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Skin Irritation Test Of A 20% Gel Formulation Of Sunflower Seed Oil (Helianthus Annuus L.) Using A Single Closed Patch Test And Repeated Open Applications Test

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Abstract:
Background: *Sunflower seeds are widely used in the food, cosmetic, and pharmaceutical industries due to their numerous benefits. However, before introducing new products or ingredients into the market, safety evaluations such as irritation tests and risk assessments are necessary. To date, no clinical trials have been conducted on the topical application of sunflower seed oil and its potential to cause irritant contact dermatitis (ICD) or allergic contact dermatitis (ACD). This study aims to provide an overview of whether sunflower seed oil gel (Helianthus annuus L.) with a concentration of 20% causes ICD/ACD or not and determine if it has irritant potential, using the single closed patch test and repeated open application test.*
Methods: *Experimental clinical research was conducted by dividing subjects into two groups: the single closed patch test and repeated open application test (ROAT) groups. The simple irritation index for the skin scheme was used to evaluate potential irritant.*
Result: *One subject in the single closed patch test group experienced an irritant reaction at the 24-hour evaluation. However, all subjects in the ROAT group showed good results with no irritation reactions. The test material of the 20% sunflower seed oil gel was not classified as an irritant.* ***Conclusion:*** *In the single closed patch tets group, one out of five subjects experienced ICD. However, in the ROAT group, there were no irritant reactions. Therefore, sunflower oil gel (Helianthus annuus L.) at 20% concentration is not classified as an irritant.*
Keywords: *Sunflower Seed Oil, Patch test, Repeated open application test, Skin Irritation test*

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I. Introduction

Sunflower (*Helianthus annuus L.*) is the world's second most important source of vegetable oil. The oil content in sunflower seeds remains constant at 45–48%. Research has found that sunflower seeds contain 1.23% alkaloids, 0.04% glycosides, 1.46% saponins, 0.02% flavonoids, 0.64% terpenoids, and 0.34% phenolic compounds. Linoleic acid is the most abundant fatty acid in sunflower seeds, making up 57–70% of the total oil content. Sunflower seeds are highly beneficial and play a vital role in the food, cosmetic, and pharmaceutical industries. These seeds have been reported to have antipyretic, diuretic, expectorant, antimalarial, anticancer, antimicrobial, asthma prevention, antidiabetic, antidiarrheal, antihistamine, antioxidant and analgesic properties.[1] Sunflower seed oil is also used topically and has moisturizing, antibacterial, anti-acne, and protection effects against ultraviolet rays.[2]

Safety evaluation, including irritation tests and risk assessments, is required before introducing new products and their ingredients. The use of cosmetics should be evaluated for potential skin irritation. Skin irritation is a local toxic effect that occurs after exposure to cosmetic products applied to the skin. This refers to a type of skin damage that can recover spontaneously within 4 hours post-application. Skin corrosion refers to the irreversible damage caused by exposure to chemicals that can result in necrosis of the epidermis to the dermis within 3 minutes to 4 hours post-application.[3] In the past, skin testing was mostly conducted on experimental animals, but there is now a debate surrounding its use. Various skin irritation test methods have been approved, including the repeated open application test (ROAT) or cumulative irritation patch test, as well as the patch test. Human patch tests are viewed as a valid and more ethical alternative to animal testing for assessing the potential irritants from chemicals.[4]

Previous in vitro research found that a 20% concentration of sunflower seed oil (*Helianthus annuus L.*) in emulgel form demonstrates strong antibacterial activity.[5] Recent research has revealed that utilizing sunflower seed oil with high linoleic acid as an emollient for children suffering from severe acute malnutrition effectively improves the damage to their stratum corneum. Remarkably, there were no adverse effects observed, such as

hypersensitivity reactions, nosocomial infections or sepsis.[6] Derivatives of Helianthus annuus L. were found to contain allergenic sesquiterpene lactones, according to a study.[7] At present, there is a lack of clinical trial studies examining the efficacy of utilizing topical sunflower seed oil for the prevention of ICD/ACD.

The purpose of this study is to determine whether a 20% concentration of sunflower seed oil gel (*Helianthus annuus L.)* causes ICD/ACD or both. Additionally, the study aims to evaluate the potential irritant properties of the sunflower seed oil gel (*Helianthus annuus L*.) with a concentration of 20% using the Single Closed Patch Test and Repeated Open Application Test methods.

