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Test the Effectiveness of Carrot Tuber Ethanol Extract Cream Preparation Formulation in Preventing Increased Melanin in Male Wistar Rats Exposed to UVB Light

Wulan Dhari[1], Ali Napiah Nasution[2], Djohan[3], Widyaningsih Oentari[4], Widya Pasca Amir[5], Anita Merry Cisca[6], Ermi Girsang[7*]

Universitas Prima Indonesia Medan

Corresponding Author: Ermi Girsang ermigirsang@unprimdn.ac.id

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ABSTRACT

This study was conducted with the aim of proving carrot tuber extract (Daucus carota L.) formulated in cream form can prevent an increase in the amount of melanin in the skin tissue of male wistar rats (Rattus norvegicus) exposed to UVB light and see histopathological changes due to carrot tuber extract given as a prevention of increasing the amount of melanin. This study is experimental with a post-test only control group research design. The test method used was testing the amount of melanin modified using a 13 watt Exoterra UVB 200 lamp. The animals tested were rats (Rattus norvegicus) male wistar strain aged 2-3 months, weighing ± 200 grams. Male rats were randomly divided into 6 groups. Each group consists of 5 male rats. The test area on the back of the back is 2x2 cm2. Group I male rats without cream administration as a negative control group, Group II male rats for Parasol Face Sunscreen Cream as a positive group. Group III is the group with the provision of basic ingredient cream and group IV-VI is the group with the administration of 25% carrot tuber extract cream; 50 %; 75 %. topically for 4 weeks.. The evaluation was performed by observing the amount of melanin at week 4 of histopathology analyzing in the test area. The results will be processed with IBM SPSS 21.0 and analyzed with the steps of Descriptive Analysis, Normality Analysis with data using Shapiro-Wilk test and Homogeneity using Levene's test, Comparative Analysis using one way Anova test, followed by Post-hoc test with LSD test. If the data is abnormal, use non-parametric analysis of the Kruskal-Wallis test followed by the Mann-Whitney test. The results showed that extra ethanol of carrot tubers can prevent the increase in the amount of melanin. This was shown by its ability to reduce melanin and histopathological changes in a group of male rats applied carrot tuber ethanol extract cream within 4 weeks

INTRODUCTION

In the process of life, growing old is a natural thing. Maturing (Aging) could be a physiogical alter that happens with expanding chronological age and will occur in all components. In maturing there's a progreddive dysfunction of all organs that happens in people, plants, creatures, conjointly single-celled life forms. Maturing starts when a modern human is born. Physiological marvels that happen are a diminish within the number of tissue cells, a diminish in metabolic rate, as well as an increment within the frequency of malady (pankahila, 2007). All organs of the body undergo the aging process, the skin comprises of three layers to be spesific the epidermis, dermis, and subcutis tissue. The epidermis, the furthest layer of skin, is composed of four sorts of cells; keratinocytes, which are the foremost inexhaustible cells that deliver keratin; melanocyte cells, phagocytic cell play a part in taking and handing antigents: and Merkel cells, neuroendocrine cells whose work is obscure (SNDER, 2003). The skin is the foremost uncovered to environmental influences. Environmental factors that play a role in the aging process of the skin are ultraviolet light radiation. Continuous exposure to ultraviolet light causes changes in skin structure and function, ranging from acute side effects such as sunburn, tanning and hyperpigmentation, to chronic side effects such as photoaging and skin cancer (Bernerd et al., 2012; Pandel et al., 2013). Pathologically, hyperpigmentation can be caused by an increase in the amount of melanin in the epidermis such as lentigo, an increase in the amount of melanin in the epidermis and the upper dermis spread in melasma. Melanin is a natural pigment that produces color on the skin. Melanin is produced in melanosomes produced by melanocytes. The formation of melanin can be stimulated by intrinsic factors such as endocrine (Hormonal), immune system, inflammatory, and central nervous system, as well as extrinsic factors such as UV radiation, drugs, pollution, and cigarette smoke (Ichihashi et al., 2009). The process of melanin synthesis (Melanogenesis) starts from the hydroxylation of tyrosine to 3,4 dihydroxyphenylalanine (DOPA) and the oxidation of DOPA to dopakuinone by the enzyme tyrosinase. Dopakuinone will polymerize spontaneously to form melanin. Tyrosinase enzyme will work directly when stimulated by UV light (Baumann and Saghari, 2009).

UV light is needed in the process of melanin formation, but excessive UV exposure causes the formation of reactive oxygen species (ROS) or free radicals that increase protein oxidation and accumulation of lipid peroxidation. In human skin, hydrogen peroxide levels more than double which catalyzes the reduction of oxygen molecules to superoxyd anions. Hydrogen peroxide can quickly form other ROS, such as hydroxyl radicals, resulting in a chain reaction. In the end, ROS will cause oxidative stress on the skin which can stimulate excessive melanin formation (Baumann and Saghari, 2009). Alatas (2004) explained that UV light is often referred to as the sunburn spectrum which is able to damage cell membranes. This results in sunburn and redness, damaging the skin cells which further results in damage to the regeneration mechanism of the skin cells. UV A rays can also cause burning effects on the skin but are weaker when compared to the effects of UV B exposure. Loss of skin elasticity, dilation of blood vessels, and thickening of the skin (keratosis) are biological effects that can be caused by exposure to UV radiation. While the long-term effects are skin cancer, melanoma and premature aging. There are 3 kinds of UV light, namely UVA, UVB and UVC. Ultraviolet (UV) A has a wavelength of 320 – 400 nm can cause tanning effects caused by excessive melanin production in the epidermis. Ultraviolet (UV) B has a wavelength of 290 – 320 nm which can cause sunburn. The ozone layer can hold about 90% of UVB, but the depletion of the ozone layer by chlorofluorocarbons (CFCs) can cause UVB to penetrate the ozone layer to the earth. Ultraviolet (UV) C has a wavelength of 220 – 290 nm which has been filtered by the ozone layer in the atmosphere (Mishra, Mishra and Chattopadhyay, 2011; Ministry of Health, 1985; Zeman, 2007). UVA and UVB rays in conditions that are not excessive are very useful for the body for the formation of vitamin D and can help activate vitamins, hormones and

enzymes (Ministry of Health RI, 1985; Jellinek and Stephan, 1970). Excessive UVB rays can cause wrinkles, dullness, melasma, skin cancer, cataracts, and immune system suppression (Ministry of Health, 1985; 2 Zeman, 2007). Protection against excessive UVB rays is very necessary for the prevention of negative effects caused.

Antioxidants are substances that work by hindering the oxidation of oxidant atoms. To anticipate the event of ROS, antioxidants work to ensure cells with different instrumens. Based on the defense instrument (Tandon, 2005). Antioxidants are recognized by their dissolvability, such as fat-soluble antioxidants (vitamin A, vitamin E, adnd CoQ10) and air-soluble antioxidants (vitamin C and glutathione). The antioxidant α lipoic corrosive (ALA) breaks down fat and discuss (Bauman, 2002). Antioksidant can moreover be separated into natural antioxidants and syntetic antioxidant. Natural antioxidants such as flavonoids, coumarins, phenilic acids, linoleic acids, omega-3, vitamin E, vitamin C, β carotene, and others.

