

The effect of variation of tetrahydropentagamavunon-0 concentration in lotion and emulgel formula toward acute dermal irritation study

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ABSTRACT

Tetrahydropentagamavunon-0 (THPGV-0) is a curcumin metabolite analog which known has a higher antioxidant activity than Vitamin E. This compound has been formulated in lotion as antiaging cosmetics, but the irritation effect is unknown yet. This study aims to analyze the effect of variation of THPGV-0 concentration in lotion and emulgel formula toward acute dermal irritation. The concentrations of THPGV-0 varied to 0.1%, 0.2%, and 0.4% in lotion/emulgel and tested for their physical properties such as organoleptic, homogeneity, pH, spreadability, and adhesion. Acute dermal irritation test is done in accordance with the *In vivo* Nonclinical Toxicity Test Guidelines by the Indonesian Food and Drug Administration (BPOM). This study showed that Primary Irritation Index (PII) value concentrations of 0.1%, 0.2%, and 0.4% THPGV-0 in lotion formula are 0.014, 0.014, and 0.028 (close to 0) and in emulgel formula are 0.08 at concentration of 0.1%, 0.33 at concentration of 0.2%, and 0.25 at concentration of 0.4%. PII of 0.1%, 0.2%, and 0.4% THPGV-0 in lotion and emulgel is included in negligible irritation response. Hence, the both of THPGV-0 formula in lotion and emulgel are considered safe to use on skin according to this study.

Key words: Emulgel, irritation, lotion, tetrahydropentagamavunon-0

INTRODUCTION

Nowadays, various kinds of cosmetics have been made from herbal ingredients to overcome various skin problems like that caused by the sun. Even though the sun plays the most important role as life source energy, the sun is a source of ultraviolet light which can cause erythema, hyperpigmentation, and skin cancer.^[1] Free radicals raised

by sunlight exposure can damage skin collagen, matrix dermal, induce wrinkled, scaly, and premature aging.^[2] Skin protection and care are considered necessary to avoid skin damage due to sunlight exposure, dust, and pollutant. There are several ways to protect human skin from UV exposure, either physical protection or chemical protection. Wearing a cap and cover body skin with long sleeve clothes are considered as physical protection, while chemical protection can be used in some kinds of sunscreens and antiaging cosmetics.

One of the cosmetics ingredients that popular is curcumin compound which has been known to

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have antioxidant, anti-inflammatory, and anticancer activities.^[3] A new analog of curcumin active metabolite which has a higher antioxidant activity than Vitamin E, called tetrahydropentagamavunon-0 (THPGV-0) [Figure 1], also has been found.^[4] ES_{50} value of THPGV-0 is 29.19, PGV-0 is 64.56, and Vitamin E is 47.87 which show that THPGV-0 antioxidant activity is better than PGV-0 and Vitamin E.^[5] Due to its potential activity as a strong antioxidant, some research starts to formulate THPGV-0 into lotion and emulgel dosage form. It can be used more easily and may become a great antiaging cosmetic. A previous study has been performed to prove the effectiveness of both formulas, but the irritation effect is unknown yet. Hence, this study aimed to determine the acute dermal irritation effect (Primary Irritation Index [PII]) of THPGV-0 (varied concentrations) in lotion or emulgel formula of THPGV-0 compound in an *in vitro* study. In long term, we would like to establish this new antiaging can be formulated in many different formulas of antiaging cosmetics.

MATERIALS AND METHODS

Materials

The active compound in this research is THPGV-0 which is synthesized by the Faculty of Pharmacy Universitas Gadjah Mada with purity 99%, cetyl alcohol (pharmaceutical grade), glycerin (pharmaceutical grade), liquid paraffin (pharmaceutical grade), triethanolamine (pharmaceutical grade), tween 80 (pharmaceutical grade), stearic acid (pharmaceutical grade), citric acid (pharmaceutical grade), DMDM hydantoin (1,3-Dihydroxymethyl-5,5-dimethylhydantoin) (pharmaceutical grade), span 80 (pharmaceutical grade), propylene glycol (pharmaceutical grade), methylparaben (pharmaceutical grade), propylparaben (pharmaceutical grade), Carbopol 940 (pharmaceutical grade), triethanolamine (pharmaceutical grade), aquadest, perfume, and Ethanol.

