

Oral provocation of patients allergic to sesquiterpene lactones with German chamomile tea in order to demonstrate a possible systemic allergic dermatitis

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Summary

Background: Most patients with contact allergy to Asteraceae plants are patch test positive to sesquiterpene lactone mix (SLM). There are several reports among these patients of a flare-up of hand eczema after ingestion of food and beverages originating from Asteraceae plants.

Aim: To investigate whether German chamomile tea can elicit systemic allergic dermatitis.

Material / methods: Individuals with or without contact allergy to SLM were patch tested with an extract of German chamomile tea. Six weeks later, they were provoked with capsules containing either freeze-dried German chamomile tea or placebo capsules containing lactose in a double-blind randomized study. A numerical rating scale (NRS) was used to get the volunteers' opinion of the hand eczema status. The study individuals were examined to detect a possible flare-up of healed patch test reactions to chamomile.

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Results: None of the subjects had a flare-up of healed patch test reactions. According to NRS, SLM-positive individuals experienced a significant worsening of hand eczema, independently of whether they received chamomile or lactose capsules.

Conclusion: No evidence suggestive of systemic allergic contact dermatitis was found.

Key words: Asteraceae, Compositae, contact allergy, delayed hypersensitivity, vesicular hand eczema, patch testing, flare-up, pompholyx, sesquiterpene lactone mix, systemic allergic dermatitis

1 Introduction

Systemic allergic dermatitis (sometimes also “systemic contact dermatitis”) is the term used to describe a flare-up of a previous allergic contact dermatitis or de novo dermatitis after systemic exposure to the same allergen. It has been demonstrated in patients with nickel allergy and with many other allergens (1, 2, 3, 4). Vesicular hand eczema (pompholyx) is a common clinical feature in systemic allergic dermatitis (5).

Patients with contact allergy to Asteraceae plants often present the clinical symptoms of vesicular hand eczema (5,6). Furthermore, some of these patients report worsening or flare up of their hand eczema after ingestion of, for example lettuce, herbal tea and sesquiterpene lactone (SL) containing spices. (7, 8, 9, 10). Patch-testing with SLM in the baseline series will detect many of these patients.

We have an ongoing research project on German chamomile (*Matricaria chamomilla* L.) tea with regard to (i) investigation of the significance for hand eczema of systemic exposure to it in SLM-allergic individuals, and (ii) identification of substances in German chamomile tea responsible for contact dermatitis. In our first study (11), patients allergic to SLM were patch-tested with extracts of herbal teas from 6 different species. German chamomile tea yielded the largest number of positive reactions. Therefore, we chose German chamomile for our further investigations. This study presents the results of oral provocation with German chamomile tea in individuals hypersensitive to SLM.

Fig.1

German chamomile (*Matricaria chamomilla*). Photo: Arne Anderberg

2 Materials and Methods

2.1 Part A/ Patch testing

Forty-five individuals from southern Sweden who had been found patch-test positive to SLM earlier were asked to participate in the study. Of these, 35 individuals, 26 women and 9 men (age 37-77 years, mean 59 years) agreed to join the study. In addition, 22 dermatitis patients (17 women and 5 men, 36-71 years, mean age 52 years), patch tested at our department in Malmö and found negative to SLM, were recruited as controls.

The flow chart in Fig. 2 describes the study design. The investigative period lasted 45 days and included 6 visits at the department. There were 3 visits in the first part of the study (Part A) from day 0 (D0) to D7. The same number of visits took place in the second part of the study (Part B) from D42 to D45.

The study was based on a standardized medical history, physical examination of the hands by a dermatologist, patch testing, and oral provocation. The dermatologist examining the hands did not know the answers on the questionnaire questions, neither whether the individual examined was allergic to SLM or not. All elements of the study were completed by 54 volunteers.

The study was approved by the ethics Committee at Lund University Medical Faculty. All patients were informed of the purpose of the study and had to give their signed informed consent before being included.

2.1.1 Questionnaire

At their first visit on day D0 the individuals had to answer questions in a questionnaire on occupational and non-occupational plant exposure, history of atopic dermatitis, asthma or hay fever as well as if they had experienced worsening of their hand eczema after ingestion of coffee, chocolate, potatoes, herbal tea, beer or lettuce. To avoid leading questions, food and beverages both containing and not containing sesquiterpene lactones were included. They were also asked about ongoing medication.

Fig. 2
Study design.

Fig. 3
Flow chart

2.1.2 Patch test materials

An aqueous extract of tea made from German chamomile in stock solution and 4 dilutions, petrolatum preparations of German chamomile 2.5% w/w, Roman chamomile (*Chamaemelum nobile* All.) 1.0 % w/w, and the sesquiterpene lactone parthenolide 0.1% w/w were used. SLM 0.1% w/w from the European baseline series containing alantolactone 0.033% and the combination of costunolide and dehydrocostus lactone, each at 0.033% was also tested. The test preparations of German chamomile were supplied by Molab, Hermal, Reinbeck, Germany and the others by Chemotechnique Diagnostics, Vellinge, Sweden.

