ORIGINAL ARTICLE

A comparison of the effect of certain inorganic salts on suppression acute skin irritation by human biometric assay: A randomized, double-blind clinical trial

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Background: Strontium, zinc, and potassium salts have been demonstrated to inhibit irritation and inflammation when applied topically. Particularly, strontium chloride (SC) and potassium nitrate (KN) are reported to reduce skin and tooth sensitivity. The aim of the present study was to compare the anti-irritant effects of four inorganic salts and assign the ingredient which can suppress skin irritation due to chemical or environmental exposure, more effectively. We compared the anti-inflammatory effects of SC, strontium nitrate (SN), KN, and zinc chloride (ZC). **Materials and Methods:** This double-blind trial was conducted on 32 healthy volunteers with sensitive skin. Irritation was induced by 24 h exposure with 1.0% sodium lauryl sulfate on arms.. Treatments were applied by an ointment of SN, SC hexahydrate, KN, and ZC and their 1%, 3%, and 5% (w/v) concentrations were prepared. The dosage was twice daily for 6 days to the irritated areas. Skin reactions were evaluated instrumentally. **Results:** SC had a beneficial effect that was significant overall. All other treatments exert a protective effect in skin barrier function but not significantly. With the exception of ZC, all test substances improved skin hydration but the effect of SC was significant. In respect of colorimetric assessment, all treatments, excluding ZC, reduced erythema significantly compared with an untreated control 7 days after treatment start. There was no support for a dose-response effect. **Conclusion:** Analysis of the biometric measurements revealed that the strontium salts are best, not treating is worst, and there is little difference between the other treatments. Hence, the skin care products containing SC and SN may reduce the signs and symptoms of irritant contact dermatitis.

Key words: Anti-irritation, contact dermatitis, potassium nitrate, sensitive skin, strontium chloride

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INTRODUCTION

Approximately, 40–50% of the population believes to possess the characteristics of sensitive skin. Itching, burning, stinging, and tightness are the most common complaints.[1-3] In addition to chemical and environmental causes of skin irritation, many people have an inherent sensitivity or genetic predisposition to skin irritants.[4,5] The sensory reactions of consumers to common products such as health and beauty products strongly influence their purchasing decisions.[6] Manufacturers of skin care products have made available a large variety of products which are designed for persons with sensitive skin.[2] So-called anti-irritants (AIs) are widely used in cosmetic formulations, with the aim of reducing irritation from substances in the formulation. It may also be claimed that they are "soothing" and "healing" ingredients.[7,8]

Some of the most popular ingredients used in these products are minerals and botanical extracts. Despite the media attention and consumer popularity that

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these ingredients have generated, there have been few scientific studies to support these claims. Furthermore, the consumer with self-assessed sensitive skin has no way of judging which products are likely to be most beneficial and least harmful.[2,9] Several studies indicate that inorganic salts inhibit irritation and inflammation when applied topically.[5,10] The therapeutic and cosmetic properties of Dead Sea mud and water have been well-established. Their unique composition is especially rich in magnesium, calcium, sodium, potassium, zinc, and strontium.[11]

The aim of this study was to compare the effect of four inorganic salts (strontium chloride [SC], strontium nitrate [SN], potassium nitrate [KN], and zinc chloride [ZC]) on suppression chemically-induced skin irritation in human volunteers.

EXPERIMENTAL

Subjects

Thirty-two volunteers were enrolled in this double-blind study. G*Power - Statistical and Qualitative Data Analysis Software version 3.0.10 (Heinrich-Heine University, Düsseldorf, Germany) used for sample size calculation. Subjects were 28–52 years old, 19 males and 13 females, and all classified as sensitive skin type by a prescreening lactic acid facial challenge.[12] The study protocol was approved by Ethics Committee of Isfahan University of Medical Sciences (No: IR.MUI.REC.93.3.378). All volunteers signed informed consent. Volunteers were excluded if pregnant or breastfeeding. Subjects were not taking any oral or topical corticosteroid and antihistamine medications and were free of any skin disease.

Irritant

To induce irritation, 60 µl aqueous sodium lauryl sulfate (SLS) 1.0% (Sigma, 99%, USA) were applied in Large Finn Chambers (inner diameter, 12-mm; Epitest Ltd., Helsingfors, Finland).

Before induction of irritant, a pilot study was performed to determine an appropriate irritant concentrations of SLS (1%, 2%, and 3%) on volunteers. Seeing unpleasant effects of the higher concentrations in the pilot, we chose to use 1% SLS in the subsequent studies.