II. Material And Methods

Research Type and Design

This research is a hospital-based experimental clinical study with pretest design and postest *control group design* conducted at the outpatient polyclinic of Dermatology and Venereology Dr. Moewardi Hospital Surakarta in June 2023. The study received permission from the Research Ethics Commission at Dr. Moewardi Surakarta Regional General Hospital with the number 360/III/HREC/2023. The study was conducted in accordance with the Helsinki declaration and patient confidentiality was ensured throughout the study.

The study was conducted in two stages, with the first stage testing the efficacy of a 20% concentration of sunflower oil gel (*Helianthus annuus L*.) in inducing ICD/ACD through a closed patch test and repeated open application test. In the second stage, the same concentration of sunflower oil gel was evaluated for its potential to act as an irritant using the simple irritation index for the skin (SIISKIN) method.

$$\text{SIISKIN} = \frac{\text{The total number of erythema present in all samples at 24, 48, and 72 hours}}{\text{3 x total evaluation}}$$

Subject

The participants in this study were men and women aged between 15-49 years, who did not have any skin diseases or lesions in the test area, and were not pregnant or breastfeeding. They willingly agreed to participate in the study and signed a consent form. To be included in the study, participants had to meet certain criteria. Those who had a history of atopy or known allergies to the test product, and those who had received immunosuppressive therapy, corticosteroids, antihistamines, or chemotherapy in the previous two weeks were excluded from the study. For the single closed patch test group, participants were instructed to avoid activities that may cause excessive sweating and to keep the test area dry until the final reading. All participants were provided with information about the purpose of the study, testing procedures, and potential side effects, and signed consent forms. The requirements for the ROAT group were the same as those for the single closed patch test group.

Test Material

For both the single closed patch test and ROAT procedures, the study utilized a 20% concentration of sunflower seed oil gel (Helianthus annuus L.) as the test preparation. The ingredients used are pure sunflower seed oil, which has been tested and certified by the Badan Pengawas Obat dan Makanan. It is processed with a mixture of emulgator ingredients, including carbomer 940, triethanolamine, tween 80, span 80, glycerin, nipasol, and nipagin. This results in the formation of sunflower seed oil gel preparations with a concentration of 20%. The preparation has undergone stability testing, including organoleptic tests, homogeneity, pH, viscosity, dispersion, and adhesion, as well as freeze-thaw testing, which has shown stable results.

Single Closed Patch Test Procedure

For a single closed patch test, the optimal area is the back. A 20% sunflower seed oil gel (Helianthus annuus L.) preparation of 0.1 mg is placed in the Gamma chamber patch test unit and attached to the clean skin of the back. It is then securely fixed with hypoallergenic plaster. Following a 24-hour period, the gamma chamber is opened, and the test area is marked and cleaned. Skin reactions are evaluated at 30 minutes, 24 hours, and 48 hours after the gamma chamber is released, and the scoring scheme is outlined in Table 1. If an erythema reaction is obtained within the first 30 minutes, an assessment is still conducted without aborting the subject. Further assessments are carried out over the next 24 and 48 hours to determine if the resulting skin irritation reaction has subsided, with the erythema score taken at 24 hours post-test. There is no variation.[8] ICD is enforced when there is a decrescendo phenomenon or a decrease in reaction at 48 and 72 -hour readings, while ACD is for a crescendo phenomenon or an increase in reaction.[9]

Repeated Open Application Test Procedure

Repeated open application test is performed on the forearm using 20% sunflower seed oil gel (*Helianthus annuus L.*) at a dosage of 0.1mg on 5x5cm2 area.[10] The process of smearing should be done twice a day, once in the morning and once at night, for 10 consecutive days. During this time, the patient's progress will be monitored and evaluated on days 2 to 7. Further observations will be made on day 14 and day 21. In case of any adverse reactions, patients are advised to stop using the test material immediately.