In order to ensure a double-blind reading of the tests and to control the specificity of any positive flare-up reactions following oral provocation, 27 individuals patch-tested earlier and found positive to nickel (16 SLM-positive and 11 controls) were tested with nickel sulfate hexahydrate 5.0 % pet. w/w (Chemotechnique Diagnostics). The remaining 30 individuals (19 SLM-positive and 11 controls) were tested with sodium lauryl sulfate (SLS) 2.0% aq. w/v. (Acros organics, Geel, Belgium).

The 35 SLM-positive individuals (Fig.3) were also tested epicutaneously with thin-layer chromatograms (12) of German chamomile tea. The 22 controls were also tested with "thin-layer chromatograms" but only with blank strips of the plastic roll with silica gel, ie without chamomile tea, because it was not possible to manufacture freshly prepared thin-layer chromatograms for all of the 57 participants.

To prepare an aqueous extract of tea made from German chamomile, we used dried flowers labelled to be of Swedish origin. They were acquired in a health-food shop. It was not possible to confirm the botanical

identity and quality. According to the health shop dealer, 1.5 g tea is needed for 1 cup (=200 mL) of tea (Online supplemental Fig. 4). Thus, 3 cups of tea were prepared as follows: 600 mL boiling water was poured on 4.5g tea. The extract was allowed to draw for 10 minutes. The flowers were strained off and the tea was filtrated through filter paper no 3 (Munktell Filter, Grycksbo, Sweden). The tea was then evaporated in a rotary evaporator to a volume of 2 mL constituting the stock solution. The stock solution (100%) was then diluted with water to 32% v/v, 10%, 3.2%, and 1.0%.

Fig.4

Preparation of tea made from dried flowers of German chamomile

2.1.3 Thin-layer chromatography (TLC) and TLC patch testing

Three cups of tea were prepared and evaporated under vacuum as described above. Ten mL ethanol (99.5% v/v, Kemetyl, Haninge, Sweden) was added to the residue and the mixture was treated in an ultrasonic bath for 30 seconds and the ethanol extract was collected. The residue was once more treated with 10 mL ethanol and this second ethanol extract was combined with the first one. Not all of the residue could be dissolved in ethanol. The extract containing 20 mL ethanol was evaporated under vacuum to a final volume of 2 mL resulting in a solution in which substances that had been extracted from the tea residue and dissolved completely in the ethanol had the same concentration as in the patch test preparation of the tea.

A plastic roll 500 x 20 cm with silica gel 60F254 (Merck, Darmstadt, Germany) was cut into 18 x 18 cm strips. Three μ L of the ethanol solution of chamomile tea was deposited with a pipette for every 2.5 cm of the baseline. This was repeated 25 times which makes 75 μ L on each spot. The chromatogram was developed in a glass tank with chloroform (Scharlau Chemie, Barcelona, Spain)/acetonitrile (Labscan, Dublin, Ireland) 86:14 v/v as the mobile phase. The chromatograms were developed for about 45 minutes and finally examined in UV radiation. All spots detected with 254 and/or 360 nm were marked with a pencil. Nine different spots were identified including the application spot. The spots were numbered from 1 to 9, where No1 indicates the site of application. The chromatogram was cut into 2.5 x 18 cm strips with a band of spots on each. The chromatograms were prepared one day before use and kept refrigerated in aluminium foil.

Twenty mg each of the petrolatum test preparations and 15 μ L of each of the 5 dilutions of the German chamomile tea were applied on the back with Finn chambers with a diameter of 8 mm (SmartPractice, Phoenix, Arizona) on Scanpor tape (Norgesplaster, Vennesla, Norway) and removed after 48 hours (13, 14). The distances between the 7th cervical vertebra and the patch tests, respectively the spinal cord and the patch tests were marked out in a figure for each tested individual.

All individuals were also tested epicutaneously with thin-layer chromatograms (15). The 35 individuals with contact allergy to SLM were tested with German chamomile tea chromatograms while the 22 controls were tested with blank strips of the plastic roll with silica gel, i.e. without chamomile tea, because it was not possible to manufacture freshly prepared thin-layer chromatograms for all of the 57 volunteers. Before applying the TLC strips, the localisation of the different spots of the chromatograms was marked out on the individual subject's back. The chromatograms in all 57 individuals were attached with Scanpor tape and removed after 48 hours.

The tests were read and scored according to the International Contact Dermatitis Research Group (14, 16) on day D3 and D7. During the patch test reading, the dermatologist did not know to which group the volunteer belonged (with or without contact allergy to SLM), and there was no communication between the individual and the reading dermatologist. The dermatologist was only allowed to look at the back of the volunteer.

2.2 Part B/ Oral provocation

There were 3 drop-outs among the individuals with contact allergy to SLM after the patch testing (Fig. 3). In 2 of the 3 drop-outs the reason was an experience of worsening of hand dermatitis after drinking herbal tea. Two of the controls were shown to be hypersensitive to chamomile tea extract, so they joined the test group (34 individuals with previous and/or present contact allergy to SLM and/or chamomile tea). Of these 34 volunteers, 26 were women and 8 men. The age ranged between 37 and 77 years. Mean age was 59 years. There were 20 controls, 15 women and 5 men; range 36-71 years; mean age 51 years. The SLM-positive individuals and the 2 volunteers with contact allergy to chamomile tea were randomized to chamomile tea capsules or placebo capsules. The SLM-negative controls all obtained chamomile tea capsules.

