Avocado (Persea americana Mill.) Seed Extract Cream 10% Inhibited The Increase MMP-12 Enzyme Levels And Elastosis In UVA Exposed Hairless Male Wistar Rats Skin

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Abstract

Background: Skin is the main target of environmental influences, especially chronic exposure to the sun's UVA rays which will result in wrinkling, decreased skin elasticity, the appearance of loose skin, and a rough clinical appearance. In the dermal tissue, not only affects collagen fibers but also degradation of elastin fibers occurs through an increase in the MMP-12 enzyme levels called photoaging and is characterized by the accumulation of dystrophic elastin fibers throughout the dermal tissue in a process called Elastosis. Avocado seed (Persea americana Mill.) is a part of the fruit that contains high concentrations of flavonoids, phenols, and tannins, it has the potential as a powerful antioxidant. The aim of the present study is to evaluate the effectiveness of avocado (Persea americana Mill.) seed extract cream 10% inhibiting the increase in MMP-12 enzyme levels and elastosis in hairless male Wistar rats (Rattus norvegicus) which were exposed to ultraviolet A rays.

Methods: A Randomized post-test-only control group design was conducted among 30 male Wistar rats for 10 consecutive days. They were divided into 3 groups as follows: group (K) as the negative control without any intervention, group (P1) with UVA rays exposure, and group (P2) with application of avocado (Persea americana Mill.) seed extraxt cream 10% and UVA rays exposure. The UVA dosage used is 5J/cm^2 twice a day among P1 and P2 groups. Avocado (Persea americana Mill.) seed extract 10% was applied to the hairless Wistar rats 4 hours after the exposure of UVA rays. MMP-12 was calculated with Rat MMP-12 ELISA Test. Elastin fiber density was calculated by Imageraster software. Statistical analysis was carried out using SPSS version 25 in measure means, normality test, homogeneity test, and comparative test.

Results: The results showed that the average MMP-12 enzyme level in the 10% avocado seed cream (P2) group 18.138 ± 0.168% was significantly different from the (K) group 1.073 ± 0.1553% as the negative control and the (P1) group 33.454 ± 1.068%. The mean density of elastin fiber in the cream of avocado seed extract group (P2) 12.42 ± 3.672% was significantly different from the group (K) 20.08 ± 4.994% and group (P1) 26.30 ± 6.822%. Comparative analysis using the Kruskal Wallis test for MMP-12 and the One Way Annova test for elastin fibers showed a p-value of < 0.05, thus indicating a significant difference between groups after treatment. The Least Significance Difference (LSD) test showed that there was a significant difference between the cream of avocado seed extract (P2) group and the other groups ($p<0.05$).

Conclusions: The administration of avocado seeds (Persea americana Mill.) extract cream 10% effectively inhibits the increase in MMP-12 enzyme levels and elastosis in hairless male Wistar rat skin which was exposed to ultraviolet A irradiation.

Keywords: UVA; avocado seed extract; Persea americana Mill; MMP-12; elastin fibers

1. Introduction

The skin is a visible organ that assesses a person's health and well-being and reflects many aesthetic parameters (D'Orazio et al., 2013). Like other parts of the biological body, the skin will also experience a complex and cumulative aging process throughout life due to structural and physiological changes caused by intrinsic and extrinsic factors in the body. The main extrinsic factor affecting the skin's aging process is ultraviolet radiation.Ultraviolet radiation is one of the

external factors that leads to the cause of skin damage, known as photoaging. UVA has more significant role than UVB in photoaging, both because of the deeper penetration of UVA into the dermal layer and because of the higher sensitivity of fibroblasts to UVA. Induction of elastase activity does not occur with UVB exposure (Tewari et al., 2014).

Elastin fiber is the main component contributing to the function of elasticity and resistance. Although the amount of elastin fibers are only 2 – 4% of the total skin protein, elastin degradation in the skin will reveal aging skin. Solar elastosis is a degenerative change in elastin fibers characterized by dystrophic elastin accumulation in the dermis due to chronic exposure to ultraviolet radiation. Matrix Metalloproteinase (MMP-12) is a protease enzyme that is most active in elastin degradation and secreted by several cells, including keratinocytes, fibroblasts, and inflammatory cells (Pittayapruek et al., 2016; Weihermann et al., 2017; Biskanaki et al., 2021) Elastosis is one of the primary markers of the photoaging process in the skin, characterized by irregular elastin fibers and the accumulation of non-functioning elastin fibers (Weihermann et al., 2017).