Preparation of THPGV-0 Lotion

The formula of THPGV-0 lotion in this study was referred to published method.^[6] The Oil phase which consisted of liquid paraffin and cetyl alcohol were put in the first cup. The second cup contains an aqueous phase such as glycerin, triethanolamine, stearic acid, and distilled water. Then, the two cups were heated at 65°C–75°C. The active substance THPGV-0 is dissolved in tween

80 solution, sonicated, and then mixed in the oil phase. The water phase is transferred into the beaker glass and mixed using a stirrer in warm conditions. The oil phase is added little by little in the aqueous phase while still being mixed using a constant-speed stirrer. When mixture becomes homogeneously and the temperature decreased, the citric acid in water is added. Then DMDM hydantoin and perfume are also added. Stirring is continued (for 15–20 min) and put the finished lotion into the primary packaging [Table 1].

Preparation of THPGV-0 Emulgel

Emulgel formula of THPGV-0 was formulated according to a published method.^[7] Emulgel was prepared in three concentrations of THPGV-0: 0.1% as Formula 1, 0.2 as Formula 2, and 0.4% Sebagai Formula 3. THPGV-0 in ethanol was mixed with the oil phase (span 80 in liquid paraffin). The water phase was prepared by dissolving tween 80 in water. Methylparaben and propylparaben as preservatives were dissolved in propylene glycol and then added into water phase. Each phase was heated up to 70°C, mixed in water phase, stirred, and then allowed to reach room temperature until emulsion was formed. Then, gelling agent 1% of Carbopol 940 was dissolved in water phase and stirred, and then, triethanolamine was added. Emulsion formula of HPGV-0, paraffin (liquid), span 80, and tween 80 was mixed into Carbopol 940 water phase [Table 2].

Acute dermal irritation test

Animal and sample preparation

This test used healthy New Zealand albino rabbits (white hair skin, red eyes) weighed ± 2 kg to identify the toxic effects that may arise after sample exposure. Rabbits were collected from animal farm, Trimulyo, Jetis, Bantul. This evaluation has done by shaving the hair of the rabbits and then applying sample on the rabbits' back that has been shaved, so the sample comes in direct contact with the skin of the test animal (rabbits). The irritation response is measured using erythema and edema response parameters which can be observed on the skin for 1, 24, 48, and 72 h after application. Erythema and edema responses are assessed by score, based on severity.^[8]

Ethics

The certified number of ethical clearance for this research is 00060/04/LPPT/XII/2019.

Animal handling protocol for rabbit

Rabbit is very susceptible to lumbar spinal luxation which can cause paralysis. Picking and lifting a rabbit is by grasping the loose skin over the shoulder with one hand and the other hand gasping the loose skin along the back or supporting its abdomen. Rabbit should never be lifted by the ears.

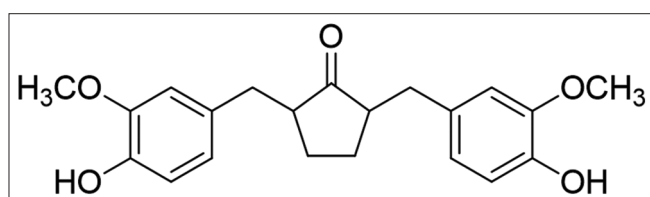


Figure 1: Structure of tetrahydropentagamavunon-0 (THPGV-0)

Determination of acute dermal irritation responses to THPGV-0 lotion formula

The responses were recorded in 1, 24, 48, and 72 h after exposure.

PII can be determined based on the Indonesian Food and Drug Administration acute dermal irritation toxicity test^[9] by applying this formula shown below:

$$PII = \frac{A - B}{C}$$

Annotation:

- A is total erythema and edema score of all sample observation at 1, 24, 48, and 72 h divided by the number of observations
- B is total erythema and edema score of all control observation at 1, 24, 48, and 72 h divided by number of observations
- C is number of animals.

Annotation:

1. Head
2. Exposure area of lotion 0.1% THPGV-0
3. Exposure area of lotion 0.2% THPGV-0
4. Exposure area of lotion 0.4% THPGV-0
5. Without exposure (control)
6. Exposure area for base
7. Tail.

PII value is then categorized according to Table 3.

The erythema and edema's responses were observed and summarized then determined according to Table 4.

RESULTS

Formulation and physical properties of tetrahydropentagamavunon-0 lotion and emulgel

This preparation was conducted based on Amalia's optimized formula.^[6] The optimized formula modified its THPGV-0 concentration to 0.1%, 0.2%, and 0.4% and added with citric acid to adjust pH value, which shows in Table 5.