2.2.1 Clinical assessment

A numerical rating scale (17) was presented to the individuals before the oral provocation as well as 24 and 72 hours after it. The individual's opinion of the severity of their hand eczema was marked on that visual analogue scale. The individuals were allowed to see their former markings. We used a 100 mm horizontal scale. The left end at 0, was marked out with "no symptoms at all" and the right end at 10 with "very severe symptoms".

The hands of all participating individuals were investigated by a dermatologist before the oral provocation as well as 24 and 72 hours after it. The anatomical site and presence of morphological features such as erythema, papules, vesicles, fissures, and scaling were registered.

2.2.2 Test material for oral provocation

Since the study was designed as a randomized, controlled, double-blind study, the volunteers could not drink chamomile tea or placebo as is during the study. Capsules containing freeze-dried German chamomile tea and placebo capsules containing lactose were manufactured. This method made it possible to perform a double-blind oral provocation.

One thousand mL boiling water was added to 7.5 g dried flowers of German chamomile, labelled to be of Swedish origin. After 10 minutes the extract was filtrated, first through a strainer, then through filter paper no. 3 (Munktell Filter) into a glass bottle. Thirty-two batches were manufactured in the same way on three different occasions. The 32 bottles were shut tight with a cap and transported to the Department of Food Technology, Engineering and Nutrition, Lund University, Lund, Sweden.

The bottles containing water extract of tea were poured into stainless steel containers, 1000 mL in each container, and placed in a freezer room. The next day, when the extract was frozen, the containers were placed in a freeze-dryer. After a week, the freeze-dried extract was first collected in plastic storage jars, and later pulverized and stored in dishes in the desiccator. The total amount of freeze-dried tea was 58.7 g. based on 240 g dried flowers of German chamomile. For manufacturing of capsules, 55 g of the freeze-dried extract was used.

The capsules with German chamomile tea were manufactured by the Hospital Pharmacy, Malmö, Skåne University Hospital, Sweden. Threehundred chamomile capsules were needed for the study, each containing 175 mg freeze-dried German chamomile tea corresponding to $\frac{1}{2}$ cup of tea (1 cup= 200 mL). In addition, 150 placebo capsules containing lactose were manufactured. The capsules were made from gelatine, and coloured brown in order to camouflage the content. The capsules were manufactured 2 weeks before the provocation and kept refrigerated until used.

2.2.3 Oral provocation

The provocation took place in the morning of D42 (Fig. 2). The individuals were instructed not to eat or to drink anything but water 6 hours before and 30 minutes after the provocation. The individual's opinion of the severity of their hand eczema was marked out individually on a numerical rating scale (NRS). The hands of the individuals were investigated by a dermatologist not knowing whether the individual was allergic to SLM/chamomile tea or not. The backs of all individuals were also inspected by a different dermatologist from the one investigating the hands to assure that there were no remaining visible test reactions. Each individual was given either 6 capsules corresponding to 3 cups of German chamomile tea or 6 capsules containing lactose. Neither the individual nor the dermatologist knew whether the individuals had received capsules containing freeze-dried chamomile tea or placebo capsules.

On the next visit (Fig. 2), after 24 hours, D43, the hands and backs of the individuals were examined by different dermatologists concerning any flare-up or worsening of the hand dermatitis as well as possible flare-ups of healed patch test reactions. In addition, all volunteers were asked about symptoms and signs such as redness, itching and eczema from other parts of the body. The individual's opinion of the severity of their hand eczema was again marked on a NRS. An additional reading and examination was performed 72 hours (D45) after the oral provocation (Fig. 2).

2.2.4 Statistics

Fisher's exact test, 2-sided, was used to investigate differences between groups of individuals such as atopics versus non-atopics, individuals with a history of worsening of dermatitis after ingestion of food versus those without worsening, individuals with contact allergy to SLM versus those without, individuals provoked with chamomile tea versus those provoked with placebo. The two-sided McNemar test was used to examine intra-individual changes in the NRS concerning the status of hand dermatitis within groups, such as those with and without contact allergy to SLM, before and after oral provocation.

3 Results

At the time of the oral provocation on D42, hand dermatitis was present in 32 individuals; 15 of those with contact allergy to SLM and provoked with chamomile tea and in 12 of those with SLM allergy and provocation with placebo. 5 of the controls had hand dermatitis. Localization of the hand dermatitis to the palms was noted in 22 of the SLM-allergic individuals and in 2 controls. A present hand dermatitis was more common in individuals with SLM allergy as compared to the controls; $P < .001$ for those provoked with chamomile tea and $P = .009$ for those provoked with placebo. Table 1 contains information on the answers in the questionnaire, results of the patch testing including the test with the thin layer chromatograms, and the individuals' opinion on worsening of hand eczema after the oral provocation.

3.1 Questionnaire

Among the SLM-positive participants, 2/34 (6%) had occupational contact with plants and 21/34 (62%) had plant contact in leisure activities, mainly through gardening. Fewer of the controls had occupational and non-occupational contact with plants; 1/20 (5%) and 9/20 (45%), respectively. The difference in plant exposure between SLM-positive individuals and controls was not significant ($P = .25$).