Carrots are one of the natural ingredients that can be developed in the drug industry. Besides being rich in nutritional content, especially vitamin A, carrots are also efficacious for healing various diseases (Rukmana, 1995). Some information about the efficacy of carrot plants including anti-cancer, inflammation, diseases in digestion, preventing heart attacks and narrowing of blood vessels and much more (Cahyono, 2002). Several studies have also scientifically proven the efficacy of carrots, including as hepatoprotective (Widari, 2004), analgesic (Putra, 2003), anthelmintic (Rahayu and Sundari, 2007) and anti-inflammatory (Widarsih, 2003).

In this study, a thick extract from carrot tubers will be used. Carrot tubers (Daucus carota) contain several active compounds, namely flavonoids, saponins, and tannins (Rahayu and Sundari, 2007). Carrot tubers are extracted using the maceration method with ethanol solvent. Extracts from plants or vegetables are chosen because extracts from natural ingredients have smaller side effects compared to chemicals. Carrot tuber extract is formulated in the form of cream preparations. The dosage form chosen is cream because it spreads evenly on the skin, is not sticky, and comfortable to use (Anwar, 2012; Ministry of Health, 1995; Voight, 1994; Schmitt, 1996; Harry and Ralph, 1982)

Based on the above facts, the ethanol extract of carrot tubers formulated in the form of cream preparations is interesting to be scientifically proven its efficacy as a preventer of increasing the amount of melanin in the skin. Inthis study, the effectiveness of carrot tuber ethanol extract cream preparation formulations will be tested in preventing an increase in the amount of melanin in male wistar rats (Rattus norvegicus) exposed to UVB light.

METHODS

1. Type and Design of Research

The type of research conducted was experimental, using a post-test only control group research design (Marczyk, et al., 2005). This study included the determination of carrot tubers (Daucus carota L.) , extraction, and preparation of creams using carrot tuber extract with concentrations of 25%, 50%, and 75%. Experimental research includes testing the effectiveness of carrot tuber extract cream preparation formulations in preventing an increase in the amount of melanin. The research design can be described as follows:

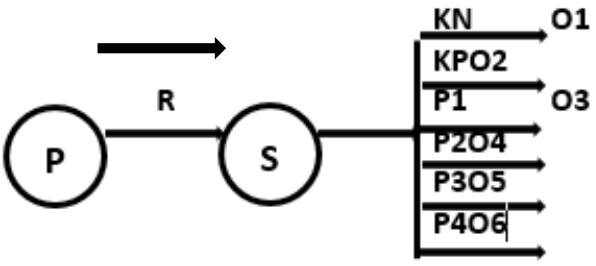

Figure 1. Type and Design of Research

Information:

P : Population

R : Random

S : Sample

KN: Treatment without giving base ingredient cream or tuber extract cream carrots and exposure to UVB rays

KP : Treatment with Parasol Face Sunscreen Cream and exposure to UVB rays

P1: Treatment with basic ingredients cream and exposure to UVB rays

P2: Treatment with cream of carrot tuber extract (Daucus carota L.) 25% and UVB exposure

P3: Treatment with 50% carrot tuber extract cream (Daucus carota L) and exposure to UVB light

P4: Treatment with cream of carrot tuber extract (Daucus carota L) 75% and exposure to UVB light

O1: Observation of the amount of melanin pigment group 1, after KN treatment

O2: Observation of the amount of melanin pigment group 2, after KP treatment

O3: Observation of the amount of melanin pigment group 3, after P1 treatment

O4: Observation of the amount of melanin pigment group 4, after P2 treatment

O5: Observation of the amount of melanin pigment group 5, after P3 treatment

O6: Observation of the amount of melanin pigment group 6, after P4 treatment

2. Time and Place of Research

The time of this study was conducted from April 2021. The manufacture of carrot tuber extract is carried out at the Pharmaceutical Pharmacology and Toxicology Laboratory, Faculty of Pharmacy, University of North Sumatra. The manufacture of the cream is carried out at the Pharmacology and Toxicology Laboratory, Faculty of Pharmacy, University of North Sumatra. Treatment of experimental animals was carried out at the Pharmacology and Toxicology Lab, Faculty of Pharmacy, University of North Sumatra. Histopathological examination of skin tissue is carried out at the Anatomical Pathology Laboratory of the Faculty of Medicine, University of North Sumatra. The histopathological examination time was carried out in April 2021.

3. Population and Research Sample

1. Population

The population in this study was rats (Rattus norvegicus) male wistar strain that did not suffer from illness and wanted to eat and drink. These rats were obtained from the Pharmacist White Rat Laboratory, Jalan Ngumban Surbakti, Simpang Akper Elisabeth, Medan. The inclusion and exclusion criteria of this research sample are as follows.

Inclusion Criteria

- Rat (Rattus norvegicus) male wistar strain
- Weight ± 200 grams, age 2-3 months
- Healthy, willing to eat and drink

Exclusion Criteria

- Wistar rats died at the time of the study

In addition to inclusion and exclusion criteria, it is also necessary to calculate the number of samples needed. Using the formula from Federer (Federer, 2008), the sample size can be calculated as follows:

$$(n-1)(t-1) \geq 15$$

$$(n-1)(6-1) \geq 15$$

$$(n-1)(5) \geq 15$$

$$5n-5 \geq 15$$

$$n \geq 4$$

Figure 2. Exclusion Criteria

Information:

t: Number of test groups

n: Sample size per group

The ideal sample size according to Federer's formula above is 4 or more wistar rats. Because the samples are divided into 6 groups, the minimum number of samples required is 24 individuals. Then the sample is added by 10% so that each group is added 1 head. Thus, the total research sample required is 30 heads to avoid dropping out during the study.

2. Samples

The sample used in the study was carrot tubers obtained from the traditional market center of Medan city.

RESULTS AND DISCUSSION

Results of Making Extracts

The results of making extracts can be seen in table 1 below:

Table 1. Results of Making Extracts

Simplisia Powder Weight	Extract Weight (Grams)	Yield (%)
500 grams	68.9 grams	3,78%

With a simplisia weight of 500 grams, an extract weight of 68.9 g was obtained with a yield value of 3.78%. Yield is a comparison of the weight of the extract produced with the weight of simplisia as raw material. The higher the yield value indicates that the larger the resulting extract. Yield is a comparison of the dry weight of the resulting product with the weight of raw materials (Yuniarifin, et al, 2006). Nurhayati et al, (2009) stated that a high yield value indicates the number of bioactive components contained in it. According to Dewastisari (2018), the yield value is related to the amount of bioactive content contained in plants. Budiyanto (2015) stated that the higher the yield of extract, the higher the content of substances attracted to a raw material.

The results of this study prove that the length of extraction time affects the yield. Mardina (2011) states that the longer the extraction time, the higher the yield obtained, because the opportunity to react between the material and the solvent is longer so that the process of solvent penetration into the material cell is better which causes more compounds to diffuse out of the cell.

