DOES THE PALM TOCOTRIENOL-RICH FRACTION INDUCE IRRITANT CONTACT DERMATITIS?

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ABSTRACT

In Malaysia, the tocotrienol-rich fraction (TRF) is used as one of the functional ingredients in cosmetics products. The topically applied vitamin E plays a role in protecting the skin from free radicals damage induced by the ultraviolet radiation. The formulation of new topical products always includes risk of unexpected cutaneous side effects in certain individuals. Although the irritant contact dermatitis (ICD) has been reported for many compounds including active substances in cosmetics, the local topical ICD of TRF preparations has not been reported. This article describes the safety evaluation of palm TRF on skin. The primary irritation potential was assessed through animal study while the ICD was determined using patch testing technique on healthy as well as highly sensitize patients. The sensitization potential was evaluated using human repeated insult patch test on healthy subjects. Results indicated that the palm TRF did not induced any cutaneous irritation or sensitization at 1%, 2.5% and 5%.

Keywords: tocotrienol-rich fractions, irritant contact dermatitis, skin irritation, cumulative irritation, skin sensitization.

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INTRODUCTION

Irritant contact dermatitis (ICD) is the inflammatory reaction by the skin to contact with a chemical substance (Mathias and Maibach, 1978). The clinical symptoms are extremely variable with the cutaneous damage depending on the irritant with generally a dose-effect relationship. The clinical morphology presented is characterized by erythema, oedema, vesicles that have coalesced, bullae and oozing fluids. Although any part of the skin may be affected, the most frequent are the hands which are more likely to come in contact with the irritant. Most adverse reactions to cosmetics occur in the face due to the particular sensitivity of the skin.

There are many types of ICD, for example, acute ICD, delayed acute ICD, cumulative ICD, traumatic ICD, pustular and acne form ICD, non-erythematous and sensory irritation (Berardesca and Distante, 1995). The most common type is cumulative ICD. In contrast to acute ICD caused by a single contact to a potent irritant, cumulative ICD is the result of multiple subthreshold damages to the skin when time is too short for restoration of the skin barrier function (Malten, 1981). Cumulative ICD is linked to exposure to several weak irritants and water rather than to repeated exposure to a single potent irritant.

Different individuals differ in their skin susceptibility to irritation with several individual factors influencing the development of irritant dermatitis, such as age, genetic background, exposed anatomic region and pre-existing skin disease. Although experimentals have not supported sexual difference of irritant reactivity, females may be more prone in epidemiologic studies (Hogan *et al*., 1990; Meding, 1990). The increased exposure to irritants at home, manual dish washing and high risk occupations contribute to the higher incidence of ICD in females. The type and concentration of the irritant, solubility, vehicle and length of exposure to it, as well as temperature and mechanical stress also influence the clinical manifestation of ICD. Low humidity and temperature and decreased water content of the stratum corneum can also lead to increased in irritant reactivity (Uter *et al*., 1998).

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ICD has been reported in many biologically and chemically synthesized compounds. Therefore, it is not surprising to find several reports of topical contact allergy to vitamin E containing preparations (Goldman and Rapaport, 1986; Aeling *et al*., 1973) and positive results of vitamin E preparations with patch testing and erythema multiformed-like eruptions (Roed and Hjorth, 1975; Saperstein *et al*., 1984). These reports were based on derived vitamin E, such as DL-α-tocopheryl linoleate, DL-α-tocopheryl acetate and tocopheryl PEG-Succinate 1000. In 1994, there was an epidemic of papular and follicular contact dermatitis due to the use of tocopheryl linoleate in cosmetics (Perrenoud *et al*., 1994). Investigations on healthy control subjects failed to detect any irritant effect by ingredients other than vitamin E derivatives, when tested at the concentrations used in the products (Perrenoud *et al*., 1995). Vitamin E linoleate positive patch test reactions were specifically observed in the patient population and not in the healthy control subjects.