Assessment of acute dermal irritation response of tetrahydropentagamavunon-0 lotion and emulgel

Rabbits were prepared by shaving its back and applied a 0.5 g sample for 4 h in each marked area. Exposure is conducted for 4 h because the sample is suspected not to be irritating/corrosive.^[9] The sample sites are applied bases, lotion/emulgel THPGV-0 0.1%, lotion/emulgel THPGV-0 0.2%, and lotion/emulgel THPGV-0 0.4%, and the area without treatment was also prepared as a control for irritating effects. The application area is shown in Figure 2. Observations were made at 1, 24, 48, and 72 h after exposure, as well as monitoring the reversibility effects for 2 weeks.

Table 1: THPGV-0 lotion formula

	Formula 1	Formula 2	Formula 3
THPGV-0	0.1	0.2	0.4
Cetyl alcohol	2.0	2.0	2.0
Stearic acid	10.45	10.45	10.45
Glycerin	10	10	10
Citric acid	0.5	0.5	0.5
Triethanolamine	1.55	1.55	1.55
Paraffinum (Liq)	10	10	10
Tween 80	1.5	1.5	1.5
Perfume (drops)	4	4	4
DMDM hydantoin	0.4	0.4	0.4
Aquadest (ad)	100	100	100

Formula 1: Lotion of THPGV-0, 0.1% (w/w), Formula 2: Lotion of THPGV-0, 0.2% (w/w), Formula 3: Lotion of THPGV-0, 0.4% (w/w)

Table 2: THPGV-0 emulgel formula

	Formula 1	Formula 2	Formula 3
THPGV-0	0.005	0.01	0.02
Ethanol	1.0	1.0	1.0
Span 80	1.25	1.25	1.25
Paraffin (liquid)	3.75	3.75	3.75
Propylene glycol	2.50	2.50	2.50
Tween 80	0.25	0.25	0.25
Methylparaben	0.015	0.015	0.015
Propylparaben	0.004	0.004	0.004
Carbopol 940	0.25	0.25	0.25
Triethanolamine	0.25	0.25	0.25
Aquadest (ad)	50	50	50

Formula 1: Emulgel of THPGV-0, 0.1% (w/w), Formula 2: Emulgel of THPGV-0, 0.2% (w/w), Formula 3: Emulgel of THPGV-0, 0.4% (w/w)

Table 3: Primary Irritation Index category

Average response	Category
0.0-0.4	Negligible
0.5-1.9	Slight
2.0-4.9	Moderate
5.0-8.0	Severe

Body weight record of rabbit

Based on paired *t*-test, analysis shows a significant value >0.05 which means that rabbit weight before and after are not significantly different.

DISCUSSION

Lotion and emulgel of THPGV-0 in this study were formed in white color, semi-solid, homogeneous, and with an *Aloe vera* odor (from its perfume). Physical property tests to both formulas were then conducted. The pH value measurements are made using universal pH paper and produce a value of 6 for all preparations. This shows the degree of acidity of THPGV-0 lotion corresponding with facial skin pH, which is between 4.5–6.5.^[10] Preparations which have a very low pH value can irritate, while too high pH value may cause dry skin.^[11]

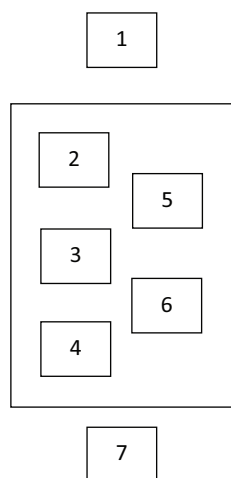


Figure 2: Application area

Table 4: Reaction assessment guideline

	Score
Erythema formation	
No erythema	0
Very slight erythema (almost indistinguishable)	1
Visible erythema	2
Moderate to severe erythema	3
Severe erythema (red meat) to eschar formation which inhibits erythema assessment	4
Edema formation	
No edema	0
Very slight edema (almost indistinguishable)	1
Slight edema (boundary area clearly visible)	2
Moderate edema (increasing area about 1 mm)	3
Severe edema (increasing area >1 mm and widened out of exposure area)	4

Table 5: Physical Properties

Sample	Formula	pH	Adhesion (S)	Spreadability (cm ²)
Base	Lotion	6	1.47±0.15	52.80±1.86
1		6	1.12±0.11	66.23±5.50
2		6	0.95±0.22	60.32±1.00
3		6	1.12±0.03	56.30±1.08
Base	Emulgel	6	3.00±1.06	17.00±1.39
1		6	1.90±1.27	20.60±1.21
2		6	3.70±1.86	19.50±0.69
3		6	2.10±0.32	17.70±0.39

