The Study on Effects and Safety of *Spongilla lacustris* in 3% Hydrogen Peroxide Solution on Rat Skin

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Objective: *To study the effects and safety of Spongilla lacustris (SL) in 3% hydrogen peroxide (H_2O_2) on rat skin.*
Material and Method: *An experimental study was conducted on 3 groups of Wistar-Furth adult rats. The first group was applied with SL in H_2O_2; the second group with SL in 0.9% normal saline (NSS) and the control group with NSS. These agents were applied on and wiped off the rat skin weekly for four weeks, then the skin biopsies were done. The number of SL spicule and the depth of spicule penetration were examined by scanning electron microscope and by polarized light microscope respectively. Skin histopathology was determined by hematoxylin-eosin staining. The gross skin changes were observed.*
Results: *Under electron microscopic examination, SL was demonstrated as spicule which was sharp-edged, rod-shaped and smooth surface with approximate 150-300 microns in length and 10-20 microns in diameter. Spicule retention was found in the rat skin lasted until day 3 but was undetectable on day 7. The spicules could be detected deep into stratum basalis. Comparing among three groups, the thickness of epidermis in the second group was decreased with statistically significant difference ($p = 0.044$) by the end of week 7. The dermal thickness of all groups was increased by age. No any gross skin alteration of all groups was observed.*
Conclusion: *The authors hypothesized that the spicule causes puncture that enhances H_2O_2 penetration into the skin. This solution was safe in the short term usage. However, the long term safety regarding granulomatous formation is still questionable.*

Keywords: *Spongilla lacustris, Hydrogen peroxide, Acne vulgaris, Rat skin*

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Acne vulgaris is a common skin disease of pilosebaceous unit. The etiology is multifactorrial[1]. The pathogeneses are follicular epithelial hyper-proliferation, sebaceous gland hyperfunction, inflammation and *Propionibacterium acne*[1-3].

Antibiotics and retinoids are the standard treatments of acne. Topical therapy alone (benzoyl peroxide, clindamycin, erythromycin, retinoic acid) or in combination with oral therapy (antibiotics, retinoic acid) is recommended according to the severity of acne. Currently, new modalities of acne treatment have been developed including light therapies which are blue light, intense pulsed light and lasers[4,5].

Spongilla lacustris is a freshwater sponge in the phylum of Pohfera where its habitations are in Europe, Asia, Russia and Canada[6,7]. The main composition of this sponge is silica or silicon dioxide[7-10]. In the past few years, *Spongilla lacustris* (SL) sponge powder extract in 3% hydrogen peroxide (H_2O_2) solution was used for the treatment of acne vulgaris in many countries. However, it provides no scientific report on pharmacological evidence as well as its safety for the treatment of acne. As a result, the authors conducted the present study concerning the effects and safety of the sponge extract in 3% H_2O_2 solution on rat skin.

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Material and Method

An experimental study was conducted on 32 Wistar-Furth adult rats from the National Animal Model Center, Mahidol University. The age was 8 weeks and the mean starting weight was 258 grams. They were raised in the same condition and categorized into 3 groups. The first group would be applied with SL extract in 3% H_2O_2 solution, the second group with SL in 0.9% normal saline solution (NSS) and the control group

with 0.9% NSS. The number of rats used in the present study was 15 in first group, 15 in second group and 3 in control group.

Anesthetic procedures were done by putting the rats into a jar filled with ether. Once the rats were fully unconscious, they would be shaved on week 0 at their backs creating a 10 cm^2 area. After that, the prepared solutions were thoroughly applied by massaging in circular motion for 10 minutes and leaved on for another 20 minutes before wiping off with 0.9% NSS. Then one cm^2 of rat's skin biopsy was performed for scanning electron microscopic examination and histological examination with hematoxylin-eosin (H & E) staining and polarized light microscope. The solution application was done every week for 4 weeks and biopsies were performed on day 1, 2, 3, 7, 14, 21, 28, 35 and 49 after solution application and wiping off to evaluate spicule retention and skin.

Histopathology

The amount of spicule retention was studied with scanning electron microscope by counting the numbers of spicule per 1 cm^2. Levels of spicule penetration into the skin was determined by using polarized light microscope. The skin histopathology was examined by H & E staining and the thickness of epidermis and dermis was measured. All of the histopathological examinations were interpreted by the author number 2 and confirmed by the author number 1 and 3. The gross skin alterations such as erythema, edema and infection were also subjectively observed on day 1, 2, 3, 7, 14, 21, 28, 35 and 49.