15/34 volunteers hypersensitive to SLM and/or chamomile tea and 6/20 controls had a history of atopic dermatitis ($P > .3$). A history of asthma or hay fever was reported by 10/34 individuals hypersensitive to SLM and/or chamomile tea and by 2/20 controls ($P = .17$). Nineteen of 34 SLM-positive patients had a history of atopic dermatitis and/or asthma/hay fever.

Concerning the questions about food and beverages, worsening of hand eczema was reported only by the SLM-positive individuals. Nine of 34 individuals reported worsening of hand eczema after ingestion of chocolate ($P=.019$) and 12/34 from eating lettuce and/or drinking herbal tea ($P=.002$). Eight of 34 individuals each for lettuce and herbal tea reported worsening of their hand dermatitis after ingestion of lettuce and herbal tea, respectively ($P=.020$). The 2 controls who reacted positively to the chamomile tea but not to SLM did not report any food intolerance. The individuals were also asked about any reaction to coffee, beer, and potatoes, but neither the test individuals nor the controls had observed worsening of their eczema after ingestion of these items.

There was no association between reported worsening of dermatitis after ingestion of tea, lettuce or chocolate with regard to the individual degree of patch test reactivity to German chamomile tea. Nor was there any relationship between the individual's experience of worsening of hand eczema according to NRS and the degree of patch test reactivity to chamomile tea.

3.2 Patch testing and testing with chromatograms

The results of patch testing and testing with thin-layer chromatograms 6 weeks prior to the oral provocation are shown in Table 1. The results have been described in detail in our previous paper (12).

Of the 35 originally SLM-positive volunteers, 31 (88%) tested positive to SLM. 30/35 (86%) of the SLM-positive individuals and 2/22 (9%) of the SLM negative controls, had a positive patch test reaction to the water extract of chamomile tea. The degree of patch test reactivity judged as the lowest concentration giving a positive reaction to 100%, 31.2%, 10%, 3.1% and 1% was 6, 9, 2, 5 and 10 respectively (table 2a + 2b). The 2 controls testing positively to chamomile tea reacted to the stock solution (100%) and down to 1%, respectively.

15/35 (43%) of the SLM-positive individuals patch-tested with thin-layer chromatograms of German chamomile tea tested positively to one or more spots on the chromatogram.

The controls were tested with blank strips, which did not cause any reaction in anyone.

3.3 Flare-up reactions

No flare-up reactions of healed patch test reactions to nickel, SLS or chamomile tea 24 h and 72 h after oral provocation with chamomile tea capsules or placebo were observed in any individual, neither the SLM-positive individuals nor the controls. No flare-up of the healed test reactions of the thin-layer chromatograms was observed in the 15 individuals (10 of these individuals were provoked with chamomile tea and 5 with placebo) who had had positive test reactions to the chromatogram.

The physical examination of the hands by the dermatologist did not reveal any flare-up of previous hand dermatitis, neither worsening of a hand dermatitis existing at the time of the oral provocation. Based on self-evaluation using NRS, the comparison between those with and without contact allergy to SLM and/or chamomile tea was based on those with hand dermatitis at the physical examination on D42 before the oral provocation. Worsening 24 h and/or 72 h after the oral provocation was reported by 15 individuals, 14 with and 1 without contact allergy to SLM (14 of 27 versus 1 of 5; $P=.34$). Significant values resulted in those with contact allergy independent of type of provocation; $P=.016$ and $P=.001$ at 24 h and 72 h, respectively. For those with allergy and tea provocation the P -values concerning worsening of hand eczema were .13 and .046 at 24 h and 72 h, respectively. The corresponding P -values for those with contact allergy and placebo provocation were .13 and .041, respectively. Within the group of contact allergy to SLM there was no difference between those provoked with tea or placebo at 24 h, neither at 72 h ($P>.3$ for both evaluations).

3.4 Other observations

Eight of 34 individuals with contact allergy to SLM and/or chamomile tea reported signs such as redness, itching and eczema from parts of the body other than the hands. Three of them had received placebo

capsules. Most of the symptoms were subjective. One of the controls also reported symptoms as itch and redness on the body and in the face (8/34 versus 1/20, $P=.13$; Fisher's exact test, two-sided).

4 Discussion

There are many reports in the literature and from SLM-positive patients at our clinic who have experienced worsening of their hand eczema after oral intake of Asteraceae products, e.g. through drinking herbal tea and/ or eating lettuce (7, 10), now confirmed in our questionnaire. The significant association concerning chocolate was surprising, and difficult to explain. Those experiencing worsening after oral intake of herbal tea and lettuce were virtually the same patients, while those experiencing worsening after intake of chocolate were a different group, which argues against the same culprits in herbal tea and lettuce on the one hand, and chocolate on the other. The fact that no SLM-allergic individual had a history of worsening after coffee, beer, and potatoes together with the finding that the controls did not report any intolerance with worsening of eczema after any oral intake of these food items as well as of herbal tea, lettuce, and chocolate may indicate that the latter food items carry some property which causes worsening of the eczema in those who are SLM-hypersensitive.

