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[1]School of Traditional Medicine, [2] School of Biomedicine, [3]School of Traditional Medicine,
[4]School of Pharmacy, Health Sciences University, Ulaanbaatar, Mongolia

D. ZANDANSUREN[1*], D. AMGALANBAATAR[2] , S. OLDOKH[3],
U. OTGONBAATAR[4], S. TSETSEGMAA[4]

The effect of sinder (Limoniterra) on external wounds as analyzed by hystomorphology

Wpływ getytu *(Limoniterra)* na rany zewnętrzne według analizy histomorfologicznej

Sinder has been used for centuries as a raw material for traditional medicine. According to the Mongolian traditional medicine practitioners, there have been over 3000 types of drugs that have used sinder as a medicinal ingredient [1]. The main components of sinder used were the various spars and minerals containing iron and silicon mud and salts. This knowledge is recorded in ancient drug scriptura (sutra) by Ishbaljir (Lhantiv-chandal), Jambaldorj (Oinjin zetsermegjin), Lubsanchutem (Erdes shoroon emiin sudar), Dandar (Emiin talaar bichsen zuils), and by the Tibetan doctors Yondongombo (Rashaan zurhnii naiman gishuunt nuuts uvidasiin undes) and Desrid Sanjaajamts (Biderombo) who described the traditional methods for prescribing and preparing the raw drug material [2–5]. According to the books and sutras, sinder is formed by pressed waterweed on the beach of lakes and seas. In some writings, sinder is described as a reddish- brown, delicious fluffy thing in the cave of a rock which is always wet". Sinder is also described as having the colour of blood and which can be used for medicinal purposes. Sinder is effective in healing wounds of freezing or burning. Mongolian doctors found sinder in Mongolia and included it in various prescription [6]. Traditional medical doctors clean, refine, dry, roast and fry sinder before using it [7–9]. It stops bleeding and diarrhea, expel heat from meridians and collaterals, treat visceral wound, dry pus and blood, relieve pain, dispel swelling, dry pathological essence and heal burns [10].

Sinder is a combination of iron oxide, mud, zinc oxide, coal, carbonate, sulphate, phosphoric oxide, and manganese oxide [11]. Stickness is associated primarily with Fe_2O_3, AL_2O_3 and SiO_2 and is referred to as sinder [12]. Sinder most commonly forms in the cave of rock as a solid, fluffy mass. It has a reddish colour. Sinder has been widely used in traditional medicine as a component of prescription drugs [13–14]. Sinder powder and ointment preparations are also used to treat animal cuts or burns [15–16].

The discovery of ribonucleic acids on the surface of the germ cell that contain calcium ions assists in connecting new cells with wound edges (Weis Meier). The substance coordinates tissue growth and regeneration and penetration from one cell to another in the callositic layers cell of the skin (Ballag Aiverson) [17].

MATERIAL AND METHODS

A randomized case-controlled experimental study was undertaken in a pharmacological laboratory of the Scientific Centre of Traditional Medical Science Technology and Production in cooperation within the Ulaanbaatar Histological Laboratory of the School of Traditional Medicine, the School of Biomedicine of the Health Sciences University, and the Central Laboratory of Geology (an accredited laboratory). The research was conducted on 80 mice and 30 rabbits of the shinshilla strain. The Khunkheriin sinder was obtained from Jargalant somon (administrative district) in Gobi-Altai aimag. Ointment and powder material was refined in its natural form.

To refine the sinder, it was finely ground in water and filtered to remove larger particles. The solution was left to evaporate in the shade to produce a residue. The residue was crushed into a powder, sifted, and warmed in a cast iron pot. After warming the sinder, soybean powder was added. Powdered sinder the size of 0.2 mm was sifted in lots of 250–300 g from 63 μm through 100 μm and packed into a powder. Subsequent renderings were repeated using the same method.