The present study was approved by the ethic committee for animal study, Faculty of Medicine, Srinakarinwirot University.

Statistical analysis

The weights, epidermal and dermal thicknesses of the rat skin were compared between groups by using unpaired t-test with statistically significant difference when p-value was less than 0.05. The gross skin reaction was reported with descriptive method.

Results

The rats gained their weights every week without statistically significant group difference (Table 1). By 350 times of magnification of electron microscopic examination, the authors could differentiate between the rat hair and SL. The hair was demonstrated as a laminated pattern of keratin, while SL was sharp edged, rod-shaped and smooth surface spicule with approximate 150-300 microns in length and 10-20 microns in diameter (Fig. 1). From histological examination of the rat skin with H & E staining, the authors found that the stratum corneum of rat skin applied with SL showed more exfoliation than applied with normal saline solution. By using polarized light microscope, the background could be seen in purplish-red and the tip of SL spicule could be seen piercing into stratum basalis (Fig. 2), no spicule was detected in the dermis.

Regarding epidermal thickness, both the experimental and control groups, showed no statistically significant difference from week 0-5. However, by the end of week 7, the epidermal thickness of the SL + NSS group was less than those of the SL + H_2O_2 group and the control group with statistically

Table 1. Weights of the rats were demonstrated no statistically significant group difference

Week	Groups	Mean weight (grams) (mean $\pm$ SE)	p-value
0	1st group	258.69 $\pm$ 1.52	0.69
	2nd group	258.31 $\pm$ 1.39	
	control group	257.00 $\pm$ 0.00	
1	1st group	291.10 $\pm$ 3.80	0.78
	2nd group	292.44 $\pm$ 2.42	
2	1st group	329.50 $\pm$ 4.25	0.49
	2nd group	325.86 $\pm$ 2.41	
3	1st group	355.33 $\pm$ 6.99	0.60
	2nd group	350.80 $\pm$ 3.13	
5	1st group	402.75 $\pm$ 6.95	0.22
	2nd group	390.33 $\pm$ 3.71	
7	1st group	423.00 $\pm$ 20.00	0.94
	2nd group	424.00 $\pm$ 0.00	
	control group	422.00 $\pm$ 2.00	

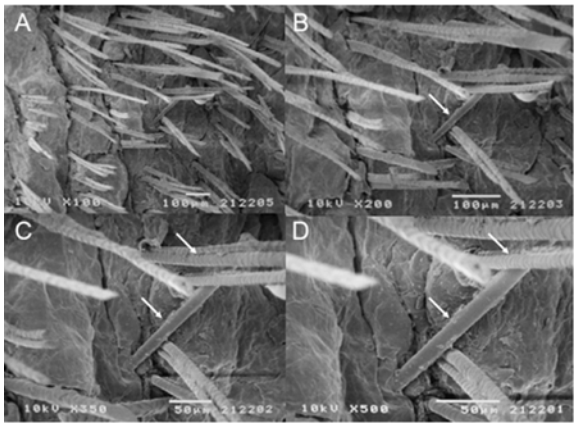

Fig. 1 Electron microscopic examination of the SL demonstrated sharp-edged, rod-shaped and smooth surface spicule with approximate 150-300 microns in length and 10-20 microns in diameter (as identified by arrows). Magnification by 100, 200, 350 and 500 times in A, B, C and D respectively

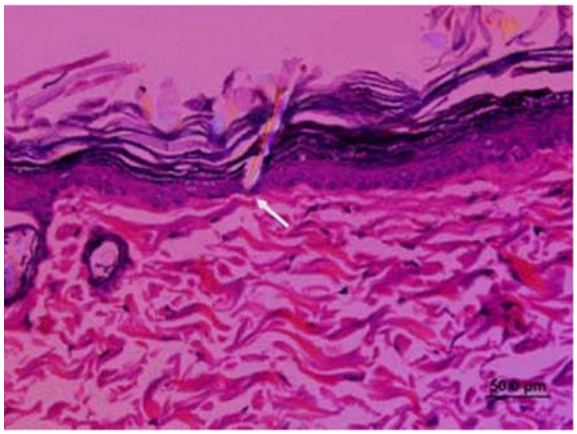

Fig. 2 Polarized microscopic examination of the SL spicule magnified by 40 times, demonstrated purplish-red and the tip of SL spicule could be seen piercing into stratum basalis

significant difference (p = 0.044) (Table 2). As the authors observed that rat dermal thickness of both experimental groups showed more significant difference (p = 0.047) than those of the control group on week 7 (Table 3), the authors then statistically evaluated the correlation between rat weight and the dermal thickness in every group. It was found that there was a statistical correlation relevant with $R^2 = 0.6768$ which can be concluded that the increasing in dermal thickness was a result of weight gaining of the rats as they grew up during the experimental time, not a result of the treatment.