Compared to other extraction methods, in terms of time the infundation method is relatively shorter than other methods, but in terms of temperature this method uses the addition of heat with a temperature of >90oC so as to accelerate the extraction process. The use of a short time aims to prevent damage to compounds in samples due to prolonged heating (Wijaya et al, 2018).

Phytochemical Screening Results

The results of phytochemical screening can be seen in table 2 below:

Table 2. Phytochemical Screening Results

Compound Classes	Result
Flavonoids	+
Saponins	+
Tannins	+
Alkaloids	+
Steroids/Tripenoids	+
Glycosides	+

Results showed that carrot tuber ethanol extract contained flavonoids, alkaloids, glycosides, tannins, saponins, steroids/triterpenoids.

Research on qualitative phytochemistry of carrot tubers has been conducted by Dotulong et al, (2018) on young leaves using methanol and ethanol solvents with maceration and soxhlet extraction methods, and by Wonggo et al, (2017) on carrot tubers using methanol, ethyl acetate, water, and hexane solvents. The results of research by Dotulong et al, (2018) showed that carrot tuber extract contains flavonoids, tannins, saponins, steroids, and alkaloids. The results of research by Wonggo et al (2017) showed that carrot tuber extract only contains phenolics, flavonoids, and tannins.

The research data above with this study both use samples from mangrove species S. alba taken at the same location, based on these similarities supported by existing research data, old leaf extract of this study has the potential to qualitatively contain bioactive compounds.

Results of Evaluation of Carrot Tuber Ethanol Extract Cream Preparations

Homogeneity Check Results

Table 3. Homogeneity Check Results

Formula	Examination Results
Blanks	Homogeneous
F1	Homogeneous
F2	Homogeneous
F3	Homogeneous

Description:
F1= Carrot Tuber Ethanol Cream Preparation 25%
F2= Carrot Tuber Ethanol Cream Preparation 50%
F3= Carrot Tuber Ethanol Cream Preparation 75%

The results showed that cream preparations of ethanol extract of carrot tubers with concentrations of 25%, 50%, and 75% showed homogeneous results. Homogeneity is needed to ensure that the extracts in the preparation are evenly distributed and homogeneous.

pH Examination Results

Table 4. pH Test Results

Formula	pH + SD
Blanks	5.60 ± 0.10
F1	5.43 ± 0.11
F2	5.90 ± 0.10
F3	5.83 ± 0.06

Description:
F1= Cream Preparation of Carrot Tuber Ethanol Extract 25%
F2= Cream Preparation of Carrot Tuber Ethanol Extract 50%
F3= Cream Preparation of Carrot Tuber Ethanol Extract 75%

The examination results showed that the pH of the preparation was stable and normal, namely at blank 5.6, concentration 25% 5.43, concentration 50% 5.90 and concentration 75% 5.83. This indicates that the preparation is stable.

Emulsion Type Examination Results

Table 5. Emulsion Type Results

Formula	Emulsion Type
Blanks	Oil/Water
F1	Oil/Water
F2	Oil/Water
F3	Oil/Water

Description:
F1= Cream Preparation of Carrot Tuber Ethanol Extract 25%
F2= Cream Preparation of Carrot Tuber Ethanol Extract 50%
F3= Cream Preparation of Carrot Tuber Ethanol Extract 75%

The results showed that cream preparations of ethanol extract of wortek tubers with concentrations of 25%, 50%, and 75% showed the type of oil-in-water emulsion. An emulsion is a thermodynamically unstable system containing at least two immiscible liquid phases, one of which is dispersed as globules in the other liquid phase. The instability of these two phases can be controlled using an emulsifier or emulgator. There are several types of emulsions, ranging from simple to complex (Pawlik et al., 2013). An oil-in-water (M/A) or oil-in-water (O/W) emulsion system is an emulsion system with oil as the dispersed phase and water as the dispersing phase. The emulsion can be found in several foods, namely mayonnaise, milk, cream and bread dough. In contrast to M/A, a water-in-oil (A/M) emulsion is an emulsion with water as the dispersed phase and oil as the dispersing phase. This type of emulsion can be found in margarine and butter products (Winarno, 1997). Duplex emulsion is a type of emulsion that is more complex than W/O and O/W emulsions. Double emulsion is an emulsion composed of complex microstructures where dispersed droplets contain droplets with smaller sizes inside. This emulsification method is used in the pharmaceutical, cosmetic, food and chemical separation industries. This type of emulsion consists of a double emulsion and a multiple emulsion (Acerin, 2008).

Antiaging Test Results

Skin Analyzer Examination Results

1. Melanin Test Results

Table 6. Melanin Test Results

Group	Melanin (Average ± SD)				
	Beginning	Week I	Week II	Week III	Week IV
Usual	14.20 ± 2.59	15.40 ± 2.07[c]	15.80 ± 1.92[b.c]	17.00 ± 1.58[b.c]	15.40 ± 2.97[b.c]
Blanks	16.60 ± 2.07	33.40 ± 2.30[a,b]	48.20 ± 3.27[a,b]	64.40 ± 3.21[a,b]	76.40 ± 5.18[a,b]
Krim Parasol Face Suncream	17.00 ± 1.58	19.60 ± 2.41[C]	22.40 ± 2.70[a.c]	25.20 ± 2.59[a.c]	28.20 ± 2.59[a.c]
EEUW Cream 25%	15.00 ± 2.74	23.40 ± 3.72[a.c]	30.20 ± 1.79[a,b,c]	35.80 ± 2.59[a,b,c]	43.00 ± 2.24[a,b,c]
EEUW Cream 50%	16.00 ± 2.55	22.60 ± 3.21[a.c]	27.60 ± 2.88[a,b,c]	30.20 ± 1.92[a,b,c]	32.80 ± 1.92[a.c]
EEUW Cream 75%	15.60 ± 2.41	19.80 ± 2.59[C]	22.80 ± 2.59[a.c]	25.40 ± 2.70[a.c]	27.60 ± 2.30[a.c]

Remarks : EEUW = Carrot Tuber Ethanol Extract
a = There are significant differences with the normal group
b = There is a significant difference with the blank group
c = There are significant differences with the Parasol Cream group