One can evaluate positive results by using a degree assessment system for the involved areas (refer to **Table 1**). The areas may show erythema, infiltration, vesicles, or other clinical signs.[11]

Table 1. Assesment of reactions for irritant patch test.[12]

Grading	Description of skin response
0	No reaction
1	Doubtful reaction: glazed appearance of site or barely perceptible erythema
2	Weak reaction: slight erythema or dryness across most of treatment site
3	Moderate reaction: moderate erythema, possibly spreading with barely perceptible edema at the margin; papules may be present
4	Strong reaction: moderate erythema with generalized edema
5	Severe reactions: severe erythema with severe edema, with or without vesicles, pustules or ulcer

III. RESULT

In this study, 10 subjects were involved and all of them completed the study without dropping out. The single closed patch test group had 5 subjects, while the ROAT group had 5 as well. It is worth noting that no significant complaints were reported by any subjects in the ROAT group. In the single closed patch test group, one subject experienced a mild erythema reaction after 30 minutes of occlusion for 24 hours, as seen in **Figure 1**. The subjects who experienced irritation reported feeling slightly itchy and sore. However, the erythema disappeared within 24-48 hours, indicating a DKI. Daily evaluations were conducted from day 2 to day 7, followed by re-evaluations on days 14 and 21. According to the evaluation results, no skin reactions were observed in any of the participants.

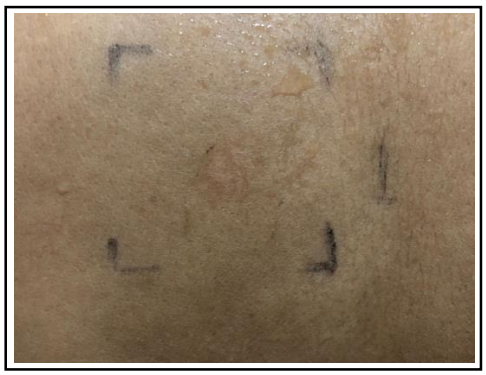

Figure 1. *Positive patch test* result

According to the SII SKIN formula in Table 2, the irritation test results show that sunflower seed oil gel (*Helianthus annuus L.*) with a concentration of 20% has an irritant potential score of 0.06. This means that it does not meet the criteria for classification as an irritant under either the Classification, Labelling and Packaging Substances and Mixtures or the Globally Harmonized System of Classification and Labelling of Chemicals.[13]

Table 2. SIISKIN ranges corresponding to CLP and GHS classifications. [13]

SIISKIN range	CLP Interpretation	GHS Interpretation
0 - < 1,5	Not irritant	Not irritant
1,5 - < 2,3	Not irritant	Potentially irritating (category 3)
2,3 - < 3	Irritant (category 2)	Irritant (category 2)
3 – 4	Corrosive (category 1)	Corrosive (category 1)

SIISKIN : *Simple Irritation Index for The Skin,* CLP: *Classification, Labelling and Packaging Substances and Mixtures*, GHS*: Globally Harmonized System of Classification and Labelling of Chemicals*. Potentially irritating corresponds to the hazard statement are divided into: category 1: causes severe skin burns and eye damage, category 2: causes skin irritation, category 3: causes mild irritation.

IV. DISCUSSION

When introducing new topical products for skin use, it's crucial to ensure safety and minimize skin irritation. The potential for irritation can depend on several factors, including the ingredients, concentration, absorption rate, quantity, skin condition, and frequency of application. To prevent skin irritation, it's necessary to conduct a compatibility study on the skin that comes into contact with the product during human use before marketing the product.[14]

The patch test is a highly effective diagnostic method utilized for identifying the root cause of ACD and assessing the potential for acute skin irritation caused by cosmetics or topical medications[15]. The Patch test can determine if a reaction is allergic or irritant. The ROAT is often used to confirm if a positive patch test result is relevant to products containing suspected allergens in low concentrations.[16] ROAT is a test that imitates the everyday use of cosmetics and can help assess the significance of patch test reactions. By combining patch tests with ROAT, a comprehensive understanding of the potential risk of skin irritation from preservatives can be obtained.[15] The ROAT test is conducted with open exposure to simulate daily living conditions. It is specifically designed for volatile materials such as liquids, gels, or creams. The test is conducted to evaluate skin safety and irritation, and only healthy individuals with no known skin disease or previous cosmetic allergies are allowed to participate.

In a single closed patch test group, weak irritant reactions were observed in one out of five subjects, while no reactions, including irritants and allergies, were detected in the ROAT group. Based on the SIISKIN scheme, the overall evaluation concluded that sunflower seed oil gel (*Helianthus annus L.)* with a 20% concentration is not an irritant.

V. CONCLUSION

Of all the participants who underwent the single closed patch test, only one out of every five had ICD. Nonetheless, the ROAT group did not show any irritant reactions. It has been concluded that *Helianthus annuus L*. gel containing a 20% concentration of sunflower seed oil is not an irritant.

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