METHOD AND TECHNOLOGY OF OINTMENT

P r e p a r a t i o n : Vaselin was used as the base substance of the sinder ointment. Vaseline was melted at 40-500 C, and then mixed with the sinder at a ratio of 2:1. After mixing, the substance was poured in either a plastic or a glass container and put in the refrigerator and the mixture was allowed to congeal. The study was conducted according to the international regulation (khelsink declaration) and the Republic of Mongolia. The aqueous refined in a 2:1 ratio was injected into the abdomen of mice as determined using LD_{50} in the accelerated method described by V.B.Prozrovskii (1978). The mice were anaesthetized with ether and their 4 legs secured. To create an external wound, the hair on the back of each mouse was removed, an incision was made with a 10:10mm instrument sterilized with 5% iodine solution, and the skin pulled back with forceps (P. S. Zorikov, 2005).

The rabbits were anaesthetized with ether, their legs secured, and hair removed from their backs. The area was sterilized with a 5% iodine solution. An incision was made with 10:10mm scalpel and the skin pulled back by forceps to create an external wound. To create a burn, wet gauze was placed over the external wound (4 doubled) and pressed with a 30:30mm copper scorcher for 15 seconds (A.A.Zakrividoroga 1995). For histological analyses we used an Olympus microscope. To conduct the histological analysis we took tissue samples from the wound, stabilized them in a formalin solution of 10%, dehydrated degreased it, and casted it in suppository. The suppository block was cut to the thickness of 5 microns that contained all the layers of the skin. The preparation was painted using the method of hemaktoxsilin eosin and vangizon.

RESULTS

The sinder used for this study was taken from the cave of the Khunkheriin spar and refined, dried, and roasted at a high temperature, rendering a clear reddish brown substance with a slightly yellow tinge.

Table 1. Determing acute poisonous quality of sinder preparation

Result	0	1	1	2
ml	0.1	0.3	0.5	0.7
Gram/kg	7.14	2.3	37	50
DL50 = 9.35(6.3–13.5) g/kg				

According to K.K.Sidorov's classification, the preparation was considered non-poisonous (i.e. harmless) as determined through the accepted method of LD_{50}=9.35(6.3-13.5) developed by V.B.Prozorovskii (1978).

Sixty experimental white mice with the weight between 20–30 grams and of the same gender were selected for the study on the action of Khunkheriin sinder when applied to external wounds. The mice were divided into 6 groups (10 mice in each group). The first group of mice was the control group and received no treatment to their wound. The second group of mice was treated with vaseline only. The remaining groups of mice were all treated with sinder, but each group had a slightly different composition of sinder. The third group of mice received treatment with-unrefined powder, the fourth group were treated with refined powder, the fifth group with unrefined ointment and the sixth group with refined ointment.

Thirty rabbits of the same gender, with the weight of between 2000–3000 grams were also selected for the study. There were 6 groups of rabbits, each group comprising 5 rabbits. The first group of rabbits was the control group and received no treatment to their burn wounds. The second group was treated with Vaseline. The third group of rabbits received treatment with sinder unrefined powder, the fourth group were treated with sinder refined powder, the fifth group with sinder unrefined ointment, the sixth group with sinder refined ointment.

The animals treated with vaseline and with sinder preparation received treatment twice a day for 21 days following the formation of the external wounds. Researchers observed swelling, pain, hot and cold, secretion and wound healing. Photos of the external wound of the histological samples were taken on Day 7, Day 14 and Day 21. Changes in the skin layers were analyzed by histomorphology and the size of the wound was measured. The results were analyzed using SPSS -12.