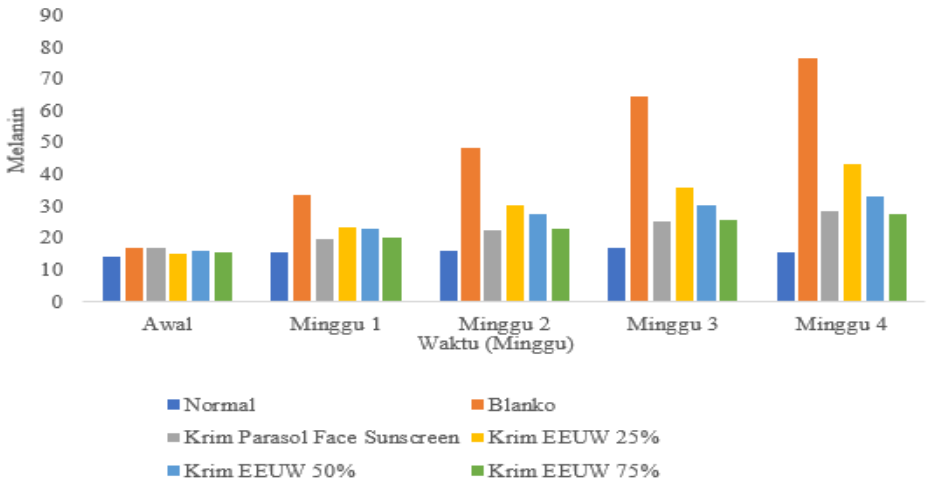

Figure 3. Face Suncream

Melanin examination was performed on mice at the beginning, week I, week II, week III, and week IV. The results showed that in blanks there was no change in melanin, while in the group given Parasol Face Suncream Cream there was a decrease in melanin, followed by the administration of EEUW

Cream preparations 25% decreased but when compared to the group given EEUW Cream 75% decrease looked better.

Table 7. Results of Percent Increase in Melanin

Group	Percent increase in melanin (%) (average ± SD)			
	Week I	Week II	Week III	Week IV
Usual	15.70 ± 13.10[b]	14.68 ± 5.58[b]	21.70 ± 15.67[b]	14.90 ± 22.86[b]
Blanks	103.74 ± 28.53[a,c]	193.93 ± 39.74[a,c]	291.20 ± 33.68[a,c]	364.87 ± 52.58[a,c]
Krim Parasol Face Suncream	15.06 ± 3.84[b]	31.50 ± 3.86[b]	48.16 ± 3.19[b]	66.00 ± 6.38[b]
EEUW Cream 25%	56.87 ± 10.72[a,b,c]	105.85 ± 31.94[a,b,c]	143.65 ± 35.39[a,b,c]	194.11 ± 51.63[a,b,c]
EEUW Cream 50%	41.87 ± 11.27[b]	74.39 ± 19.20[a,b]	91.36 ± 21.70[a,b]	107.92 ± 23.88[a,b]
EEUW Cream 75%	27.40 ± 7.12[b]	47.26 ± 14.13[b]	64.57 ± 21.22[b]	79.58 ± 26.97[b]

Remarks : EEUW = Carrot Tuber Ethanol Extract
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b = There is a significant difference with the blank group
c = There are significant differences with the Parasol Cream group
Face Suncream

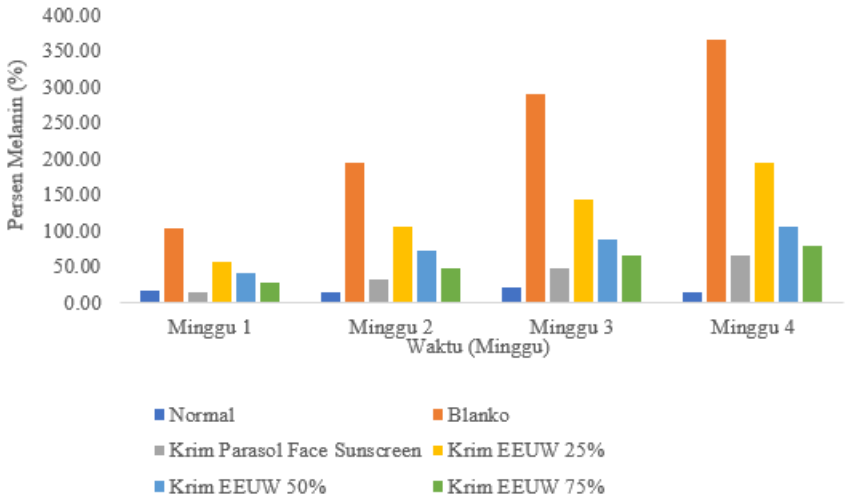

Figure 4. Face Suncream

Wrinkle examination was performed on mice at the beginning, week I, week II, week III, and week IV. The results showed that in blanks there was no change in melanin, while in the group given Parasol Face Suncream Cream there was a decrease in melanin, followed by the administration of EEUW Cream preparations 25% decreased but when compared to the group given EEUW Cream 75% decrease looked better.

2. Wrinkle Examination Results

Table 8. Wrinkle Examination Results

Group	Wrinkles (Average ± SD)				
	Beginning	Week I	Week II	Week III	Week IV
Usual	5.00 ± 0.00	5.00 ± 0.00[b]	5.00 ± 0.00[b]	5.00 ± 0.00[b]	5.00 ± 0.00[b]
Blanks	5.00 ± 2.24	12.80 ± 3.35[a.c]	22.00 ± 5.70[a.c]	30.60 ± 6.35[a.c]	46.20 ± 4.92[a.c]
Krim Parasol Face Suncream	5.00 ± 1.58	6.80 ± 2.59[b]	8.20 ± 1.39[b]	9.80 ± 4.44[b]	11.80 ± 4.44[b]
EEUW Cream 25%	6.00 ± 1.58	13.40 ± 2.70[a.c]	20.80 ± 2.59[a.c]	25.40 ± 3.21[a.c]	30.80 ± 3.11[a,b,c]
EEUW Cream 50%	5.20 ± 0.84	8.40 ± 2.30	12.40 ± 3.21[a.b]	16.60 ± 3.36[a,b]	20.40 ± 3.65[a,b,c]
EEUW Cream 75%	6.00 ± 1.43	8.40 ± 2.07	10.20 ± 2.39[b]	13.60 ± 3.43[a,b]	15.20 ± 3.56[a,b]

Remarks : EEUW = Carrot Tuber Ethanol Extract
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b = There is a significant difference with the blank group
c = There are significant differences with the Parasol Cream group
Face Suncream

