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Effectiveness of Topical Mangosteen Pericarp Extract Against Angiogenesis in Mice Exposed to Ultra Violet B

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Abstract: Exposure to ultraviolet B (UVB) can have an impact on the epidermis and dermis that triggers inflammatory processes that affect angiogenesis in the skin. Our study aimed to assess the effectiveness of mangosteen pericarp extract against angiogenesis in mouse skin after being exposed to UVB. This research was carried out with animals in the anatomic pathology laboratory of Hasanuddin University in Makassar. We used an animal experimental research design with a control group. A total of 24 mice were divided into the following 4 sample groups: normal group, UVB group, ethanol group and mangosteen group. In the mangosteen group, mangosteen pericarp extract with a concentration of 50% was smeared on the mice shortly after they were exposed to UVB. The amount of vascularization was significantly different among the groups. The greatest amount of vascularization was found in the ethanol group and the least amount was found in the normal group. The amount of vascularization in the mangosteen group was significantly lower than the UVB and ethanol groups but significantly higher than the normal group. In conclusion, the mangosteen pericarp extract concentration of 50% (2 mg/g of mouse weight) reduced the amount of angiogenesis, which is a marker of inflammation, in the skin of the mice that were exposed to UVB.

Key words: Angiogenesis, mangosteen pericarp extract, UVB

INTRODUCTION

Sunlight is a source of electromagnetic energy that mainly consists of solar ultraviolet (UV) radiation, visible light and the infrared spectrum. The ability for UV radiation to reach the Earth is significantly affected by the atmosphere in its path so that only two-thirds of the solar energy is captured (Lim, 2012).

Various acute and chronic effects from exposure to UV that causes unburn, pigmentation, premature aging and skin cancer are found in person who often exposed to it. Although data on these effects have not been published, a high incidence of these effects likely occurs in Indonesia, based on its geographical location. Additionally, many Indonesian people live in rural areas and work as farmers and fishermen and research on strategies for handling the adverse effects of sunlight has become very important (Adriani, 2014).

Angiogenesis is the process of the formation of new blood vessels (vascularity) and it can occur with wound healing, inflammation and malignancies. Furthermore, angiogenesis can also affect the aging process. Age-related reduction in the size of cutaneous blood vessels and reducing the number of blood vessels in the dermis are markers of all aging processes, including aging caused by UV (Zouboulis and Makrantonaki, 2011; Bosch *et al*., 2015).

Angiogenesis forms new blood vessels from preexisting vessels, which involves increased microvascular permeability, the degradation of extracellular matrix molecules and migration of endothelial cells, which leads to the formation of new capillaries. In normal conditions, angiogenesis in the perifollicular skin is limited to blood vessels during the growth phase of hair follicles. However, skin can have a rapid angiogenic response during wound healing and inflammation. Several studies have found that the skin also shows angiogenic responses to external environmental stimuli, such as UV light (Sawane and Kajiya, 2012).

Preventing the cellular damage associated with oxidative stress, it is important to maintain the balance of oxidants with antioxidants and this can be performed with antioxidant supplementation (Hanggono, 2004). One Indonesian plant that can be used for such purposes is mangosteen (Garcinia mangostana), especially using the pericarp extract of the fruit. Mangosteens come from tropical forests in Southeast Asia, including Indonesia. The oxidized, yellow mangosteen pericarp resin is rich of xanthones (Akao *et al*., 2008). Mangostin is a major xanthone element and it is present in mangosteen. The mangosteen pericarp extract is 95% xanthone and it also contains isoflavones, tannins and flavonoids (Priya *et al*., 2010).

The mangosteen pericarp extract, similar to antioxidants, have anti-inflammatory activity (Nakatani *et al*., 2002).

Based on the above data information, this research is needed to assess the effectiveness of mangosteen pericarp extract against angiogenesis in mouse skin after being exposed to UVB.

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MATERIALS AND METHODS

This study employed an animal experimental research design with a control group. The research was conducted in an animal anatomic pathology laboratory at Hasanuddin University, Makassar, during the period of March to April 2016. The study was conducted after obtaining approval from the Health Research Ethics Committee (KEPK) of the Faculty of Medicine at the University of Hasanuddin.

Total sample was comprised of 24 mices that met the inclusion criteria that included healthy, female swiss albino mice that were age of 6-9 weeks and 20-30 g of weight.