Avocado (Persea americana Mill.) seed extract has been reported to exhibit greater antioxidant capacity. Phytochemicals contained in avocado seeds include flavonoids, tannins, saponins, phenolics, antioxidant capacity, oxalates, phytates, and alkaloids. Antioxidants can inhibit oxidation reactions by binding to free radicals and highly reactive molecules. Organisms have an antioxidant system to protect themselves. Antioxidants can form new, more stable radicals through intramolecular hydrogen bonds and further oxidation (Petruk et al., 2018). Research with avocado seed extract cream containing antioxidants is to evaluate whether it can inhibit the increase in MMP-12 levels and elastosis in the photoaging process. In conclusion, avocado is a desirable natural source because of its phenolic-rich extract, which is high in antioxidants (Morcuende, Kylli and Est, 2011; Bhuyan et al., 2019; Zaki et al., 2020; H Y Setyawan, 2021).

2. Methods:

A Randomized post-test-only control group design was conducted among 30 male Wistar rats for 10 consecutive days at the Laboratory Animal Unit, Department of Pharmacology, Faculty of Medicine, Udayana University. They were divided into 3 groups namely: control group (K), UVA exposed group (P1), and 10% ethanolic extract of avocado (Persea americana Mill.) seed with UVA exposed group (P2).

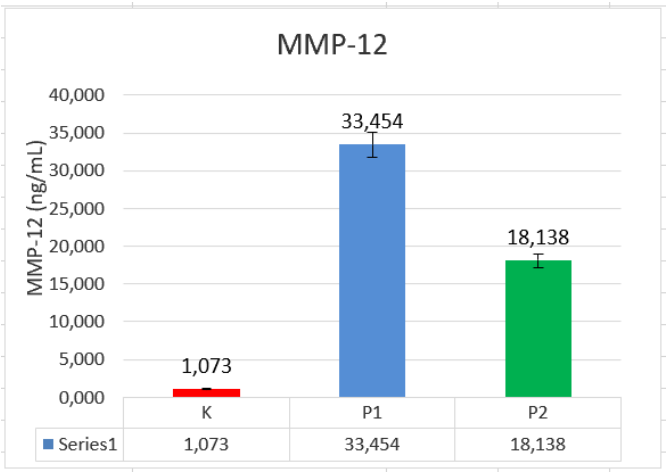
The (K) control group didn't The UVA dosage used is 5J/cm^2 twice a day among P1 and P2 groups. The first group (K) as the negative control without any intervention, the second group (P1) was subjected to UVA irradiation twice a day with a dosage of 5J/cm^2 each exposure, and the third group (P2) was subjected to UVA irradiation twice a day with a dosage of 5J/cm^2 each exposure and 10% ethanolic extract of avocado seed (Persea americana Mill.) applied topically 4 hours after each UVA irradiation. Throughout the study period, all three groups were given an exact amount of food and water. The temperature and humidity of the experiment environment are strictly controlled After 10 consecutive days of intervention, the rats were rested for 48 hours to avoid the effects of acute exposure to UVA irradiation, then a skin biopsy about 5mm thick measuring 1x1cm^2 was made, then preparations were made for biochemical and histological examination.

MMP-12 levels were calculated using the Rat MMP-12 ELISA test at the Department of Biochemistry, Faculty of Medicine, Udayana University. The mean density of elastin fibers was checked by Verhoeff Van Gieson staining and calculated by the digital fast analysis method for measuring the elastosis in the skin at the Department of Histology, Faculty of Medicine, Udayana University.

The data obtained were statistically analyzed using Kruskal Wallis Test for MMP-12 levels and the One Way Annova Test for Elastin fibers density by SPSS version 25. The results of mean, standard deviation, and significance tests were shown. A p-value less than 0,05 was considered statistically significant for MMP-12 levels and elastin fiber density.

3. Results:

The Biochemistry analysis regarding MMP-12 levels using RAT MMP-12 ELISA Test was shown in Figure 1 below. The results of MMP-12 levels showed that the highest mean of MMP-12 levels was found in group P1 (33.454 ± 1.068%), followed by group P2 (18.138 ± 0.168%) and group K (1.073 ± 0.1553%). Data of MMP-12 levels in each group were tested for normality and homogeneity by using Saphiro-Wilk and Levene Test. The results showed that MMP-12 levels data were abnormally distributed with $p<0.005$ (Table 1). The result was also analyzed by using the Kruskall Wallis test which showed that there was a significant difference among groups ($p< 0.05$). Multivariate analysis using Dunnet T3 Test also found a significant difference between groups ($p<0.05$).



