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Does Salt and Mineral Content of Dead Sea Mud Affect Its Irritation Potential: A Laser Doppler Flowmetry Study

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Synopsis

The aim of the study was to investigate skin microcirculation, flux, and temperature changes induced by the application of Dead Sea mud (DSM) formulas with different mud salts and mineral contents using laser Doppler flowmetry. Instrumental analysis of eight over-the-shelf DSM products and four different samples of nonformulated Dead Sea mud were carried out to determine their contents of various salts and elements, including K, Na, Cl, Mg, Mn, Ca, SO_3, SiO_2, Al, Br, Fe, Hg, Cr, Co, Ni, Cu, Zn, As, Cd, Pb, and Sr. Three DSM samples with different levels of salts were then used to study the influence of salt content on skin irritation potential using laser Doppler flowmetry. Fifteen healthy nonsmoking females aged 18–45 years participated in the study. Subjects were randomly assigned to either "Salted" mud group ($n = 5$), "As is" mud group ($n = 5$), or "Over-the-Shelf" mud group ($n = 5$). Five circular areas were marked on the ventral aspect of each forearm. One forearm was assigned randomly for mud treatment and the other forearm was untreated. Ten milliliters of mud was applied on the assigned forearm and left for 30 minutes. Two reading protocols were designed and used to study the effects of tested type of mud on skin blood flux and temperature during mud application (protocol 2) as well as before and after mud removal (protocol 1). All types of tested mud were not associated with a significant measurable elevation in skin temperature and skin blood flow. All types of Dead Sea mud did not cause detectable microcirculatory and skin temperature changes regardless of their different mineral and salts contents.

INTRODUCTION

Dead Sea water is the richest natural mineral source in the world with the main elements being chlorine, magnesium, sodium, calcium, potassium, and bromine (1).

The beneficial role of salt and mineral content of Dead Sea water in the treatment of various skin problems has been shown in several published studies. The anti-inflammatory activity

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of Dead Sea salt solution has been demonstrated in a number of clinical studies in patients with psoriasis (2) and atopic dermatitis (3). In addition, the clinically proven enhancement of skin barrier properties and the anti-inflammatory effect of Dead Sea salt have also been studied at the cellular level. Schempp et al. (4), demonstrated that magnesium ions inhibit the antigen-presenting function of human epidermal Langerhans cells both *in vivo* and *in vitro*. Furthermore, magnesium and potassium ions have been shown to have a specific inhibitory effect on the uncontrolled proliferation of psoriatic dermis cells grown in tissue culture (5).

The therapeutic efficacy of Dead Sea products can be attributed to the penetration of minerals into the skin where it affects its biochemical processes. Penetration of salts through healthy and damaged epidermis was observed in healthy volunteers as well as psoriatic patients after bathing in Dead Sea or in simulated bath-salt solutions for 4 weeks (6).

Besides Dead Sea water and salts, Dead Sea mud is a popular over-the-shelf product in the Jordanian market. It is an unconventional treatment modality that is used at the Dead Sea shore of Jordan. Tourists and local visitors of the Dead Sea intentionally apply mud directly onto their skin for a period of time to achieve different perceived cosmetic and therapeutic benefits.

In addition, mud pack therapy is widely used in rehabilitation centers and spas to alleviate joint pain. In such cases, the mud is preheated to 50°C and then applied to the affected area while the patient is covered with a woolen blanket protected by a film of plastic (7).

The Dead Sea mud is believed to be a rich source of various minerals and salts (8). The physical and chemical characteristics of 24 Dead Sea mud samples collected from different locations on the eastern shore of Dead Sea has shown that mud sample's composition varied because of different phenomena taking place at different sampling points (8).

Although the effects of Dead Sea salts on various skin biochemical processes were previously investigated, there are no reports, to date, that assessed, quantitatively, the effect of different levels of minerals and salts on skin microcirculation and temperature as surrogate indices of irritation.

The objective of this study was to investigate the safety of Dead Sea mud preparations with varied levels of minerals and salts and determine their irritation potential in human volunteers using the laser Doppler method. Laser Doppler method allows noninvasive, objective, and quantitative measurement of microvascular blood perfusion and skin temperature and can be used to discriminate between irritant and nonirritant substances and to detect early stages in the development of an irritant reaction before it is visible (9).