With the above-mentioned background we had expected that the dermatologist's evaluation should have resulted in flare-up reactions of previous positive test reactions to SLM, extracts of chamomile tea, as well as thin-layer chromatograms and possibly worsening of pre-existing hand dermatitis. All this did not happen. However, based on the self-evaluation after the oral provocation 8/34 individuals with contact allergy to SLM and/or chamomile tea reported signs such as redness, itching and eczema from other parts of the body while only 1/20 controls experienced the same ($P=.13$). Furthermore, worsening of hand eczema was virtually only reported in SLM-allergic individuals with significant values at 24 h and 72 h after the oral provocation. Looking separately within the SLM-allergic group based on the oral provocation with tea or placebo, there was a tendency for statistical significance at 24 h, see above. After 72 h there was a statistical significance concerning worsening of hand eczema within both the SLM-allergic individuals provoked with tea and the SLM-allergic ones provoked with placebo with P -values of .046 and .041, respectively.

These P -values may seem to support the conclusion that chamomile tea induces an worsening of hand dermatitis in SLM-allergic individuals provoked with chamomile tea. However, there was only indicated trend of a difference ($P=.16$) in the numbers of individuals experiencing worsening of hand dermatitis 24 h after the oral provocation between the SLM-allergic individuals and the controls. One limitation of the study is that hand dermatitis was only present in 5 controls. As there was a numerical difference between the groups in those experiencing worsening of the hand dermatitis at 24 h, 37% versus 0%, respectively, one can speculate that more controls with present hand dermatitis in the study would have resulted in a statistically significant difference. However, there was no difference between the SLM-allergic individuals provoked with tea and placebo concerning both worsening of hand eczema and appearance of other reported signs from other parts of the body such as redness, itching and eczema (Table 1). Thus, this study could not demonstrate any worsening effect on hand dermatitis after oral intake of chamomile tea in those testing positively to SLM and extract of chamomile tea.

The reactions reported in the SLM-allergic individuals provoked with placebo clearly demonstrate the necessity to have a design that excludes a biased result. It is likely that all SLM-allergic individuals expected an aggravation of their dermatitis independent on whether they were provoked with chamomile tea or placebo.

This study was carefully planned to exclude biased results. There were different dermatologists (i) reading the tests performed on D3 and D7, (ii) controlling the previous test sites on D42, (iii) examining the hands on D42, D43 and D45, and (iv) reading the previous test sites on the back on D43 and D45. Furthermore, no communication was allowed between the individuals and the dermatologists on D3, D7, D42, D43, and D45. To allow a double-blind provocation, indistinguishable capsules containing either freeze-dried chamomile tea or lactose were made. We are not aware of any other controlled, randomized study that has used a freeze-dried product to enable a double-blind provocation when exploring the possibility of a systemic allergic dermatitis.

How could the provocation fail to elicit flare-up reactions of previous positive tests to dilutions of the chamomile tea extract and patches on the thin-layer chromatograms as well as aggravation of a pre-existing eczema when there are quite a few case reports suggesting such an association (18, 19, 20)? The history of the SLM-allergic individuals in the present study also suggested an aggravation of hand dermatitis after oral intake of herbal tea unlike the controls without contact allergy to SLM and chamomile tea ($P=.020$). If chamomile tea can indeed elicit flare-up reactions and aggravate hand eczema, particularly of the vesicular type on the palms, in individuals with contact allergy to SLM and chamomile tea, there are a few methodological issues which may explain the negative provocation.

(1) Was the dose of chamomile tea too low?

In patients experiencing aggravation of eczema after oral intake of herbal tea, the volume of tea has not been reported to be excessive. The SLM-allergic individuals who were provoked with chamomile tea got a single dose of 6 capsules corresponding to 3 cups of tea. Furthermore, in nickel-allergic individuals with flare-up reactions of previous nickel dermatitis after provocation with oral nickel, an oral dose being approximately 100 times larger than the dose needed to elicit a positive patch test reaction requires to give flare-up reactions (21, 22). In this study the oral dose of chamomile tea was approximately 100 times larger than the dose required for a positive patch test reaction. Thus, the dose should be sufficient to result in flare-up reactions in at least a few SLM-allergic volunteers provoked with the chamomile tea.

(2) Has the freeze-dried chamomile tea lost its sensitizing potential?

After the termination of part B, 2 SLM-allergic individuals were patch tested with the content of a capsule containing the freeze-dried chamomile tea. Both of them got strong, vesiculo-bullous reactions spreading outside the tested skin area. Thus, the freeze-dried chamomile tea had kept its sensitizing capacity.

(3) Is buccal absorption overlooked?

Buccal absorption of potential sensitizers in the herbal tea may be significant for absorption on the whole. The sensitizers in the capsules were not available for buccal absorption. Moreover, the first pass metabolism in the liver may transform sensitizers into non-sensitizers. However, this possibility was explored. After completion of the study, four volunteers with contact allergy to German chamomile were once again patch tested with German chamomile tea. Six weeks later they drank 3 cups of freshly made German chamomile tea from the same batch of tea used in part A and B. No flare-up reactions were noted, neither of the hand eczema, nor of the healed patch test reactions on the back. These findings argue against the lack of buccal absorption as the explanation of the negative result of the oral provocation.

(4) Could the explanation for the negative provocation be that the capsules did not rupture so that the content could be absorbed?