The exclusion criteria comprised sick and dying mice. Those 24 mices that met the study criteria were divided into the following 4 sample groups: the normal group, the UVB group, the ethanol group and the mangosteen group.

Mangosteen pericarp extract with a concentration of 50% was made in the laboratory of Pharmacognosy Phytochemical at the Hasanuddin University Faculty of Pharmacy. Each treatment comprised 3 drops (0.1 ml) of mangosteen pericarp extract, which was equivalent to a dose of 2 mg/g of mouse mass.

The UVB radiation treatment of the mice comprised exposure to a 311 nm narrow band UVB (Dermalight 1000) radiation with the power of 450 mJ/cm^2 three times a week for 4 weeks.

Histopathologic preparations were obtained from mouse back skin tissue after biopsy excision. Each specimen was fixed with buffered formalin. The specimens were then placed on a flat surface and cut into two parts down the center. A slide was made using pieces of tissue from the middle, which were cut perpendicular to the cut down of centre and to a thickness of 4 µm. These tissue samples were then stained with hematoxylin eosin (HE) for the assessment of angiogenesis.

Angiogenesis measurements were conducted by counting the number of blood vessels (vascularity) in 5 fields of view with a 400 x magnification and the counts were then averaged (Fig. 2).

The data analysis was performed using Statistical Product and Service Solutions SPSS version 22. The analysis compared the number of angiogenesis of the four groups. A Kruskal-Wallis statistical test was also used. Significance was declared if the test p-value was <0.05. If a test was significant, an advanced test (post-hoc test) was performed to assess which groups were different. The post-hoc test method was Bonferroni's test.

RESULTS

There was a significant difference in the amount of vascularization among the sample groups (p<0.001; Table 1 and Fig. 1).

Table 3 shows the amount of angiogenesis was significantly different among the groups, with the amount of angiogenesis greatest in the ethanol group and least in the normal group. The amount of angiogenesis in the mangosteen group was significantly lower than the UVB and ethanol groups but was significantly higher than the normal group.

DISCUSSION

The UVB dose used in this study comprised450 mJ/cm^2 3 times a week for 4 weeks. The dose of UVB rays that can cause skin photo aging in mice, according to several studies, can vary greatly. Kim *et al*. (2004) used UVB at a dose of 600 mJ/cm^2, which was given in divided doses. In another study, a total dose of 840 mJ/cm^{2}UVB was given in divided doses (Wahyuningsih, 2010).A study was conducted in Makassar by Djawad (2008). In this study, mice were exposed to 343 mJ/cm^{2}UVB at a frequency of 3 times a week, which resulted in epidermal hyperplasia. Waspodo (2012) reported that the exposure of albino mice to 343 mJ/cm^2 UVB caused only minimal changes, which comprised the immune histochemical expression of MMP 1. Adriani (2014) reported that the exposure of albino mice to 450 mJ/cm^2 UVB induced the expression of 8-OHdG and PCNA. There have been no studies that have assessed how UVB affects angiogenesis in the skin.

In our study, before irradiation with UVB, the mice were shaved in the hip area and the shearing action was repeated again before the scheduled irradiation. These actions were taken to reduce the influence of hair on the transmission of the UVB rays.

Table 1: Distribution of the amount of vascularity (average number in the fifth field of view)

	Samples						
	A	B	C	D	E	F	Average
N	26.8	20.4	23.4	14.6	24.75	13.4	20.56
UV	52.8	47.4	56	63.4	86.2	81.75	64.59
ET	80.8	91	107.6	101	93	71.8	90.87
MG	40.4	59.6	36.8	25.5	45.8	46.8	42.48

Kruskal-Wallis

Table 2: Comparison of the amount of angiogenesis (n = 24)

Groups	Minimum	Maximum	Mean	Median	SD	p
Normal	13.40	26.80	20.56	21.90	5.50	0.000
UVB	47.40	86.20	64.59	59.70	15.94	
Ethanol	71.80	107.60	90.87	92.00	13.05	
Mangosteen	25.50	59.60	42.48	43.10	11.38	

Table 3: Multiple comparisons

(I) Group	(J) Group	Mean difference (I-J)	p
Normal	UVB	-44.03	0.000
	Ethanol	-70.31	0.000
	Mangosteen	-21.93	0.031
UVB	Normal	44.03	0.000
	Ethanol	-26.27	0.007
	Mangosteen	22.11	0.029
Ethanol	Normal	70.31	0.000
	UVB	26.27	0.007
	Mangosteen	48.38	0.000
Mangosteen	Normal	21.93	0.031
	UVB	-22.11	0.029
	Ethanol	-48.38	0.000

