Wound Healing Properties and Structural Analysis of Four Geographical Areas' Natural Clays

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Abstract–**Clays are fine particle materials that harden after drying. The difference in their structure is the key to their efficacy and their subsequent application. The current study aims to evaluate the wound healing property of four countries (C1:Iraq, C2:Turkey, C3:Azerbaijan and C4:Russia) clay samples by excision model using *Sprague dawley* rats also the chemical analysis of the samples was performed using X-ray diffraction (XRD) and X-ray Fluorescence (XRF) methods. Results revealed that the best wound healing activities were given by C1, C3, C4 and C2 respectively with healing percentages (76%, 71%, 62%, and 60%), respectively. XRD results revealed the presence of Calcium carbonate and Calcium-Magnesium carbonate in C1, Dolomite and Calcium-Magnesium carbonate in C2, Cobalt Tantalum Sulfide in C3, Finally Quartz and Silicon Oxide in C4. On the other hand, XRF analysis showed the appearance of different major and trace elements with different quantities in each clay type. We conclude that different countries clays enclose wound healing property with diverse ranges and this diversity is due to their chemical and mineral structures.**

Index Terms—**Natural clay; wound healing; rats; X-ray diffraction; X-ray fluorescence.**

I. Introduction

Worldwide many people are experiencing different types of skin wounds and the synthetic compounds are of less use day by day because they are environmental pollutants and take a long time circulating to the nature that is why there is an increase in the using of natural remedies for the treatment and healing of the wounds. In the developing countries, around 80% of their population are depending on the natural substances to treat their infections (Qureshi, Khatoon and Ahmed, 2015). The evolution and progress of wound therapies and advanced strategies with new techniques are observed over the years; however, skin wound treatments are categorized generally as "Regenerative" or "Conventional". Regenerative therapy aims to restore skin to its original function, reestablishing damaged cells and skin tissue without scarring while conventional therapy leads to the formation of scars (Tottoli, et al., 2020). Clay minerals healing efficacy were discovered many years ago and have been applied in Medicine for different purposes like spas, pharmaceutical formulations and aesthetic medicine. They are either orally administered for uses as laxatives, gastrointestinal protectors, anti-diarrhetics or as topical applications in cosmetics and dermatological protectors (Carretero, 2002) also clays were given to farm animals for the detoxification purposes and alleviation of gastrointestinal diseases. (Slamova, et al., 2011). Moreover, latest studies have documented the potential of clay minerals in the nanomedicine field regarding their beneficial effects on skin proliferation, cellular adhesion, and differentiation (Viseras, et al., 2019). In addition, the clay minerals were used to moisturize and clean the skin and to reduce acne, lipodystrophies and cellulite (Arab and Alshikh, 2012) (Williams and Haydel, 2010) investigated the antibacterial activity of natural clays and they found that the antioxidant state and the pH are the key of the reactions control and the chemistry of the bacterial cell wall. In addition, clays are proposed not to kill bacteria directly but may enclose soothing effects which are palliative (Williams, 2019). Other researchers proved that the clay material destroyed many bacterial strains, including Methicillin-resistant *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella enterica* (Gaskell and Hamilton, 2014). Other pharmacological uses of clay include treatment of gastrointestinal diseases, applied topically to treat skin disorders, and have been taken orally to manage chronic or urgent diarrhea, and rheumatism. The mechanism of action was suggested to be due to their high porosity, large specific surface area, high adsorption and exchange capacities (Tang, et al., 2005). The chemical composition of natural clays includes silica, magnesium, aluminum, little iron substitutes and low quantities of sodium, potassium and calcium are existent as well. Depending on their ionic structure, these minerals can be classified into nine groups: pyrophyllite-talc, kaolin-serpentine, vermiculite, mica, chlorite, smectite, interstratified clay minerals (e.g., rectorite, tosudite, and corrensite), sepiolite-palygorskite, and allophane-imogolite (Wilson, 1994). These inorganic compositions of clays has been documented to improve and treat skin wounds through three different mechanisms, (1) physically by water vapor transmission and mechanical resistance, (2) chemically by hemostasis or adsorption of

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moisture and release of drugs, and (3) biologically by their antibacterial/antimicrobial effects and improving the healing process(García-Villén, et al., 2020). The aim of the present study was to compare the chemical composition of four clay samples obtained from different geographical areas and to evaluate the efficacy of the topical application of these clay samples in accelerating excision wound healing *in vivo* in experimental rat model.