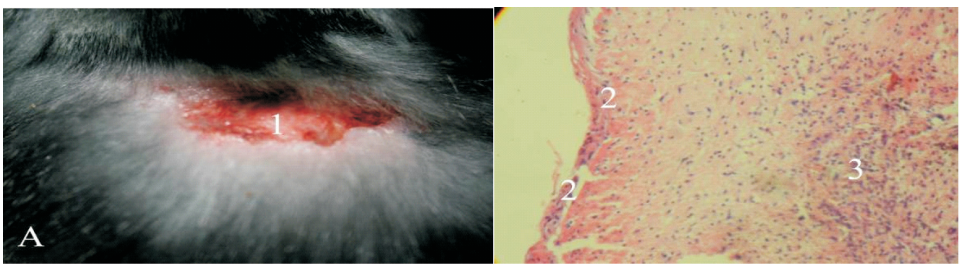

Fig. 1. Picture of the wound which was formed on the back of the rabbit on the 7th day (A) treated by Sinder picture of micro preparation prepared from the margin (B) magnification*200 paint hematoksilin-eozin; 1) healing wound, 2) layer of epithelial tissue, 3) granular leukocyte exudate of fibroid inflammation

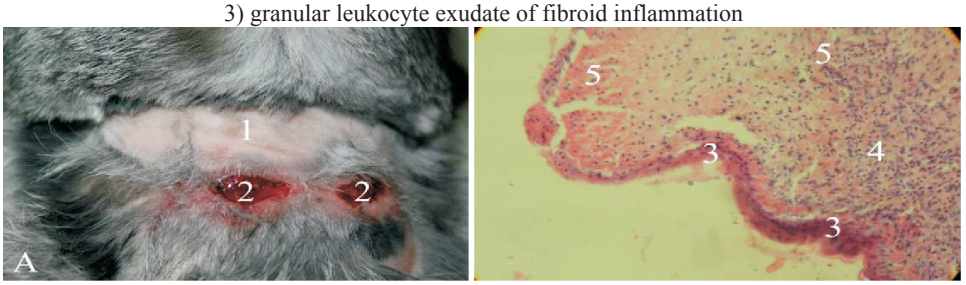

Fig. 2. Wound which was formed on the back of the rabbit treated by sinder and the wound on the 14th day (A) and picture of micro preparation from its margin (B), magnification * 200 paint hematoksilin-eozin; 1) part where hair grows, 2) centre part of the wound, 3) epithelial tissue 2–3 layers, 4) part which is pushed by the fiber of connecting tissue, 5) blood vessel

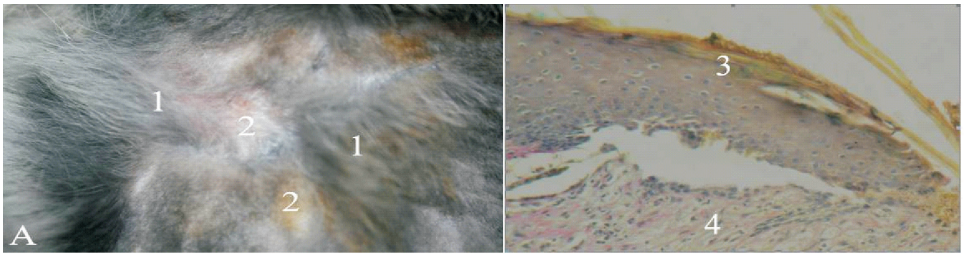

Fig. 3. Wound which was formed on the back of the rabbit treated by sinder, wound on the 21 st day (A) and picture of macro preparation (B) which was prepared from its margin magnification * 200 paint hematoksilin-eozin; 1) strip where hair grew, 2) strip where hair did not grow, 3) epithelial tissue with many layers, 4) scar formed disordered concentration of gluten fibre

According to the histomorphological analyses conducted, the external wounds of mice receiving sinder refined ointment treatment were healed by Day 7. The wounds on the mice control group (vaseline) were considered healed by Day 12. Rabbits that had their wounds treated with the sinder ointment showed healing of their burns by Day 21. The control group's burns (vaseline) were healed by Day 30.

