EFFECTIVENESS OF GLUTATHIONE (GSH) 2%, TOCOPHERYL ACETATE 1%, AND MAGNESIUM ASCORBYL PHOSPHATE 3% COMBINATION CREAM COMPARED WITH HYDROQUINONE 4% CREAM AS A SKIN LIGHTENING AGENT: A RANDOMISED STUDY

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ABSTRACT Aims This study aimed to determine the score of skin brightness using Chroma Meter on the subjects before the topical glutathione (GSH) 2%, 1% tocopheryl acetate 1%, and 3% magnesium ascorbyl phosphate 3% combination therapy compared with hydroquinone 4% cream after six weeks of administration. **Methods** A double-blind, pre- and the post-treatment randomised controlled clinical trial was conducted on 34 patients at the Dermatology and Venereology clinic of Hasanuddin University Hospital in Makassar, by applying combination cream and hydroquinone on the patient's right arm twice daily. Measurement of skin brightness and erythema using Chroma Meter performed weekly. **Results** The results of the study using Wilcoxon Signed Rank and Mann-Whitney test found that the L* (brightness) score in combination cream group was significantly higher than in the hydroquinone cream group ($p < 0.001$). As for the a* (erythema), the score of a* (erythema) in the hydroquinone cream group was significantly higher than in the combination cream group ($p < 0.001$). Measurement of ITA0 score (Individual Typological Angle) after six weeks was obtained and showed that the score in combination cream group was significantly higher than in hydroquinone cream group ($p < 0.001$). **Conclusion** Combination cream showed a higher score regarding brightening the skin than with hydroquinone cream, whereas the score of skin erythema increased significantly with the use of hydroquinone cream compared to the combination cream for six weeks. It is hoped that subsequent research could be done with a larger sample and longer time to get more representative results of the population.

KEYWORDS: Chroma Meter; Glutathione; Skin brightness; Combination Cream.

Introduction

Excessive pigmentation or hyperpigmentation is the most frequent complaint of cosmetic patients. Also, some cosmetic patients experience hyperpigmentation after chemical peeling,

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laser therapy, even after acne eruption. Melanin synthesis in melanosomes and its distribution to keratinocytes within the epidermal melanin unit determines skin pigmentation. Hyperpigmentation will occur when this system is impaired. [1]

Various skin lightening agents are available today, such as hydroquinone, azelaic acid, kojic acid, mulberry, α-arbutin, β-arbutin, glutathione, liquorice root, papaya, vitamin A (retinol), vitamin B3 (niacinamide), and vitamin C. Some of these are now widely contained in commercial skin lightening cosmetics. These agents act by inhibiting tyrosinase, ultimately inhibiting melanogenesis. Also, some agents act by inhibiting the transfer of melanosomes from melanocytes and keratinocytes as well as cytotoxic to melanocytes. [2]

Reduced glutathione (GSH) could brighten the skin in humans by the activity of tyrosinase inhibitors, but in the oxidised form of glutathione (GSSG), the effect is not very clear. A random, double-blind, placebo-controlled study conducted on 30 healthy Filipino women aged 30-50 years has provided some evidence to support a topical GSSG 2% as a temporary skin brightener. GSSG eventually converted to GSH after skin absorption. Changes in melanin index, stratum corneum moisture, skin smoothness, skin elasticity, and wrinkle formation are objectively assessed. The decline in melanin index by glutathione was statistically significant when compared to placebo. [3]

Vitamin E is known for its antioxidant properties. In nature, vitamin E is present in the form of tocopheryl and tocotrienol which have four forms, namely alpha, beta, gamma, and delta. Alpha-tocopheryl (AT) is the most active form of vitamin E in humans. The World Health Organization states that 1 mg (1%) of ATA equivalent to 1 IU of vitamin E. Most people do not experience any side effects when using topical vitamin E and its derivatives, including ATA. [1]