In order to investigate that, we intended to perform an *in vitro* test using 2.0 g of sodium chloride and 7.0 mL of concentrated hydrochloric acid added to 1000 mL water and the capsule left in the solution for 30 minutes at 37°C. Unfortunately, no capsules were available for this investigation. However, we think that this

explanation is unlikely as this type of capsules is used successfully for administration of other substances to the gastro-intestinal tract (23).

The lack of capsules after the termination of the study and the additional studies according to (2) above and (5) below made it also impossible to investigate another finding possibly related to the coloured capsules. Was there any release of substances from the capsules “cross-reacting” with sesquiterpene lactones which would explain the experience of worsening of the hand dermatitis in SLM-allergic individuals independent of type of oral provocation? - Again, we think that this possibility is unlikely.

(5) Was the gastro-intestinal absorption hampered as the provocation was done on an empty stomach, and would a repeated exposure to the chamomile tea during several days have resulted in a different result?

To explore these possibilities, we let 2 strongly chamomile-allergic individuals continue provocation with 6 chamomile tea capsules per day during one week. Again, there was no reaction, neither flare-up of hand dermatitis, nor flare-up of the healed test reactions on the back. Thus, the provocation on the empty stomach was not the cause of the negative provocation as the repeated exposure during several days did not result in systemic allergic dermatitis.

(6) Was the point of time chosen for the oral provocation optimal?

It is impossible to tell whether the time interval between the patch testing and oral provocation influenced the outcome, but it is considered very unlikely. The major reason for this is the demonstration that specific flare-up reactions can be obtained several months after the patch test reactions are healed (22). Thus, it is unlikely that the time interval between the patch testing and oral provocation influenced the outcome of the investigation.

(7) Could a different chamomile tea have resulted in a positive provocation?

At least theoretically, German chamomile teas may differ in their composition concerning sensitizers. These sensitizers may vary in their absorption from the gastro-intestinal tract. Presently, we do not have any idea whether this hypothesis is true.

In summary, this study demonstrates that SLM-allergic individuals unlike those without SLM contact allergy experience aggravation of their dermatitis after oral intake of lettuce and herbal tea as well as chocolate. After the provocation the SLM-allergic individuals experienced a statistically significant worsening of their dermatitis. However, this worsening was independent on whether the provocation was performed with chamomile tea extract or placebo which emphasizes the necessity of a study design eliminating the influence of the individuals' expectations on the provocation. The design of the present study was careful to enable a controlled, randomized, and double-blinded study. The negative provocation with chamomile tea in SLM-hypersensitive individuals does not argue in favour of a possible systemic allergic dermatitis after oral intake of herbal teas. However, neither does it exclude systemic allergic dermatitis after oral intake of chocolate, lettuce or another herbal tea than the chamomile tea used in this study. We therefore still advise our SLM-allergic patients to avoid or be restrictive with sesquiterpene-containing food items such as herbal teas and lettuce.

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Table 1a Characterization and test results of sesquiterpene lactone mix-positive patients provoked with chamomile tea capsules

Pat no.	Sex	Questionnaire					Patch testing			NRS	
		Plant contact in occupation	Plant contact in leisure activities	History of atopic dermatitis	History of hay fever/ asthma	Worsening of eczema after ingestion of herbal tea or lettuce	Test reaction SL-mix	Lowest dilution cham. tea giving pos test reaction	Patch test reaction for TLC cham. tea	Wors. hand eczema 24 h after oral prov.	Wors. hand eczema 72 h after oral prov.
4	M	-	-	-	-	-	+++	1 %	+		
5	F	-	-	-	-	+ (lettuce)	+++	1 %	+	+	
6	F	-	+	-	-	+ (lettuce)	-	31.2 %	-	+	+
7	M	-	+	-	+	+ (h tea)	+++	3.1 %	+	+	+
8*	M	-	+	-	-	-	+++	31.2 %	-	nt	nt
11	F	-	+	+	-	-	+++	100 %	-		+
26	F	-	-	+	-	+ (lettuce)	++	-	-		+
27	F	-	-	-	-	+ (h tea, lettuce)	+++	1 %	-		
30	F	-	-	-	+	-	+++	31.2 %	+		+
36	F	-	+	+	+	-	+++	1 %	+		+
38	F	-	-	+	+	-	+++	31.2 %	+	+	+
41	F	-	+	+	-	-	+++	1 %	+		
46	M	-	+	-	-	-	+++	10 %	-		
47	M	-	+	-	-	-	+++	3.1%	+		
48*	F	+	+	-	-	-	+++	3.1%	-	nt	nt
53	M	-	+	-	-	-	+++	3.1%	-		
55	F	+	+	-	-	-	+	100 %	-		
60	M	-	+	+	-	+ (h tea, lettuce)	+++	1 %	+		
61	M	-	-	-	-	+ (h tea, lettuce)	+++	10%	+		
62*	F	+	-	+	+	+ (h tea)	++	100 %	-	nt	nt

*=did not participate in the oral provocation, F= female, M= male, wors. = worsening, h tea= herbal tea, cham.= chamomile, TLC= thin-layer chromatogram, prov.= provocation, + = yes, - = no, nt= not tested

Table 1b

Characterization and test results of sesquiterpene lactone mix -positive patients provoked with placebo capsules