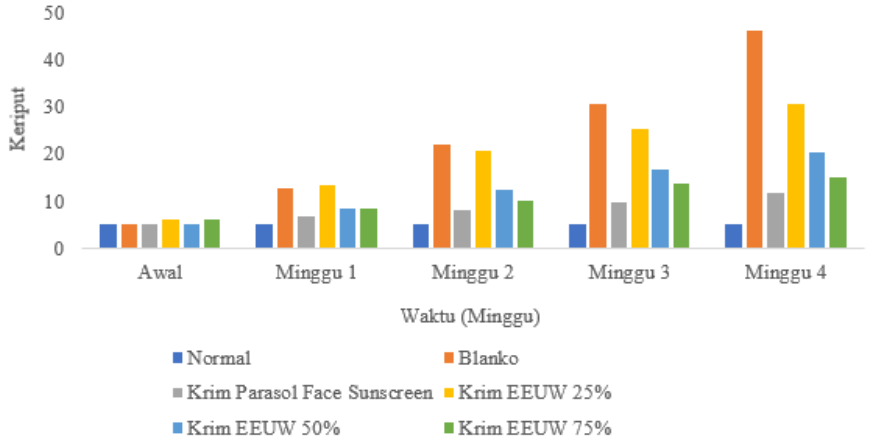

Figure 5. Wrinkle Examination Results

Table 9. Results of Percent Increase in Wrinkles

Group	Percent increase in wrinkles (%) (average ± SD)			
	Week I	Week II	Week III	Week IV
Usual	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]
Blanks	189.17 ± 106.62[a.c]	418.50 ± 258.29[a.c]	634.83 ± 402.55[a.c]	1003.00 ± 542.14[a.c]
Krim Parasol Face Suncream	34.24 ± 12.37[b]	63.29 ± 24.95[b]	89.86 ± 32.16[b]	133.57 ± 24.41[b]
EEUW Cream 25%	129.83 ± 52.46[a.c]	262.67 ± 86.78[a.c]	342.91 ± 105.91[a.c]	437.55 ± 123.38[b]
EEUW Cream 50%	60.67 ± 28.32[b]	137.67 ± 41.46[b]	219.67 ± 38.23[b]	293.33 ± 39.16[b]
EEUW Cream 75%	40.74 ± 6.25[b]	71.48 ±13.89[b]	127.21 ± 14.09[b]	155.62 ± 21.96[b]

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Face Suncream

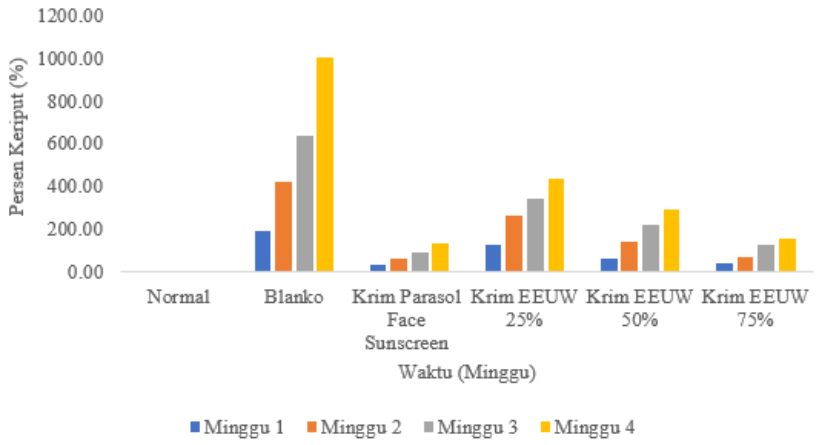

Figure 6. Wrinkle Examination Results

3. Pore Examination Results

Table 10. Pore Examination Results

Group	Pore (Average ± SD)				
	Beginning	Week I	Week II	Week III	Week IV
Usual	15.40 ± 2.41	16.40 ± 2.41[b]	16.40 ± 2.41[b]	17.40 ± 2.41[b]	18.00 ± 2.92[b]
Blanks	14.20 ± 2.59	25.40 ± 3.65[a.c]	35.40 ± 2.70[a.c]	42.40 ± 5.98[a.c]	50.60 ± 5.46[a.c]
Krim Parasol Face Suncream	14.00 ± 1.58	16.60 ± 2.07[b]	19.00 ± 2.24[b]	21.00 ± 3.39[b]	24.60 ± 4.22[b]
EEUW Cream 25%	16.00 ± 1.58	24.40 ± 2.07[a.c]	29.80 ± 3.27[a,b,c]	34.60 ± 3.65[a,b,c]	39.80 ± 4.21[a,b,c]
EEUW Cream 50%	16.20 ± 0.84	23.00 ± 2.24[a.c]	26.60 ± 2.70[a,b,c]	30.00 ± 2.24[a,b,c]	35.00 ± 1.58[a,b,c]
EEUW Cream 75%	14.40 ± 2.41	18.80 ± 2.86[b]	21.00 ± 2.55[b]	23.00 ± 2.55[b]	26.80 ± 2.86[a,b]

Remarks : EEUW = Carrot Tuber Ethanol Extract
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Face Suncream

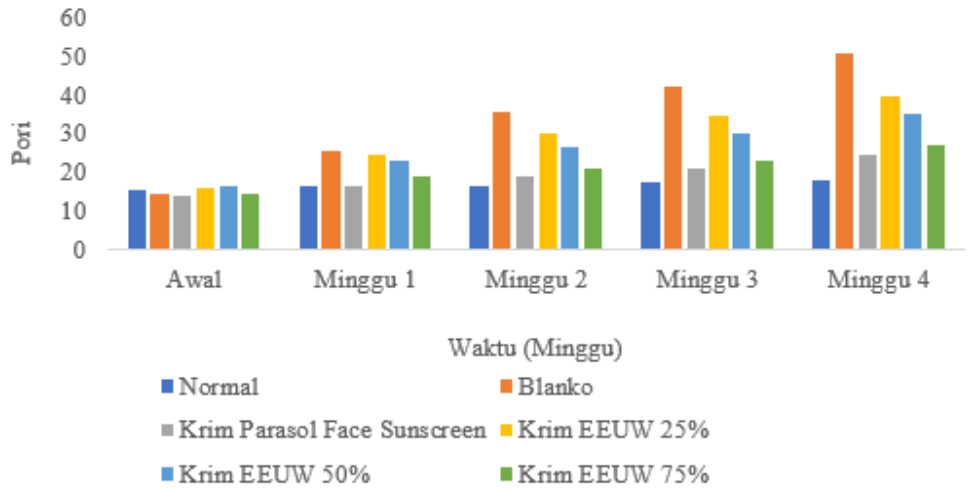


Figure 7. Pore Examination Results

Melanin examination was performed on mice at the beginning, week I, week II, week III, and week IV. The results showed that in blanks there was no change in melanin, while in the group given Parasol Face Suncream Cream there was a decrease in melanin, followed by the administration of EEUW Cream preparations 25% decreased but when compared to the group given EEUW Cream 75% decrease looked better.

Table 11. Percent Pore Increase Results

Group	Percent increase in wrinkles (%) (average ± SD)			
	Week I	Week II	Week III	Week IV
Usual	6.84 ± 7.71[b]	6.84 ± 7.71[b]	13.54 ± 9.06[b]	17.01 ± 7.82[b]
Blanks	80.69 ± 20.68[a,c]	154.24 ± 36.60[a,c]	204.00 ± 55.75[a,c]	265.94 ± 75.90[a,c]
Krim Parasol Face Suncream	18.45 ± 2.45[b]	35.67 ± 2.35[b]	49.40 ± 7.90[b]	74.98 ± 13.08[b]
EEUW Cream 25%	52.77 ± 6.33[a,b,c]	86.32 ± 11.14[a,b,c]	116.39 ± 12.60[a,b,c]	148.99 ± 16.87[a,b,c]
EEUW Cream 50%	42.03 ± 12.82[a,b,c]	64.43 ± 17.44[a,b]	85.34 ± 13.20[a,b]	116.28 ± 9.75[a,b]
EEUW Cream 75%	30.89 ± 3.54[a,b]	46.94 ± 9.63[a,b]	61.18 ± 12.04[b]	87.82 ± 13.28[a,b]

