

Original article**The Ethyl Acetate Extract of Mangosteen Peel Cream Attenuates Ultraviolet B Radiation-Induced Apoptotic Cell Death Via Antioxidant Effect By Regulation TNF- α and Caspase 3 in Guinea Pig Skin**Pasid Harlisa¹, Harijono Kario Sentono², Bambang Purwanto³, Paramasari Dirgahayu⁴, Soetrisno⁵**Abstract**

Background: UV radiation induced ROS cause DNA damage mainly due to photoaging. Lipid peroxidation due to UV exposure also produces non-toxic metabolite compounds namely malonyldialdehyde (MDA). Upregulation of TNF- α is key to the initial response to ultraviolet B (UVB) exposure by keratinocytes. The basal cell layer is the most affected undergoes to apoptosis and can be detected through caspase 3 which is the main executor of apoptosis. Mangosteen peel (*Garcinia mangostana* Linn.) contains xanthone known as alpha mangosteen, the most attention today is as an antioxidant. The purpose of this study is to determine the antioxidant effects of ethyl acetate extract in mangosteen peel cream on MDA levels, anti-inflammatory effects on TNF- α levels and anti-apoptosis effects on caspase 3 levels, tested in guinea pig skin exposed to UVB rays. **Methods:** This study uses a post test only control group design using female guinea pig (*Cavia porcellus*) who obtained, cared, and treated at the Wates Veterinary Center, Yogyakarta. Consisting of 20 animals divided randomly into 4 treatment groups. P1: normal group, without any treatment. P2: exposed to UVB light. P3: exposed to UVB light and given base cream. P4: exposed to UVB light and given 12% extract mangosteen peel cream. MDA were analyzed by the TBARS method (nmol/ml). Levels of TNF- α epidermis were obtained by the TNF alpha Elisa antibodies Kit (pg/ml). Levels of caspase 3 epidermis were obtained by the caspase antibody 3 Elisa Kit (pmol / L). **Results:** Post Hoc statistical analysis showed that MDA serum levels of P4 group was significantly lower compared to P2 group (p<0.01), TNF- α epidermis levels of P4 group was significant lower compared to P2 group (p<0.001) and caspase 3 epidermis levels of P4 group was significant lower compared to P2 group (p<0.01). **Conclusion:** Administration of 12% dose ethyl acetate extract of mangosteen peel cream is proven to significantly lower MDA levels, TNF- α levels and Caspase-3 levels in the epidermal tissues of guinea pig skin exposed to ultraviolet B light.

Keyword: The Ethyl Acetate Extract Of Mangosteen Peel Cream; antioxidant; UVB; ROS; MDA; TNF- α ; caspase 3.

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Introduction

Located in the equator, the formation of free radicals is most often caused by the induction of ultraviolet (UV) rays from the sun that cause various changes

to the skin such as inflammation, apoptosis and skin aging, ^{1,2}UVR-induced ROS cause DNA damage or poor DNA repair in intrinsic and extrinsic aging, mainly due to photoaging. Repeated DNA damage to keratinocytes and fibroblasts leads to apoptosis

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or cell aging. Apoptosis is a protective factor against UVB damage to epidermal keratinocytes, which aims to eliminate pre cell malignant. Upregulation of TNF- α is key to the initial response to ultraviolet B (UVB) exposure by keratinocytes and becomes an important component in the inflammatory cascade and apoptosis events in the skin. Keratinocyte epidermis primarily expresses TNFR1 (surfacereceptor TNF α) which is a death receptor in the apoptosis process. The TNF- α bond in TNFR1 will trigger a series of intra-cellular events that act on transcription factors including NF κ B and AP-1 and are responsible for gene induction of various biological processes including extrinsic pathways of apoptosis. The basal cell layer is most affected because it is the part that first undergoes apoptosis immediately after UV exposure and can be detected through caspase 3 which is the main executor of apoptosis^{5,7}. The enzyme Caspase-3 is a member of the endoprotease family that regulates inflammatory signaling and apoptosis processes, coordinating and destroying cell structures such as DNA fragmentation or degradation of the cytoskeleton protein⁸.

Lipid peroxidation due to UV exposure also produces non-toxic metabolite compounds namely malonyldialdehyde (MDA) that can be detected in serum or blood⁹.