MATERIAL AND METHODS

SAMPLES

Eight samples of over-the-shelf Dead Sea mud from popular Jordanian brands of personal care products were purchased, two different samples of raw Dead Sea mud were generously provided by Numeira Mixed Salts & Mud Company Ltd. (Amman, Jordan). Raw Dead Sea mud samples were treated in our laboratory by removing stones and made spreadable either by mixing with distilled water or Dead Sea water. Raw mud samples mixed with distilled water were called "As is" mud and the one mixed with Dead Sea water was called "Salted" mud. Then, both were autoclaved for 15 min at 121 C.

Samples were sent to the Royal Scientific Society Instrumental Analysis Laboratories (Amman, Jordan) for analysis of their salts and element contents. Laboratory tests and analyses performed included moisture content; loss on ignition (BS EN 196-2); total Al, K, Ca, Cr, Co, Ni, Cu, Zn, As, Cd, Pb, Li, Sr, Na, Mg, Mn, Fe, and SiO_2 (SOP No. 3/01/04-005, atomic absorption spectroscopy); total Hg and As (SOP No. 3/01/04-006, atomic absorption spectroscopy); total Br (ASTM D-3869-79, gravimetric analysis); and Cl water soluble (BS EN 196-2).

SUBJECTS

Fifteen healthy, normotensive, nonsmoking female volunteers aged 18–45 years participated in the study after giving their informed consent. Subjects with any lesions on their forearms or receiving any local or systemic treatments were excluded from the study.

The study protocol was officially approved by the Hashemite University Institutional Review Board. Subjects were randomly assigned to either "Salted" mud group ($n = 5$), "As is" mud group ($n = 5$), or "Over-the-Shelf" mud group ($n = 5$). Each subject served as her own control.

INSTRUMENT

Skin blood flow and temperature were monitored pre-, during, and postmud application using moorVMS-LDF dual channel—laser Doppler and temperature monitoring (Perimed® PF4001, wavelength 82 nm, Perimed, Moor, UK) and recorded by the system software with a 3-s time constant downstream from a broadband filter (12 MHz).

Laser Doppler is a gold standard for dynamic microvascular blood flow assessment. Laser Doppler flowmetry (LDF) provides continuous noninvasive measurements of microvascular perfusion in terms of flux, which represents the movement of erythrocytes through the microvasculature (10). It was used to assess the effect of local treatment in improving skin microcirculation, thus improving the healing of skin ulcers (11) and to assess the effect of a topical product on microcirculation (12). In addition, LDF data correlate well with visual scores of patch test reaction and is used in dermatology to predict irritancy and to assess topical products such as anti-inflammatory, vasoactive drugs, and detergent barrier creams (10).

A dual channel laser Doppler monitor (VMS-LDF2) with two (2) laser probes for simultaneous recording of skin microcirculation and skin temperature was used. VMS-LDF2 was computer-controlled with software (MoorVMS-PC V2.2). The sampling depth of the probe is around 1 mm. Low-power laser light is transmitted via the optic probe to the tissue, and the light is scattered by the tissue and moving blood cells and their frequency is Doppler-broadened. Some of the scattered light is collected by the optic probe and transmitted to a photodetector. The data are electronically processed by the system software to produce the laser Doppler (blood flow) flux signal. The probes were calibrated just before the start of the study according to the manufacturer's guidelines. Perfusion values were measured for each tested time points at a sample rate of 33 recordings per second and were analyzed by averaging over 1-min intervals for protocol 1 and averaging over 10 min before and 30 min during intervals for protocol 2 using the manufacturer software.

STUDY DESIGN

The participants were randomly assigned into three groups of five subjects each. Each group ($n = 5$) was used to test one of the aforementioned mud types. According to the published guidelines for measurement of cutaneous blood flow (13), the subjects were asked not to drink caffeinated beverages, not to eat a high-calorie meal at least 2 h before the experiment, and not to exercise at least 24 h before the experiment. The subjects were asked to relax for 20 min in a supine position while their forearms are relaxed and in an extension position in a controlled temperature and a controlled relative humidity room. Lighting conditions in the room were kept constant by closing window blinds and turning off ceiling lights, and there was typically sufficient light to be able to read. Five circular areas were marked on the ventral aspect of each forearm (Figure 1). One forearm was