Magnesium ascorbyl phosphate (MAP) is an ascorbic acid derivate (vitamin C). It is produced from the chemical modification of ascorbic acid, by introducing the phosphate group into position 2 in the enediol system. When used as an active ingredient for the cosmetic formulation, the concentration used is usually 1-2%, and when used as an antioxidant, the concentration used is 0.05-0.1%.[4]

A large and various number of current commercially available skin lightening products are known to use a combination of different skin-lightening agents. Some of it including niacinamides, α-arbutin, and kojic acid, as well as vitamins A, C, and E. Some products contain extract of ingredients which work as tyrosinase inhibitors (Mulberry, liquorice, sophora, and peonia extracts) and others which act as antioxidants and anti-inflammatory. [2]

Water-based GSH such as solution and cream are very unstable and easily decompose. To overcome this, additional substances that can maintain the stability of GSH is required. One study found that the addition of Ascorbic Acid (Vitamin C) to a GSH concentrate could reduce the oxidation rate of the GSH. [5] Hwang et al. (2012) stated that MAP could increase GSH levels to protect keratinocytes in the skin against ultraviolet radiation. [6] Vitamin E (one of its active forms is alpha tocopherol acetate) has a role in gene regulation by interacting with cell receptors to modulate the levels of various proteins, one of which is glutathione. [7]

Based on this, our study was aimed to determine the score of skin brightness using Chroma Meter of the subjects before the topical glutathione (GSH) treatment 2%, tocopheryl acetate 1%, and magnesium ascorbyl phosphate 3% combination cream compared with hydroquinone 4% cream after six weeks of administration.

MATERIALS AND METHODS:

Anamnesis and physical examination are done in polyclinic of Dermatology and Venereology of Hasanuddin University Educational Hospital in Makassar. The study was conducted in January 2018.

This study used a double-blind, pre- and post-treatment randomised controlled clinical trial design. The research variables consisted of independent variables (glutathione (GSH) 2% combination cream, hydroquinone 4% cream, skin type), dependent variable (skin brightness), intermediate variables (melanogenesis), and control variables (UV, hormonal and skin type according to Fitzpatrick).

The population is all female aged 25-35 years' old who come to Dermatology and Venereology clinic of Hasanuddin University Educational Hospital in Makassar. The sample of the study were all individuals who met the criteria of the study; the total sample was 34 people aged 25-35 years.

Data collection includes 1) Anamnesis: subject identity, history of sunscreen use, daily activity and duration of daily sun exposure, history of hormonal contraceptive use, first and last menstrual date. Past medical history (epilepsy, malignancy, contact dermatitis due to cosmetics), history of other skin diseases, family history, and social history. 2) Physical examination includes vital signs, general status, and dermatologic status. Skin brightness measurements were performed using Chroma Meter. The subjects were evaluated for six weeks with one visits every week (T0, T7, T14, T21, T28, T35, and T42). At each visit, a clinical therapeutic evaluation, measurements with Chroma Meter, and the results were recorded as well as any side effects that occurred, followed by a documented photograph of the cream-applied portion of the right forearm using a Sony 16.1-megapixel digital camera, ordinary light, 20 cm distance, and dark blue background.

All of the collected data will be analysed using a statistical program. The statistical methods used are descriptive statistics and Wilcoxon Signed Rank and Mann-Whitney statistic test. A p-value <0.05 considered as statistically significant.

RESULTS

Table 1 Characteristics of Study Population

Characteristics		**N**	**%**
Age(year)	25-30	15	44.1
	31-35	19	55.9
Occupation	Student	12	35.3
	Employee	22	64.7
Office Hours	8 -12	13	38.2
	>12	21	61.8
Abbreviations: N: Number of Sample			

The total sample included in this study were 34 people aged 25-35 years. Most common age group were 31-35 years old with 19 persons (55.9%), while in the 25-30 years' age group only 15

people (44.1%). Regarding occupation, the study sample was taken mostly from the private sector, such as housewives and household assistants with 22 people (64.7%), while from the students' group as many as 12 people (35.3%). Regarding the number of in-room working hours, the most frequents were the group with working hours > 12 hours a day, as many as 21 people (61.8%), while in the group with 8-12 hours working hours a day only 13 people (38.2%) (Table 1).