The evaluation of Palm TRF for irritation or allergic contact dermatitis can easily be carried out via common patch testing on human subjects. However, for all new topical raw materials, assessment of their irritant potential must be first determined on animals before any study on humans. One of the most important animal studies is the primary skin irritation, which determines the undiluted compound irritation potential on rabbit skin. In this article, several safety concerns are addressed to determine whether or not Palm TRF induce ICD.

MATERIALS AND METHODS

Materials

Palm TRF was obtained from Carotech (M) Sdn Bhd. The TRF specifications are as in *Table 1*. Sodium dodecyl sulphate (99%, BDH), prepared as a 10% (w/w) in petrolatum (99%, Fluka), was the positive control. Six healthy New Zealand albino rabbits (Animal Lab, UKM), weighing 2-3 kg were used.

For the patch test studies, TRF at 1%, 2.5%, 5%, 7.5%, 10% and 20% were prepared in petrolatum and placed in Finn Chambers on Scanpore (Epitest Ltd, Finland). A total of 30 healthy subjects participated in the study as volunteers.

In the human repeated insult patch test study, TRF at 2.5% and 5% was prepared in petrolatum. Sodium lauryl sulphate (99%, Sigma), which is the positive control, was prepared as 0.5% solution. Empty Finn Chambers served as the negative control and a total of 25 healthy subjects volunteered for the study.

TABLE 1. TOCOTRIENOL-RICH FRACTION SPECIFICATIONS

Component	Specifications	*Analysis – results
Tocotrienol/tocopherol complex	50.0% min	50.5%
d-γ-Tocotrienol	20% – 24%	20.11%
d-δ-Tocotrienol	4% – 6%	5.09%
d-α-Tocotrienol	10% – 14%	13.01%
d-α-Tocopherol	10% – 14%	12.26%
Squalene	8% – 12%	11.67%
Sterol	3% – 5%	4.23%
Carotenoid	0.05% – 0.09%	0.039%
Moisture	1.0% max	0.4671%
Peroxide value	10 meq kg^{-1} max	25.25 meq kg^{-1}
Lead	≤ 10 ppm	Not present
Copper	≤ 25 ppm	Passes
Zinc	≤ 25 ppm	Passes

Note: * The supplier provided the specifications and analysis results.

Primary Skin Irritation

The dermal irritation potential of the undiluted TRF to intact and abraded skin of rabbit was determined on six healthy New Zealand albino rabbits (males and non-pregnant females) weighing 2 to 3 kg. Adjacent area of untreated skin of each animal served as control for the test. The rabbits were housed at room temperature of 28°C and relative humidity of 30% to 70%. In this study, the dorsal area of the albino rabbits were clipped free of hair prior to the commencement of the test. Care was taken so as to avoid abrading the skin. The skin was abraded in one area and left intact in the other. Abrasions were

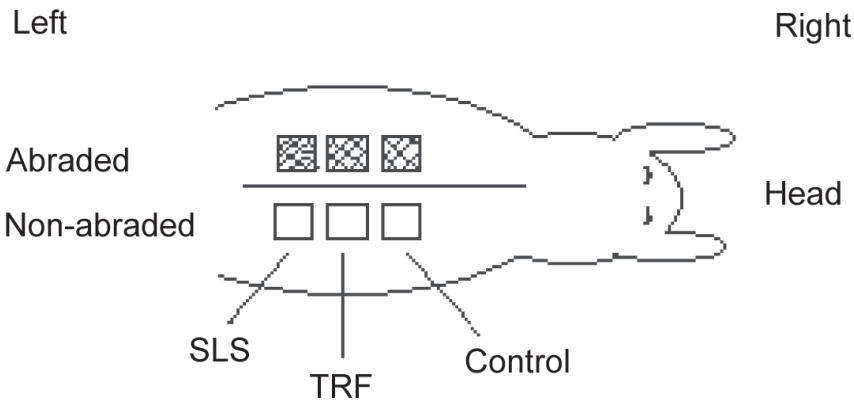

Figure 1. Position of the test materials on the rabbit skin.