Anti-irritants

An ointment consisting of 10% polyethylene and 90% paraffin oil, was prepared as vehicle for the AIs.

The following chemicals were purchased from Sigma-Aldrich and used: SN, SC hexahydrate, KN and ZC and their 1%, 3%, and 5% (w/v) concentrations were prepared; in all studies, the pH was adjusted to be equivalent in all test materials.

Induction of irritation

Guidelines on SLS exposure tests was used for induction of acute irritation.[13] On day 0, after baseline biometric measurements, eight circular areas of 20 mm diameter were framed on both volar forearms of volunteers with a marker pen. Eight sites include Two AIs in three concentrations, vehicle and "no treatment." On each site, a 12 mm diameter Finn Chamber was applied. Each chamber includes 60 µl of 1.0% SLS on filter paper discs. The patches were removed after 24 h, and skin reactions were evaluated by biometric measurements within 1 h of patch removal.

Application of substances

About 5 mg of ointment per cm^2 (corresponding to a total of approximately 160 mg) of each of the formulations, was applied twice daily for 6 days (days 1–6) to the treatment areas. Morning applications were performed immediately after measurements by a technician, whereas evening applications (at least 6 h later) were self-administered by the volunteer.

Design of experiment

The study was conducted according to double-blind, vehicle-controlled, random treatment assignment protocols in which both the subject and the assessor being blind to the treatment modality applied to each site (apart from the untreated site).

Biometric measurements

Measurements were performed once daily on days 1–4 immediately before treatment application and at the end of treatment on day 7.[14]

Transepidermal water loss

Transepidermal water loss (TEWL), a measure of skin barrier integrity, was determined with a TEWL probe (MPA 9 System, Courage + Khazaka electronic GmbH, Cologne, Germany), according to the published guidelines.[15-17] The instrument is made of two pairs of sensors to measure the humidity and temperature gradients in two different spacings.

Regarding the resulting humidity and temperature gradients, the TEWL is automatically calculated and shown.

Skin hydration

Hydration of the stratum corneum was determined using a Corneometer Probe (MPA 9 System). This probability measures the change in the dielectric constant due to skin surface hydration changing the capacitance of a precision capacitor.

The measurement is based on capacitance measurement of a dielectric medium, according to published guidelines.[18]

Skin colorimetry

The skin color was assessed by a Mexameter Probe (MPA 9 System), to measure the melanin (pigmentation)/redness (erythema). The measuring principle for the melanin and erythema readings is based on a source of light with three specific wavelengths whose radiation is absorbed by the skin and diffusely reflected. Measurements were made according to the guidelines provided by the manufacturer.[19,20]

Statistical analysis

All statistical analyses were performed using SPSS v. 18.0 (IBM Corp., Armonk, NY, USA). Statistical significance was considered when $P < 0.05$. The structure of the data is based on repeated measurements. In addition to estimation of any overall treatment effects, identification of any time-related differences between the treatments was of interest. For all biometric assessments, a parametric method, the one-way analysis of variance (ANOVA) followed by the Tukey test, was used to compare data among all groups.

RESULTS

The biometric measurements before induction of acute irritation were normal in healthy volunteers. The vehicle could not exert any significant effect compared to nontreated areas (data not shown). Sample raw data are presented in Tables 1-4. The results from the ANOVA model matched to both control value, and location of the test site are summarized in Tables 5-7. Analysis of the biometric assessments supported the finding that the strontium salts are best, not treating is worst, and there is little difference between the other treatments. Furthermore, there was no support for a dose-response effect.

The underlining of each AI salt and no treatment group at day 7 (end of treatment) indicates that with statistical testing ($P < 0.05$), no difference in treatment effect was seen. Moreover, the underlining of some of the AI salts shows the observed difference in effect seen between these AIs was not statistically significant.

The results can be interpreted as follows: The order SC5, SC3, SN5, SC1, KN5, KN3, KN1, SN3, ZC1, ZC5, ZC3, no treatment, SN1 indicates that the observed effect of SC5 was better than SC3 and the following AI salt, while the effect of SC3 was better than SN5 and SC1.