Remarks : EEUW = Carrot Tuber Ethanol Extract
a = There are significant differences with the normal group
b = There is a significant difference with the blank group
c = There are significant differences with the Parasol Cream group
Face Suncream

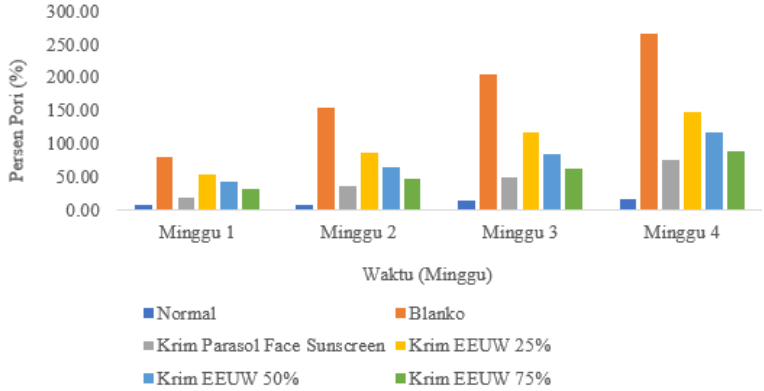


Figure 8. Pore Examination Results

4. Fineness Check Results

Table 12. Fineness Check Results

Group	Fineness (Average ± SD)				
	Beginning	Week I	Week II	Week III	Week IV
Usual	26.60 ± 5.68	26.20 ± 5.36[b]	25.40 ± 4.16[b]	24.00 ± 3.54[b]	23.00 ± 4.00[b]
Blanks	24.00 ± 3.16	37.80 ± 3.90[a.c]	54.20 ± 3.70[a.c]	70.80 ± 3.11[a.c]	84.80 ± 4.49[a.c]
Krim Parasol Face Suncream	24.60 ± 2.70	28.80 ± 3.42[b]	32.60 ± 3.78[b]	36.80 ± 3.19[b]	41.60 ± 2.07[b]
EEUW Cream 25%	26.00 ± 1.58	35.80 ± 3.56a	44.40 ± 4.67[a,b,c]	49.80 ± 3.27[a,b,c]	54.60 ± 3.36[a,b,c]
EEUW Cream 50%	25.60 ± 2.97	33.00 ± 4.47	38.20 ± 3.11[a,b]	43.60 ± 3.05[a,b,c]	49.00 ± 3.24[a,b,c]
EEUW Cream 75%	24.20 ± 1.92	29.80 ± 2.39[b]	33.40 ± 2.41[a,b]	38.00 ± 1.58[a,b]	43.80 ± 2.59[a,b]

Remarks : EEUW = Carrot Tuber Ethanol Extract
a = There are significant differences with the normal group
b = There is a significant difference with the blank group
c = There are significant differences with the Parasol Cream group
Face Suncream

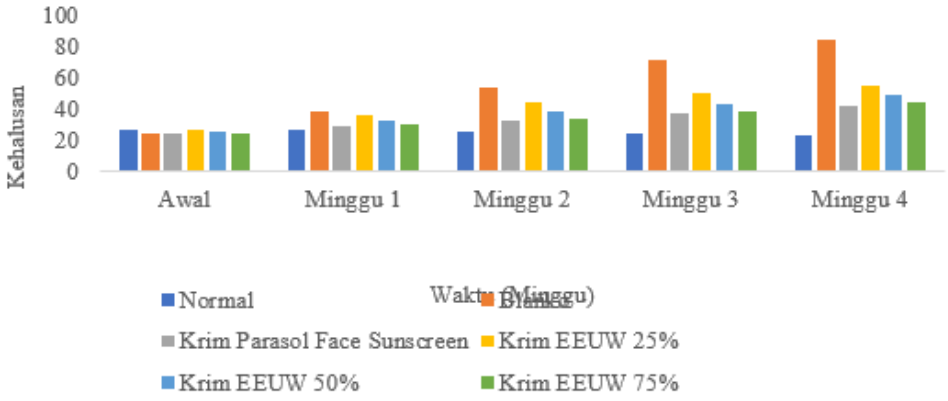


Figure 9. Fineness Check Results

Fineness checks were performed on mice at base, week I, week II, week III, and week IV. The results showed that in blanks there was no change in melanin, while in the group given Parasol Face Suncream Cream there was a decrease in melanin, followed by the administration of EEUW Cream preparations 25% decreased but when compared to the group given EEUW Cream 75% decrease looked better.

Table 13. Percent Results of Fineness Decrease

Group	Percent decrease in fineness (%) (average ± SD)			
	Week I	Week II	Week III	Week IV
Usual	1.34 ± 1.87[b,c]	3.75 ± 4.67[b]	8.78 ± 6.42[b]	12.89 ± 6.10[b,c]
Blanks	58.36 ± 13.15[a,c]	129.15 ± 35.65[a,c]	200.07 ± 49.05[a,c]	257.87 ± 46.88[a,c]
Krim Parasol Face Suncream	17.05 ± 5.01[a,b]	32.62 ± 8.01[b]	50.01 ± 8.24[b]	70.01 ± 11.07[a,b]
EEUW Cream 25%	37.49 ± 7.70[a,b,c]	70.57 ± 12.23[a,b,c]	91.73 ± 10.86[a,b]	110.51 ± 16.32[a,b]
EEUW Cream 50%	28.80 ± 6.78[a,b]	49.96 ± 10.61[a,b]	71.23 ± 11.31[a,b]	92.49 ± 12.77[a,b]
EEUW Cream 75%	23.33 ± 7.92[a,b]	38.33 ± 9.73[a,b]	57.57 ± 10.26[a,b]	81.42 ± 10.17[a,b]

Remarks : EEUW = Carrot Tuber Ethanol Extract
a = There are significant differences with the normal group
b = There is a significant difference with the blank group
c = There are significant differences with the Parasol Cream group
Face Suncream

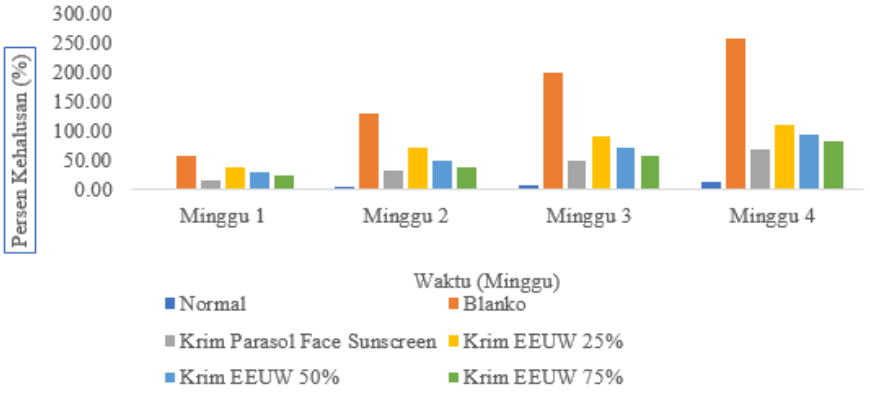
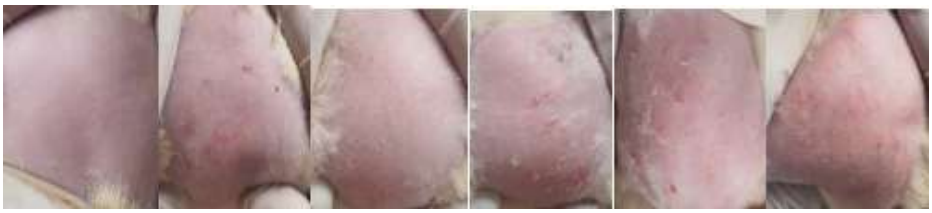