II. Materials and Methods

A. Clay Preparation and Analysis

Four types of natural clays were collected from four different countries: Iraq (C1), Turkey (C2), Azerbaijan (C3), and Russia (C4), their different external views or appearances are shown in Fig. 1. The clays were grinded and prepared freshly every day by mixing with local mineral waters, which contain a unique balance of salts in a ratio of 2:1 water and clay, respectively. The mineral content of the clays were tested at Civil Engineering laboratory, Research Center, Koya University, to perform X-Ray Diffraction (XRD) technique type Panalytical Empyrean with CuKα radiation. The elemental composition and their energies were determined using energy dispersive X-ray Fluorescence (EDXRF).

B. In Vivo Wound Healing (Excision Model)

Forty male Wistar rats (weighing 250–300 g) were randomly divided into 5 groups. Round seal was used to excise a uniform area of 2 cm in diameter from the nape of dorsal neck of all the rats. Then different treatments were applied as described in following:

Group 1 (Control) rats were topically applied with MEBO cream twice a day

Group 2 rats were topically dressed with 2 mL of C1 sample twice a day.

Group3 rats were topically dressed with 2 mL of C2 sample twice a day.

Group 4 rats were topically dressed with 2 mL of C3 sample twice a day.

Group 5 rats were topically dressed with 2 mL of C4 sample twice a day.

The wound area was noted by measuring the contraction area. Manually, the wound areas were traced and calculated in square millimeters. The wound closure area and closure percentage rate were measured at days 0, 5, and 10 of the experiment. The wound area was measured immediately by placing a transparent tracing paper over the wound and tracing it out. The tracing paper was placed on 1 mm 2 graph sheet and traced out. The squares were counted and the area recorded. Then, the percentage of wound closure was calculated following the formula: Wound closure (%) $=1-(Ad\ A0) * 100$, (1) where $A0$ is wound area at day zero and Ad is wound area on corresponding day (Amin, et al., 2015).

C. Statistical Analysis

All values are reported as mean ± SEM. and the statistical significance of differences among groups was assessed using one-way ANOVA. A value of (0.05) was considered significant.

III. Results

Table 1 shows the effects of different treatments on the percentage of wound healing at different days after surgery, at day 0 no significant difference was found between groups that shows the accuracy of the work whereas significant results were found on day 5 of the treatment in which C1 and C3 clays showed wound closure percentage of (48% and 45%), respectively, which are comparable to MEBO group (43%). Moreover, remarkable results were observed at day 10th of the surgery in which the wound closure percentage were (76%, 60%, 71%, and 62%) for C1, C2, C3, and C4 groups, respectively. The C1 and C3 group's results were better than the MEBO control group (64%). These results confirmed macroscopically (as shown in Fig. 2), as seen groups C1

Fig. 1. The external view of the clay samples; C1 clay from Iraq, C2 clay from Turkey, C3 clay from Azerbaijan and C4 clay from Russia.

Table I
Wound Healing Measurements of Experimental Rats after Exposure to Different Treatments.

Groups	Day 0	Day 5		Day 10	
	Wound area	Wound area	Wound closure %	Wound area	Wound closure %
MEBO	332±2.4	190±2.1*	43	120±2.1*	64
C1	339±0.9	175±1.7*	48	81±1.6*	76
C2	340±0.7	231±1.4	32	137±1.5*	60
C3	342±2.1	187±1.1*	45	100±1.3*	71
C4	338±1.4	225±3.1	33	130±3.3*	62

Data expressed as Mean±SEM, *P* value considered significant when is <0.05

and C3 showed a significant re-epithelialization with sign of dermal healing as compared to MEBO group.