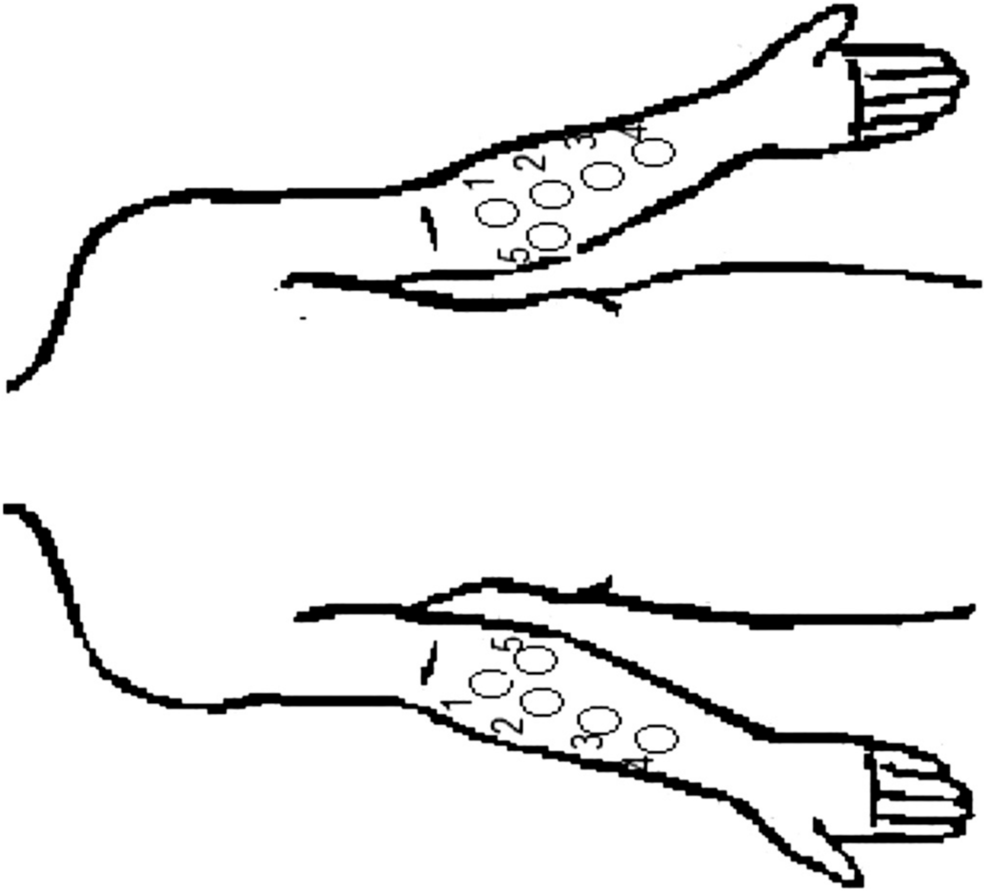

Figure 1. Five circular areas were marked on the ventral aspect of each forearm. Areas 1–4 were used for protocol 1 and area 5 for protocol 2. For protocol 1; laser Doppler readings (flux and temperature) were taken for areas 1–4 at baseline before mud application and then directly and 15 min after mud removal.

assigned randomly for mud treatment and the other forearm was untreated. All mud samples were left at room temperature for 24 h before application.

Ten milliliter of mud was gently applied using feeding syringe on the assigned forearm and left for 30 min. Then, the mud was gently removed and washed using warm tap water. The untreated forearm was also washed in a similar fashion to exclude the effect of washing on skin temperature and blood flow.

Two reading protocols were designed and used to study the effects of tested types of mud on skin blood flux and temperature during mud application as well as before and after mud removal.

Protocol 1 (Figure 1): laser Doppler readings (Flux and temperature) were taken for areas 1–4 at baseline before mud application and then directly and 15 min after mud removal.

A ring of a double-sided tape was applied to hold the laser Doppler probes in place and to allow the removal and reattachment of the probes without applying pressure or otherwise disturbing the microvascular structure under each site. The procedure was simultaneously performed on the left and right forearms using the dual channel monitor with two probes.

Perfusion values for each circular area at the tested time point were measured over 1-min intervals using the manufacturer software, and the mean flux and skin temperature for each area at each time point were used for data analysis.