minor incisions in the stratum corneum, but not sufficiently deep to disturb the dermis or to produce blood. The abrasion was made by making eight cross hatch mark of the skin using the tip of a sterile needle. A total 0.5 g of undiluted TRF was applied with a sterile cotton swab to each of two sites per rabbit. A double layer of surgical gauze covers the TRF sample. The same procedure was repeated for 10% SLS. The gauze was then covered with non-reactive hypoallergenic transpore adhesive tape and the entire test site was wrapped with an impervious material cloth (rubberized cloth) to maintain the test patches in position. The rabbits were returned to their cages. The condition of the skin was then evaluated after 24 hr of exposure following the removal of the test material and again at 72 hr (*i.e.* 48 hr after removal of the test material). The position of the undiluted TRF, 10% SLS and control sites on the rabbit were shown in *Figure 1*. The test materials were randomly changed among the six rabbits to minimize site-to-site variations. The reactions were scored according to the skin reaction values as stated under ASTM (American Society for Testing and Materials) method F719-81 as shown in *Table 2*.

The scores for erythema and oedema of the abraded and intact sites at the two time intervals for each preparation were totalled and divided by six to obtain the individual primary irritation index (PII) for each test rabbit. The average PII was then calculated by dividing to the sum of the PII for each preparation for all test animals with the number of test animals (six in the present experiment). The average PII was calculated as follows:

$$\text{PII average} = \frac{\text{sum } (PII_i)}{\text{Number of rabbits (6)}};\ i = 1 \text{ to } 6 \text{ (rabbits)}$$

where,

$$PII_i = \frac{\begin{array}{c}\text{Sum (erythema at 24 hr and 72 hr) +} \\ \text{Sum (oedema at 24 hr and 72 hr)}\end{array}}{6}$$

Ratings corresponding to the following definitions were derived from data obtained from the test methods as described in ASTM method F719-

TABLE 2. EVALUATION OF SKIN REACTION AND SCORING CRITERIA

Reaction	Description	Score
Erythema (E)	Erythema and eschar formation	
	No erythema	0
	Very slight erythema (barely perceptible)	1
	Well defined erythema (pale red in colour)	2
	Moderate to severe erythema (red and area well defined)	3
	Severe erythema (beet redness to slight eschar formation - injuries in depth)	4
Oedema (O)	Oedema formation	
	No oedema	0
	Very slight oedema (barely perceptible)	1
	Slight oedema (edges of the area well defined by definite raising)	2
	Moderate oedema (raised approx 1 mm)	3
	Severe oedema (raised more than 1 mm and extending beyond the area of exposure)	4

81:

1. Practically non-irritating. The undiluted product causes no noticeable irritation, or causes slight inflammation (oedema and erythema skin reaction values of 0 or 1) of intact or abraded skin of rabbits during the study period. PII of 0- 1.9.
2. Moderately irritating. The undiluted product causes well-defined inflammation (oedema and erythema skin reaction values of 2) during the study period. PII of 2-4.9.
3. Primary skin irritant. The undiluted product causes moderate to severe inflammation (oedema and erythema skin reaction values of 3 or 4) of the intact or abraded skin of rabbits during the study period. PII of 5 or more.
4. Corrosive. The undiluted product causes visible destruction or irreversible alterations of the tissue structure at the site of contact on intact or abraded skin of rabbits during the study period.

Patch Test

The TRF at six different concentrations, *i.e.* 1%, 2.5%, 5%, 7.5%, 10% and 20% were placed in Finn Chambers (aluminum cells with a diameter of 8 mm). Petrolatum was used as a matrix for TRF dilution. The cells were put on plaster strips Scanpor (2 x 5 cells per strip) of 60 cm^2 area. All the six TRF concentrations as described above were put directly into the cells. The six cells in a patch containing increasing TRF concentrations were then applied on the back of subjects as shown in *Figure 2*.