Table 1: Biometric measurements in sites either left untreated or treated with strontium nitrate

Treatments	TEWL units: g/m² h	Skin hydration units: Arbitrary corneometer® units	Colorimetry units: Arbitrary mexameter® units
Postinduction (day 1)			
No treatment	18.2±1.1	42.8±7.5	338.6±19.7
Day 4			
No treatment	12.6±2.7	52.5±10.2	336.6±16.0
1% SN	11.6±2.0	59.1±2.9	272.6±14.3**
3% SN	10.9±1.4	60.2±3.4	262.6±10.5**
5% SN	8.6±1.4**	65.6±5.7	246.0±9.5**
End of treatment (day 7)			
No treatment	7.6±1.9	49.7±5.4	298.0±4.0
1% SN	7.7±1.1	55.7±5.4	249.3±7.0**
3% SN	6.9±0.5	56.3±4.5	241.3±10.2**
5% SN	5.0±1.5**	58.3±4.3	233.3±15.7**

Data are expressed as mean±SD; **P<0.01 compared with no-treated group values. TEWL = Transepidermal water loss; SD = Standard deviation; SN = Strontium nitrate

Table 2: Biometric measurements in sites either left untreated or treated with strontium chloride

Treatments	TEWL units: g/m² h	Skin hydration units: Arbitrary corneometer® units	Colorimetry units: Arbitrary mexameter® units
Postinduction (day 1)			
No treatment	18.2±1.12	42.8±7.57	338.6±19.7
Day 4			
No treatment	12.6±2.7	52.5±10.21	336.6±16.0
1% SC	9.3±1.1**	49.1±5.0	272.0±12.6**
3% SC	8.7±0.4**	51.6±6.2	268.3±13.0**
5% SC	8.3±0.5**	53.5±5.7	265.3±12.5**
End of treatment (day 7)			
No treatment	7.6±1.95	49.71±5.4	298±4.0
1% SC	5.5±1.0*	58.2±3.7	252.3±6.1**
3% SC	4.5±0.6**	62.4±2.5**	243.0±9.2**
5% SC	4.0±0.3**	65.2±2.9**	238.3±8.5**

Data are expressed as mean±SD; *P<0.05 and **P<0.01 compared with no-treated group values. TEWL = Transepidermal water loss; SD = Standard deviation; SC = Strontium chloride

DISCUSSION

Sensitive skin is described as being hypersensitive to stimulants, and its symptom seems to occur due to increased permeability of the stratum corneum and aggravation of the nerve response in skin. There is a manifestation of a less hydrated, less elastic, and more erythematous skin, compared with normal people.[3,21]

Classical and subjective methods of sensory testing, such as stinging test and sensibility of consumers, have been utilized to provide information on sensitive skin reactions. Bioengineering or noninvasive biophysical methods enabling the most accurate quantification of different skin biometric parameters are now replacing subjective

Table 3: Biometric measurements in sites either left untreated or treated with potassium nitrate

Treatments	TEWL units: g/m² h	Skin hydration units: Arbitrary corneometer® units	Colorimetry units: Arbitrary mexameter® units
Postinduction (day 1)			
No treatment	18.2±1.12	42.8±7.5	338.6±19.7
Day 4			
No treatment	12.6±2.7	52.5±10.2	336.6±16.0
1% KN	10.5±1.6	44.2±7.0	280.0±13.2**
3% KN	10.1±1.1*	46.7±6.6	269.3±14.6**
5% KN	9.9±0.4*	47.7±6.3	262.6±17.6**
End of treatment (day 7)			
No treatment	7.6±1.9	49.7±5.4	298.0±4.0
1% KN	6.8±1.1	50.5±6.7	246.6±15.7**
3% KN	6.6±0.6	53.5±7.6	238.3±18.5**
5% KN	5.7±1.0*	54.0±5.3	236.6±15.7**

Data are expressed as mean±SD; *P<0.05 and **P<0.01 compared with no-treated group values. TEWL = Transepidermal water loss; SD = Standard deviation; KN = Potassium nitrate

Table 4: Biometric measurements in sites either left untreated or treated with zinc chloride

Treatments	TEWL units: g/m² h	Skin hydration units: Arbitrary corneometer® units	Colorimetry units: Arbitrary mexameter® units
Postinduction (day 1)			
No treatment	18.2±1.1	42.8±7.5	338.6±19.7
Day 4			
No treatment	12.6±2.7	52.5±10.2	336.6±16.0
1% ZC	12.5±0.5	50.5±5.2	336.3±12.5
3% ZC	13.0±0.5	51.7±5.4	324.0±10.8
5% ZC	12.3±0.4	53.5±6.1	304.6±9.2**
End of treatment (day 7)			
No treatment	7.6±1.9	49.7±5.4	298.0±4.0
1% ZC	7.0±0.4	46.3±5.4	288.3±14.9
3% ZC	7.6±0.4	45.3±6.8	285.0±13.0*
5% ZC	7.1±0.7	49.5±4.2	269.1±15.6**

