International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2019): 7.583

Administration of Papaya (Carica Papaya Linn) Seeds Extract Cream 40% Decrease MMP-1 Expression and Increase Dermal Collagen of Male Wistar Rats (Rattus Norvegicus) Exposed to UV-B Ray

Dyah Ratih Pramesti [1], Anak Agung Gde Wiraguna[2], A.A.A.N. Susraini[3]

[1]Magister Biomedical Science, Postgraduate Program, University of Udayana
email: *drratihdyah[at]gmail.com*

[2]Department of Dermatology and Venerology, University of Udayana,

[3]Department of Pathology Anatomy, University of Udayana

Abstract: ***Background and purpose: Exposure to UV-B may induce free radicals in the form of ROS, which cause oxidative stress on the skin. This will trigger the activation of AP-1 (activator protein-1) which will induce matrix metalloproteinase (MMPS) especially MMP-1 so that it can degrade dermal collagen and cause photo aging. Papaya seeds contain antioxidant compounds namely flavonoids (quercetin), tannins, and phenols. Flavonoids can prevent an increase in MMP-1 by inhibiting phosphorylation of MAPK (mitogen-activated protein kinases). The purpose of this study was to proof whether giving papaya seeds extract cream 40% decrease MMP-1 expression and increase the amount of dermal collagen in male Wistar rats exposed to UV-B ray. Methods: Experimental research was conducted with a randomized posttest only control group design. Subjects were white male Wistar rats (Rattus norvegicus). 36 animals were divided into two group (n=18 individuals). The control group (mice were given a cream base and were exposed to UV-B). The treatment group (rats were given a 40% Bali papaya seed extract cream and exposed to UV-B rays). The total UV-B dose given was 840mJ/cm^2 for 4 weeks. After 28 days of treatment, MMP-1 levels were examined using the immunohistochemistry method from average of three microscope field of view while the amount of dermal collagen were examined digitally by calculating the average percentage of three microscope field of view of collagen pixels that appear in the histopathological preparations using Picro Sirius Red staining. Results: The comparative analysis results of independent sample t-test showed the mean MMP-1 expression of the control group was higher (27,46 ± 4,29 %) compared to treatment group (9,72 ± 3,46%) (p<0,001). While the mean amount of collagen in the control group was lower (57,82 ± 3,05%) compared to the treatment group (82,40 ± 2,62 %) (p<0,001). Conclusion: Based on the results of this study it can be concluded that the cream of Bali papaya seed extract 40% (Carica papaya linn) decreased MMP-1 expression and increased of dermic collagen on skin of male Wistar rats (Rattus norvegicus) exposed to UV-B rays.***

Keywords: cream of Bali papaya seed extract, MMP-1 expression, collagen dermis, ultraviolet-B

1. Introduction

Every living thing will surely experience an aging process, but the aging process does not just happen but rather gradually. Initially it might not show any complaints from the aging process, but along with increasing age and the presence of risk factors, symptoms and complaints will appear.

There are several factors that cause the aging process, generally divided into two factors, which are external factors and internal factors. Some external factors that cause the aging process are unhealthy lifestyles, stress, wrong habits, unhealthy diets, poverty (Pangkahila, 2017), environmental pollution, cigarette smoke, and sunlight / ultra violet (Baumann and Saghari, 2009). Internal factors of the aging process are free radicals, the process of glycosylation, methylation, apoptosis, changes in hormone levels, genetic, and decreased immune system (Pangkahila, 2017).

Lately, there have been many uses of chemicals substance to protect the skin from the dangers of UV radiation. One of them is a polyphenol compound from plants. The use of this material aims to prevent, restore, slow down the adverse effects of UV radiation on the skin. The photo protective effect on the skin from polyphenols is obtained from its ability as an anti-inflammatory, antioxidant and mechanism of Deoxyribose Nucleic Acid (DNA) repair (Nichols and Katiyar, 2010).

Polyphenols are a group of chemicals found in plants, characterized by the presence of more than one phenol unit per molecule. Phenolic in human food consists of phenolic acid, flavonoids and tannins. The most studied polyphenols are the flavonoid group, which is divided into 6 subclasses namely flavones, flavonols, flavafonolols, isoflavones, flavanols, anthocyanins (Kim et al., 2013).

Indonesia is a country which have various kinds of plants, such as papaya (Carica papaya linn), papaya is a type of tropical plant that has antioxidant activity. Recent findings are beginning to lead to the use of antioxidants derived from papaya seeds, both oral and topical, which can fight aging on the skin, but there is still very little publicity about this.