For each new subject, the patches were randomly positioned on one site (P1 to P4) to reduce site-to-site variation. The application of the Finn Chambers was made immediately after filling. After the application of plaster strip, the cells were pressed upwards in order to press out the air from the Finn Chambers and make the plaster adhere to the skin. Another plaster was then applied to ensure adhesion of the cells. Subjects were required to return for reading 48 hr and 96 hr after patch. At day 3 hr or 48 hr after patch, all patches were removed and they were required to relax for 30 min prior to reading. This was to eliminate all possible skin redness due to plaster strips occlusion. Readings at 96 hr were carried out immediately. The skin reaction was scored as in *Table 3.* (Reiche *et al.,* 1998).

Human Repeated Insult Patch Test

The procedure were as follow: a 0.5% solution of sodium lauryl sulphate served as positive control while an empty Finn Chamber was used as a negative control. The TRF samples were prepared at 2.5% and 5% active. Finn chambers of 8 mm diameter with 20 ml filling volume were used in the study. The TRF samples, positive and negative sample were applied under occlusive patches to skin sites on the scapular back. The procedures were repeated daily on the same test site for 21 days, or

TABLE 3. SCORING CRITERIA FOR SKIN REACTION IN THE PATCH TEST

Reaction	Score
No visible reaction	0
Doubtful/negligible erythema	0.5
Mild/just perceptible erythema	1.0
Moderate & confluent erythema area (red & well defined)	2.0
Strong erythema and spread beyond test area	3.0
Papule	0.5
Oedema	0.5
Vesicle	0.5
Bullae	0.5

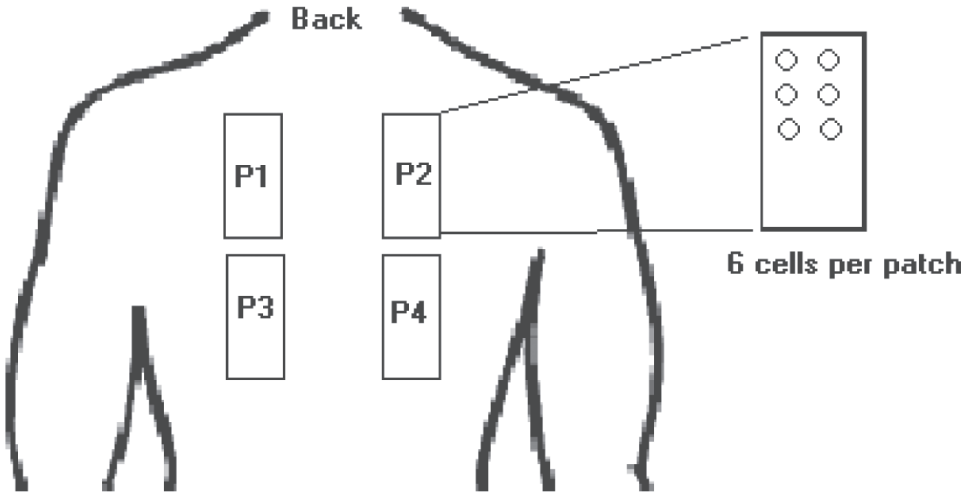

Figure 2. Position of the patches on the subject back.

until an irritation score of 3.0 or greater was observed. The test site was carefully examined for irritation, scored 30 min after removal of the patch, and then re-patched with fresh test material. In cases where re-application of the test material was discontinued because of the severity of the irritation, the scores were carried through to the end of the induction phase. Each subject was instructed to keep the patches as dry as possible and remove and discard them at 24 ± 2 hr. Patch removal was carried out half an hour prior to grading. A rest period of two weeks after the induction phase was allowed, after which, a single challenge patch was placed on the back of the subject at different site for 48 hr. The readings were taken at 48 hr and 96 hr after removal of the patch. The score and classification were based on the modified 21 days cumulative irritation test according to Berger and Bowman (1982) as shown in *Tables 4* to *6*.