Protocol 2 (Figure 2): a ring of a double-sided tape was applied to hold the laser Doppler probes in place on areas numbered 5 on both forearms simultaneously. Baseline readings of the microvascular blood perfusion and skin temperature were performed for areas numbered 5 for 10 min before mud application (Figure 2) and then 10 ml of mud was gently

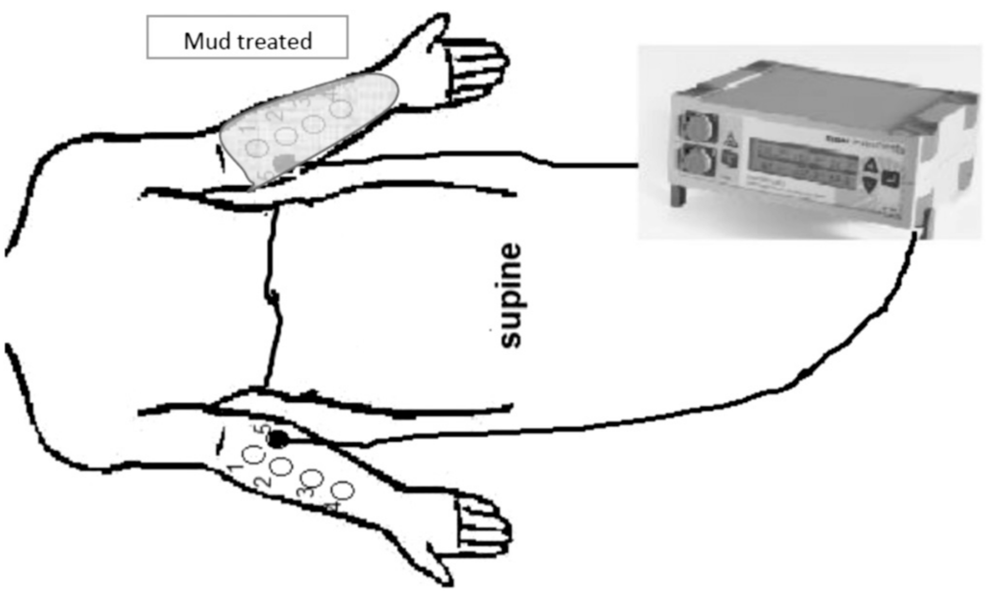

Figure 2. Protocol 2; laser Doppler probes were fixed simultaneously on areas 5 on both forearms and baseline readings were recorded over 10 min before mud application and then during the whole mud treatment time for both the treated and untreated forearms.

applied using feeding syringe on the assigned forearm and readings were recorded during the whole mud treatment time (i.e., 30 min). The mean flux and skin temperature for the baseline readings and for the 30 min during mud treatment readings were used for data analysis.

DATA ANALYSIS

The flux and skin temperatures at different time points for each area were summarized as mean ± SEM for both the treated and untreated forearms. The three groups of Dead Sea mud were treated as one group ($n = 15$) and the statistical analysis was performed using the software Minitab© (LLC, State College, PA).

Multiple paired t-tests were used, and its use was justified by the normality test for the differences between the treated and the untreated forearms.

The paired t-tests were used to compare the flux and the temperature measurements' difference for each area (of the five circular areas) between the treated and untreated forearms. No significant differences were found between the treated and untreated forearm measurements for both the flux and the temperatures for any of the five circular areas.

Furthermore, comparison between treated and untreated forearms for each mud type ($n = 5$) was performed using nonparametric testing, and no significant differences were found for the variable flux and temperatures for each mud type.

All statistical tests were performed with 5% level of significance.

RESULTS AND DISCUSSION

The chemical analysis results of the Dead Sea mud are presented in Table I. Samples 1–4 are native Dead Sea mud samples that were treated in our laboratories. Samples 1 and 2 were mixed with distilled water and then autoclaved. Samples 3 and 4 were mixed with Dead Sea water and then autoclaved. Samples 5–12 were over-the-shelf Dead Sea mud products available in the Jordanian market.

Potassium levels (%) ranged from 0.23 for RV Body Mud to 0.79 for "As is" mud-1. Calcium contents (%) ranged from 11.59 for BS Mud Mask up to 18.3 for "As is" mud-1. Sodium levels (%) ranged from 0.53 to 1.86. The highest levels were for AT Body Mud and "As is" mud-1 (1.86% and 1.85%, respectively) and the lowest for RV Body Mud. Water-soluble chloride levels (%) ranged from 0.68 to 11.24. The highest levels were for "Salted" mud-1 and 2 (11.1% and 11.24%, respectively) and the lowest level was for RV Facial Mask (0.68%). Manganese levels were below 0.03% for all tested mud samples. Aluminum levels (%) ranged from 0.41 to 0.7 and were highest for BS Mud Mask and lowest for NC Body Mud. Iron (Fe) contents ranged from 0.56% for NC Body Mud to 1.7% for BS Mud Mask. Magnesium levels (%) ranged from 0.82 to 3.57 and were the highest in salted mud samples (3.22% and 3.57%) and lowest for RV Facial Mask (0.82%).