Mangosteen peel (*Garcinia mangostana* Linn.) contains xanthon known as alpha mangosteen has a lot of benefits. Its use that gets the most attention today is as an antioxidant^{10,11,12}. Research proves that alpha mangosteen has the potential as an antioxidant through the mechanism of warding off free radicals and the scavenger's ability against the ONOO⁻ group through nitration or electron donor^{12,13}. The purpose of this study is to determine the antioxidant effects of ethyl acetate extract in mangosteen fruit cream on MDA levels, anti-inflammatory effects on TNF- α levels and anti-apoptosis effects on caspase 3 levels, tested in guinea pig skin exposed to UVB rays.

Material and methods

Materials And Chemicals

Mangosteen fruit used in this study were obtained from Bogor botanical garden Indonesia. Alpha mangosteen with 98% purity were purchased from Sigma Aldrich (USA). DPPH was purchased from Sigma-Aldrich(USA), Quercetins were purchased from Sigma-Aldrich(USA). Dionex-UltiMate® 3000, spectrophotometer UV-Vis (Analytical Jena, specord 200). Ketamine 10% injection was obtained from Kepro B.V (Deventer, NL), TNF α and CASP3 Elisa kit were purchased from MyBioSource (San Diego, USA).

Animal Models And Research Procedure

This study uses a post test only control group design using female guinea pig (*Cavia porcellus*) as experimental animal with inclusion criteria of aged 2-4 months, weight 250-600 grams. Guinea pigs were obtained, cared, and treated at the Wates Veterinary Center, Yogyakarta, consisting of 20 animals divided randomly into 4 treatment groups.

P1: normal group, without any treatment.

P2: induction group, exposed to UVB light

P3: negative control group, exposed to UVB light and given base cream.

P4: treatment group, exposed to UVB light and given 12% extract mangosteen peel cream

Animal Conditioning and Adaptation

Animals were conditioned for seven days before treatment. All Guinea pigs are given standard feedings, water spinach and given aquadest for drinks during the adaptation period.

UVB exposure to animals

To all groups except P1, after hair removal, the dorsal skin of the guinea pigs was exposed to 65 mJ / cm² UVB radiation with a source of Exo Terra Reptile UVB150-25 W Desert Terrarium Bulb that has been measured using UV meter to ensure its power, 3 times a week, so that the total UVB received for 4 weeks were 780 mJ / cm² ¹⁴. After 28 days of treatment, blood sampling for serum MDA examination and euthanasia were conducted to take a sample of guinea pig's back skin tissue for ELISA examination.

The treatment of experimental animals in this study has fulfilled the 3R principals in accordance with the provisions of the National Centre for the Replacement, Refinement and Reduction of Animal in Research (NC3Rs) and has received approval from the health research ethics committee of Universitas Sebelas Maret Surakarta Number 011/UN27.06/KEPK/EC/2020.

Mangosteen peel extract cream

Mangosteen fruits were obtained from mangosteen plantations in Bogor area. Extraction of mangosteen peel (*Garcinia mangostana* L.) was conducted at the pharmacognosy laboratory pharmaceutical biology department of the Faculty of Pharmacy, Gajah Mada Yogyakarta University. Ethyl acetate extract of mangosteen peel (*Garcinia mangostana* L.) is obtained by extraction of maceration with ethyl acetate solvent protocol ¹⁵.

The α -mangosteen contains of the ethyl acetate extract of mangosteen peel determined used

α -mangosteen standard quality High Performance Thin Layer Chromatography /HPTLC α -mangosteen method and antioxidant activity with the DPPH method^{16,17}. Antioxidant capacity was determined using quercetin as a gold standard.

Formulation of mangosteen peel extract cream is made in accordance with the modification of formulation cream in the preliminary study with a 12% dose of mangosteen peel extract^{18,19}.