Concerning the scanning electron microscope and polarized light microscopic studies on spicule retention, the authors found that the number of spicule were decreased everyday on day 1, 2 and 3. The first group contained less spicule than the second group. Moreover, there was no retention of spicules in both groups on day 7, the number of spicule also decreased each week after reapplying the agent on day 14 and 21. There was no retention of the spicule on day 35 and 49. With gross observation, no skin inflammation (*e.g.* vesiculation, erythema and edema) and infection were detected. The histological examination showed that there was no inflammatory reaction (*e.g.* spongiotic dermatitis, granulomatous reaction).

Discussion

The present study of *Spongilla lacustris* in 3% hydrogen peroxide solution on rat skin demonstrated the desquamation of stratum corneum and thinning of the epidermis. Moreover, the increase in dermal thickness correlated with the rat weight gain.

Firstly, the authors hypothesized that the mechanism of actions of SL spicule may be either microablative technique[11] or puncture-resembling mesotherapy due to the small and sharp thorn-like nature of the spicule (150-300 micron in length, which was nearly the size of aluminum hydroxide crystals used in microdermabrasion). But the evidence from previous studies on the effect of microdermabrasion on human skin demonstrated that there was the increment in thickness of epidermis and dermis of the treated skin[12,13]. Furthermore, various inflammatory markers of dermal collagen remodeling through wound healing process were also detected *e.g.* transcription factor AP-1, NF-Kβ, IL-1β and metalloproteinase 1 and 3[14]. In present study, the change in the rats' epidermal and dermal thickness did not support the mechanism by which microdermabrasion acts on the treated skin. Moreover, the rat epidermis in the SL + H_2O_2 group was decreased; the epidermal thickness of the SL + NSS was unremarkable and the rats' dermis of both groups were unchanged. In addition, the authors detected the exfoliation of stratum corneum. Therefore, the effect of spicule may only cause deeper puncture into the skin hence facilitating hydrogen peroxide to be penetrated deeper into the skin which can eventually eradicate *Propionibacterium acne*. There was some evidences which demonstrated the role of H_2O_2 stabilized cream for acne treatment[14,15]. It has shown to be as effective as benzoyl peroxide in reduction of both inflammatory and non-inflammatory acne lesions in mild to moderate

Table 2. Epidermal thickness of all groups was demonstrated no statistically significant difference from week 0-5. Epidermal thickness of SL+NSS group was less than those of SL+H_2O_2 and control group with statistically significant difference (p = 0.04) on week 7

Week	Groups	Epidermal thickness (μm) (mean ± SE)	p-value
0	1st group	25.82 ± 0.93	0.75
	2nd group	26.36 ± 1.34	
	control group	25.00 ± 0.00	0.68
1	1st group	26.30 ± 0.50	0.69
	2nd group	26.70 ± 0.70	
2	1st group	28.00 ± 1.80	0.38
	2nd group	25.60 ± 1.20	
3	1st group	25.40 ± 0.60	0.53
	2nd group	25.90 ± 0.30	
5	1st group	24.70 ± 0.90	0.39
	2nd group	26.10 ± 0.90	
7	1st group	24.10 ± 1.00	0.04*
	2nd group	21.60 ± 0.00	
	control group	25.10 ± 0.30	0.20

Table 3. Dermal thickness of all groups was demonstrated no statistically significant group difference from week 0-5. The dermal thickness of SL+NSS and SL+H_2O_2 groups was increased with statistically significant difference comparing to control group (p = 0.04) on week 7

Week	Groups	Dermal thickness (μm) (mean ± SE)	p-value
0	1st group	978.40 ± 36.55	0.85
	2nd group	969.80 ± 24.28	
	control group	990.00 ± 0.00	0.83
1	1st group	1,146.00 ± 66.00	0.44
	2nd group	1,083.00 ± 0.00	
2	1st group	1,157.50 ± 57.50	0.40
	2nd group	1,220.00 ± 15.00	
3	1st group	1,472.50 ± 57.50	0.09
	2nd group	1,252.50 ± 37.50	
5	1st group	1,280.00 ± 5.00	1.00
	2nd group	1,280.00 ± 45.00	
7	1st group	1,317.50 ± 32.50	0.86
	2nd group	1,305.00 ± 0.00	
	control group	1,265.00 ± 60.00	0.04*

severity.

