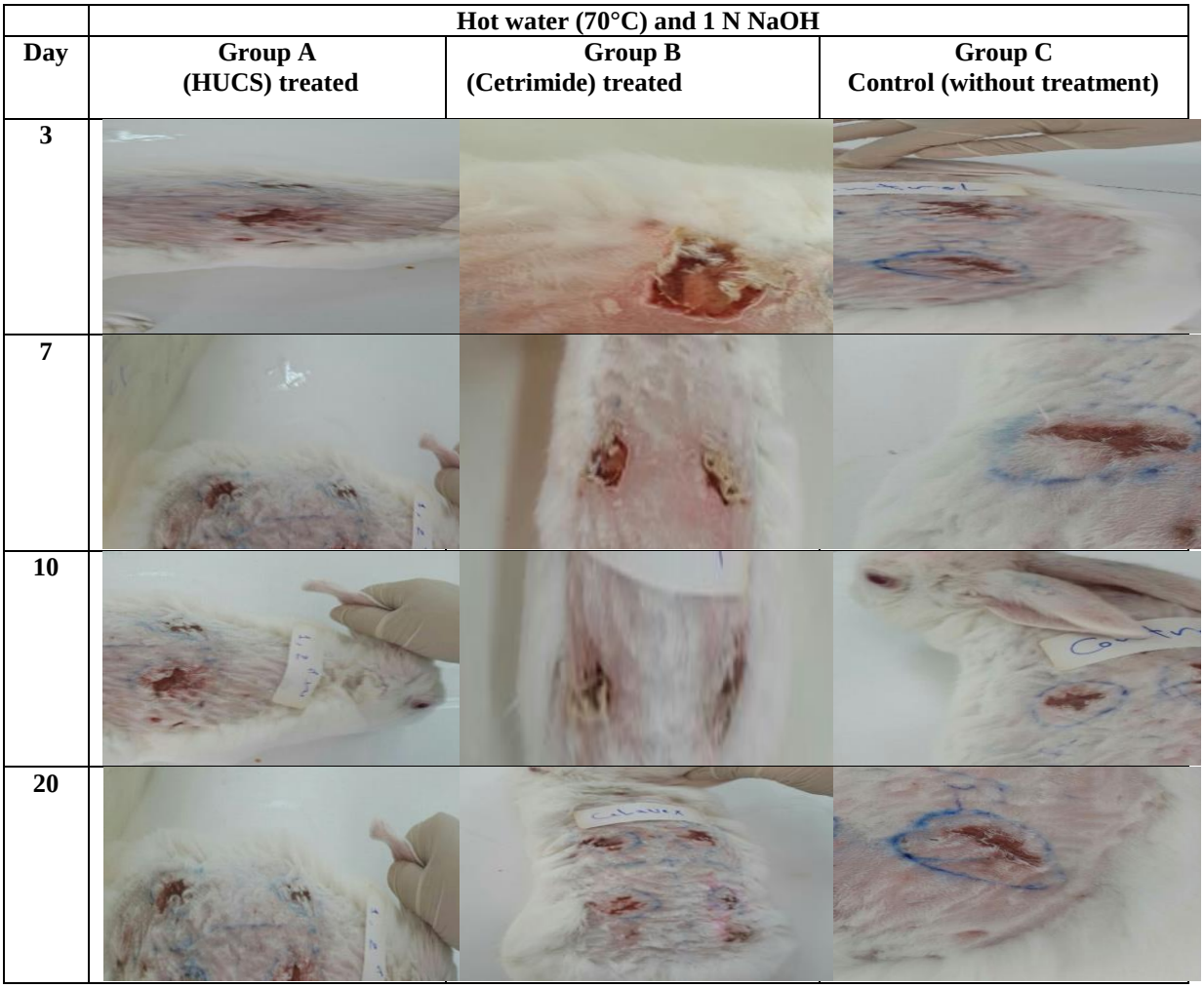


Figure 4. The difference in healing process between rabbit group subjected to both hot water (70°) and 1 N NaOH burns treated with HUCS in comparison with those treated with 0.5% cream and control without treatment

The use of HUCS topically accelerated the burn healing without any side effects or any sign of injury microbial contamination. Wharton's jelly are reported to possesses biological properties (collagen 1, hyaluronic acid, laminin, and fibronectin) that stimulate cellular responses important for soft tissue healing and promote scar-less wound healing [(11)].
The level of satisfaction with the HUCS treatment was very high, for all animals tested, with improvement of their skin injury as early as day 3, reaching full satisfaction at endpoint of 3 weeks of the experiment. The HUCS had been shown to have anti-inflammatory effects which promote angiogenesis and enhance healing. Therefore; HUCS was recommended for burnt human.

Conclusion

In conclusion, this work confirmed that HUCS is a promising therapy for the healing of burns by alkali substance and/hot or boiling water. The area exposed to boiling water or hot water with alkali decreased in size and the burn improved after 10 days of treatment. The extent of area of burn injury rather than its duration was the main factor determining the early response and repair process. The HUCS need to be prepared by well-equipped and trained laboratory staff. Preparation steps include efficacy and microbiology controls for a reproducible quality of the final product. More clinical trials are needed to further explore the long-term effects with the HUCS and ultimately provide safer and more effective therapies for future clinical applications.

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