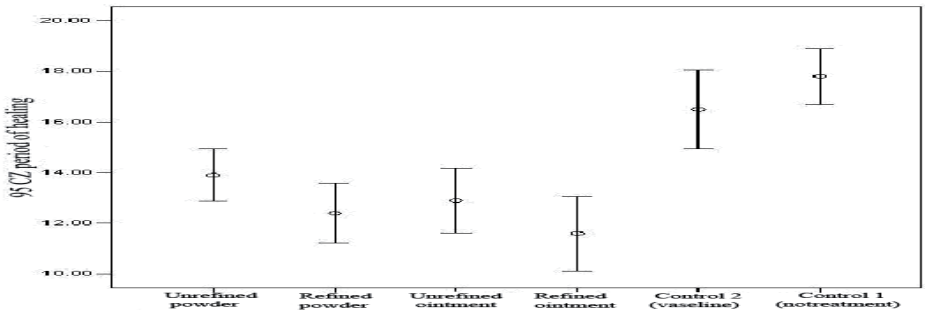

Fig. 4. Effect of sinder in the period of aseptic wound healing formed on white mouse

Table 2. Effect of sinder in the period of aseptic wound healing formed on white mouse

Peroid of healing	N	Average day	Standard inclination	Standard mistake	95 percent trusting interval		The least meaning	The most meaning
					the lowest meaning	the highest meaning		
Unrefined powder	10	13.9	1.45	0.46	12.86	14.94	11	15
Refined powder	10	12.4	1.65	0.52	11.22	13.58	10	15
Unrefined ointment	10	12.9	1.79	0.57	11.62	14.18	10	15
Refined ointment	10	11.6	2.07	0.65	10.12	13.08	7	14
Control 2 (vaseline)	10	16.5	2.17	0.69	14.95	18.05	11	18
Control 1 (notreatment)	10	17.8	1.55	0.49	16.69	18.91	15	20
Total	60	14.18	2.84	0.37	13.45	14.92	7	20

Tissue slides of the strip of epithelial tissue layers that was formed were taken from the wounds. Micro preparation of tissue taken from healed portions of the wounds revealed a clean, granular base

layer of the epidermis. Areas in the centre of the wound (i.e. the part furthest away from the wound margins) were of greatest depth and extended to the base of the lower connective tissue cell layer. As the base differentiated, it formed a round structure for hair nodule formation and hairs. The barky layer became apparent and scar tissue formed at the base of the wound. Disordered gathering of gluten fiber was also observed.

The most effective one to external wound of experimental mouse is refined out ointment of sinder and the average day of wound healing is 11.6±2.1 (Fig. 4, Table 2).

Table 3. Comparing days of wound healing of refined out ointment of sinder with control

Refined ointment 11.6	Average day	Difference	95 percent trusting interval of difference		P
			the lowest Meaning	the highest meaning	
Control 2 (vaseline)	16.5	4.9	2.86	6.93	0.000
Control 1 (no treatment)	17.8	6.2	4.94	7.45	0000

When we compared the average day of wound healing of refined out ointment of sinder with control 2 (vaseline), the difference of average days of wound healing is 4.9 days (Table 3).

Table 4. Effect of sinder to aseptic wound size formed on white mouse (100 mm^2)

Experiment	The 3rd day	The 7th day	difference	95 percent trusting interval of difference		p	Decreased percentage
				The lowest meaning	the highest meaning		
Unrefined powder	84.5	64.7	19.8	15.06	24.54	0.000	23.43
Refined ointment	71.3	39.0	32.3	24.44	40.16	0.000	45.30
Unrefined Ointment	81.2	53.3	27.9	21.08	34.72	0.000	34.36
Refined powder	79.5	52.0	27.5	20.03	34.97	0.000	34.59
Control 2 (vaseline)	99.0	90.8	8.2	-2.17	18.57	0.107	8.28
Control 1 (no treatment)	98.0	92.3	5.7	-1.76	13.16	0.118	5.82