All samples were applied with cream 1 (combination of glutathione 2%, tocopherol acetate 1%, and magnesium ascorbic phosphate 3%) and 2 (hydroquinone 4%) in the right forearm every day according to the study procedure and measured L* (skin brightness), a* (erythema) and ITA^0 (individual typological angle) with Chroma Meter every week for 6 weeks.

L* (skin brightness) score for cream 1: there were a significant increase in L* (skin brightness) scores each week during the study period. At the beginning of the study (T0), the L* (skin brightness) score of the sample was 58.88 (SD 3.11). The following week the score grew to 59.76 (SD 2.98), and this score continues to increase until at the end of the study period (T42) to 62.40 (SD 2.98). For cream 2, after each week during the study period, the scores tend to be constant. At the beginning of the study (T0), the L* (skin brightness) score of the sample was 57.93 (SD 3.12). The following week, the score changed to 57.60 (SD 2.70), and this score tends to be the same until the end of the study period (T42) of 57.70 (SD 2.58). Comparison of L* scores (skin brightness) between cream 1 and 2 showed that at the beginning of measurement (T0), the L* score between the two groups did not differ significantly (p = 0.212). However, in the following weeks (T7-T42), there was a significant difference between the two groups, where the L* (skin brightness) score in the cream 1 group was higher than cream 2 (p <0.001) (Table 2). The a* (erythema) score for cream 1 each week during

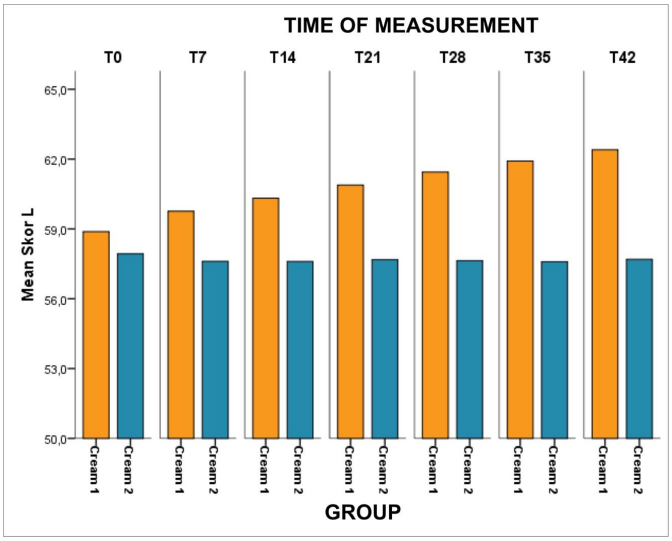

Figure 1: Comparison of L* Score (Skin Lightness) Between Cream 1 and Cream 2.

the study period tends to be constant. At the beginning of the study (T0), the average a* (erythema) score of the sample was 9.82 (SD 1.33). The following week the score changed to 9.73 (SD 1.33), and this score tends to be the same until the end of the study period (T42) with 9.29 (SD 1.43). For cream 2, there were a significant increase in a* (erythema) scores each week during the study period. At the beginning of the study (T0), the average a* (erythema) score of the sample was 10.31 (SD 1.23). The following week, the score grew to 10.71 (SD 1.27), and this score continues to increase until at the end of the study period (T42) to 11.13 (SD 1.03). Comparison of a* scores (erythema) between cream 1 and 2 during the study period showed that at the beginning of measurement (T0), the a* score (erythema) between the two groups did not differ significantly (p = 0.121). However, in the following weeks (T7-T42), there was a significant difference between the two groups, where the score of a* (erythema) in the cream 2 group was higher than that of cream 1 (p <0.001) (Table 3). There was a significant increase in

