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Vitamin E (Alpha Tocopherol) Level in the Dorsal Skin of Sunda Porcupine (*Hystrix javanica*)

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Abstract

Alpha tocopherol, as the most active component of Vitamine E, is identified as antioxidant present in some parts of the skin and the concentration is variesdepends on regions of the body due to differences of the skin characteristic of the region. Sunda Porcupine (Hystrix javanica) is one of the indigenous rodents of Indonesia with quill as its spesific skin characteristic. The porcupine quills are distributed in various shapes and sizes in body, while dorsal region and the tail are the body part that have the most developed forms. This study aimed to measure alpha tocopherol content in the dorsal region skin of male and female Sunda porcupine. The study used skin samples of thoracodorsal and lumbosacral regions of 4 males and 4 females of Sunda porcupine. Samples were prepared and processed for analysis through high performance liquid chromatography. The results showed that the level of alpha tocopherol in the thoracodorsal region of males and females is significantly higher than in the lumbosacral region.In conclusion, the results of this study suggested that characteristics of the dorsal skin region of Sunda Porcupine presumably contribute to the level of alpha tocopherol.
***Keywords**: antioxidant, dermis, epidermis, quill cluster, quill follicle, sebaceous gland*

Introduction

The skin as the outermost organ of the body plays a role in protecting the body from various enivromental elements. The skin is often exposed to environments that contain many oxidants, such as ultraviolet radiation, drugs and air pollution. Various layer of the skin (stratum corneum, epidermis, dermis and subcutaneous) are equipped with enzymatic and non enzymatic antioxidant activity systems (Thielle *et al.*, 1999) to counteract damage to lipid and protein structures due to oxidative reactions.

Alpha tocopherol is the most active form of antioxidant from vitamin E (Traber and Sies 1996). Alpha tocopherol was identified as the dominant antioxidant in rats and human stratum corneum with various concentrations in each layer (Thiele*et al.*, 1999). Levels of alpha tocopherol in the human dermis and epidermis are known to be several times higher than the dermis and epidermis in the hairless mice (Shindo *et al.* 1993, 1994). Stratum

corneum in human skin is also known to have alpha tocopherol levels 10 times higher compared to rat stratum corneum (Thiele *et al.*, 1998). Levels of vitamin E (including alpha tocopherol) in rats and humans are known to be higher in the epidermal layer compared with the dermis layer. It is related to activity and secretion in the epidermal layer by the sebaceous glands (Freedberg *et al.*, 2003).

In addition to its role in prevent free radicals, alpha tocopherol also plays a role in various cellular and molecular processes through the non-antioxidant portion of this molecule. Alpha tocopherol is known to inhibit the proliferation of smooth muscle cells (Chatelain *et al.*, 1993; Azzi *et al.*, 1995),to facilitatemonocyte adhesion to endothelial (Islam *et al.*, 1998; Martin *et al.*, 1997; Wu *et al.*, 1998; Yoshikawa *et al.*, 1998), to release of reactive oxygen species (ROS) and cytokine monocytes (Devaraj *et al.*, 1996; Kanno *et al.*, 1995; Cachia *et al.*, 1998), to facilitateadhesion and platelet aggregation (Freedman *et al.*, 1996, Williams *et al* 1997), and the expression of the enzyme collagenase of fibroblasts in the skin (Ricciarelli *et al.*, 1999). These processes is known to take place at the molecular level, such as transcription and post transcriptional stages.

Sunda Porcupine is one of the mammals in the order of Rodentia, family Hystricidae that has specific characteristic with the quill as the main derivate of the skin. The porcupine quills are distributed in various shapes and sizes on body, while dorsal regions and the tail is the body part that has the most developed forms. Quills in the dorsal region are used for defense mechanism (Myers, 2001; Mohr, 1965; Van Weers, 1983). Skin in the dorsal region of Sunda Porcupine showed a specific characteristic of protrusion of quill follicles to the skin surface in the form of cluster quills, and the quill follicle as the dominant structure in the skin (Prawira *et al.,* 2018a).