XRD results showed that the main peaks were related to magnesium calcite (chemical formula $C_1Ca_{0.94}Mg_{0.06}O_3$) and magnesium calcium carbonate (chemical formula $Mg_{0.06}Ca_{0.94})(CO_3)$) in C1 sample (density = 2.75 g/cm^3) as shown in Fig. 3a. Whereas, the peaks of C2 sample (density = 2.88 g/cm^3) were related to Dolomite (chemical formula $C_2Ca_1Mg_1O_6$) and Calcium Magnesium Carbonate (chemical formula $CaMg(CO_3)$) Fig. 3b. In addition to Cobalt Tantalum Sulfide (chemical formula $CO_2S_6Ta_9$) in C3 (density = 10.77 g/cm^3), finally Quartz (chemical formula

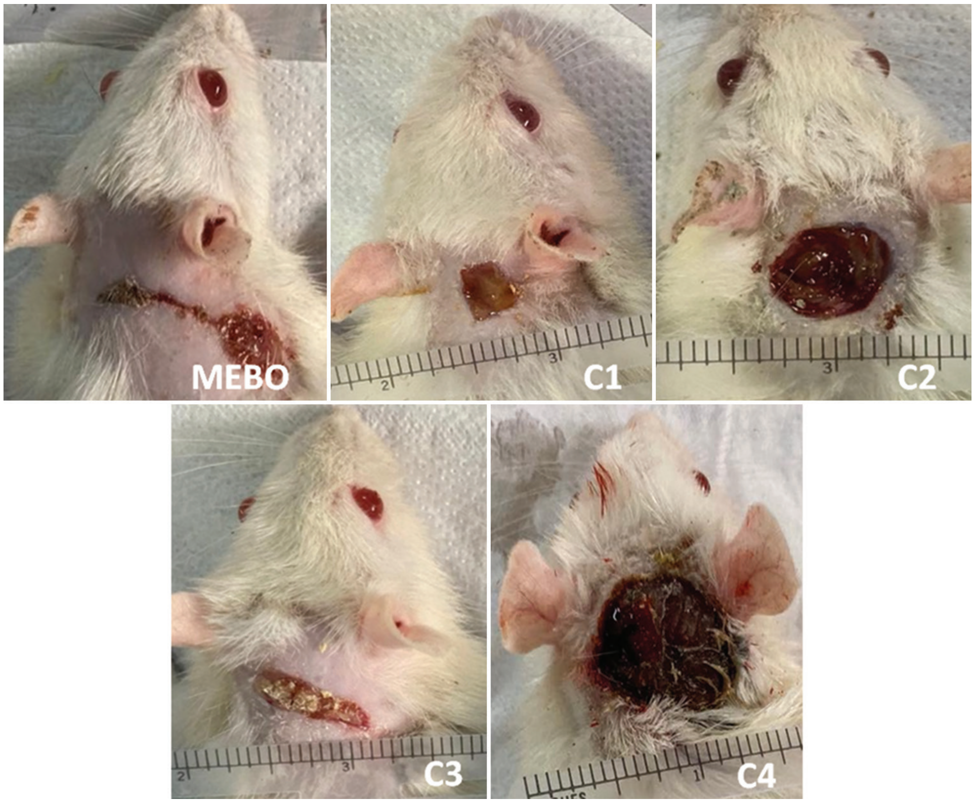

Fig. 2. Macroscopic appearance of excision wound healing area on rat skin at day 10 after surgery within different groups of treatments.