Post-hoc test (Bonferroni's test)

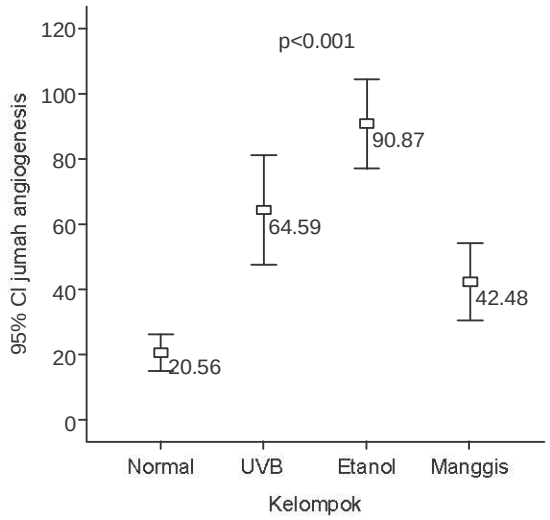

Fig. 1: Comparison of the extent of Angiogenesis

The experimental animals were healthy, normal, albino strains of mice that were 6-9 weeks of age and weighed 20-30 g. Mice, as mammalian vertebrates, have a similar skin structure to human skin. The mouse skin structure is similar to that of human young adults who have not experienced intrinsic aging. Albino mice are a strain of mice that have no pigment, including no pigment in the hair follicles. Vayalil *et al*. (2004) used SKH-1 hairless mice in his research on green tea and its preventative effects against UV exposure. Hairless mice are ideal for research that requires treatment involving exposure of the skin because the shearing step is not required. However, strains of mice without hair cannot be found in Indonesia. Angiogenesis is the process of the formation of new blood vessels (Yuhernita *et al*., 2014) Angiogenesis occurs in wound healing and the aging process. Age related reduction in the size of cutaneous blood vessels and reduction in the number of blood vessels in the dermis is a marker of all aging processes, including aging caused by UV (Zouboulis and Makrantonaki, 2011).

The presence of new blood vessels at the sites of inflammation serves as a supplier and transporter of nutrients and oxygen, which are needed by cells that are in the repair process involving the destruction of harmful substances as well as the formation of granulation tissue (Yuhernita *et al*., 2014).

When inflammation of the skin occurs, the healing process is characterized by epidermal regeneration, angiogenesis and proliferation of fibroblasts to form a fibrin clot with debris, which is then replaced by granulation tissue. Granulation tissue is very smooth and bleeds easily because it contains many new blood vessels but has little connective tissue. During the development of granulation tissue, new capillaries invade the area of inflammation and the adjacent blood vessels regulate the microvascular circulation into the tissue. At the same time, fibroblasts proliferate and form the granulation tissue collagen and other connective tissue components to strengthen the dermis as an attempt to repair the damaged connective tissue. Not all of these new capillaries eventually develop into functional blood vessels. When the mature granulation tissue has eventually formed, the whole cellular network and the density of blood vessels were reduced (Couffinhal *et al*., 2011).

In some studies on UVB, the skin changes after exposure to 290-320 nm UVB have produced erythema, vascularity per permeability, dilution of the blood vessels in the dermis, dermal edema and hyperplasia of the epidermis. The vascular changes after exposure to UVB indicate that the skin blood vessels play an important role in mediating photo damage. The vascular and lymphatic systems form a dense network in the dermis of the skin. The blood vessels function in the maintenance of body temperature, supply of oxygen, nutrients and hormones and transportation of inflammatory cells throughout the body, which plays a role in organogenesis and tissue maintenance (Sawane and Kajiya, 2012).

Repeated blood vessel leakage caused by chronic UV radiation can inhibit blood vessel repair, as with intrinsic aging and photo aging, as well as damage to the function of blood vessels. These phenomena result in a reduced oxygen supply to the peripheral tissues and chronic inflammation (Sawane and Kajiya, 2012).

The main effect of UV radiation is DNA damage, inflammation and immunosuppression. This effect is related to the production of reactive oxygen species (ROS). ROS are very dangerous to other molecules and promote chain reactions that damage biomolecules quickly, such as telomere shortening, mitochondrial damage and membrane degradation. Oxidative structural and enzymatic proteins are initial characteristics of angiogenesis (Fig. 3) (Bosch *et al*., 2015).