Regarding the safety aspect of the SL, the spicule retention was detected after application of the agents in the first 3 days, but after one week, no spicule could longer be detected. This might be due to the wipe-off technique of the procedure that diminished the spicule. Moreover, the authors observed that the spicules pierced into stratum basalis but not into the dermis. This may be according to the application pressure of the author on the rat skin. Mild pressure may contribute to the fact that the spicules were only be detected deep into stratum basalis and if more pressure was applied they may be found deeper into the dermis.

No rash and no histopathological change of the rat skin were demonstrated (*e.g.* spongiotic dermatitis and granuloma formation). However, if there was any retention of the spicule which its main component is silicon dioxide, granulomatous reaction in dermis might occur both acutely and in long term[16,17]. As described in the report of Kenmochi A et al, granulomatous reaction was observed as early as 3

days after indwelling catheter containing silica was removed from the patient[16]. Furthermore, there was a report of granulomatous reaction from glass particle retention after 10 years of trauma[17]. Since the spicule retention and depth of spicule piercing might depend upon the pressure application of the author and despite the fact that the present study demonstrated no retention and skin alteration both clinically and histologically after 7 week follow-up, they were safe to use in clinical practice with mild and gentle application in short term treatment. The major concern is that the granulonatous reaction from silica can occur as lately as 10 years. So the long term safety is guarded.

In conclusion, the authors hypothesized that spicule of *Spongilla lacustris* showed effects on the rats' skin by causing poration-facilitating penetration of hydrogen peroxide to effectively treat acne vulgaris. *Spongilla lacustris* was safe to be used on the rats' skin in short term. While the long term safety cannot be concluded particularly on granulomatous reaction.

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Potential conflics of interest

None.

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การศึกษาผลและความปลอดภัยของ *Spongilla lacustris* ใน 3% hydrogen peroxide บนผิวหนังของหนูทดลอง

มนตรี อุดมเพทายกุล, มนตรี วงศ์นิราศภัย, อุดมศรี โชว์พิทธพรชัย, อัมพร จริยะพงศ์สกุล

วัตถุประสงค์: *Spongilla lacustris (SL) ใน 3% hydrogen peroxide (H_2O_2) ได้ถูกนำมาใช้ในการรักษาสิวแต่ยังขาดการวิจัยถึงประสิทธิภาพและผลข้างเคียง*

วัสดุและวิธีการ: *เพื่อศึกษาถึงผลของ SL และ H_2O_2 ในผิวหนังของหนูทดลอง โดยแบ่งหนูเป็น 3 กลุ่ม กลุ่มแรกทาด้วย SL + H_2O_2 กลุ่มที่สองทาด้วย SL + 0.9% normal saline (NSS) และกลุ่มที่สามทาด้วย NSS เป็นกลุ่มควบคุม โดยทาทุกสัปดาห์เป็นเวลา 4 สัปดาห์ จากนั้นทำการตัดชิ้นเนื้อ เพื่อส่องหาจำนวนของ spicule ของ SL และความลึกในการฝังตัวของมันโดยใช้กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราดและกล้องโพลาไลซ์ตามลำดับ*

ผลการศึกษา: *โดยกล้องจุลทรรศน์อิเล็กตรอนพบว่า SL มีลักษณะเป็นผิวเรียบขนาดความยาว 150-300 ไมครอน และขนาดเส้นผ่าศูนย์กลาง 10-20 ไมครอน พบมีการค้างของ spicule ที่ผิวของหนูจนถึงวันที่ 3 และหายไปในวันที่ 7 ของการศึกษา และพบว่า spicule แทงทะลุถึงชั้น stratum basalis*

สรุป: *คณะผู้นิพนธ์ได้ตั้งสมมติฐานว่า spicule ของ SL ทำให้เกิดรูเป็นผลให้ H_2O_2 ซึมลงผิวได้ดีขึ้น สารนี้ปลอดภัยในการใช้ระยะสั้น แต่ผลในระยะยาวโดยเฉพาะการเกิด granuloma คงต้องติดตามต่อไป*