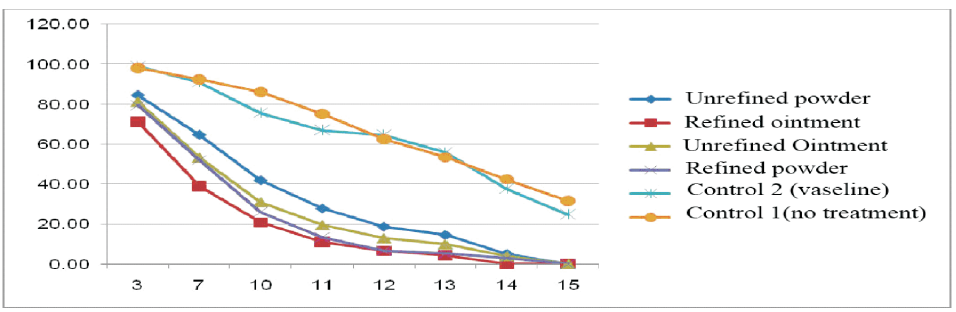

Fig. 5. Effect of sinder preparation on the size of aseptic wound formed on white mouse (100 mm^2)

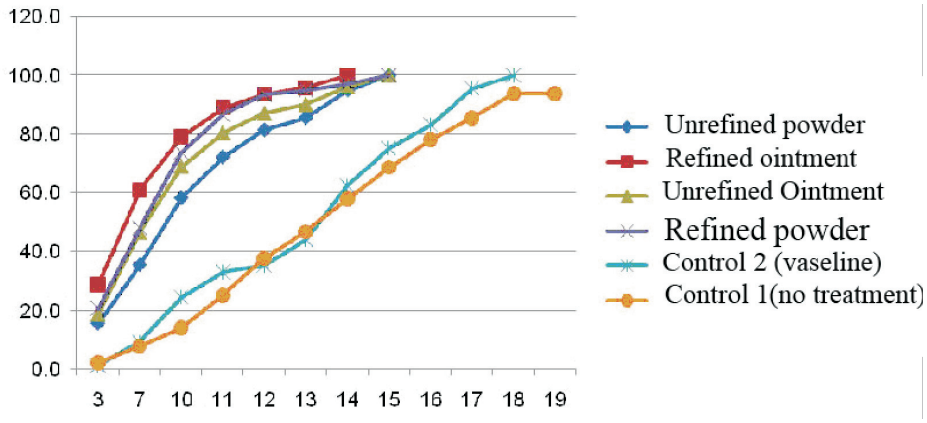

Fig. 6. Effect of sinder preparation on the size of aseptic wound formed on white mouse (100 mm^2) (by percentage)

The refined sinder ointment was the most effective treatment in decreasing the wound size on mice. When comparing Days 3 and 7 there was a decrease in the wound size by 32.3 mm^2 or (45.3 percent) (Table 4, Fig. 5, 6). Groups treated with vaselin showed a smaller decrease in the wound size averaging 24.1 mm^2.

Refined sinder ointment was also the most effective treatment for healing burns on rabbit. The average time for healing of burns among rabbits treated with refined sinder ointment was 17.8± 2.2 mm^2 (Fig. 7, Table 5) When comparing the sinder treatment group to the vaseline treatment group, the difference in average days of wound healing was 15.3 days (Table 5, Fig. 7)

The refined sinder ointment used on the rabbit was most effective when comparing 3 and 7 days, where by the wound decreased in size by 280.2 mm^2 (34.4 percent of wound size) (Table 6, Fig. 8). When action of sinder on rabbit burns was compared to the action of vaseline on rabbit burns, there was a decrease by 304.4mm^2 (Table 6, Fig. 8). When comparing days 7 and 14, there was a decrease of 273.0 mm^2 or 53.5 percent of the wound (Table 6, Fig. 8). In this period the experimental animals of this group healed in 100 percent.