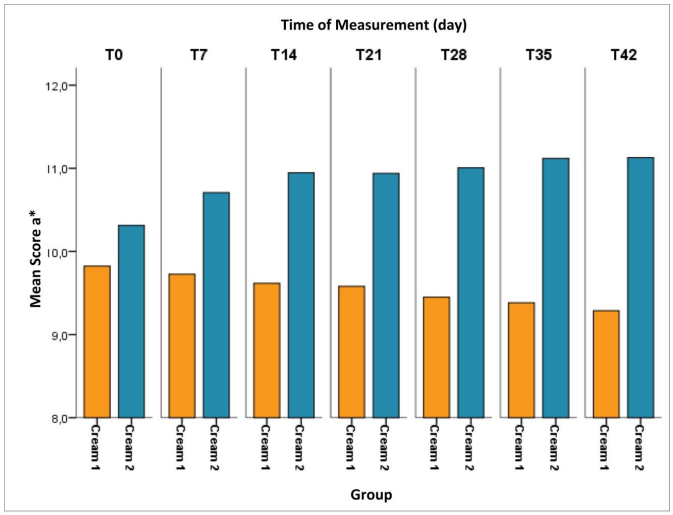

Figure 2: Comparison of a* Score (Erythema) Between Cream 1 and Cream 2.

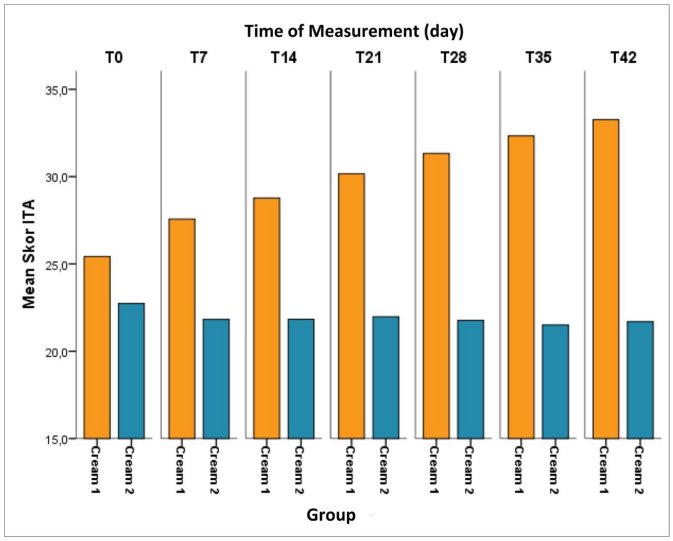

Figure 3: Comparison of ITA0 Score (Individual Typological Angle) Between Cream 1 and Cream 2.

the ITA^0 (Individual Topological Angle) score for cream 1 each week during the study period. At the beginning of the study (T0), the average ITA0 score of the sample was 25.42 (SD 8.97), and this score continued to increase until the end of the study period (T42) to 33.27 (SD 7.78). For cream 2, ITA^0 scores per week during the study period are likely to be constant. At the

Table 2 Comparison of L* score (Skin Brightness) between Cream 1 and Cream 2

Time of Measurement	Group	n	Mean	SD	p
T0*	Cream 1	34	58,88	3,11	0,212
	Cream 2	34	57,93	3,12	0,212
T7*	Cream 1	34	59,76	2,98	0,003
	Cream 2	34	57,60	2,70	0,003
T14*	Cream 1	34	60,32	2,88	0,001
	Cream 2	34	57,60	2,70	0,001
T21*	Cream 1	34	60,89	2,92	0,001
	Cream 2	34	57,68	2,68	0,001
T28*	Cream 1	34	61,45	2,95	0,001
	Cream 2	34	57,64	2,67	0,001
T35*	Cream 1	34	61,92	2,97	0,001
	Cream 2	34	57,59	2,60	0,001
T42*	Cream 1	34	62,40	2,98	0,001
	Cream 2	34	57,70	2,58	0,001
Abbreviations: n: Number of Samples; SD: Standard Deviation; p: Probability *Days					