UV radiation induces pro-inflammatory genes and inflammation is an important mediator of photo aging and photo carcinogenesis. Inflammatory mediators are released from keratinocytes, fibroblasts, tumor cells,

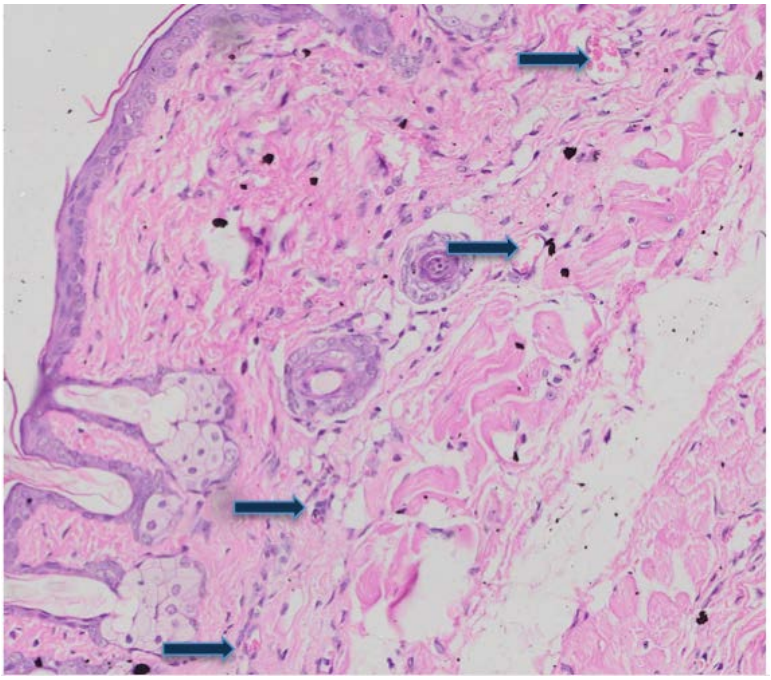

Fig. 2: Histopathology showing the extent of Angiogenesis; Vascularization (arrows); Hematoxylin eosin staining. 400X magnification

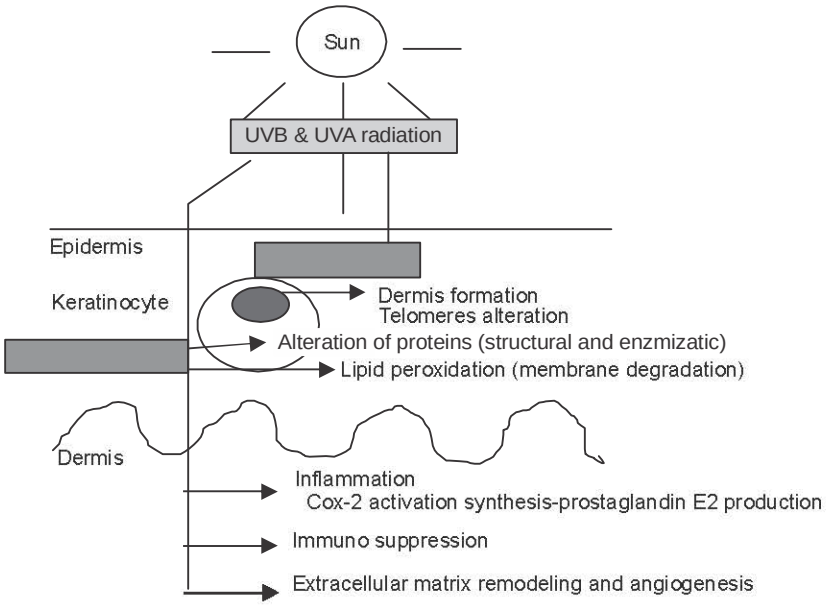

Fig. 3: Harmful effects of ultraviolet (UV) radiation in the skin (Bosch *et al*., 2015)

leukocytes and the endothelial lining of blood vessels. The mediators include the plasma (bradykinin, plasmin and fibrin), mediator lipids (prostaglandins, leukotrienes and platelet activating factor) and inflammatory cytokines [interleukin-1 (IL-1), IL-6 and tumor necrosis factor (TNF) -α]. Lipid mediators, COX-2 (cyclooxigenase-2) and prostaglandin E2 (PGE2), are also activated by ROS. The inflammatory processes that occur after UV exposure trigger skin angiogenesis (Bosch *et al*., 2015).