Papaya seeds contain antioxidants phenol, flavonoids, tannins, terpenoids, DPPH (2,2-diphenyl-1-picrylhydrazyl), carotenoids, B, vitamin C, tocopherol, and zinc (Dada, 2016). Previous studies have shown that 2-month-old raw papaya seeds have better antibacterial ability than 5-month-old ripe papaya seeds (Martiasih et al., 2014). The levels of flavonoids and phenols in raw papaya seeds are higher than ripe papaya and very mature ones (flavonoid and phenol levels in raw papaya seeds (unripe) 0.89% and 0.16%, hard ripe papaya seeds 0.83%, and 0.13%, seeds papaya is very ripe (very ripe) 0.71% and 0.01%) (Chukwuk et al., 2013). Based on this, raw papaya seed extract was selected as an ingredient in this study. To ensure the concentration of papaya seed extract cream which is the most effective in decreasing MMP-1 expression and increasing the amount of collagen dermis of male Wistar rats (Rattus norvegicus) exposed to UV-B ray, a 4-week preliminary study was conducted at the Integrated Biomedical Laboratory Unit of the Udayana University FK. By using doses of 30%, 40% and 50% and the results obtained papaya seed extract cream 40%is the most effective in decreasing expression of MMP-1 and increasing dermal collagen of male Wistar rats exposed to UV-B ray.

The purpose of this study was to proof whether giving papaya seeds extract cream 40% decrease MMP-1 expression and increase the amount of dermal collagen in male Wistar rats exposed to UV-B ray.

2. Method

Thirty-six white male Wistar rats (Rattus norvegicus) were enrolled in this study. Animal Ethics Committee of the Faculty Veterinary Science, University of Udayana had approved this study. The subjects were selected to undergo the initial screening for inclusion criteria: healthy male white rats (Rattus norvegicus), age: 10-12 weeks, weight: 300-350g.

Subjects were randomized into two groups. Control group receive a cream base and were exposed to UV-B and Treatment group receive a 40% Bali papaya seed extract cream and exposed to UV-B rays. The total UV-B dose given was 840mJ/cm^2 for 4 weeks

After 28 days of treatment, MMP-1 levels were examined using the immunohistochemistry method while the amount of dermal collagen were examined digitally by calculating the percentage of collagen pixels that appear in the histopathological preparations using Picro Sirius Red staining.

3. Result

The statistical analysis results showed that the average percentage of MMP-1 expression in treatment group was significantly lower (9.722± 4.29 %) compared to control group (27.467 ± 4.29 %). The mean difference was 17.74 ± 1.3(15.10- 20.38). The independent T-test showed significant difference in the average expression MMP-1 between two groups (Fig 1).

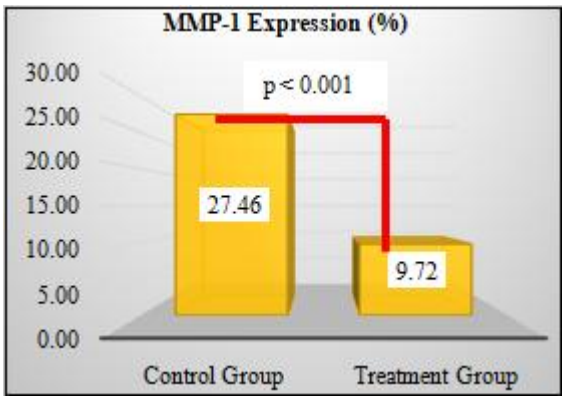


Figure 1: Quantitative comparison of MMP-1 expression between groups, graphs illustrate mean, p value was calculated using the Independent Sample T test.

Meanwhile, the average percentage of collagen expression in treatment group (82.406 ± 2.62%) was higher than the control group (57.828 ± 3.05 %). Independent sample T test showed p<0.001, which means that there was significant difference in the average amount of dermal collagen between two group (Fig 2).