A score of > 3 were capped at 3. If the reaction again scored 3 or more the next day, the test was stopped with subject accorded the maximum score of 3 for the study. A persisting reaction score of > 1 with the challenge site may be indicative of irritation, as allergic responses normally do not improve markedly at 72 hr to 96 hr. Oedema or infiltration, which persists or increases in intensity, is suggestive of allergy responses. Other indicators are flares at the former application sites, which develop between induction and the challenge. The total cumulative scores were then calculated and classified according to Berger and Bowman's classification of cumulative irritation as shown in *Table 6*.

TABLE 4. SCORE OF REACTIONS TO THE TEST MATERIALS IN THE INDUCTION PHASE

Reaction	Score
No irritation	0
Minimal redness, barely perceptible	1
Definite erythema, readily visible, minimal oedema/popular	2
Erythema and papule	3
Definite erythema	4
Erythema, oedema and papule	5
Vesicular eruption	6
Strong reaction spreading beyond test site	7

TABLE 5. SCORE OF REACTIONS TO THE TEST MATERIALS IN THE CHALLENGE PHASE

Reaction	Score
No irritation	0
No reaction	0
Macular erythema	0.5
Indurated erythema	1.0
Erythema, infiltration and redness	2.0
Bullous reaction or ulcer	3.0

RESULTS AND DISCUSSION

Primary Skin Irritation

The PII are shown in *Table 7* and *Figure 3*, which showed the value of average PII of undiluted TRF compared to sodium lauryl sulphate [SLS; 10% (w/w)], a known skin irritant as a positive control and untreated site (negative control). The results showed that TRF had induced slight erythema to a well-defined erythema on the six rabbits. However, the positive control results showed that SLS had caused mostly severe erythema and oedema on the rabbits. The average PII values obtained for undiluted TRF and SLS were noted to be higher than control. The mean PII of undiluted TRF was 1.0 ± 0.1 compared to SLS which had higher PII values of 4.8 ± 0.2. Control sites, which are untreated, had a low PII score of 0.2 ± 0.3.

According to the ASTM (American Society for Testing and Materials) classification of skin irritation potential, both control and undiluted TRF falls into the first category of non-irritating which causes no noticeable irritation, or causes slight inflammation (oedema and erythema skin reaction values of 0 or 1) of intact or abraded skin of rabbits during the

TABLE 6. CLASSIFICATION OF OBSERVED RESPONSES TO THE TEST MATERIAL*

Category	Total cumulative score	Responses	Conclusion from test
1	0 – 69	No cumulative irritation	Mild material – no irritation
2	70 - 276	Very mild cumulative irritation	Probably mild in normal use
3	277 - 621	Moderate cumulative irritation	Possibly mild in normal use
4	621 - 805	Strong cumulative irritation	Experimental cumulative irritant
5	805 - 874	Primary irritation	Experimental primary irritant

Source: * Berger and Bowman (1982).

TABLE 7. PRIMARY IRRITATION INDEX FOR CONTROL, TOCOTRIENOL-RICH FRACTION (TRF) AND SODIUM LAURYL SULPHATE (SLS)

Rabbit	Primary irritation index (PII)		
	Control	Tocotrienol	10% SLS
1	0	0.8	4.7
2	0	1.0	4.5
3	0.7	1.2	4.8
4	0	1.2	4.7
5	0	1.0	5.0
6	0.7	1.0	4.8
Average PII	0.2	1.0	4.8
S.D	0.3	0.1	0.2

Note: s.d – standard deviation.

study period. While SLS, although it scored an average of 4.8, most of the reactions had scored 3 or 4 for oedema and erythema. Therefore, according to the ASTM classification, it is classified as a primary skin irritant. This substance causes moderate to severe inflammation (oedema and erythema skin reaction values of 3 or 4) of the intact or abraded skin of rabbits during the study period. The results suggested that the undiluted TRF causes slight inflammation to the rabbits skin. In contrast to TRF, 10% SLS could be categorized as skin irritant, which may induce moderate to severe inflammation of intact and abraded skin.