Figure 10. Fineness Check Results

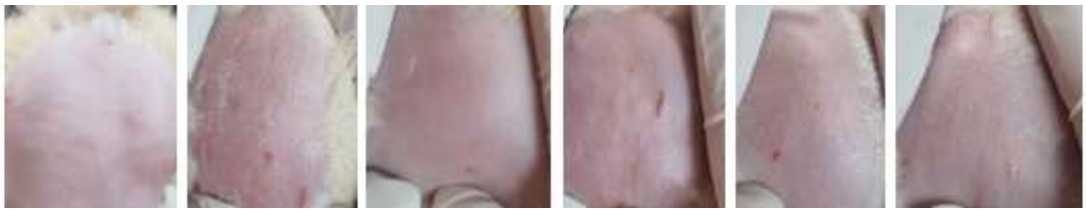
2. Results of Macroscopic Examination of Mouse Skin



A B C D E F

Figure 11. Before Exposure to UVB Light

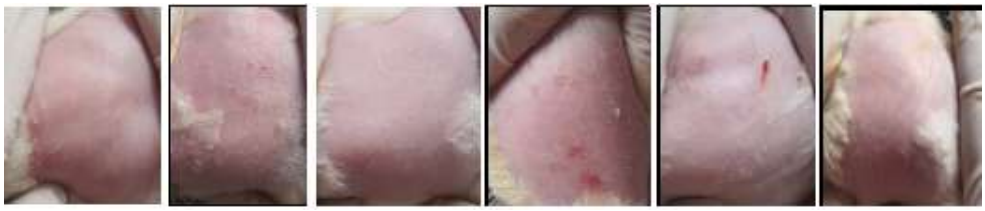
Remarks :A = Normal Group
B = Blank Group
C = Parasol Sunscream Group
D = EEUW Group 25%
E = EEUW Group 50%
F = EEUW Group 75%



A B C D E F

Figure 12. Week I After Exposure

Remarks :A = Normal Group
B = Blank Group
C = Parasol Sunscream Group
D = EEUW Group 25%
E = EEUW Group 50%
F = EEUW Group 75%



A B C D E F

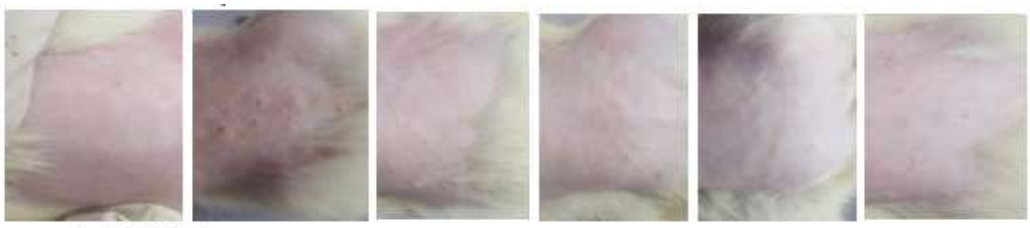
Figure 13. Week II After Exposure

Remarks :A = Normal Group
B = Blank Group
C = Parasol Sunscream Group
D = EEUW Group 25%
E = EEUW Group 50%
F = EEUW Group 75%

A B C D E F

Figure 14. Week III After Exposure

Remarks :A = Normal Group
B = Blank Group
C = Parasol Sunscream Group
D = EEUW Group 25%
E = EEUW Group 50%
F = EEUW Group 75%



A B C D E F

Figure 15. IV Weeks After Exposure

Remarks : A = Normal Group
B = Blank Group
C = Parasol Sunscream Group
D = EEUW Group 25%
E = EEUW Group 50%
F = EEUW Group 75%

From macroscopic examination, it can be seen that the skin of rats improved especially in the Parasol Sunscream group and the EEUW group 75%, this shows that the antiaging activity of carrot tuber ethanol extract is very high and contributes to the improvement of mouse skin.

Aging or aging is one of the problems in everyone, especially there are those who have entered upper middle age. Sun exposure, air pollution that continues to rumble, the use of cosmetics, as well as physiological changes of the skin itself can trigger the aging process (Cunningham, 2003). Aging can be caused by various factors, namely intrinsic factors and extrinsic factors. Intrinsic factors include the activity of certain enzymes. Increased activity of certain enzymes involved in the aging process of the skin including elastase, hyaluronidase, collagenase and tyrosinase. Collagen is the main component of the skin with a percentage of as much as 70-80% of the total skin weight. In addition, elastin has an important role in seeing skin elasticity (Thring et.al, 2009). While extrinsic factors include environmental factors such as sun exposure, air temperature or humidity, and free radicals. Free radicals appear in the body through normal body metabolic processes and due to external exposure such as cigarette smoke, pollution, and UV rays (Brenneisen et.al, 2002). Aging skin is mostly caused by free radicals, one of which is sunlight radiation. UV A and B in sunlight induce the formation of reactive oxygen species (ROS) in the skin and cause oxidative stress when the amount of ROS exceeds the antioxidant defense ability in skin cells (Almeida, 2008). Free radicals are unstable and highly reactive atoms or molecules because they contain one or more unpaired electrons in their larar orbitals. To achieve atomic or molecular stability, free radicals will react with surrounding molecules to obtain electron pairs. This reaction will take place continuously in the body and if not stopped will cause various diseases such as cancer, heart, premature aging and other degenerative diseases (Kikuzaki et.al, 2002). Carrot tubers contain lycopene carotenoid compounds, the potential of lycopene as an antioxidant and free radical catcher is a very beneficial effect on human health. Lycopene can also interact with ROS such as H2O2 and NO2 (Lu, 1995 & Woodall, 1997). Tomatoes also contain phenolic compounds such as quercetin, naringenin, rutin and chlorogenic acid. Phenolic compounds can capture peroxide radicals and can chelate ferrous metals.