Table 3 Comparison of L* score (Skin Brightness) between Cream 1 and Cream 2

Time of Measurement	Group	n	Mean	SD	p
T0*	Cream 1	34	9,82	1,33	0,121
	Cream 2	34	10,31	1,23	0,121
T7*	Cream 1	34	9,73	1,33	0,003
	Cream 2	34	10,71	1,27	0,003
T14*	Cream 1	34	9,62	1,30	0,001
	Cream 2	34	10,95	1,20	0,001
T21*	Cream 1	34	9,58	1,30	0,001
	Cream 2	34	10,94	1,17	0,001
T28*	Cream 1	34	9,45	1,38	0,001
	Cream 2	34	11,01	1,13	0,001
T35*	Cream 1	34	9,38	1,39	0,001
	Cream 2	34	11,12	1,07	0,001
T42*	Cream 1	34	9,29	1,43	0,001
	Cream 2	34	11,13	1,03	0,001
Abbreviations: n: Number of Samples; SD: Standard Deviation; p: Probability *Days					

Table 4 Comparison of ITAo score between Cream 1 and Cream 2

Time of Measurement	Group	n	Mean	SD	p
T0*	Cream 1	34	25,42	8,97	0,221
	Cream 2	34	22,73	8,98	0,221
T7*	Cream 1	34	27,57	8,26	0,005
	Cream 2	34	21,82	8,01	0,005
T14*	Cream 1	34	28,77	8,06	0,001
	Cream 2	34	21,83	8,01	0,001
T21*	Cream 1	34	30,17	7,96	0,001
	Cream 2	34	21,97	8,04	0,001
T28*	Cream 1	34	31,33	7,88	0,001
	Cream 2	34	21,77	7,90	0,001
T35*	Cream 1	34	32,33	7,91	0,001
	Cream 2	34	21,50	7,64	0,001
T42*	Cream 1	34	33,27	7,78	0,001
	Cream 2	34	21,69	7,54	0,001
Abbreviations: n: Number of Samples; SD: Standard Deviation; p: Probability *Days					

beginning of the study (T0), the average ITA0 score of the sample was 22.73 (SD 8.98), then decreased a week later to 21.82 (SD 8.01). Furthermore, this score tends to be the same until the end of the study period (T42) which is 21.69 (SD 7.54). Based on these values, at the time of measurement T0, ITA^0 score in cream 1 group did not differ significantly with cream 2 group ($p > 0.05$). At the time of measurement of T7-T42, it was found that the ITA^0 score in the cream 1 group was significantly higher than in the cream 2 group ($p < 0.001$) (Table 4).

Discussion

The results showed that combination cream a higher score regarding brightening the skin than hydroquinone cream, while the score of skin erythema increased significantly with the use of hydroquinone cream compared with combination cream for six weeks.

In the dermatologic practice and clinical research, visual clues such as colour are a significant factor for proper diagnosis and assessment of skin damage. Quantification of erythema and pigmentation are essential for in vivo assessment for skin reactions against external stimuli such as ultraviolet radiation and skin lightening effect. The colour measurement of skin damage is also useful for the quantitative evaluation of the therapeutic efficacy.[8]

However, visual inspection (eyes) can be influenced by subjective perceptions and interpretations that are difficult to measure. This, because there is such a variety of ways to express colours, it is become very difficult to describe the colour or its differences. Therefore, objective methods are required, based on science and do not damage skin tissue. Chroma Meter or spectrophotometer from Konica Minolta can be used to observe skin colour, as was done in this study.[8] L^* (skin brightness) scores are often used to evaluate the number of epidermal melanin, while the a^* (erythema) score is used to assess the amount of erythema (also referred to as hemoglobin) in the superficial plexus, whereas ITA0 (Individual Topological Angle) is a skin color classification system based on the formula (arctan (L^*-50/b^*) x 180/π.[9]

In this research, subjects were applied with cream 1 which is a combination of glutathione 2%, tocopherol acetate 1%, and magnesium ascorbic phosphate 3% on the right forearm and application of cream 2 containing hydroquinone 4% on the right forearm below cream 1 on the same sample. The results were then observed using Chroma Meter, then compared against each other.