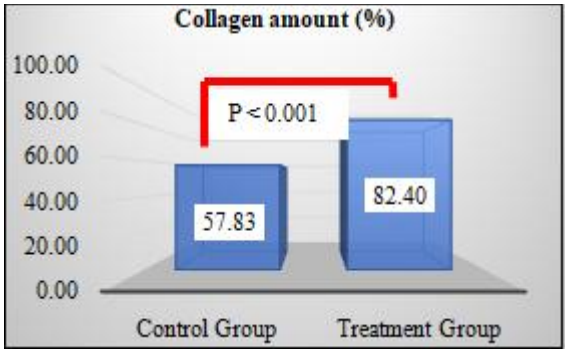
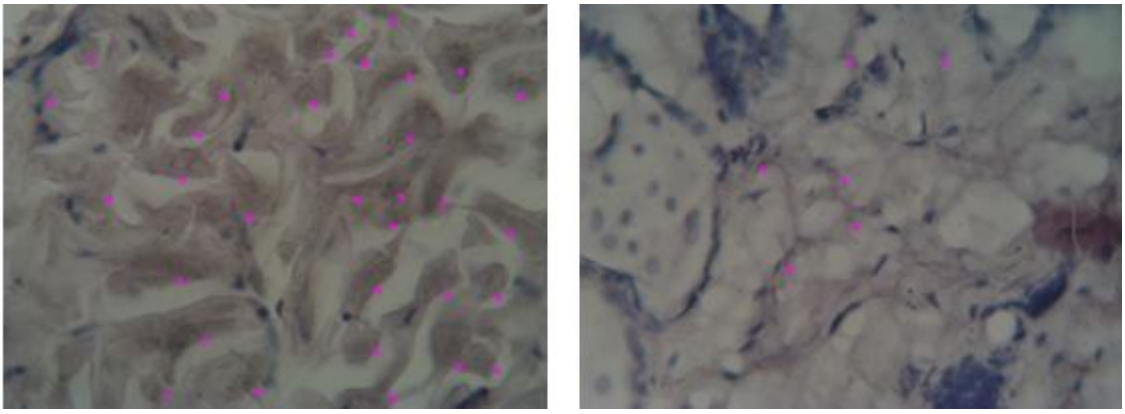


Figure 2: Quantitative comparison of Collagen amount between groups, graphs illustrate mean, p value was calculated using the Independent Sample T test



(A) (B)

Figure 3: Expression of MMP-1 on dermis tissue of rats with IHC staining. The expression of MMP-1 (brown) in the control group (A) was higher than treatment group (B). Purple arrows indicate fibroblast cells expressing MMP-1.

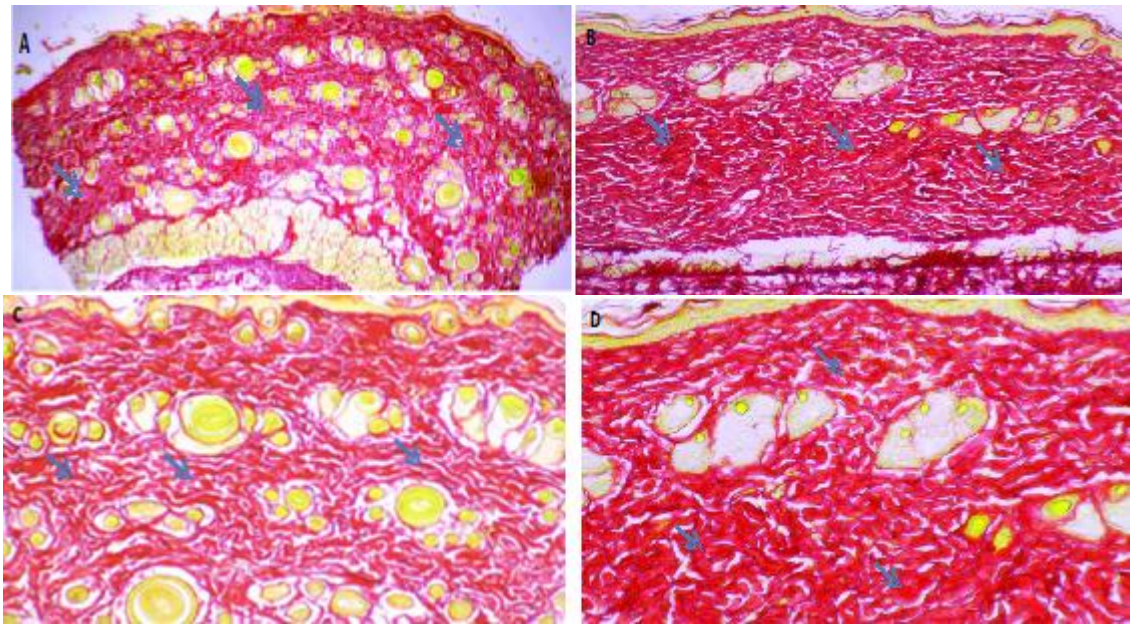


Figure 4: The Collagen examination on the dermis of Rats dermis with Picro Sirius Red Staining
The picture showed the collagen structure with red collagen fibbers that appear thin in the control group (A, C). the treatment group (B, D) showed a wider and thicker collagen fiber. Blue Arrows indicate intact collagen fibers. (A&B 40x magnification), (B&C 100x magnification)