Data are expressed as mean±SD; *P<0.05 and **P<0.01 compared with no-treated group values. TEWL = Transepidermal water loss; SD = Standard deviation; ZC = Zinc chloride

Table 5: Statistical rating of anti-irritant salts by pairwise comparisons with Tukey adjustment in the transepidermal water loss measurements at day 7 (end of treatment)

Best												Worst
SC_5	SC_3	SN_5	SC_1	KN_5	KN_3	KN_1	SN_3	ZC_1	ZC_5	ZC_3	NO	SN_1

SC = Strontium chloride; SN = Strontium nitrate; KN = Potassium nitrate; ZC = Zinc chloride; NO = No treatment; 1, 3 and 5 shows the percent of anti-irritants

Table 6: Statistical ranking of anti-irritant salts by pairwise comparisons with Tukey adjustment in the skin hydration assessment at day 7 (end of treatment)

Best												Worst
SC_5	SC_3	SN_5	SC_1	SN_3	SN_1	KN_5	KN_3	KN_1	NO	ZC_5	ZC_1	ZC_3

SC = Strontium chloride; SN = Strontium nitrate; KN = Potassium nitrate; ZC = Zinc chloride; NO, no treatment; 1, 3 and 5 shows the percent of anti-irritants

Table 7: Statistical ranking of anti-irritant salts by pairwise comparisons with Tukey adjustment in the colourimetric assessment at day 7 (end of treatment)

Best												Worst
SN_5	KN_5	SC_5	KN_3	SN_3	SC_3	KN_1	SN_1	SC_1	ZC_5	ZC_3	ZC_1	NO

SC = Strontium chloride; SN = Strontium nitrate; KN = Potassium nitrate; ZC = Zinc chloride; NO = no treatment; 1, 3 and 5 shows the percent of anti-irritants

judgments. They are typically used as methods to measure the efficacy of dermal preparations.[2,3,22]

Inorganic salts have been shown to inhibit irritation and reduce inflammation when used topically. In this work, we investigated the effects of certain inorganic salts on skin sensitivity in individuals exposed to SLS exposure test.[13,22,23]

We herein reveal that only one of four supposed AIs showed any improvement in healing rate when compared with "no treatment" in regard to all biometric parameters. SC had a beneficial effect that was statistically significant overall. All other treatments had reduced TEWL and probably exerted a protective effect in skin barrier function but not significantly. With the exception of ZC, all test substances improved skin hydration but the effect of SC was significant. With regard to colorimetric assessment, all treatments, excluding ZC, reduced erythema significantly compared with an untreated control 7 days after treatment start.

As mentioned previously a dose-response relationship was not observed between the salts. The lack of dose-response in the test substances could be due to too small a sample size.

In accordance with previous studies, strontium salts were found to have AI effect in experimentally induced irritant contact dermatitis. It seems that strontium's suppressive activity is not due to the nitrate or chloride anion alone since sodium nitrate and sodium chloride were inactive at concentrations equimolar to active concentrations of SN.[5,24]

The exact mechanism supporting the AI effects of strontium salts is not well understood. In fact, strontium ions can interact with calcium sensing receptors; a G-protein coupled receptor on type C nerve fibers. As a result of similarities in the chemical nature, size, and charge between Sr^{2+} and Ca^{2+} ions, strontium ions could demonstrate the ability to compete with calcium for receptor binding and transfer through calcium channels. It can finally lead to inhibition of calcium-dependent depolarization of C-type nerve fibers that normally transmits the sensory signal to the brain.[25] It is assumed that strontium salts could induce the release of neurotransmitters in synapsis or could antagonize the usual calcium-induced depolarization. It is also possible that strontium salts could influence keratinocytes or inflammatory cells and regulate the release of some cytokines.[26,27]

CONCLUSION

This study reveals that strontium salts can effectively improve the skin biometric parameters in comparison to other tested salts and can significantly reduce the signs and symptoms of irritant contact dermatitis.

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Conflicts of interest

There are no conflicts of interest.

AUTHORS' CONTRIBUTION

SF contributed to the conception of the work, conducting the study, drafting and revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. AJ contributed to the conception of the work, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. VH contributed to the conception of the work, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. AA contributed to the conception of the work, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work. MN contributed to the conception of the work, approval of the final version of the manuscript and agreed for all aspects of the work.

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