MMP - 12

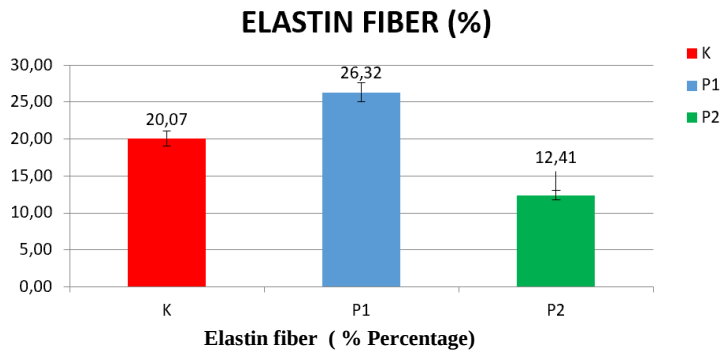
Figure 1. The Mean of MMP-12 in different groups of hairless male Wistar Rats

Table.1. The mean of MMP-12 in different groups of hairless male Wistar rats

Variable	Group	n	Mean	SD	Median	Min	Max
	K	10	1,07	0,16	1,04	0,88	1,34
MMP-12	P1	10	33,45	1,07	33,52	30,99	34,72
	P2	10	18,14	0,19	18,13	17,88	18,44

Footnotes :
K : Control group
P1 : UVA exposure group
P2 : UVA + cream extract avocado seed 10%

The results are also similar to Image raster software measurement where the highest mean of dermal elastin fiber density was found in group P1 (26.30 ± 6.822%), followed by group K (20.08 ± 4.994%) and P2 (12.42 ± 3.672%) (Table 2). Data of the dermal elastin fiber in each group were tested for normality and homogeneity by using Saphiro-Wilk and Levene Test. The results showed that the elastin fiber data were normally distributed with p>0.005 (Table 2). The result was also analyzed by using the One Way Annova test which showed that there was a significant difference among groups (p< 0.05). Multivariate analysis using LSD Test also found a significant difference between groups (p<0.05).



Elastin fiber (% Percentage)

Figure.2 The Mean of Elastin Fiber in different groups of hairless male Wistar Rats

Table 2. The mean of Elastin Fiber in different groups of hairless male Wistar rats

Variable	Group	N	Mean	SD	Min	Median	Max
Elastin Fiber	K	10	20,08	4,99	19,90	13,90	29,20
	P1	10	26,30	6,82	25,15	18,70	39,20
	P2	10	12,42	3,67	11,90	8,50	18,80

Footnotes :
K : Control group
P1 : UVA exposure group
P2 : UVA + cream extract avocado seed 10%

The histological analysis regarding elastin fiber density was shown in Figure 3 and Figure 4 below. Skin tissue was stained by Verhoeff Van Gieson (VVG) dan observed by using a light microscope 10x 40 magnification. Macroscopically, the thickest of dermal elastic fiber was found in group P1 with UVA exposed only group, followed by K and P2 groups.

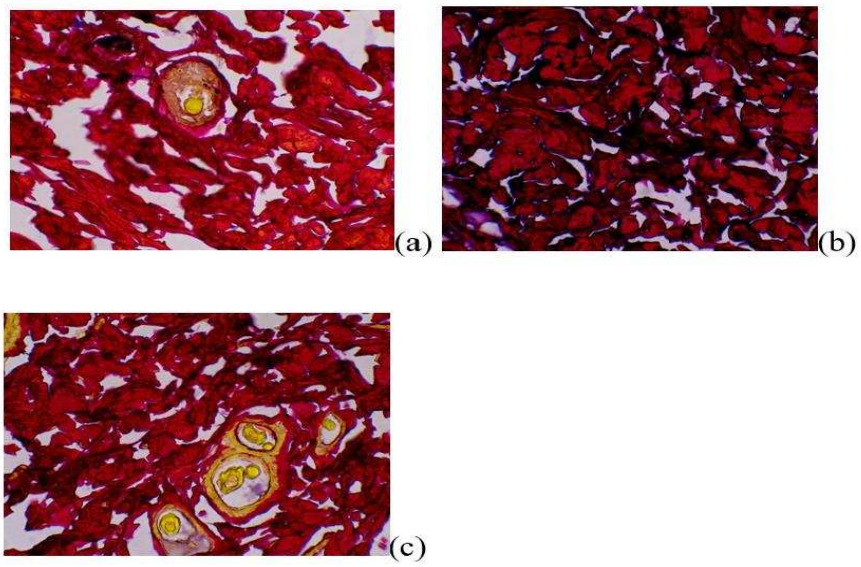


Figure 3. The microscopic histology preparations using VVG stain of the dermal layer, elastin fibers (black), collagen fiber (red) (Light microscope; 10 x 40 magnification). Group (a) K = control group; (b) P1 = UVA exposure and (c) P2 = avocado (Persea americana Mill.) seed extract cream 10% and UVA exposure.