To the best of our knowledge, studies inspiny animals such as porcupine (*Hystricidae* and *Erethizontidae*), tenrec (*Tenrecidae*), hedgehog, and others received more attention in the aspect of the spine structure (Roze *et al.,* 1990; Zherebtsova, 2000), while the skin biochemical aspect received less attention. Alpha tocopherol has an important role in the physiology and defense of the body in the skin. Therefore, due to lack of published data regarding this biochemical characteristic (i.e. level of alpha tocopherol) of the skin of Sunda Porcupine, we considered the importance of this study. This study aimed to measure alpha tocopherol content in the dorsal region skin of male and female Sunda porcupine.

Materials and Methods

Animals

The subject of this study were 6 adults Sunda Porcupine (3 males and 3 females) weighing 6-8 kg and 2 frozen specimens of Sunda Porcupine (1 male and 1 female).This study obtainded ethical approval from The Ethical Clearance Subcommitee of of Life Science, Indonesian Institute of Sciences No. B-12695/K/KS.02.04/XII/2017. The animals were sampled for excisional skin biopsy and the procedures were conducted under anesthesia (10% HCl ketamine and 2% Xylazine HCl with a dose of 2.5 mg / kg BW and 1 mg / kgBW). The biopsy skin samples were obtained from two dorsal regions, Thoracodorsal (TD) and Lumbosacral (LS) (Figure 1). All samples then were freeze and sectioned to separate and sectioned to separated the skin surface (epidermis and papilary layer of dermis) from other parts (reticular layer of dermis and subcutaneous). Then, samples were grouped into 4 categories, i.e. male TD, male LS, female TD, and female LS with each sample weighting 5 gram (1.5 gram of skin surface and 3.5 gram of other parts).

Analysis of alpha-tocopherol with High Performace Liquid Chromatography (HPLC)

The method of analysis refers to DeVries & Silveira (2002). The 5 gram of tissues from each samples were homogenized, and 2 grams of homogenized samples collected from each group for the HPLC analysis into Erlenmeyer container. Pyrogallic acid (50 mg) were added into the container and boiled until all the ingredients were mixed in solution. Potassium hydroxide 50% solution were then added as much as 10 ml into each container and then mixed and cooled. The next step was the addition of 10 ml glacial acetic acid into the container and then mixed and cooled to room temperature. The solution was then transferred to a volumetric container and diluted to 100 ml with a mixture of 95% tetrahydrofuran-ethanol (1: 1). The solution were centrifuged to form a fatty acid salt precipitate.

The standard solution of vitamin E (Alfa-tocopherol) used as a comparison was vitamin E acetate. The standard working solution (concentration 2,627 gram/ml) was optimized in 5 concentrations with each volume was 0.5 ml, 1 ml, 2 ml, 5 ml and 10 ml in diluted volume of 10 ml. Samples and standard (1 µl each)were then injectedinto the HPLC (Aqcuity UPLC H class). The HPLC device has a C8, 10 µm (4.6 × 250 mm) inverted column capable of detecting alpha tocopherol with a resolution of 1.5 or more. The detector used is a 280 nm wavelength fluorescence detector. The detection was carried out for 15 minutes with vitamin E retention time being at about 5 min. Vitamin E levels on the skin were calculated by comparing the area of the standard and the sample solution. The data were analyzed statistically with student T test analysis and described in table.