In vivo Patch Test

All patch test reactions and scores were according to Rieche *et al*. (1998) who also followed the method of the International Contact Dermatitis Research Group (ICDRG), for evaluating erythema. A summary of the results is given in *Figure 4*. TRF at 1%, 2.5% and 5% concentrations did not induce any irritation reactions after 48 hr and 96 hr of patching. Similar observations were also recorded for the control sample (petrolatum) and empty Finn Chamber. The positive control was SLS at 0.5% and intense erythema with bullae was observed. SLS is used in human study as a positive patch and skin irritation model (Lee and Maibach, 1995). In this study, SLS recorded total skin reaction scores of 44 and 32 after 48 hr and 96 hr of patch removal, respectively, indicating its high irritant potential. *Figure 4* showed that by increasing TRF concentrations, higher level of cutaneous damages were observed. From just doubtful erythema at 7.5% TRF, moderate and well-defined erythema was seen to appear at elevated TRF concentration of 20%. However, at 5% TRF and below, no cutaneous reactions were seen indicating possible safe use of TRF at 5%. The TRF concentration of 5% was chosen as the possible safe level or maximum concentration that did not induce any cutaneous reactions.

In vivo Human Repeated Insult Patch Test

Human repeated insult patch test (HRIPT) was used to determine the incidence and severity of cumulative irritation and allergic contact dermatitis by predictive patch test techniques. Repeat patching of the test material has been shown to produce both cumulative irritation and allergic contact dermatitis. A total of 25 female subjects, aged between 20 and 47 with a mean of 28.4 ± 6.8 (mean ± s.d) and average age of 26, in good health and free from skin diseases, were tested. They were clearly informed of the study and the possible risks involved. *Table 8* and *Figure 5* showed that both TRF samples at 2.5% and 5% showed very low cumulative score of 4 and 7 while the empty Finn Chamber (negative control) also recorded a low cumulative score of 14. While the

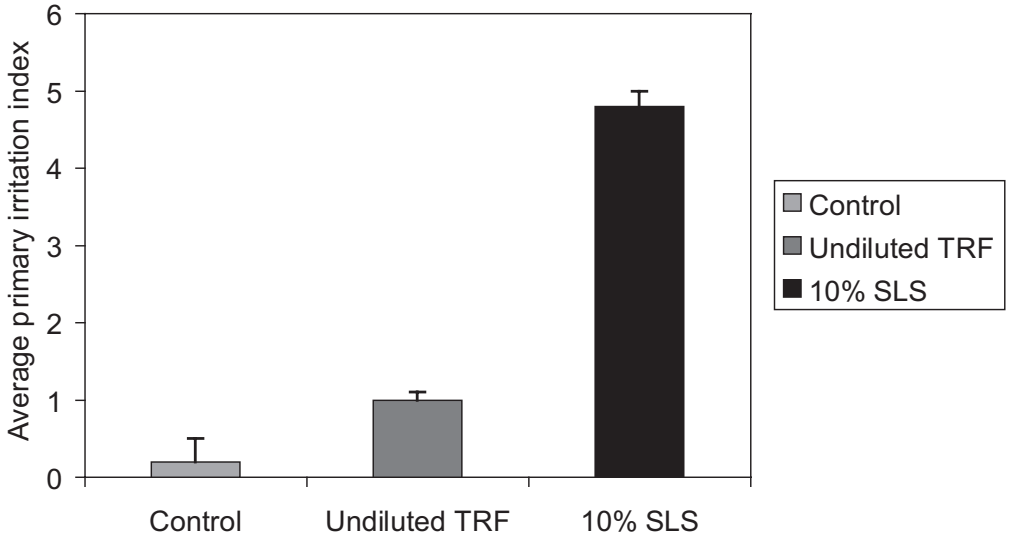

Figure 3. Average primary skin irritation index values of tocotrienol-rich fraction (TRF) (Mean ± SD, n=6).