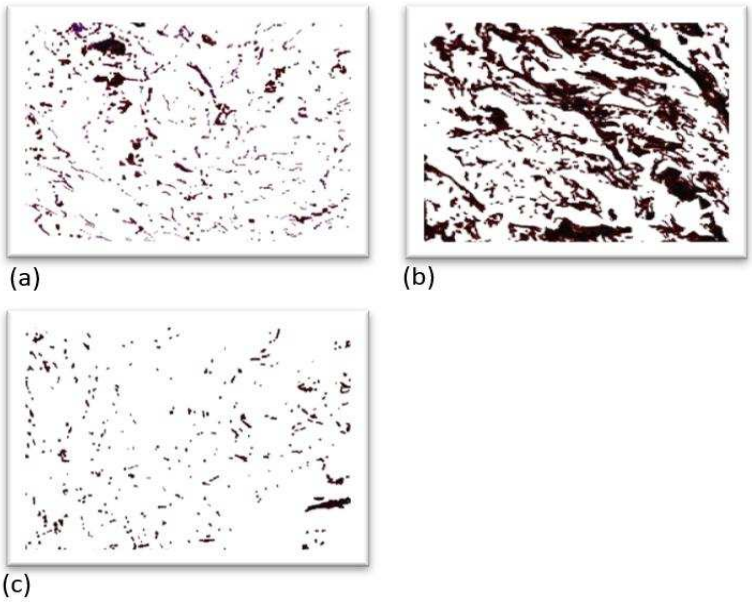


Figure 4. The microscopic histology preparations using VVG stain of the dermal layer, elastin fibers (black), collagen fiber (red) (Light microscope; 10 x 40 magnification). Group (a) K = control group; (b) P1 = UVA exposure and (c) P2 = avocado (Persea americana Mill.) seed extract cream 10% and UVA exposure.

4. DISCUSSION

This study proved that 10% ethanolic extract cream of Avocado (Persea americana Mill.) seed were effective inhibits the increase in MMP-12 enzyme levels and elastosis in hairless male Wistar (Rattus norvegicus) rat skin which was exposed to ultraviolet A irradiation.

The results of MMP-12 levels showed that the highest mean of MMP-12 levels was found in group P1 (33.454 ± 1.068%), followed by group P2 (18.138 ± 0.168%) and group K (1.073 ± 0.1553%). The result was also analyzed by using the Kruskall Wallis test which showed that there was a significant difference among groups ($p< 0.05$). Multivariate analysis using Dunnet T3 Test also found a significant difference between groups ($p<0.05$).

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Chronic UVA exposure elicits gradual increase in MMPs levels that will breakdown the extracellular matrix. In the dermal layer, this process lead to the degradation of elastin fibers through an increase of MMP-12 enzyme levels induced by AP-1 (Activator Protein-1) and NFkB (Nuclear Factor Kappa Betta) (Torres-Contreras et al., 2022).

In the photoaging process, one of the main characteristics is the occurrence of elastosis, namely the accumulation of non-functional dystrophic elastin fibers in the degraded, fragmented and thick dermal layer. Changes in the elastin network were caused by increased levels of MMP-12, elastase-derived skin fibroblasts, and neutrophil elastase.

The phytochemical test of seed extract of avocado (Persea americana Mill.) shows an IC50 result of 49.2732 (ppm), so it can be called a powerful antioxidant, with antioxidant capacity of 20699.34 mg /L, Flavonoids 6081.82 mg/100gram, Phenol 1760.96 mg/100gr, Tannins 2203.21 mg/100gr. One of the mechanisms of action of flavonoids in inhibiting the occurrence of elastosis is as an MMPs Inhibitor.

Flavonoids, as photoprotection, absorb UVA/UVB rays, bind ROS, and inhibit the inflammatory process. Phenol can stop free radical reactions in lipid oxidation(Petruk et al., 2018). Phenol has a photoprotective action by inhibiting pro-inflammatory mediators, modulating NF-kB, and modifying eicosanoid synthesis. Phenol inhibits ROS formation and

neutralizes RNS (Reactive Nitrogen Species). Phenol also has anti-aging properties where phenolic compounds can inhibit the formation of MMPs, induce collagen and elastin, and carry out cell renewal.

Tannins in avocado seed extract can prevent oxidative damage to DNA in two ways: binding to metal iron and directly counteracting free radicals. Figure 2.10 shows tannins as polyphenols inhibiting the activation of increased MMPs due to increased AP1 (Activator Protein 1) and NFkB (Nuclear Factor Kappa B) caused by the formation of Reactive Oxygen Species by chronic exposure to UVA and UVB radiation (Działo et al., 2016).

Acknowledgement

This paper presents The Administration of Avocado **(**Persea americana Mill.) seed extract cream 10% inhibited The Increase of MMP-12 enzyme levels and Elastosis in UVA-Exposed Hairless Male Wistar (Rattus norvegicus) Skin.

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