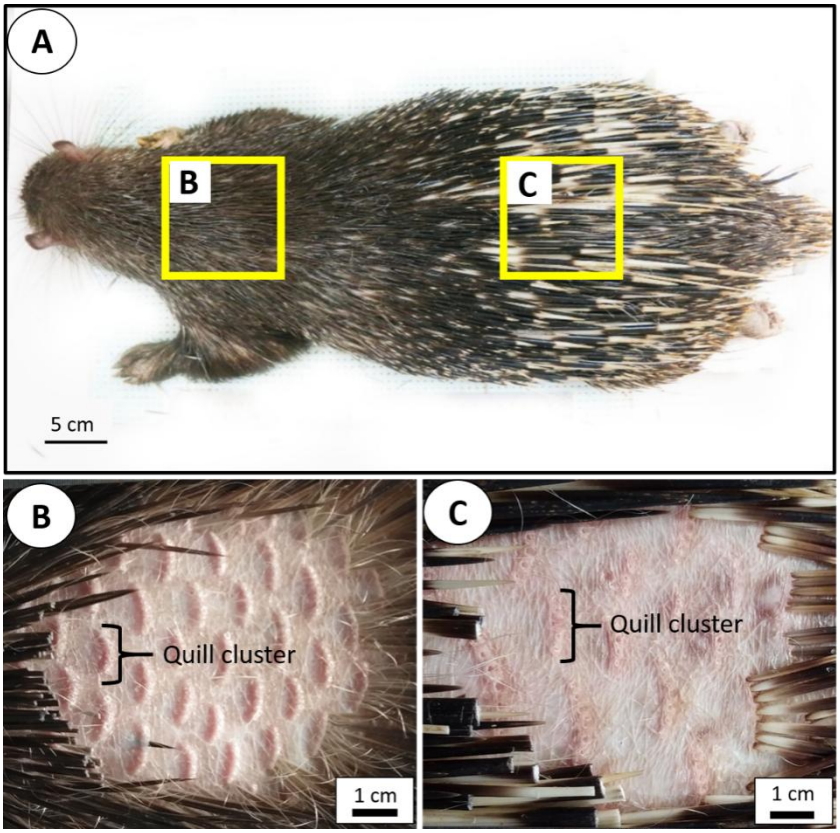

Figure 1. Region of body skin sampling. The dorsal region's Sunda Porcupine (A) showed the appearance of different spines in the thoracodorsal, TDregion (B) at the anterior of the body and the lumbosacral, LS region (C) at the posterior of the body. Quills in the TD (B) and LS (C) regions grow in the form of quill clusters.

Results

The results of HPLC showed that alpha tocopherol level in the skin of TD region of both males and females was significantlyhigher than in the LS region (Table 1). The levels of alpha tocopherol in the LS region is very low, less than 0.21nmol / grams of skin tissue. Levels of alpha tocopherol in the male TD region (60nmol / gram tissue) were much higher and differ significantly than the female TD (37.49nmol / gram tissue). Comparative values of alpha tocopherol in Sunda Porcupine and some animal species and human show a specific value based on the region and the skin (Table 2).

Table 1. Alpha Tocopherol Level in Sunda Porcupine

Group	Level Alpha Tocopherol (nmol/g tissue)
Male TD	60[a]
Male LS	<0.21[b]
Female TD	37.49[c]
Female LS	<0.21[b]
Mean TD region	48.75
Mean LS region	<0.21

Note: different superscript font in a column showed significant difference at $p<0.05$

Table 2 Comparison Of Alpha Tocopherol Level by Species, Region and Layer

Skin parts	Sunda Porcupine (nmol/g jaringan)	Hairless mice (nmol/g jaringan)	Human (nmol/g jaringan)	Dog ($\mu g/cm^2$)	Cat ($\mu g/cm^2$)
Skin surface	-	-	-	0,1-03[e]	0,02-0,2[e]
Epidermis	-	4,81[a]	31,1[c]	-	-
• Face			102[d]		
• Fore arm			72,7[d]		
Dermis	-	3,3[a]	16,2[c]	-	-
Epidermis + dermis	-	8,11	47,3	-	-
Whole skin		192.9[b]	-	-	-
• TD	48,75	-	-	-	-
• LS	<0,21	-	-	-	-

[a] (Shindo *et al.* 1993), b (Fuchs *et al.* 1989), c (Shindo *et al.* 1994), d (Thiele *et al.* 1999), e (Jewell *et al.* 2000)

Discussion

Epidermis is a layer in the skin with high metabolic rate and always exposed to pro-oxidant conditions, such as ultraviolet light, inflammatory responses, and chemicals. Most of the exposed antioxidant in the skin will be neutralized in the epidermal layer and only a small part penetrates the dermis layer (Epstein, 1977).