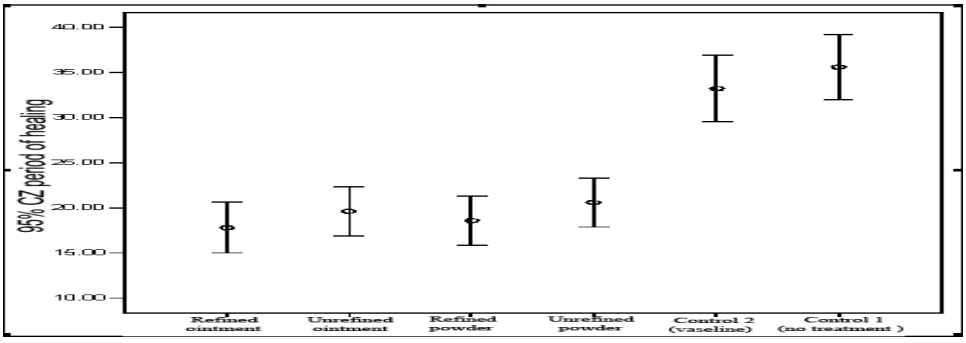

Fig. 7. Effect of sinder preparation on the healing of burned wound formed on experimental rabbit of Shinshilla strain

Table 5. Effect of sinder preparation on the healing of burned wound formed on experimental rabbit of shinshilla strain

Experiment	N	Average period of healing	Standard inclination	Standard mistake	95 percent trusting interval		The least meaning	The most meaning
					The lowest meaning	The highest meaning		
Refined ointment	5	17.80	2.28	1.02	14.97	20.63	15	21
Unrefined ointment	5	19.60	2.19	0.98	16.88	22.32	17	23
Refined powder	5	18.60	2.19	0.98	15.88	21.32	16	22
Unrefined powder	5	20.60	2.19	0.98	17.88	23.32	18	24
Control 2 (vaseline)	5	33.20	2.95	1.32	29.54	36.86	30	38
Control 1 no treatment)	5	35.60	2.88	1.29	32.02	39.18	32	40
Total	30	24.23	7.73	1.41	21.35	27.12	15	40

Table 6. Effect of sinder preparation on burned wound healing square formed on experimental rabbit of Shinshilla strain (900mm^2)

	3rd day	7th day	difference	95 percent trusting interval of difference		P	Decreased percentage
				The lowest meaning	The highest meaning		
Refined ointment	789.6	509.4	280.2	15.7	544.7	0.042	35.49
Unrefined ointment	835.0	692.0	143.0	-23.3	309.3	0.075	17.13
Refined powder	852.8	646.6	206.2	-45.0	457.4	0.085	24.18
Unrefined powder	882.0	784.6	97.4	39.7	155.1	0.009	11.04
Control 2 (vaseline)	918.2	942.4	-24.2	-74.0	25.6	0.249	-2.64
Control 1 (no treatment)	924.2	1005.0	-80.8	-125.6	-36.0	0.007	-8.74
Refined ointment	509.4	236.4	273.0	212.0	334.0	0.000	53.59
Unrefined ointment	692.0	510.4	181.6	63.2	300.0	0.013	26.24
Refined powder	646.6	340.2	306.4	223.4	389.4	0.001	47.39
Unrefined powder	784.6	531.0	253.6	123.9	383.3	0.006	32.32
Control 2 (vaseline)	942.4	806.2	136.2	90.4	182.0	0.001	14.45
Control 1 (no treatment)	1005.5	899.8	105.2	71.2	139.2	0.001	10.47

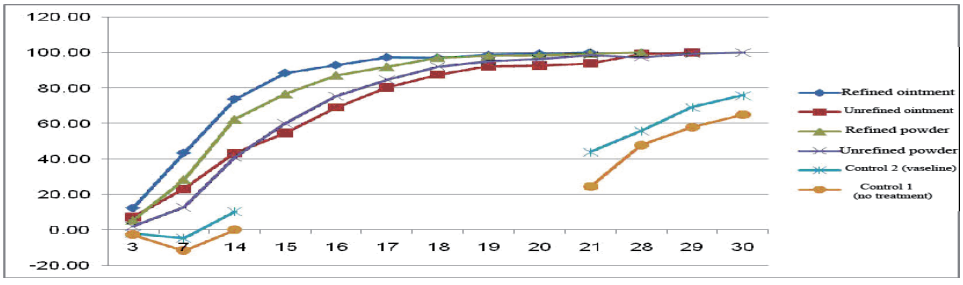

Fig. 8. Effect of sinder preparation on burned wound healing square formed on the experimental rabbit of Shinshilla strain (by percentage)