Pat no.	Sex	Questionnaire					Patch testing			NRS	
		Plant contact in occupation	Plant contact in leisure activities	History of atopic dermatitis	History of hay fever/ asthma	Worsening of eczema after ingestion of herbal tea or lettuce	Test reaction SL-mix	Lowest dilution cham. tea giving pos test reaction	Positive test reaction for TLC cham. tea	Wors. hand eczema 24 h after oral prov.	Wors. hand eczema 72 h after oral prov.
1	F	-	+	+	-	-	+++	1 %	-		
2	F	-	+	+	+	+ (h tea)	+++	31.2 %	-		
10	F	-	+	-	-	-	+++	31.2 %	+	+	+
18**	F	-	+	+	-	-	-	100 %	nt		
19	F	-	+	+	+	+ (h tea)	-	-	-	+	+
20	F	-	+	+	-	+ (h tea, lettuce)	++	31.2 %	-		
21**	F	-	-	-	+	-	-	1 %	nt		
23	F	-	-	+	-	-	++	100 %	+	+	+
25***	F	-	-	-	-	+ (lettuce)	+++	1 %	+	+	+
29	F	-	-	-	+	-	+++	-	-	+	+
35	M	-	+	+	-	-	-	31.2 %	-		
44	F	-	-	-	-	-	++	31.2 %	+		
50	F	-	+	-	-	-	+++	1 %	-	+	+
52	F	+	-	-	-	-	+++	3.1%	-		
54	F	-	+	-	-	-	++	-	-		
58	F	-	+	+	+	-	+	100 %	-		
64	F	-	+	-	-	-	-	-	+		

=former controls found to be SL-pos patients, *= medication with methotrexate , F= female, M= male, wors.= worsening, h tea= herbal tea, cham.= chamomile, TLC= thin-layer chromatogram, prov.= provocation, += yes, -= no, nt= not tested

Table 1c

Characterization and test results of controls provoked with chamomile tea capsules. Patch test reaction to sesquiterpene lactone mix, chamomile tea and TLC strips with chamomile tea negative in all patients.

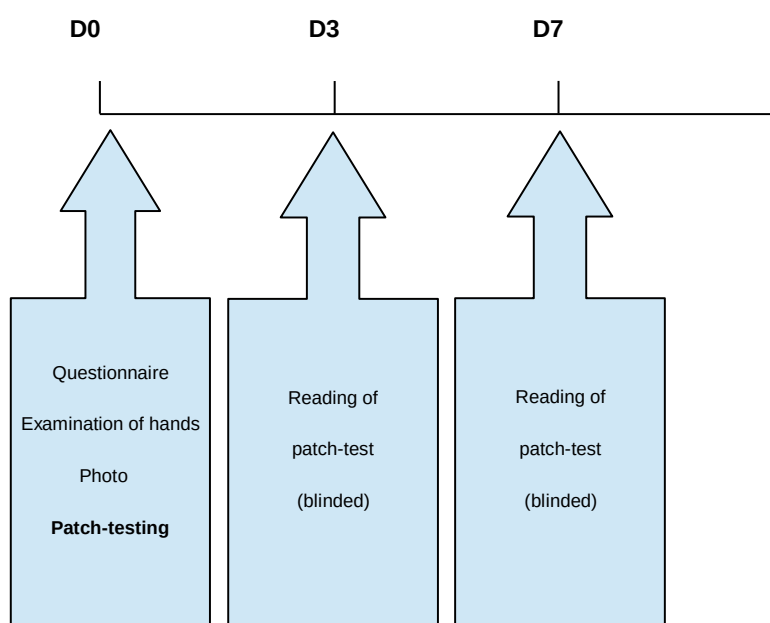
Pat no.	Sex	Questionnaire					NRS	
		Plant contact in occu- pation	Plant contact in leisure activities	History of atopic dermatitis	History of hay fever/ asthma	Worsening of dermatitis after ingestion of herb. tea or lettuce	Wors. hand eczema D 43 after oral prov.	Wors. hand eczema D 45 after oral prov.
3	F	-	-	-	-	-		
9	M	-	-	-	-	-		
12	F	-	-	+	-	-		
14	F	-	-	+	+	-	+	
15	F	-	-	-	-	-		
17	F	-	+	+	-	-		
28	F	-	+	-	-	-		
31	F	-	+	+	-	-		
32	F	-	-	-	-	-		+
39	M	-	-	-	-	-		
40	F	-	+	-	-	-		
42	M	-	+	-	+	-		
43	F	-	-	+	-	-		
45	F	-	+	-	-	-		
49	M	-	+	-	-	-		
51	F	-	-	+	-	-		
56	F	-	-	-	-	-		
63	F	-	+	-	-	-		
65	F	-	+	-	-	-		
66	M	+	-	-	-	-		

F= female, M= male, herb.=herbal, cham.= chamomile, TLC= thin-layer chromatogram, prov.= provocation, nt= not tested; wors.= worsening