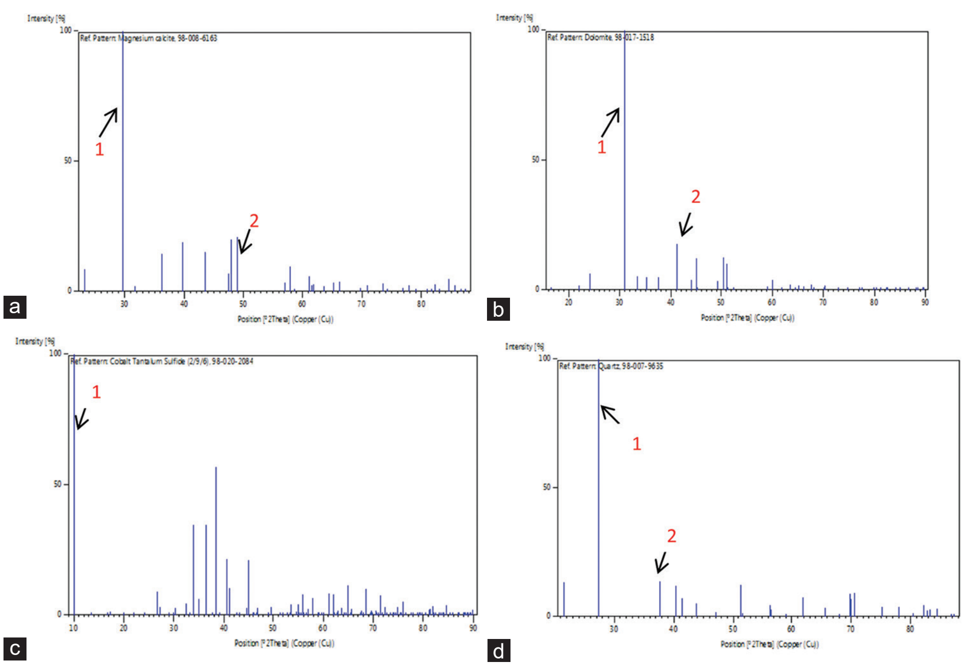

Fig. 3. The X-Ray Diffraction analysis of the clay samples. (a) C1 sample: peak 1 Magnesium Calcite peak 2 Magnesium Calcium carbonate, (b) C1 sample: peak 1 Dolomite peak 2 Calcium Magnesium Carbonate, (c) C3 sample: peak 1 Cobalt Tantalum Sulfide peak 2 Silica Oxide (d) C4 sample: peak 1 Quartz.

O_2Si_1), and Silicon Oxide (chemical formula SiO_2) in C4 sample (density = 2.75 g/cm^3), respectively.

On the other hand, the X-ray Fluorescence (XRF) analysis of the clay samples showed that the Silica or Quartz (SiO2), Calcium Oxide (CaO), Aluminum Oxide (Al2O3) and the Iron III or Ferric Oxide (Fe2O3) are the main elements of all types of clays with the appearance of few other trace elements in a lower quantities (Table 2).

IV. Discussion

Experimental animals wound healing models have been studied widely in the last years. Nowadays, 80% of population depend on natural remedies to maintain wound care management (Qureshi, Khatoon and Ahmed, 2015). Indeed, the wound healing property of clays was well defined (Ferrell, 2008) *in vitro* and *in vivo* studies showed the therapeutic activities of clays (Ghadiri, Chrzanowski and Rohanizadeh, 2014b, Marinelli, et al., 2021). The inorganic contents of clay minerals, cations, zeolites, etc., were documented to have the ability to enhance cell proliferation, adhesion and cellular differentiation and uptake (García-Villén, et al., 2020). The results of the present study revealed the effective activity of four types of clays collected from different countries to the skin wounds induced *in vivo* on *Sprague Dawley* rats. The healing process characterized by declined wound area measurements and greater wound size reduction percentage as shown in Table 1 and Fig. 2. Whereas, it is obvious from Table 2 and Fig. 3 that all types of clays enclose different quantities of materials as performed by XRD and XRF analysis. Clay collected from Iraq (C1) and the one collected from Azerbaijan (C3) showed the best two results in healing rat's skin wounds (76% and 71%), respectively. The analysis of C1 clay showed the presence of Magnesium calcite and Magnesium calcium carbonate (Fig. 3a) whereas Cobalt Tantalum Sulfide was seen in C3 sample (Fig. 3c). Whereas elements analysis by XRF showed the presence of Calcium and Ferric Oxides C1 sample and Aluminum Oxide in C3 sample respectively. Other studies reported the same chemical ions and elements in clays (Ghadiri, Chrzanowski and Rohanizadeh, 2014c, Morrison, et al., 2014, Maniatis, et al., 1983) Williams et al. suggested that the biological effects of clays are due to their large surface area that buffers the oxidation state and the water pH also controlling the solubility of clay derived materials (Williams, et al., 2008).