In this study, the L^* (skin brightness) score was higher in the cream-1-applied area compared to the cream-2-applied area. This indicates that the glutathione 2%, tocopherol acetate 1%, and magnesium ascorbic phosphate 3% combination had a higher score on the brightness of the skin than hydroquinone 4%.

Glutathione, vitamin C, and vitamin E can increase skin brightness through the antioxidant effects of free radicals and the inhibitory effects of melanin synthesis by reaction with tyrosinase and L-DOPA. Glutathione in reduced form (GSH) is unstable when in the water-based form such as solution and cream, therefore it is easy to decompose. To overcome this issue, additional substances that can maintain the stability of GSH are required.

In the colourimetric tristimulus system, the L^*score shows the brightness gradient of the skin between bright white (high value) and black (low value), with a range between 0-100. The a^* score is the colour spectrum gradient between green (value-) and

red (value +). From Chroma Meter results, L* (skin brightness) is the dominant factor in determining the brightness of skin tone. At the cream-1-applied area, at each measurement week (T7-T42) there was a significant increase in L* (skin brightness) score, while the a* (erythema) score did not show much difference. This indicates that the substances used in cream 1 such as glutathione, tocopheryl acetate, and magnesium ascorbyl phosphate have been shown to increase the L* (skin brightness) score which is a differentiating factor in skin brightness, whereas the a* (erythema) score is not significantly affected

In this study, the L* (skin brightness) score was lower in the cream-2-applied area compared to cream 1. This indicates that hydroquinone 4% has a lower value on skin brightness than the glutathione 2%, tocopherol acetate 1%, and magnesium ascorbic phosphate 3% combination.

Hydroquinone effect is evident after 5-7 weeks of usage and should be continued for at least three months to 1 year.[10] In this study, it was applied only for six weeks so that its effect on increasing skin brightness shown as L* (skin brightness) was not obtained significantly.

Hydroquinone can cause skin irritation due to its effect on the skin; especially hydroquinone concentrates 4% or more. Common side effects after skin exposure to hydroquinone are irritation, red skin (erythema), and burning sensation. This effect occurs immediately after the use of high concentration hydroquinone above 4%. Hydroquinone also more often cause erythema than other lightening creams. It is proved in this study, a* (erythema) scores on Chroma Meter of cream 2 higher than cream 1 after six weeks of use.[11]

Individual Typological Angle (ITA0) is a skin colour classification system which is divided into six groups, from very bright, bright, intermediate, tan, to dark. ITA0 calculations are considered an objective parameter to assess skin colour and have been used in various studies.[9] In this study, it was found that the ITA^0 score in the cream 1 group was significantly higher than in the cream 2 group, wherein cream 1 there was an increase of skin type from tan (group-4) to intermediate (group-3).

During the study, some of the cream 1 samples had a yellowish discolouration. This possibility is caused by the oxidation process in the ingredients contained in the cream, as it is known that glutathione is very unstable and easily decompose, resulting in discolouration. However, the combination of vitamin C and E derivatives can help maintain glutathione stability.

Conclusion

The researchers concluded that the value of skin brightness significantly increased with the use of glutathione 2%, tocopheryl acetate 1%, and magnesium ascorbyl phosphate 3% combination cream for six weeks. Skin brightness values tend to be constant after using hydroquinone 4% cream for six weeks. Glutathione 2%, tocopheryl acetate 1%, and magnesium ascorbyl phosphate 3% combination cream showed higher values regarding skin brightening compared to hydroquinone 4% creams. The value of skin erythema increased significantly with the use of hydroquinone 4% cream compared with combination cream after six weeks of use. The limitation of this research is the packaging of cream using a pot with a white lid that may cause the oxidation of the cream material. The author recommends for subsequent studies to be performed with a larger sample and longer time to get more representative results of the population. It is hoped that subsequent research not only assessing skin brightness and the side effects of erythema quantitatively (using Chroma Meter) but also qualitatively using other tools such as Spectrophotometer or Mexameter.

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Authors' Statements

Competing Interests

The authors declare no conflict of interest.

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