DISCUSSION

Drugs used in traditional Oriental medicine are prepared first by extracting the minerals. The exact method of extraction selected is based on the type of disease the drug is intended to treat and the formulas can be found in the oriental medical sutras [18]. In general, Sinder extraction takes place by first dissolving the sinder in water, filtering it, drying the filtered solution, and roasting it at a high temperature [19-20]. During this process the mineral structure of Sinder breaks down. Chemical bonds are broken resulting in a special change in the physical and chemical quality. Elements are changed into dissolvable ion isotope form, energy is released, the crystal loses its water, and poisonous ingredients are separated. In the process some biogenetic elements are activated and a useful combination if formed. The biologically active new mineral's taste, smell and colour have changed in quality from the original sinder and it is now in a form that is useful for medicinal purposes, such as the external wound treatment studied in this research.

Before using any substance for medicinal purposes, its poisonous quality must first be ascertained. According to the standard of LD_{50}=9.35(6.3–13.5)g/kg, the sinder used in this experiment was considered non-poisonous. After refining the sinder unrefined powder and ointment, and refined powder and ointment were formulated for treatment purposes on the external wounds of mice and rabbits. There was a high effectiveness of sinder when treatment and control groups were compared. When refined powder preparations were used, secondary infection decreased. The conclusion reached from this observation is that the refined powder molecules combined with wound secretion form a protective coat over the wound. This protective coat altered the wound environment in such a manner that protected against infection from bacteria, decreased pain and soreness, decreased stress and it promoted optimal conditions for wound healing. These results confirm the hypothesis of this study. The advantage of sinder is that it softened the olid wound covering facilitated absorption of the medicinal component of sinder, and improved healing conditions. This process of wound healing using sinder was studied at the level of tissue structure.

In the case where refined ointment and powder preparation were used in the first week, the surface of the wound was pushed from the edge and covered by square epithelial tissue. At the base of the wound, infiltration of inflammation consisting of fibrin and leukocyte was seen. The micro preparation of tissue taken from the wounds during the healing process showed that after 14 days of treatment with sinder, the single layer of epithelial tissue of covering the surface of the wound became thick and developed into 2–3 layers. Also, when observing the cells of the thickened layer of flat epithelial tissue a granular layer and double callosity were seen. Subsequent slides of wound tissue revealed epithelial tissue infiltration of the inflamed wound base and formation of connecting tissue and capillaries in the deepest part of the wound. Micro preparations obtained from the wounds on Day 21 showed that epithelial tissue and epidermis callositical layers were formed in the centre of the wound, 5 layers of epidermis were formed at the edge of the wound, hair nodules were pushed into the real layer of the skin, and hair covering was formed.

When we compared sinder ointment and powder preparation, ointments resulted in intensified vessel growth at the base of the wound, promoting epithelial tissue growth that was balanced and regenerated. It accelerated wound healing and reduced scar formation at the base of the wound. The refined preparations of ointment and powder on external wounds act to regenerate epithelial tissue at an appropriate proportion to normal tissue. By using sinder ointment and powder on the wounds, the calcium and magnesium oxide in the sinder became the link for the regeneration of epithelial tissue recognition between cell and strengthening connection.

The result of this study was similar to the above mentioned scientists' discoveries (Weis Meier and Ballag Aiverson). Subsequent research is needed to prove this hypothesis [21].

CONCLUSIONS

1. Refined sinder transformed into a reddish brown colored substance with a hint of yellow. Sinder is more effective in its refined form.

2. According to research and pharmacological standards, the sinder used in this study was non-poisonous – LD_{50}=9.35(6.3–13.5)g/kg.