Angiogenesis is the growth of new blood vessels from preexisting blood vessels to the area of hypoxia. The formation of new capillaries during angiogenesis increases

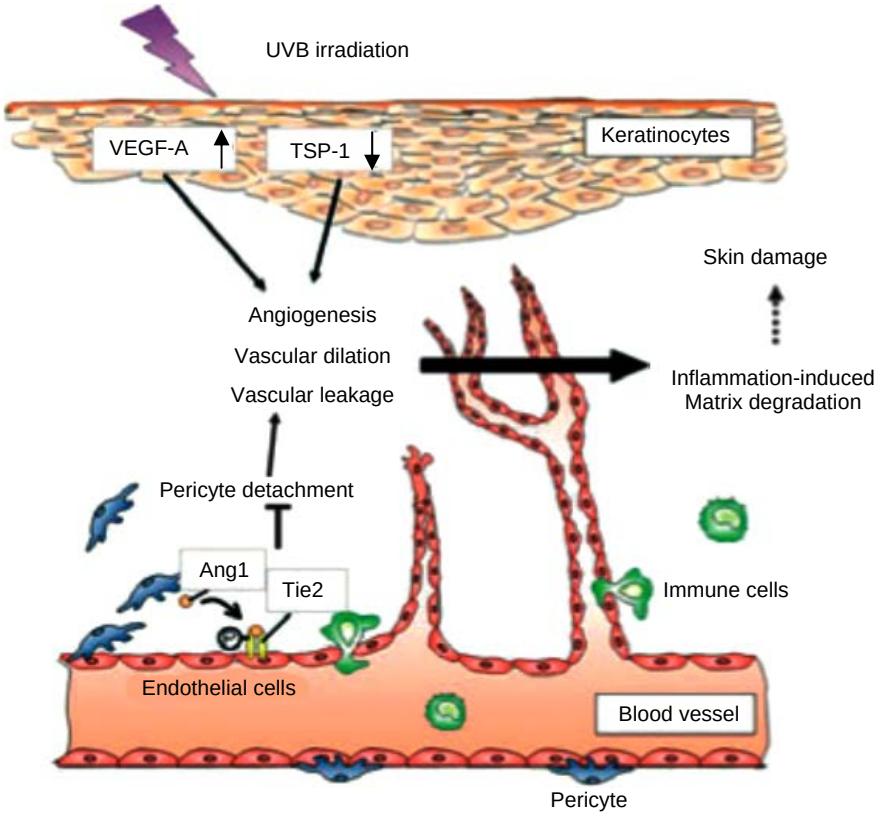

Fig. 4: Molecular Mechanism resulting from UVB angiogenesis and vascular changes in skin. The down regulation of thrombospondin-1 (TSP-1) and the upregulation of vascular endothelial growth factor (VEGF)-A in keratinocytes acted as inducers of angiogenesis molecules. Additionally, UVB radiation caused widening blood vessels and leakage, which caused the infiltration of immune cells, including neutrophil recruitment, which resulted in the degradation of the extracellular matrix via the secretion of matrix metalloproteinases (Sawane and Kajiya, 2012)

vascular permeability, extracellular matrix degradation and the proliferation and migration of the endothelium. Although the normal blood vessels in the skin are constant, except for the hair cycle, angiogenesis in the skin has a quick start in pathological conditions, such as in wound healing and inflammation. Angiogenesis induced by acute UVB radiation is accompanied by infiltrating leukocyte elastase into the skin, exacerbating the degradation of elastin. Radiation UVB to human skin showed erythema, epidermal hyperplasia and vascular hyper permeability and dermal edema. Studies have indicated that angiogenesis is a major mediator of skin damage caused by UVB (Sawane and Kajiya, 2012).

Angiogenesis can be used as a benchmark for inflammatory markers. Several studies have shown that angiogenesis and the inflammatory process have a close connection. The blood vessels at the site of inflammation were found to exist in large quantities and were hyper permeable to maintain blood flow to meet the needs of an increased metabolic network (Zhang *et al*., 2004).