3. Results of Microscopic Examination of Mouse Skin

Cardiac histopathology examination can be seen in the picture below:

a. Normal group

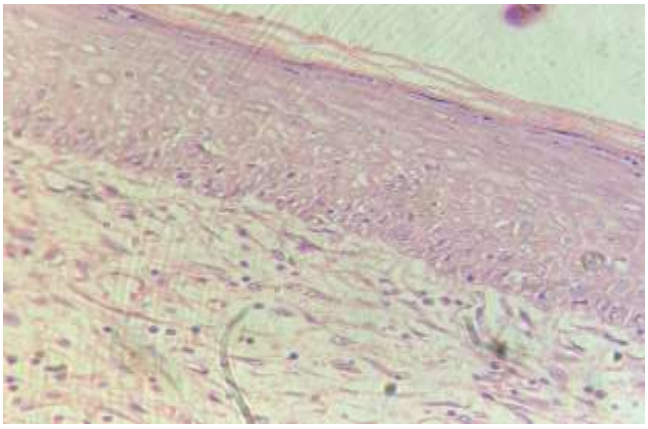


Figure 16. Normal Group

b. Blank Group

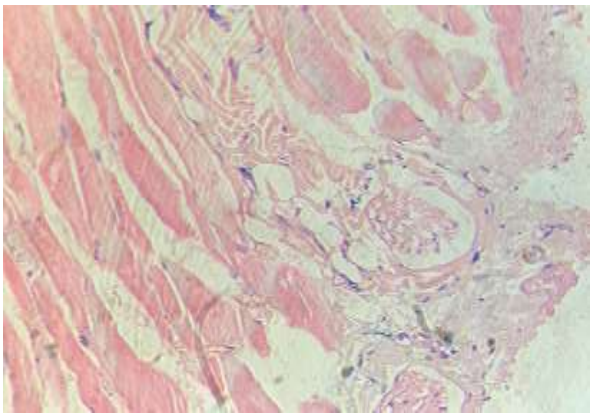


Figure 17. Blank Group

c. Parasol Sunscreen Group

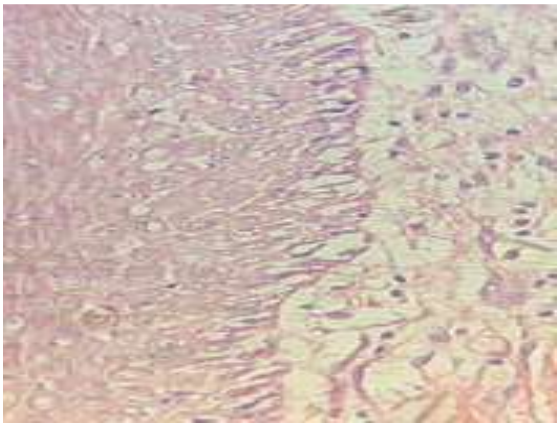


Figure 18. Parasol Sunscreen Group

d. Extract Group Concentration 25%

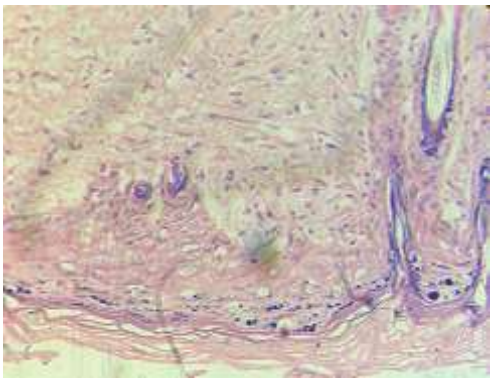


Figure 19. Extract Group Concentration 25%

e. Extract Group Concentration 50%

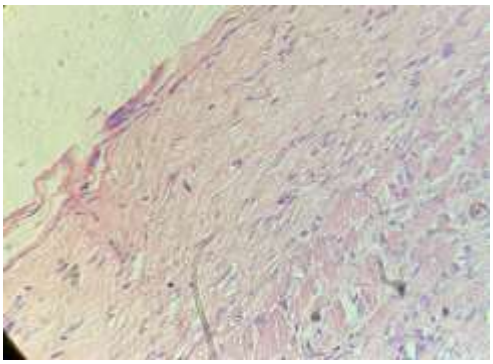


Figure 20. Extract Group Concentration 50%

f. Extract Group Concentration 75%

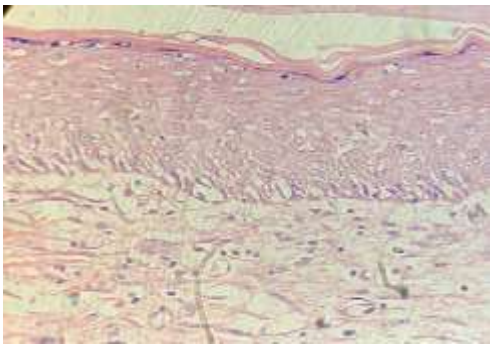


Figure 21. Extract Group Concentration 75%

The description on the black color shows fibroblasts while the blue color indicates the dermis layer, in the group given the extract has improved it can be seen that fibroblasts have increased while in the blank group that is not given treatment has damage to the tissue. The skin is the largest organ of the human body, protecting it from external attacks, such as microorganisms, chemicals, and physical agents, including sunlight. The skin also plays a role in thermal regulation, water retention, and cell regeneration. This protective barrier is formed by skin and epidermal cells, including special glands for the secretion of sebum and sweat, which form a special layer as a real protective coat. Apart from these protective features, the skin is permeable and absorbs substances that can benefit or impair its function, such as those involved in skin aging. Aging is a complex and ongoing biological process characterized by cellular and molecular changes, with a progressive decrease in the body's capacity to maintain homeostasis and an increase in aging and/or apoptosis. This process varies between individuals and from organ to organ, and the skin shows the most obvious effects of time travel. In addition, the use of ultraviolet radiation, excessive alcohol consumption, tobacco abuse, environmental pollution and other factors can influence and accelerate this physiological process, leading to premature skin aging. In this context, finding mechanisms that restore adolescent aspects of skin is an area of research of interest to the scientific community. Therefore, the cosmetic industry acts in this process, constantly looking for new compounds to prevent and reduce skin aging. Growth factors have become an important therapeutic option to avoid aging, as they are responsible for cell differentiation and

maturation, which directly correlates with the minimization of topical aesthetic changes resulting from the advancement of age. Growth factor proteins are naturally secreted by the cell and interact directly or are sequestered by the surrounding extracellular matrix to be presented to cell surface receptors. Events such as cell migration, survival, adhesion, proliferation, growth, and differentiation are triggered by binding to specific growth factor receptors, which stimulate cell signal transduction pathways. This growth factor-stimulated cellular response in greater proportions is involved in organ development, angiogenesis, and wound healing (Chandle, 1987).

CONCLUSION

1. Carrot Tuber Extract (Daucus carota L.) formulated in cream form can prevent an increase in the amount of melanin in the skin tissue of male wistar rats (Rattus norvegicus) exposed to UVB light
2. Carrot tuber ethanol extract contains phytochemical compounds namely flavonoids, tannins, saponins, glycosides, streoids, and triterpenoids.
3. Cream preparations of ethanol extract of carrot tubers with a concentration of 75% have better effectiveness in preventing an increase in the amount of melanin compared to concentrations of 25% and 50%
4. Microscopic picture shows thatcream preparations of ethanol extract of carrot tubers concentration of 25%, 50%, 75% can repair skin tissue.

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