This study showed that alpha tocopherol level in the TD region of Sunda Porcupine skin was higher compared to epidermis and dermis part of the skin of hairless mice (Shindo *et al.*, 1993), but lower than in the LS region. Levels of alpha tocopherol skin of Sunda Porcupine in TD region also higher when compared with levels of tocopherol in human skin (epidermis and dermis part). In contrast, levels of alpha tocopherol in human skin is higher than the level of tocopherol skin of Sunda Porcupine in LS region, bothin male or female. This is presumably influenced by the activity of sebaceous glands as glands that deliver vitamin E and its homolog to the skin surface. Skin with high density of sebaceous glands will have higher alpha tocopherol content (Thiele *et al.*, 1999). Our previous studies showed that sebaceous gland

density in TD region is higher than in LD region (Prawira *et al.*, 2018b). The difference in level between male TD and female TD may also be influenced by different hormonal activity between the sexes affecting the activity of the sebaceous glands (Cunliffe & Forster, 1987; Fritsch *et al.,* 2001). The estrogen shows inhibition of the activity of the in vivo sebaceous glands (Strauss *et al.*, 1962). Moreover, study conducted by Jewell *et al.* (2000) in dogs and cats show that feeding rich in vitamin E can increase vitamin E levels in blood plasma and skin surfaces.

The significant difference in the level of alpha tocopherol among dorsal regions might be related to skin characteristics in the region. The skin of Sunda Porcupine has quills as main derivatives of varying sizes and densities in various parts of the body. The quill clusters in the TD region is smaller but higher density compared to the LS region which is larger but lower density(Prawira *et al.*, 2018b) resulting in broader skin surface exposed to the environment. In addition, higherquillcluster density in the TD region has implications for higher sebaceous gland density when compared to the LS region due to the sebaceous gland are found only in quill and hair follicle (Prawira *et al.*, 2018a; 2018b) and might be resulting in different sebaceous gland activity in eachregion.

Skin characteristics in the LS region showed lower density of the quill clusters than in the TD region (Prawira *et al.*, 2018b) allow many parts of the skin to be exposed to the environment such as sunlight, soil, dust, etc. more than the TD region. This condition might be contributes to antioxidant activity in both regions. Levels of alpha tocopherol in the stratum corneum of the skin are known to decrease if exposed by ultra violet light to 50% in humans and 85% in mice (Thiele *et al.*, 1998). Levels of alpha tocopherol decreased by exposure to oxidants (free radicals) can be recycled if there is sufficient amount of ascorbic acid on the surface of the skin (Kagan *et al.*, 1992; Kitazawa *et al.*, 1997). Research conducted by Weber *et al.* (1999) found that ascorbic acid was found only in very small amounts in the stratum corneum of human skin and mice when compared with epidermal layers and dermis. Another factor that may affect the difference of alpha tocopherol levels between regions of the body is the presence of Tocopherol Associated Protein (TAP) in tissues (Yamauchi *et al.*, 2001). This protein is probably responsible for levels of alpha tocopherol in skin tissue and others and the cellular response in the tissues (Azzi *et al.*, 2000).

Conclusion

The level of alpha tocopherol in the skin of dorsal body of Sunda Porcupine is significantly higher in the thoracodorsal region when compared with the lumbosacral region in both males and females. The results of this study indicate that characteristics of the skin region of Sunda Porcupine presumably contribute to the level of alpha tocopherol.

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