Intrinsic skin aging can be observed as the reduction of the microvasculature of the skin, causing decreased skin temperature and a decreased nutrient supply, which can cause thinning of the nail plate and skin. Several studies have reported that the aging process is comparable to a decrease in skin vascularization in sun-exposed areas as well as areas that are rarely exposed, such as the buttocks, but a significant decrease in vascularization can be found in areas of the skin frequently exposed to the sun (photoaged skin). With intrinsic skin aging, there were no significant differences in vascular density, although a decrease in blood vessel size was found between young skin and old skin. In one study on papillary dermis skin photo aging, donors' aged skin showed significant differences in the size of blood vessels and the number of blood vessels when compared to young skin (Ichihashi *et al*., 2009).

Acute UV exposure stimulates angiogenesis through vascular endothelial growth factor (VEGF) upregulation via the MEK-ERK pathway activation and down regulation of thrombospond in-1 (TSP-1) through activation of the P13K-Akt pathway in the human epidermis. However, in chronic exposure, decreased blood vessels have been found on skin damaged by UV (Ichihashi *et al*., 2009).

UVB radiation induces VEGF-A in the basal layer of the epidermis, whereas the expression of TSP-1 is downregulated in the epidermis. Thus, the down regulation of TSP-1 and the upregulation of VEGF-A appear to contribute to a molecular level trigger of the induction of angiogenesisin the skin due to UVB radiation (Sawane and Kajiya, 2012).

UVB induces an increase in VEGF-A leakage and the widening of blood vessels, which result in the infiltration of inflammatory cells, including neutrophil recruitment, leading to the degradation of the extracellular matrix via the secretion of matrix metalloproteinases. Together, these results indicated that the result of UVB exposure is skin damage and wrinkle formation and preventing the occurrence of angiogenesis could be a new approach to prevent photo damage to the skin (Fig. 4) (Sawane and Kajiya, 2012).

Mangosteen pericarp extract oxidizes the yellow resin-rich xanthone (Akao *et al*., 2008). The mangosteen peel extract was found to contain 95% xanthone and it also contains isoflavones, tannins and flavonoids (Priya *et al*., 2010). In addition, mangosteen pericarp also contains anthocyanins (Pradipta *et al*., 2009). Xanthones are a group of yellow pigments that are found in some families of higher plants, fungi and moss plants. Mangostin is a major xanthone element and it is found in mangosteen plants (Peres *et al*., 2000). Xanthones are polyphenolic compounds with a chemical structure that contains a tricyclic aromatic ring. This structure has biologically active molecules such as antioxidants and anti-inflammatory, antibacterial and anticancer factors (Nakagawa *et al*., 2007). The antioxidant molecules of the skin of mangosteens are very strong as catchers of free radicals (radical scavenging) (IPB, 2009). Therefore, the antioxidant properties of mangosteen pericarp extract can inhibit the formation of ROS, which can further inhibit the destruction of collagen by exposure to UVB rays and increase collagen in the dermis.

A study conducted by Ericson (2014) using a solution of mangosteen pericarp extract with a concentration of 95% showed significant differences in MMP-1 and the amount of collagen in the dermis. In an experiment using extracts of the mangosteen pericarp in a laboratory of Pharmacognosy at the University of Hasanuddin, the maximum dose that could be made in drops was a concentration of 50% so that the mangosteen pericarp extract dose used was 2 mg/g of mouse mass.

Until now, research on the anti-inflammatory activity of the mangosteen pericarp has only been performed in an *in vitro* setting. The research results suggested that the compound that had anti-inflammatory activity was γ-mangostin. γ-Mangostin inhibits the conversion of arachidonic acid to PGE-2, which causes the inhibition of the microsomal cyclooxigenase pathway. This compound is able to inhibit the activity of the enzymes COX-1 and COX-2 (Nakatani *et al*., 2002).

In our study, we found the highest amount of vascularization in the ethanol group, so it is suspected that ethanol caused skin irritation. The amount of vascularization in the mangosteen group was found to be lower than in the UVB group and the control and ethanol groups, which was statistically significant. This was consistent with reports in the literature that showed an anti-inflammatory role for extracts of mangosteen pericarp in dampening the inflammatory process caused by exposure to UVB.

We can conclude that a mangosteen pericarp extract concentration of 50% (a dose of 2 mg/g of mouse mass) can be used as a marker of inflammation in mouse skin that was exposed to UVB. For further study, the use of 96% ethanol can be reconsidered because, based on our results, we found the highest amount of vascularization in the ethanol group, indicating the highest inflammatory response.

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