Results show that "Salted" mud were the richest in magnesium and chloride ions as compared with "As is" mud and other processed "Over-the-Shelf" mud. Sodium contents were almost similar between "As is" mud-1 and "Salted" mud-1 and between "As is" mud-2 and "Salted" mud-2. We have noticed that for mud produced from the same company,

Table I
Chemical Compositions (%) of Different Dead Sea Mud Samples. Samples 1–4 Were Native Dead Sea Mud Treated at Our Laboratory. Samples 5–12 were "Over-the-Shelf" Mud Bought from the Market. Mud Samples Used in the Clinical Study are in Gray-Shaded Rows

	Mud	K	Ca	Na	Cl	Mg	Mn	Al	Fe	SO_3	SiO_2	L.O.I	Moisture content
1	"As is" mud-1	0.79	18.3	0.78	9.62	2.96	0.01	0.55	0.66	0.61	19.78	33.55	28.26
2	"As is" mud-2	0.61	16.49	1.85	8.77	2.72	0.03	0.6	0.71	1.08	16.32	45.93	30.05
3	"Salted" mud-1	0.72	17.52	0.84	11.1	3.57	0.02	0.44	0.62	0.52	17.26	35.28	26.64
4	"Salted" mud-2	0.59	14.46	1.72	11.24	3.22	0.03	0.6	0.81	0.3	14.54	50.3	31.77
5	RV Body Mud	0.23	13.27	0.53	0.71	0.93	0.02	0.64	0.77	0.27	25.5	40.51	37.22
6	RV Facial Mask	0.25	13.77	0.56	0.68	0.82	0.02	0.57	0.75	< 0.1	24.72	40.07	35.49
7	BO Body Mud	0.5	14.05	1.31	7.14	2.22	0.02	0.6	0.77	0.73	14.12	51.58	34.68
8	BS Mud Mask	0.43	11.59	1.15	4.94	1.8	0.03	0.7	1.7	0.39	29.1	34.19	39.73
9	AT Body Mud	0.57	15.71	1.86	4.16	2.97	0.03	0.64	0.8	0.61	15.13	47.57	32.78
10	AT Facial Mask	0.59	15.38	1.76	4.13	2.87	0.03	0.42	0.61	0.68	15.42	47.14	32.07
11	NC Body Mud	0.46	12.59	1.31	3.07	1.86	0.02	0.41	0.56	0.41	19.15	49.94	29.35
12	NC Facial Mask	0.47	12.78	1.25	5.72	1.89	0.02	0.49	0.65	0.84	21.73	45.31	26.64

there were no tangible differences in mineral and salt levels between mud designed for the face and mud designed for the body.

Based on their mineralogical contents, we have chosen three muds for the clinical study: "Salted" mud-2, "As is" mud-2, and RV Body Mud (Table 1, gray-shaded rows). The "Salted" mud, "As is" mud and RV Body Mud contain the following amounts (%) of K: 0.59, 0.61, and 0.23; Na: 1.72, 1.85, and 0.53; Cl: 11.24, 8.77, and 0.71; and Mg: 3.22, 2.72, and 0.93, respectively.

RV Body Mud contained the lowest levels (%) of K (0.23), Na (0.53), Cl (0.71), and Mg (0.93).

Changes in mean skin blood flow (flux) and skin temperature during the different protocols for each tested group (n = 5) are presented as mean ± SEM in Figures 3 and 4 and Tables II and III. In addition, changes in mean skin blood flow (flux) during the different protocols for the three groups of Dead Sea mud combined together (n = 15) are presented in Figure 5.

LDF has been in clinical use since 1977; it is characterized by its unique capacity for noninvasive measurement of local cutaneous blood flow with its moment-to-moment variability (14).

The effect of Dead Sea mud on skin microcirculation according to protocol 1 and 2 is represented in Figures 3–5.

Before applying Dead Sea mud, resting skin blood flow values and skin temperatures for both treated and untreated (control) forearms were statistically similar.

There were no statistically significant differences between the flux of treated forearms directly after mud removal as well as 15 min posttreatment for both "As is" mud and RV Body Mud group compared with the control forearms at each time points as shown in Figure 3A and C.