Clay minerals were reported to establish interactions with biomolecules and permitting new possibilities for delivery of growth factors, matrix proteins and genes in tissue regeneration (Sandri, et al., 2016). The mechanism of wound healing property of the natural clays was documented to be due to regulation of macrophages and recruitment of monocytes into wounds. Moreover, in the healing wounds keratinocytes migrate through the wound area to maintain the epithelial integrity (Incledion, et al., 2021). In another study, the healing process of a synthetic clay has been studied when applied to wounds and the healing process was proposed to be through fibroblast cell proliferation (Ghadiri, et al., 2014a). Furthermore, clay mineral was investigated to promote collagen formation and angiogenesis on skin wounds (García-Villén, et al., 2020)

Table II
The X-Ray Fluorescence Analysis of the Clays samples.

	Elements	Mass %			
		C1	C2	C3	C4
1	SiO_2	42.9	47.6	63.3	98.5
2	CaO	33.8	15.6	3.96	0.226
3	Al_2O_3	11.8	12	15.5	0.874
4	Fe_2O_3	5.19	4.62	4.29	0.123
5	MgO	4.22	16.2	3.9	…
6	K_2O	1	2.96	1	0.0365
7	TiO_2	0.709	0.512	0.804	0.0065
8	MnO	0.105	0.0837	0.0817	0.005
9	Cr_2O_3	0.0993	0.0086	…	0.0028
10	SO_3	0.0545	0.0598	1.85	0.0222
11	Co_2O_3	0.0281	0.0172	0.0162	0.0011
12	SrO	0.0276	0.122	0.059	0.0006
13	NiO	0.0235	0.0083	0.0054	…
14	BaO	0.018	0.028	0.637	…
15	V_2O_5	0.0165	0.0199	0.0556	…
16	SnO_2	0.0101	0.0113	0.0091	0.0065
17	ZnO	0.0096	0.0092	0.0096	0.0006
18	CuO	0.0066	0.005	0.0071	0.0011
19	Rb_2O	0.0048	0.0117	0.0055	0.0004
20	Y_2O_3	0.0027	0.0023	0.0032	…
21	WO_3	0.0016	…	…	…
22	Ga_2O_3	0.0016	0.0012	0.0011	…
23	As_2O_3	0.0015	0.0033	…	0.0001
24	PbO	0.0012	0.0024	0.145	0.0005
25	U_3O_8	…	0.001	…	…
26	ThO_2	…	0.0016	0.0021	…
27	HfO_2	…	0.0019	…	…
28	Nb_2O_5	…	0.0023	…	…
29	Ta_2O_5	…	0.0028	0.0017	…
30	Cr_2O_3	…	0.0086	0.0124	0.0028
31	Na	…	…	3.3	…
32	Cl	…	…	0.708	0.0143
33	P_2O_2	…	…	0.251	…
34	Ir_2O_3	…	…	0.0018	…
35	PtO_2	…	…	0.001	…
36	Au_2O	…	…	0.0005	…
37	TeO_2	…	…	…	0.0008
38	GeO_2	…	…	…	0.0004

V. Conclusion

This research has demonstrated the potential of different countries clays as wound healing agents. The difference in their activities was supposed to be due to their different structures; however, more studies are recommended to be performed to reveal the exact mechanism of their action.

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