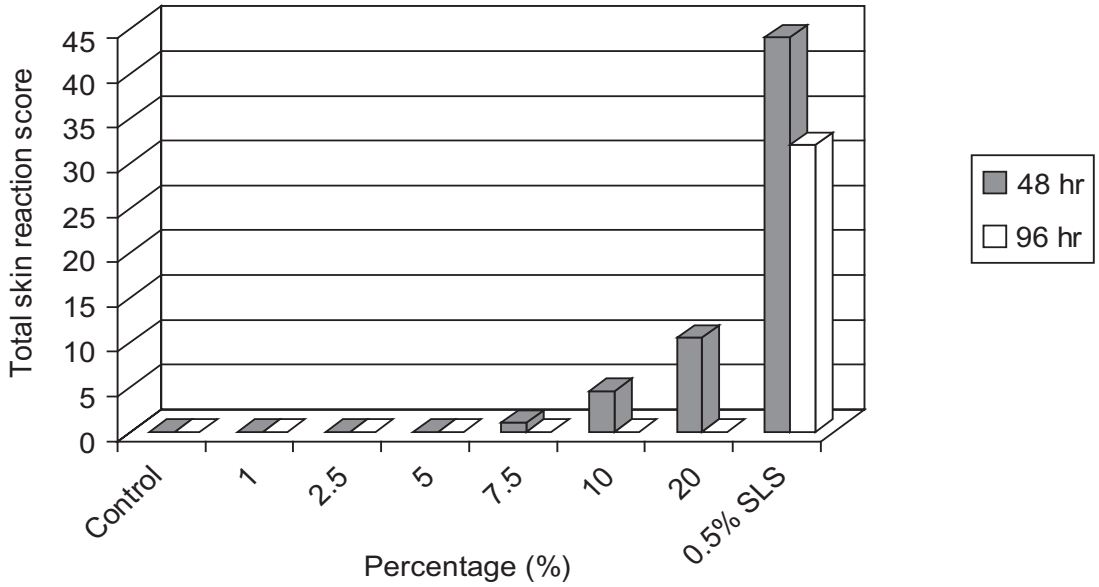

Figure 4. Total patch test score of patch for tocotrienol-rich fraction (TRF) at various concentrations (n=30).

TABLE 8. INTERPRETATION OF TOTAL CUMULATIVE SCORES FOR 0.5% SODIUM LAURYL SULPHATE (SLS), BLANK FC, 2.5% TOCOTRIENOL-RICH FRACTION (TRF) AND 5% TRF

Sample	Total cumulative score	Classification	Conclusion
0.5% SLS	1 085	4	Irritant
Blank FC	14	1	Mild
2.5% TRF	4	1	Mild
5% TRF	7	1	Mild

positive control of 0.5% SLS showed very high total cumulative score of 1085. These results indicated that both TRF samples and the negative control have minimal cumulative irritation potential compared to 0.5% SLS. The results also suggested that both TRF samples (2.5% and 5%) and the empty Finn Chamber were mild as there was no evidence of cumulative. However, SLS at 0.5% concentration recorded a total cumulative score of 1085, which was in category IV with high potential for mild to moderate cumulative irritation. The high score for SLS was in agreement with data from Lee and Maibach (1995). SLS is a known chemical skin irritant