Measurement of adhesion test showed that the average adhesion time of THPGV-0 lotion base is 1.47 s, lotion with 0.1% THPGV-0 is 1.123 s, with 0.2% THPGV-0 is 0.953 s, and lotion with 0.4% THPGV-0 is 1.123 s. The longest adhesion is the base formula, while the lowest is a lotion with 0.2% THPGV-0 form. A good lotion’s adhesion should be more than 4 s, so adhesion of all lotions which has been made in this research felt to be less long to stick on the skin surface.^[12] The average adhesion time of THPGV-0 emulgel base is 3 s, lotion with 0.1% THPGV-0

is 1.9 s, with 0.2% THPGV-0 is 3.7 s, and lotion with 0.4% THPGV-0 is 2.1 s.

The average spreadability of lotion base is 52.80 cm², while lotions which contain THPGV-0 0.1%, 0.2%, and 0.4% THPGV-0, respectively, 66.23 cm², 60.32 cm², and 56.30 cm². These results indicate that the spreadability decreased while its concentration increased. This is related to the increased amount of active substances, so it becomes thicker and also the distribution becomes narrower. Low spreadability value requires more pressure to apply, so the spreadability of lotion obtained is quite good because the average is above 25 cm².^[13] The average spreadability of emulgel base is 17.0 cm², while emulgel contains THPGV-0 0.1%, 0.2%, and 0.4% respectively are 20.6 cm², 19.5 cm², and 17.7 cm² [Table 5].

The irritation effect is determined based on the erythema and edema score [Table 4] caused by assessing the PII and then categorized according to the level of irritation based on ISO 10993-10,2002.^[13] The negative control has 0 value because it did not experience any changes or treatment. The irritation value of lotions which contain THPGV 0.1% and 0.2% produce the same irritation response due to appearance of erythema in rabbits 1, at hour 1 after exposure. But, the irritation value of lotions which contain THPGV 0.1% and 0.2% are lower than the irritation response value of lotion contained THPGV 0.4% which shows erythema in rabbit 1 at hour-1 and hour-24 after exposure. Besides that, the lotion base actually has the highest PII value among all samples. This may possible because of the presence of DMDM preservatives with sufficient concentration (0.4%), without the antioxidant content in the base [Table 5].

PII values occurred due to erythema, and not because of edema. In addition, the skin condition of the rabbits themselves can also affect the results because most erythema occurred in rabbit 1 and rarely occurred in the other rabbits. The results obtained indicate a very small IIP value (even close to 0), so the result is included in the negligible category [Table 6]. All erythema that occurs in rabbit skin has been reversed to its normal condition in <72 h after exposure. Also the literature study conducted by Basketter *et al.*^[14] states that testing the irritating effect on rabbits can be overpredicted. This research result can be said that lotion and emulgel with 0.1%, 0.2%, and 0.4% THPGV-0 are safe to use, although still must be careful to use, especially on sensitive skin. Before and after exposure, the test animals are weighed and their weights are recorded. The increased weight in rabbit 1 which is 0.2 kg is the highest compared to rabbit 2 (0.15 kg) and rabbit 3 (0.1 kg). Rabbit 1 also has the most erythema animals had edema, so the weight increase happens because of rabbits eat and drink a lot during treatment, not due to an edema appearance [Table 7].



Table 6: Primary Irritation Index result

Sample	Formula	PII	Category
Control	Lotion	0.000	-
Base		0.042	Negligible
Lotion 0.1%		0.014	Negligible
Lotion 0.2%		0.014	Negligible
Lotion 0.4%		0.028	Negligible
Control	Emulgel	0.000	-
Base		0.000	Negligible
Lotion 0.1%		0.080	Negligible
Lotion 0.2%		0.330	Negligible
Lotion 0.4%		0.500	Negligible

PII: Primary Irritation Index

Table 7: Rabbit weight

Rabbit	Formula	Before	After
		exposure (kg)	exposure (kg)
1	Lotion	2.000	2.200
2		2.200	2.350
3		2.300	2.400
1	Emulgel	1.663	2.0400
2		2.0745	2.3079
3		1.6134	1.5604

CONCLUSION

The study showed that lotion and emulgel formula of THPGV-0 are safe to use in cosmetics application. There is no irritation effect considered hazardous to skin during the study.

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Conflicts of interest

There are no conflicts of interest.

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