Table 1. Formulation of mangosteen peel extract Cream

Material	(%)
Extract	11,94
Karbopol	1,19
Cetyl Alcohol	2.19
Stearic acid	3.8
Isopropyl Miristat	7,96
Glyceril monostearate	2.39
Propylene Glycol	7.96
TEA	3.18
Metyl Paraben	0.12
Propyl Paraben	0,04
Water	57,76
Span	0.80
Tween 80	0.48

Determination of alpha mangosteen invitro levels

Mangosteen 0.3973mg/ml

Table 2. absorbance gold standard percentage of alpha mangosteen

No.	Volume of administration (μL)	Mangosteen Weight(μg)	Final Concentration (μg/mL)	Abs
1	4	1.5892	1.5892	13590.18
2	6	2.3838	2.3838	19904.22
3	8	3.1784	3.1784	23832.17
4	10	3.9730	3.9730	27470.25

Table3. Percentage of α -mangosteen content in mangosteen peel extract

No.	Sp	Weight (μg)	Vol. (μL)	Vol. Test (μL)	Fp	Abs	Power Level (μg/mL)	Level %	Average (%)
1	Extract 1	10060	10000	2	5000	16513.93	1.9641	97.6202	
2	Extract 2	10020	10000	2	5000	15349.56	1.7611	87.8782	88.62
3	Extract 3	10210	10000	2	5000	14660.13	1.6409	80.3554	

Determination of invitro antioxidant capacity

Gold Standard of Quercetin 10.32mg/1000ml (10ppm) Control : 0.421

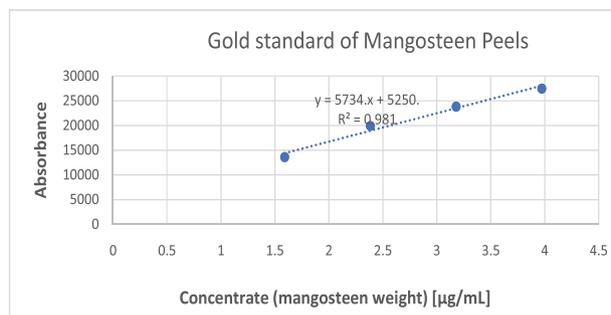


Figure 1. Calibration curve of α -mangosteen with ranged concentration (1,5-4 μg/ml)

Table 4. Inhibition Concentration from Quercetin Gold Standard Percentage

Quercetin (ppm)	Abs	Inhibition (%)	IC ₅₀
3	0.300	0.287	28.741
5	0.233	0.447	44.656
7	0.169	0.599	59.857
9	0.119	0.717	71.734

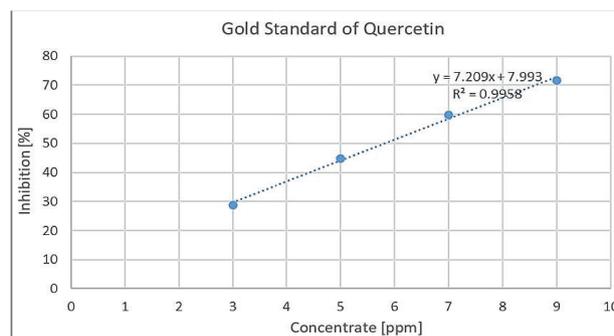


Figure 2. Antioxidant power VS gold standard of Quercetin levels curve

Sample of Mangosteen Peel Extract 10.02mg/100ml (100ppm)

Control : 0.421

Table 5. Percentage of inhibition and IC₅₀ from ethyl acetate of mangosteen peel extract

Sample (ppm)	Abs	Inhibition (%)	IC ₅₀
40	0.329	0.219	21.853
50	0.306	0.273	27.316
60	0.285	0.323	32.304
70	0.248	0.411	41.093
80	0.194	0.539	53.919
90	0.176	0.582	58.195
100	0.137	0.675	67.458

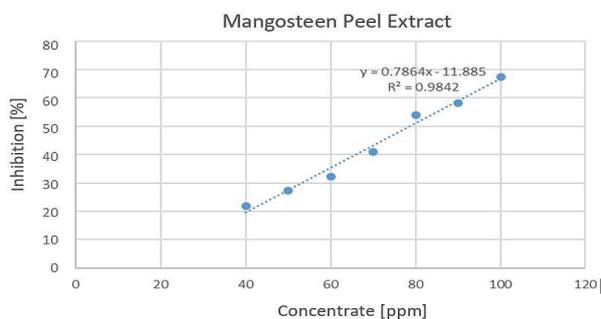


Figure 3. Antioxidant Power VS Ethyl Acetate Extract of Mangosteen Peel Levels Curve

Malondialdehyde (MDA)

As a result of the interaction between free radicals with lipid components of cell membranes, their levels can be used to measure ROS activities. Analyzed from blood serum performed through the retro-orbital plexus Guinea pig (*Cavia porcellus*) by the TBARS method (nmol/ml).