Interestingly, salted Dead Sea mud group showed slight increase in flux directly after mud removal (Δ 1.94), which was maintained 15 min posttreatment (Δ = 2.16) compared

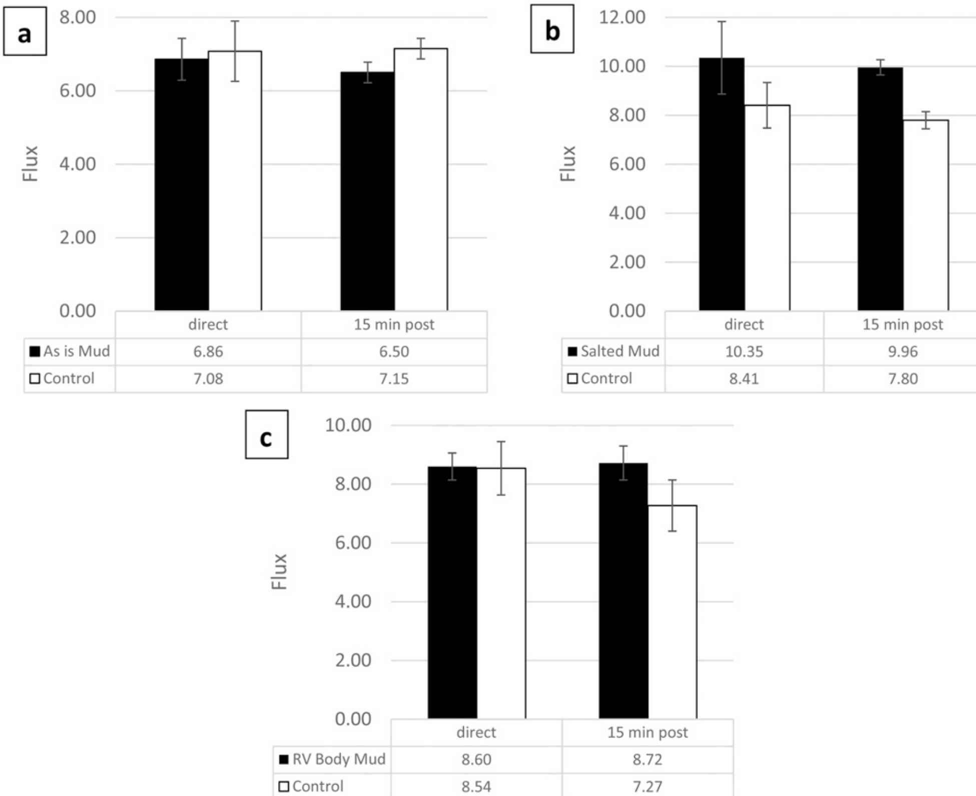

Figure 3. The effect of each type of Dead Sea mud on skin blood flow, according to study protocol 1; directly and 15 min after mud removal as compared with untreated control forearm. Mean (n = 5) ± SEM.

with control forearms at each time points as shown in Figure 3B. However, this slight increase was not statistically significant ($p > 0.05$).

The results from study protocol 1 were confirmed by study protocol 2 as shown in Figure 4. In this protocol, the effect of Dead Sea mud on skin microcirculation were monitored during the whole treatment period (i.e., 30 min) by the dual channel LDF for both treated and control forearms, simultaneously.

Resting skin blood flow and skin temperature readings (i.e., baseline) were monitored for 10 min before applying Dead Sea mud and were statistically similar for both treated and untreated (control) forearms.

Applying Dead Sea mud for 30 min did not result in a statistically significant increase in skin microcirculation for all types of Dead Sea mud compared with control untreated forearm as shown in Figure 4.

The slight increase in skin microcirculation for salted Dead Sea mud–treated forearms compared with untreated forearms was also shown in study protocol 2 in which salted Dead Sea mud caused a slight, insignificant increase in Flux (Δ 1.28) compared with control untreated forearm as shown in Figure 4B.

The effect of the three types of Dead Sea mud combined together as one group (n = 15) regardless of their salt content according to both protocols is presented in Figure 5.