4. Discussion

Matrix metalloproteinase-1 is the MMPs on the skin that are most triggered due to UV-B exposure. MMP-1 expression increased after the group of rats were exposed to UV B for 28 days, this is because UV B radiation irradiated cytokine receptors and growth factors in dermis fibroblast cells. UV exposure causes an increase in c-jun transcription factors, while the second transcription factor, constitutive c-fos is available even without exposure to UV-B. The combination of the two transcription factors (c-jun and c-fos) will induce the formation of activator protein 1 (AP 1) transcription factors which will regulate MMP gene expression which will then cause an increase in MMP production such as collagenase, stromelysin and gelatinase. Therefore,skin aging occurs due to increased AP-1 activity and MP expression and also increased collagen damage (Pittayapruek et al., 2016). In the present study, we evaluated the administration of 40% topical papaya seeds cream extract for MMP-1 expression and collagen number in male Wistar Rats UV-B exposed skin. From preliminary study with 30%, 40% and 50% concentrations, we found that a 40% concentration gave the significant results in preventing the increase of MMP-1 expression and decrease dermic collagen number. The 50% concentrations showed increasing of MMP-1 expression and decreasing of collagen number, which is contradictory antioxidant function.This happen because the higher the concentration of an antioxidant can be toxic and turn into a prooxidant. Prooxidant is a substance that trigger oxidative reactions which cause cell damage even to cell death, damage proteins and the formation of lipid peroxidase (Bouayed and Bohn, 2010).

Antioxidants are stable molecules which neutralize free radicals and reduce the damage caused by free radicals by donating electrons and eliminating ROS triggers by free radical chain initiator catalysts (Lobo et al., 2010). These antioxidants are molecules that works in the skin to reduce the effects of free radicals, such as Reactive Oxygen

Species (ROS) which are formed as a result of exposure to UV-B rays, and cause damage to collagen dermis (Alam and Harvey, 2010). The most recent findings point to the use of antioxidants derived from papaya seeds, both oral and topical, which can fight aging on the skin, but there is still very little publication. Based on previous research conducted by Kumar and Devi, papaya seed extract has the ability to eliminate free radicals which protect fibroblast cells that experience H_2O_2oxidative stress and papaya seed extract can prevent increased activity of the catalase enzyme and prevent the release of cytochrome C, so that the extract has the potential to protect against oxidative stress (Kumar and Devi., 2017).

According to previous research, it shows that the number of flavonoids and phenols in raw papaya seeds is higher than ripe and highly ripe papayas (flavonoid and phenol levels in raw papaya seeds (unripe) are 0.89% and 0.16%, hard papaya seeds (hard ripe) 0.83%, and 0.13%, very ripe papaya seeds (very ripe) 0.71% and 0.01%) (Chukwuk et al., 2013). Polyphenols are a group of chemicals found in plants. The most studied polyphenols are the flavonoid group, which is divided into 6 sub-classes namely flavones, flavonolols, flavafonolols, isoflavones, flavanols, anthocyanins (Kim et al., 2013).

One of the potential flavonols is quercetin. Papaya seeds contain 785.05 mg / 100gr QE flavonoids, tannin 731 mg / 100gr TAE, total phenol 520.65 mg / 100gr GAE and IC 50% 0.634 mg / ml. Phytochemical compounds are relatively higher than other extract phytochemical compounds that have been used in previous studies to inhibit the increase in MMP-1 in skin exposed to UV-B rays. As a comparison with Bali coffee bean extract containing flavonoids 58.94 mg / ml, IC 50 0.3630 mg / ml (Soraya, 2017), Moringa fruit extract containing phenol 137.88 mg / 100gr GAE, total antioxidant capacity of 259.54 mg / L GAEAC, flavonoid 2.10 mg / 100gr, tannin 13339.76 mg / 100gr and IC50 77.75 mg / ml (Yuziana, 2018) and black onion extract with IC50 value 2.87 mg / ml, total flavonoids 1, 06 mg / 100gr, phenol levels 8.27 mg / 100gr (Wasliati, 2018).

Quercetin has been shown to protect the skin's antioxidant system (glutathione peroxidase, glutathione reductase, and superoxide dismutase). The photo-protective of quarcetin is absorbing UV radiation, thereby preventing the formation of ROS and direct DNA damage. Quarcetin also has an effect on inhibiting collagen degradation through inhibition of MAP-kinase and MMP1 formation, thus inhibiting collagen degradation (Saewan and Jimtaisong, 2013).