TNF-α epidermis

Cytokines appear when an inflammatory reaction occurs. Levels of TNF-α epidermis were obtained by examination from guinea pig's back skin tissue (*Cavia porcellus*) with the TNF alpha Elisa antibodies Kit (pg/ml).

Caspase 3 epidermis

It is the endoprotease that regulates the process of inflammatory signaling and execution in apoptosis events. Levels of caspase 3 epidermis were obtained by examination of samples from guinea pig's back skin tissue (*Cavia porcellus*) with the caspase antibody 3 Elisa Kit (pmol / L).

Ethical clearance: This study was approved by ethics committee of Universitas Sebelas Maret,

Surakarta, Indonesia.

Results

In vitro, the ethyl acetate extract of mangosteen peel used has α-mangosteen level of 88.62% (HPLC method) and obstruct scavenger radical activity belongs to the strong category (IC₅₀ 78.69405 μg/ mL).

All parameters in the P2 group induced with UVB show a significant increase in levels (table 6).

Table 6. On average, minimum and maximum levels of MDA, TNF-α, and caspase-3 and different tests across the group.

Group	P1	P2	P3	P4	p-value
Mean					
MDA	2,269±0.26	3,697±0.32	2,287±0.37	1,664±0.37	0,003*
Min.	2,058	3,191	1,724	1,324	
Max.	2,557	4,019	2,715	2,219	
TNF-α	8,831±0.85	19,770±2.89	12,055±1.16	5,592±0.74	0,000+
Min.	7,973	15,883	10,958	4,476	
Max.	9,933	23,419	3,979	6,473	
Caspase-3	0,106±0.04	2,512±0.50	0,526±0.06	0,070±0.01	0,001*
Min.	0,072	2,085	0,474	0,063	
Max.	0,167	3,087	0,626	0,089	

+anova test *test kruskal wallis

Effect of Ethyl Acetate Extract Mangosteen (Garcinia mangostana L) Peel Cream on MDA levels

Kruskal Wallis test was conducted to assess whether or not there was any difference because shapiro wilk's test were abnormally distributed (p<0.05). The result of the difference on MDA levels can be seen in table 6, where the P4 group has the lowest MDA level, which is 1,664 ±0.37 nmol / mL and the highest is induction group P2, which is 3,697± 0.32 nmol / mL. The statistical test results get a value of p = 0.003 (p < 0.01) which means there is a significant difference on MDA levels between different treatment groups.

Effect of ethyl acetate Extract mangosteen (Garcinia mangostana L) Peel Cream Against TNF-α

The results of the Anova Test to assess the difference in TNF-α levels can be seen in table 6, where the lowest is in the P4 group, which is 5,592 ±0.74 pg / dL; while the highest is the induction P2 group, which is 19,770 ±2.89 pg / dL. The results of Anova statistical tests indicated that there are significant differences in TNF-α levels between various treatment groups.

Post Hoc Mann Whitney Test results:

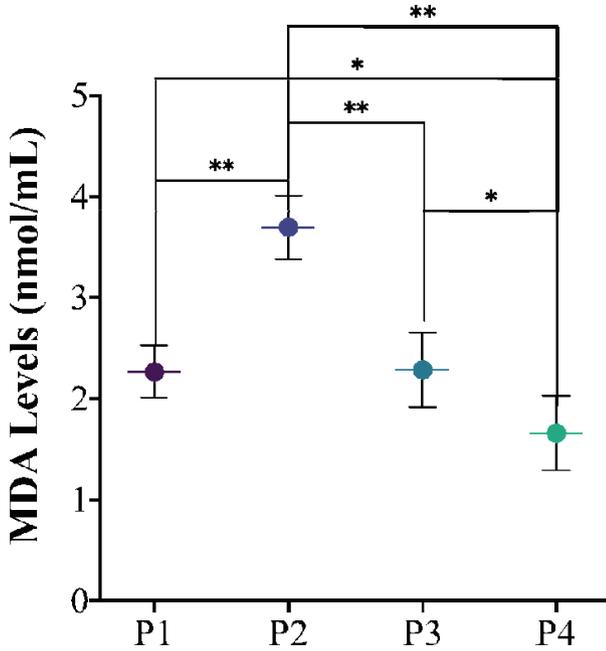


Figure 4. MDA levels - Mann Whitney test

*p<0.05

**p<0.01

Post Hoc LSD test results:

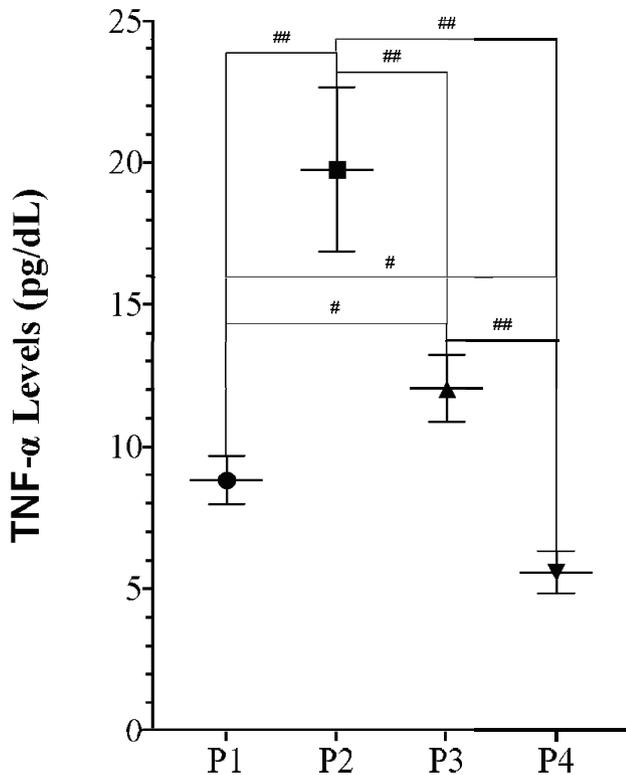


Figure 5. Levels of TNF- α - LSD test

#p<0.01

##p<0,001

Effect of Ethyl acetate extract of mangosteen (Garcinia mangostana L) peel Cream On Caspase levels 3

The result of the difference in caspase 3 levels can be seen in table 4, where the lowest is in group P4, which is 0.070 ± 0.01 pmol / L, the highest is induction P2 group, which is 2.512 ± 0.50 pmol / L. The statistical test results obtained p values of = 0.001 ($p < 0.01$) which means that there is a significant difference in caspase 3 levels between various treatment groups.

Post Hoc Mann Whitney test results:

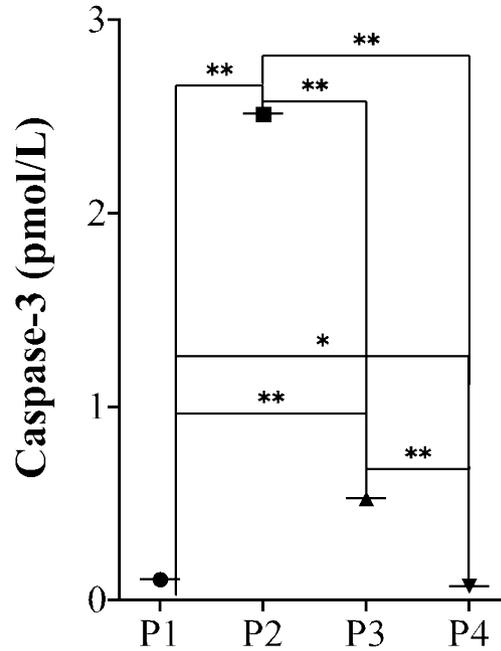


Figure 6. Caspase-3 levels - Mann Whitney's test

*p<0.05

**p<0.01

Discussion

The Administration of of ethyl acetate extract of mangosteen peel cream lowers blood MDA levels

The results of this study indicated that the induction of UVB light with a total of 780 mJ / cm on the back skin of guinea pigs (P2) can cause a significant increase in malon dialdehyde (MDA) levels compared to the normal group of P1. It was shown that the dose of uvb exposure used could induce the occurrence of oxidative stress. Malon dealdehyde is a marker that is often used to assess the occurrence of oxidative stress. MDA is the primary product of high lipid peroxidation MDA levels are markers of tissue damage caused by to free radicals^{20,21}.