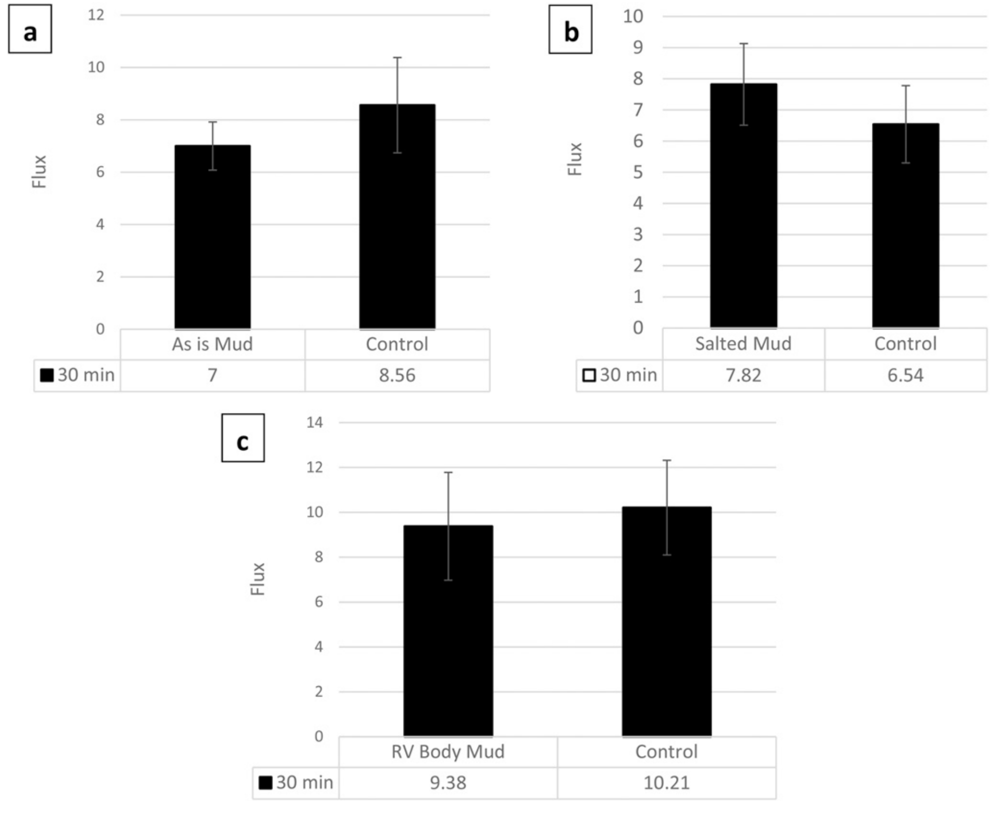

Figure 4. The effect of each type of Dead Sea mud on skin blood flow, according to study protocol 2; during mud application (i.e., 30 min) compared with untreated control forearm. Mean (n = 5) ± SEM.

Dead Sea mud application did not cause an increase in skin blood flow directly and 15 min postapplication compared with both baseline readings as well as to readings of skin blood flow of the untreated (control) forearms at the same time points as shown in Figure 5A. These findings were confirmed with protocol 2, where applying Dead Sea mud for 30 min did not result in a statistically significant increase in skin microcirculation compared with baseline readings as well as compared with control untreated forearm as shown in Figure 5B.

Table II
The Effect of Dead Sea Mud on Skin Temperature, According to Study Protocol 1, Skin Temperature before Mud Application, Directly, and 15 min after Mud Removal as Compared with Untreated Control Forearm. Mean (n = 5) ± SEM

	Skin temperature					
	Baseline		Direct		15 min after	
	Treated arm	Control	Treated arm	Control	Treated arm	Control
"As is" mud	28.11 (0.12)	28.02 (0.20)	24.61 (0.20)	25.29 (0.35)	25.32 (0.14)	26.08 (0.17)
"Salted" mud	28.03 (0.53)	27.95 (0.74)	25.04 (0.27)	26.04 (0.31)	26.29 (0.09)	26.98 (0.11)
RV Body Mud	28.75 (0.33)	29.06 (0.44)	25.61 (0.31)	26.93 (0.34)	26.68 (0.10)	27.95 (0.17)

Table III
The Effect of Dead Sea Mud on Skin Temperature, According to Study Protocol 2, Skin Temperature Baseline Value before Mud Application (i.e., for 10 min) and during Mud Application (i.e., for 30 min) Compared with Untreated Control Forearm. Mean ($n = 5$) ± SEM