Flavonoids come from large groups of low molecular weight polyphenols and benzo-γ-pyrone derivatives. Flavonoids can suppress MMP-1 expression and induce the expression of type 1 procollagen proteins in UV-induced cell culture. Flavonoids inhibit the cascade phosphorylation of MAPK namely ERK, JNK and C Jun which are involved in the induction of MMP-1 expression after UV-B exposure. Then, flavonoids affect the TGF-β/Smadsignaling pathway, which is involved in the regulation of type 1 procollagen expression (Agic et al., 2016). Flavonoids can also inhibit the activation of nf-kB which is a transcription factor of MMP1 so that MMP1 levels decrease and collagen degradation can be prevented (Jung et al., 2016).

Tannins are polymer compounds from high molecular weight polyphenols (Ignat et al., 2011). Tannin is a natural phenolic acid consisting of two different carbon chains, which are hydroxycinnamic acid and hydroxybenzoic structure. Tannins can interact with collagen through hydrogen bonds and hydrophobic interactions thereby increasing the thermal stability and enzymatic stability of collagen. The collagen hydrothermal stability increases and inhibits collagen breakdown by collagenase enzymes such as MMP-1 through the formation of hydrogen bonds and hydrophobic interactions. Tannins can bind to collagen with high affinity because the structural flexibility of collagen compensates for the structural rigidity of phenolic. Increased tannin concentrations induce significant changes in the triple helical conformation of collagen (Velmurugan et al., 2014). Phenol compounds are plant secondary metabolites. In the pathogenesis of photoaging due to UVB exposure, phenol functions as a primary antioxidant because it is able to neutralize and become a radical scavenger of free radicals and ROS, so that pathways that induce collagen degradation are not triggered (Tapas et al., 2008). The application of papaya seed extract cream containing total antioxidant capacity can neutralize free radicals, so that oxidative stress is not formed which is the main cause of the decrease in the amount of collagen in photoaging due to UVB rays and decrease MMP-1.

UV-B exposure in the control group for 28 days with a total dose of 840Mj / cm2 caused an increase in MMP1 expression (Tarawan et al., 2016). In this study, the mean MMP-1 expression in the control group was 27.467 ±4.2% and in the treatment group was 9.722±3.4%. MMP1 expression levels in the control group were higher than those in the treatment group and the p value <0.001. This means that both groups after being treated for 28 days had significantly different mean MMP-1 expressions. This shows that the papaya seeds extract cream 40% can effectively reduce MMP-1 expression of male Wistar rats exposed to UV-B ray.

This study was also showed that the average amount of collagen in the control group was 57.828 ±3.05% and in the treatment group was 82.406± 2.62%. The amount of collagen in the control group was lower than the treatment group and the p value <0.001. This means that the two groups after being treated for 28 days had significantly different amounts of collagen.

As previously explained that the application of Bali raw papaya seed extract cream can reduce MMP-1 through various mechanisms due to the content of flavonoids (Quercetin), phenols, tannins and antioxidants, the administration of Bali raw papaya seed extract cream also increases the amount of collagen. This is because the decrease in collagen in UV-B exposure is mediated by an increase in MMP-1.

Chronic exposure to UV-B rays causes the formation of ROS which will cause an increase in AP-1 and inhibit TGF-ß type 2. ROS will increase the transcription factor AP-1

which further increases the production of MMPs, which is an enzyme that triggers collagen degradation.

ROS also inhibits TGF-β type 2 which will reduce procollagen synthesis. Decreased procollagen synthesis and increased production of MMPs cause a decrease in the amount of collagen dermis. Naturally, the body has the ability to repair cells, but if there is enough damage it will result in repair of imperfect cells. This causes the formation of a collection of irregular collagen fibers (solar scar), which in the initial phase is still not visible (invisible). Repeated damage from UV-B rays will then cause visible solar scar (wrinkle) (Pinnel, 2008).

Based on the results of this study it was found that Bali raw papaya seed extract is a source of antioxidants and can be used topically to prevent skin damage from UV-B exposure. The photoprotection mechanism of Bali raw papaya seed extract cream in preventing the process of skin aging (photoaging) is proven by reducing MMP-1 expression and increasing the amount of collagen. The reduction in the amount of collagen occurs in addition to the MMP1 expression pathway can also be from the MMP-3 and MMP-9 pathways that need further investigation.

From this research, it has been proven that the cream dose of Bali raw papaya seed extract 40% decreases MMP-1 expression and increases the amount of collagen in the dermis of white rats (Rattus norvegicus) male Wistar strains exposed to UV-B rays.

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