High MDA levels occur because UVB can trigger the formation of ROS through the role of the enzyme catalase and in the regulation of increased synthesis

of nitric oxide synthase (NOS) in keratinocytes. The enzyme catalase is known to degrade hydrogen peroxidation through a reaction of $2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$ through the activity of the catalysis process. UV also decreases protein kinase C expression so that ROS production increases²². Increased production of Reactive Oxygen Species (ROS) in the skin because of UV causes oxidative stress in the body that has a target of damage to all types of biomolecules such as proteins, lipids, and DNA²³. This is in line with previous research showing that UVB exposure in animal skin attempts to induce the occurrence of oxidative stress characterized by a meaningful increase in blood MDA level^{24,25}. Fig. 4 showed the comparison of MDA content in guinea pig serum after administering a dose of 12% ethyl acetate extract of mangosteen peel cream (P4), it shows that the MDA content was generally decreased compared to induction P2 group. These data suggest that the administration of the dose 12% ethyl acetate extract of mangosteen peel cream results in an effective change in inhibition of the lipid peroxidation in guinea pig. The reduced MDA levels due to its ability as an antioxidant correspond to the results in table 5, where the potential of radical scavenger of ethyl acetate extract mangosteen peel used 78.69405, 77 indicates a strong category²⁶. Previous research proved, the administration of ethanol extract mangosteen in solution form can reduce MDA levels significantly in white male rats (*Rattus norvegicus*) wistar strain induced with e-cigarette smoke²⁷. Other studies also obtained results in the form of a significant reduction in MDA levels in wistar rats who consumed ethanol after being given ethanol extract of mangosteen peel²⁸.

The administration of ethyl acetate extract of mangosteen peel cream lowers TNF- α

Elevated levels of TNF- α are an early response to epidermal keratinocytes due to UVB exposure and are an important component in the inflammatory cascade of the skin²⁹. The results of this study showed that the total dose of UVB light for 4 weeks of 780 mJ / cm in the induction group (P2) resulted in a significant increase in TNF- α levels compared to the normal P1 group.

Inflammatory responses due to UVB exposure include increased pro-inflammatory cytokines, tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), IL-8 and IL-6, all of which lead to an increased risk of skin damage. Tumor necrosis factor- α (TNF- α) plays an important role in photo damage and photoaging events. UV radiation induces and activates the transcription factors AP-1 and NF- κ B. The activation of NF- κ B can be triggered by TNF- α and IL-1 β as well as increase

reactive oxygen species (ROS)^{31,32}. The secretion of TNF- α from keratinocytes causes the expression of adhesion molecules that result in inflammatory cells arriving and secreting the enzymes elastase and collagenase, resulting in damage and aging of the skin. It also results in apoptosis, lymphocyte activation, and skin hyperproliferation disorders³².

Research on the effects of UVB on TNF- α tissues on the back skin of mice conducted by A-Rang Im et.al, indicated that there was an increase in the expression of TNF- α mRNA after exposure to UVB light determined using reverse transcription-quantitative PCR³⁰. Several studies in vitro of UVB's effect on TNF- α levels also provided corresponding results where there was an increase in TNF- α levels after UVB exposure doses of 128 J/cm² in human skin keratinocyte cell cultures as measured by elisa method³³. Likewise, there was a significant increase in TNF- α levels in human epidermal keratinocyte cultures (HEKa cells) after uvb exposure dose h 200 mJ/cm² for 10 minutes³⁴.

Fig. 5 showed the comparison of TNF- α levels of guinea pigs' epidermal tissue after the administration of the 12% dose ethyl acetate extract of mangosteen peel cream (P4), it shows that the TNF- α level was generally decreased compared to induction group P2. These data suggest that the administration of the 12% dose ethyl acetate extract of mangosteen peel cream results as effective changes in inhibition, excretion, mediator, pro inflammation TNF- α on guinea pigs' epidermal tissue. These results are in line with the role of α -mangosteen on the skin of the mangosteen fruit which can inhibit the pro-inflammatory mediators IL-8 and TNF- α ³⁵. Table 3. indicated the alpha mangosteen (α -mangosteen) contains in extract mangosteen peel are 88.62%. Alpha mangosteen (α -mangosteen) are the polyphenol compounds of the phenolic group mangosteen peel extract that have 3 phenol rings with one or more hydroxyl substituents so as to prevent oxidative stress by capturing free radicals in ROS formed due to UV exposure at the beginning of the above sequence of events, so that the secretion of TNF- α can be suppressed³⁶. Research shows the α -mangosteen contains in the skin of mangosteen fruit can suppress the expression of inflammatory mediators TNF- α and IL-6 induced with lipopolysaccharides³⁷. Barriers to activation of MAPK, NF- κ B, AP-1 and reduced expression of cytokine genes were also observed in other studies using α -mangosteen therapy³⁸.