Fig.1
German chamomile (*Matricaria chamomilla*). Photo: Arne Anderberg

Part A



Part B

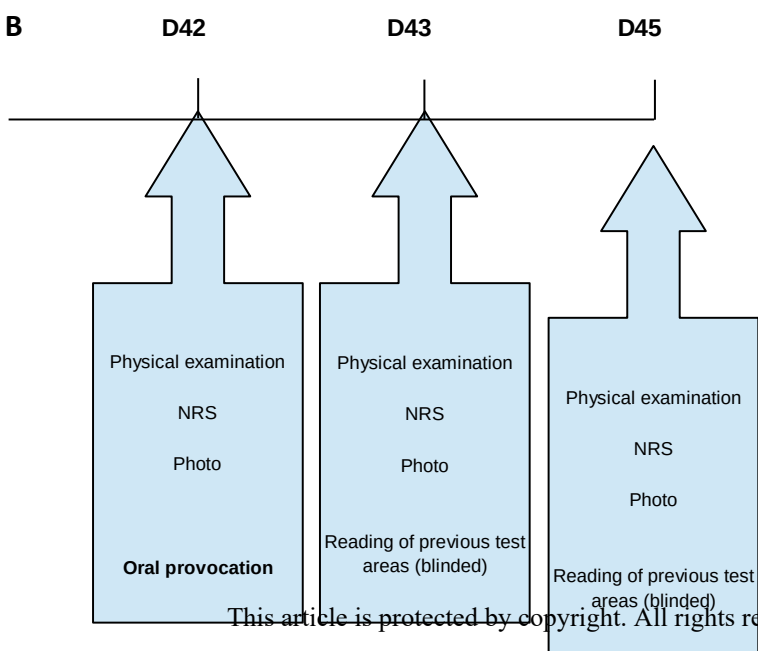


Fig. 2
Study design.

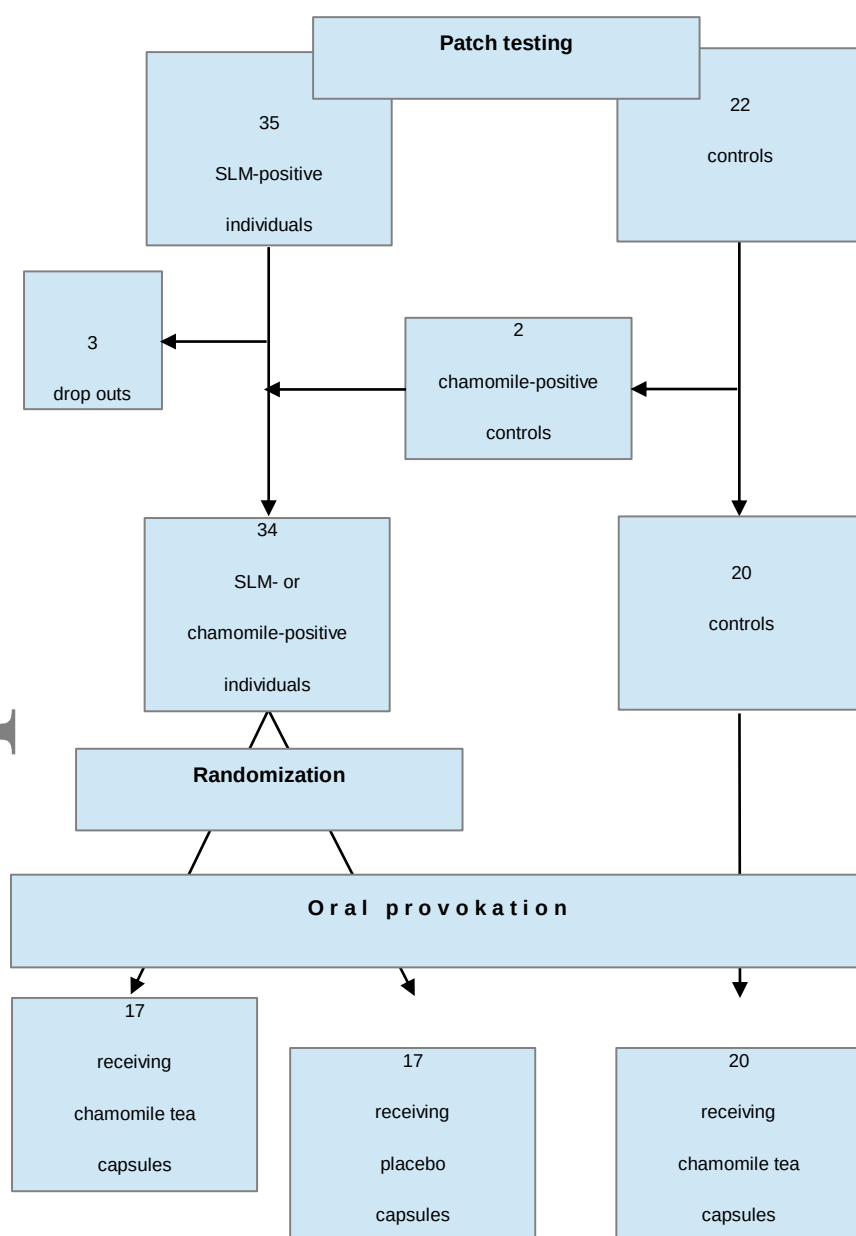


Fig. 3
Flow chart



Fig.4
Preparation of tea made from dried flowers of German chamomile