	Skin temperature			
	Baseline		30 min	
	Treated arm	Control	Treated arm	Control
"As is" mud	27.95 (0.42)	28.02 (0.49)	26.35 (0.41)	27.17 (0.51)
"Salted" mud	28.61 (0.29)	29.24 (0.39)	27.17 (0.28)	28.49 (0.38)
RV Body Mud	29.26 (0.43)	29.58 (0.48)	27.31 (0.42)	28.81 (0.51)

The effect of Dead Sea mud on skin temperature was in good agreement with the effect of Dead Sea mud on skin microcirculation. Resting skin temperatures before applying Dead Sea mud were statistically similar for both treated and untreated (control) forearms in both study protocols. In addition, all types of Dead Sea mud did not result in a significant increase in skin temperature as compared with untreated control forearms at all tested time points as shown in Tables II and III.

The slight increase in skin blood flow for salted Dead Sea mud–treated forearms as compared with control forearms was not associated with any measurable increase in skin temperature directly and 15-min posttreatment in study protocol 1 (Table II) and during the 30-min treatments in study protocol 2 (Table III) compared with control forearm at the mentioned time points.

Erythema is an important consequence of skin irritation. It is documented that erythema cannot be detected by visual observation until the skin blood flow has at least tripled (15). In addition, skin temperature is closely associated with cutaneous blood flow (16). All types of Dead Sea mud did not cause significant increase in skin blood flow and skin temperature during as well as 15 min postapplication.

It is noteworthy to mention that, in both protocols and for both treated and untreated forearms, readings for skin blood flow (Figure 5) were higher at baseline than readings

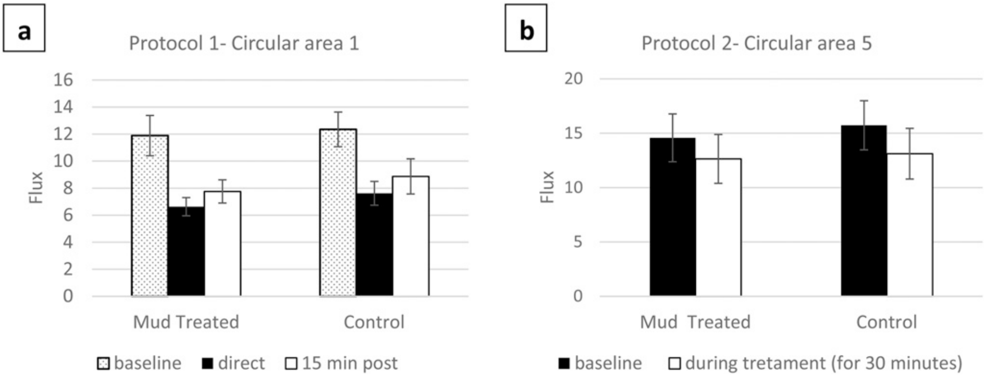

Figure 5. The effect of Dead Sea mud combined together on skin blood flow, according to study protocol 1 (A) and according to study protocol 2; during mud application (i.e., 30 min) (B) compared with untreated control forearm. Mean ($n = 15$) ± SEM.

during mud application as well as directly and 15 min posttreatment. This confirms that Dead Sea mud application for 30 min did not increase skin blood flow as compared with baseline values.

However, because cutaneous microcirculation is a physiological parameter that is subject to dynamic regulation and rapid variation and influenced by many environmental and individual factors, it is important to compare flux readings simultaneously at the same time point in a contralateral site (13). Taking this in consideration, and after confirming that baseline flux and temperature readings were stable and comparable for each circular area in both treated and control forearms, we compared flux and skin temperature readings of the treated forearms with their simultaneously recorded values of the control forearms for each circular area at each measured time points in both protocols.

CONCLUSION

Our two clinical studies showed the lack of a significant increase in skin blood flow and skin temperature during, as well as 15 min postapplication of all types of Dead Sea mud; "Salted" mud, "As is" mud, and "Over-the-Shelf" mud.

We may conclude that the increase in skin blood flow and temperature advertised by the manufacturer after mud application is not attributed to skin irritation rather, it could be attributed to the direction of use suggested by the manufacturer. The manufacturer direct mud user to warm the mud before application. This warming may enhance skin blood flow and skin temperature as it is well documented that heat increases skin blood flow in healthy subjects (17).

This LDF study confirms the mildness of all Dead Sea mud regardless of their salt content. DSM mildness previously confirmed in our laboratory by the lack of any marked differences in skin erythema as well as skin pH in mud-treated forearms compared to baseline values (18).

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