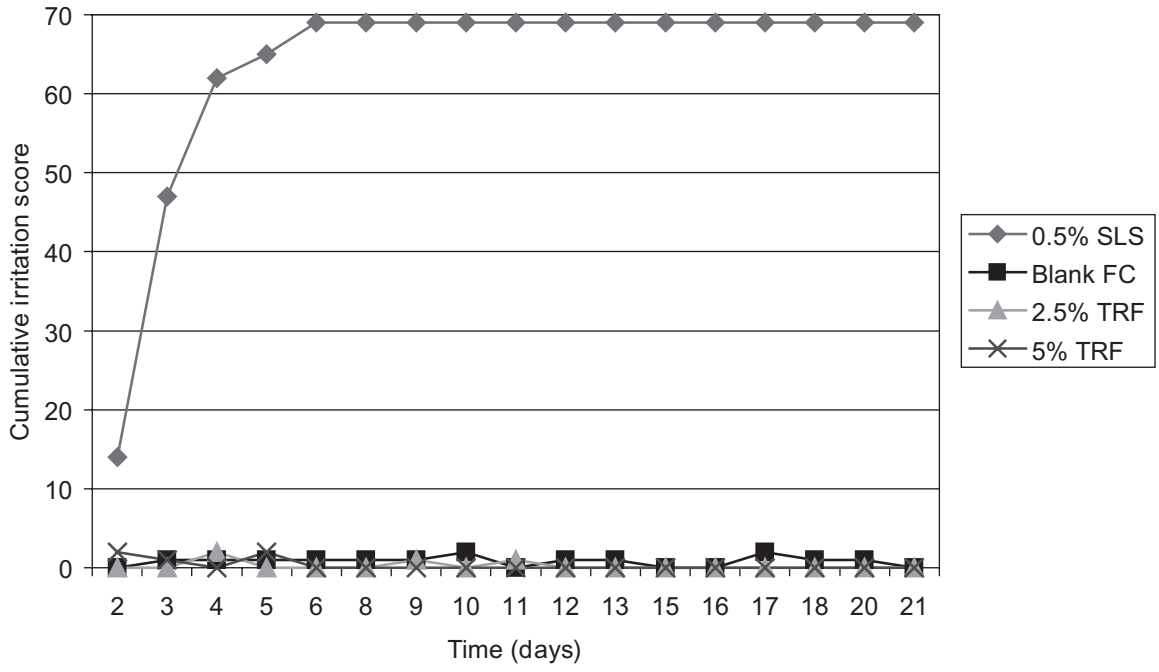

Figure 5. Total cumulative irritation of 0.5% sodium lauryl sulphate (SLS), Blank FC, 2.5% tocotrienol-rich fraction (TRF) and 5% TRF.

TABLE 9. RESULTS OF CHALLENGE PATCH FOR 2.5% TOCOTRIENOL-RICH FRACTION (TRF) AND 5% TRF

Reaction score	Reaction at 48 hr	Reaction at 96 hr
0	21	23
0.5	1	0
1	1	0

and it is used in human study as positive patch and as skin irritation model (Lee and Maibach, 1995).

After the induction phase, the test subjects were rested for two weeks. A single challenge patch with TRF at 2.5% and 5% was then applied on all subjects on a different site for 48 hr and the readings taken 48 hr and 96 hr after removal of the patch. The reactions were transient at 48 hr and 96 hr (*Table 9*). Most of the subjects did not develop any irritation reaction after 48 hr and 96 hr of patch removal. Only two subjects had scores of 0.5 and 1.0 respectively but none at 96 hr. No expressions of irritation or allergic reactions were observed on the subjects during this procedure. The results showed that TRF at 2.5% and 5% concentrations might not induce irritant contact dermatitis on healthy human subjects. Only persisting reaction of score +1, +2 and +3 at challenge site maybe indicative of ICD.

CONCLUSION

The safety evaluation of Palm TRF did not induce any cutaneous reactions. The Palm TRF may not induce IRC and it is safe to be used as an active ingredient for topical applications such as in cosmetics.

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