Study in mice pre-eclampsia model treated with mangosteen peel extract per sonde, obtained the result of a significant decrease in TNF- α expression on the examination of IHC³⁹. Other research also found reduced inflammation in human macrophages

exposed to macrophage-conditioned media because the ability of mangosteen peel extract inhibits the activation of MAPK and NF- κ B which further leads to reduced levels of TNF- α ⁴⁰.

The administration of ethyl acetate extract of mangosteen peel cream lowers caspase levels 3

Exposure to UV light on epidermal keratinocytes can cause apoptosis. Caspase-3 is known as the executor of apoptosis and is often used to observe the apoptosis process in cells due to its role in DNA fragmentation⁵. The results of this study showed that the total dose of UVB light for 4 weeks of 780 mJ / cm resulted in a significant increase in caspase-3 levels (P2) compared to the P1 group. This is according to previous studies where UVB exposure led to the formation of activated caspase 3 seen on the examination of Western blots⁴¹. Other studies also found caspase-3 expression in the epidermis tissue of the skin of the backs of mice that increased significantly post-UVB exposure with a dose of erythema for at least 30 minutes⁴². An increase in Caspase3 mRNA also occurred after exposure to UVB radiation in Sprague Dawley Rats⁴³.

Fig. 6 presented caspase 3 levels in the P4 group decreased significantly compared to the induction P2 group after the administration of the 12% dose ethyl acetate extract of mangosteen peel cream, showing anti-apoptosis effects. A potential mechanism that can explain this phenomenon is the presence of phenolic group polyphenol compounds (α -mangosteen) mangosteen peel extract has 3 phenol rings with one or more hydroxyl substituents that can inhibit the work of caspase-3 through anti-inflammatory and antioxidant properties possessed by capturing free radicals in ROS formed due to exposure to UV rays³⁶. Evidence suggests that oxidative stress can induce mitochondrial dysfunction which further triggers apoptosis resulting from the release of cytochrome c. Cytochrome c interacts with the apaf-1 and caspase 9 factors and further activates the apoptosis-related caspase. In particular caspase 3 causes DNA fragmentation and apoptosis⁴⁴. The DNA fragmentation is a transient characteristic of the dying cell, will stain by Terminal dUTP nick-end labeling (TUNEL) allows researchers to identify DNA fragmentation at the single-cell level⁴⁵. Some inflammatory mediators are involved in the activation of apoptosis pathways such as TNF- α , Fas ligand and MMP-9^{6,46,47}.

The previous studies about protective effect of mangosteen peel extract against oxidative stress of SK-N-SH cell cultures induced with hydrogen peroxide (H₂O₂) and polychlorinated biphenyls (PCBs) proved the visible cytoprotective effects of cell viability and reduced ROS levels and reduced caspase-3 activity⁴⁸. Recent research has found that

mangosteen pericarp water extract (MPW) can inhibit neurotoxicity and ROS production in mouse cortical cell primary cultures triggered by A β (25–35) or excitatory amino acids. MPW also inhibits the activation of caspase 3 and DNA fragmentation in cells treated with A β (25–35)-or N-methyl-D-aspartate demonstrating its anti-apoptosis ability⁴⁴.

Recommendation

It is the first in vivo study of topical applications of mangosteen peel extract, which demonstrated its beneficial effects against UVB irradiation. Mangosteen peel extract cream possesses antioxidant, anti-inflammatory and antiapoptotic effects which protect against acute UVB exposure.

Further studies need to be undertaken to determine the number of cells undergoing apoptosis using various examinations against proinflammatory cytokines and growth factors involved in photoaging processes such as factors activation protein-1 (AP-1) and nuclear factor-B (NF- κ B) especially in long-term UVB exposures, as well as determining the number of cells undergoing apoptosis using TUNEL assay.

Conclusion

Administration of 12% dose ethyl acetate extract of mangosteen peel cream is proven to significantly lower MDA levels, TNF- α levels and Caspase-3 levels in the epidermal tissues of guinea pig skin exposed to ultraviolet B light.

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Conflict of interest

All authors declare that they do not have any conflict of interest in the present study.

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Editing and approval of final draft : Paramasari Dirgahayu, Soetrisno, Brian Wasita

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