3. Refined sinder ointment was more effective than other medicinal preparation in this study.

4. Histological analysis index became the same on Days 7 and 12 among the control groups and on Days 21 and 30 for rabbits with burn wounds. It was observed that vascularisation was supported and there was an accelerated growth of epithelial tissue.

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SUMMARY

Sinder (*Limoniterra* or goethite) is a Mongolian term that comes from the Sanscrit word 'Sindura', meaning 'water weed'. Sinder has been used for medical purposes for many centuries. Khunkher is a place in Mongolia where sinder can be found year-round. Khunkher has spring-fed caves of sedimentary deposits originating from the cretaceous period. It is in this place that Khunkheriin sinder grows. Khunkheriin sinder has a reddish brown colour. It is both fluffy and solid and crunches when compressed. It is organic in origin and comes from an alkaline environment that includes hydrogen with calcium oxide and mineralized composition with quartz such as: CaO, Fe_2O_3, Al_2O_3, MgO, Na_2O, K_2O, F_2O_5, MnO and T,V, Cu, Sc, Be, Nb, Nd, Ni, Pr, Sb, Sm, Sr, Ta, Y, Yb, Zn and Zr. It is a sticky substance with more than 20 macra and microelements. The hypothesis for this study was the quality of wound treatment with khunkheriin sinder depends on a mineral combination of Ca, Fe, Al, K, Si, Mg, Mn, Zn, Cu, P, and a hydrogen with calcium oxide and quartz combination. V. B Prozrovski's method of extraction (LD_{50}=9.35[6.3-13.5]g/kg) rendered the ointment harmless according to the K.K.Sidorov's (1987) classification. The result showed that if the raw material of khunkheriin sinder ointment was prepared by roasting the sinder at high temperatures, then the hypothesis was supported. An external wound on a mouse was healed within 11.6±2 days and a burn wound on a rabbit was healed in 17.8±2 days. The authors of this research study concluded that Khunkheriin sinder supports the growth of vessel in the bottom of the wound and it accelerates growth of skin epithelial tissue.

STRESZCZENIE

Getyt *(Limoniterra)* jest mongolskim okresleniem pochodzącym z sanskrytu (oznaczającym „chwast wodny"). Getyt jest stosowany w celach leczniczych od wielu wieków. Khunkher jest miejscem w Mongolii, gdzie getyt można znaleźć przez cały rok. Tamtejszy getyt ma kolor czerwonobrązowy. Jest puszysty lub o konsystencji stałej i kruszy się pod wpływem zgniecenia. Z pochodzenia jest substancją organiczną i pochodzi ze środowiska zasadowego zawierającego wodór z tlenkami wapnia oraz o składzie zawierającym CaO, Fe_2O_3, Al_2O_3, MgO, Na_2O, K_2O, F_2O_5, MnO oraz T, V, Cu, Sc, Be, Nb, Nd, Ni, Pr, Sb, Sm, Sr, Ta, Y, Yb, Zn i Zr. Jest to lepka substancja z ponad 20 makro- i mikropierwiastkami. Hipotezą postawioną w niniejszej pracy jest, że jakość leczenia ran za pomocą getytu z Khunkher zależy od kombinacji minerałów: Ca, Fe, Al, K, Si, Mg, Mn, Zn, Cu, P, oraz wodoru z tlenkiem wapnia i kwarcem. Metoda ekstrakcji V.B. Prozrowskiego sprawiła, że według klasyfikacji K.K Sidorowia maść jest nieszkodliwa. Wyniki badań wykazały, że jeśli surowiec maści z getytem z Khunkher jest przygotowany poprzez działanie wysokich temperatur, to powyższa hipoteza jest potwierdzona. Zewnętrzna rana u myszy wygoiła się w ciągu 11,6±2 dni, natomiast oparzenie u królika w ciągu 17,8±2 dni. Autorzy doszli do wniosku, że getyt z Khunkher wspomaga rozwój naczyń na dużych ranach oraz przyspiesza wzrost tkanki skórnej.