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The Antioxidant Activity of Lycopene and β-Carotene of Tomato Fruit (Licopersicum Pyriform) as Anti-Aging in Rats

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Key words: Photo aging, MDA, AP-1 expression, MMP-1 activity, collagen, design

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Abstract: Photo aging is caused by a lack of collagen and skin elastin fibers due to external factors such as solar UV which may give negative effects on skin, for examples, wrinkled, pigmentation spotted, low elasticity and hard textures. The process of such an early aging may be blocked or prevented by avoiding factors that may accelerate it. However, so far no explanation has suggested that the effect of lycopene or β-carotene may give effects on ROS activity, AP-1 expression, MMP-1 activity and collagen in rats' skin radiated by UV-B with a dosage of 150 mJ cm^{-2}. The objective of this study was to investigate the effect β-caroten and lycopene on the prevention of collagen damage due to the irradiation of UV-B light. This current research used 24 rats divided into 4 groups consisting of 6 rats each. The control group was not irradiated by UV-B or not given tomato juice (P_0). The experimental group was given the following treatments: exposure to UV-B light of 150 mJ cm^{-2} (P_1), exposure to UV-B light of 150 mJ cm^{-2} +β-caroten (P_2) and exposure to UV-B irradiation with the dosage of 150 mJ cm^{-2}+lycopene (P_3). Treatments were given to each group for 6 weeks. An experimental design using a cluster random with 4 treatments and 6 repetitions was employed. AP-1 and MMP-activity were measured by immunohistochemistry and the MDA content was measured by means of NWLSS MDA Assay technique. The data were analyzed using a variance analysis and then followed by LSD test. It can be concluded that the application of lycopen and β-carotene may prevent the aging by preventing the increase in MDA levels (as an indicator of ROS activity) and the expression of AP-1, also by a decrease in MMP-1 activity in the irradiated skin by UV-B rays of 150 mJ cm^{-2}. However, it also found that the application β-carotene has shown no significant difference compared to the application of lycopene.

INTRODUCTION

This current research is a continuation of the previous researches. In the previous researches, it has been found that the tomato (Lycopersicum pyriform) contains the highest Vitamin C and carotenoids compared to the other commercial tomato fruit. Besides, it was also found that the administration of the tomato fruit extracts can prevent/protect an increase in GOT and GPT induced by CCl_4 in rats (*Rattus norvegicus*) and prevent the increase of MDA and liver cell damage. In the first stage, it was concluded that the extract of tomatoes at a dose of 7-15 g kg BW^{-1} has decreased MMP-1 and MMP-3 but can degrade collagen type-1 at a young age (4-7 months). Previous research has found that the provision of fruit juice of tomato with the dose of 11 g kg BW^{-1} can reduce the levels of MDA, the expression of AP-1 activity of MMP-1 and prevent the reduction in the expression of collagen type-1 and the levels of collagen in the skin aging process as a result of UV-B radiation with the dose of 150 mJ cm^{-2}. However, it has not been further revealed the ability of lycopene and β-carotene of tomatoes in slowing the aging process of rats' skin as a result of UV-B radiation.

From the background elaborated above, some questions were raised as follows: Can the provision of lycopene of tomatoes prevent the expression of AP-1, MMP-1 activity, MDA and the levels of collagen in the skin aging process as a result of UV-B radiation?

Can the provision of β-carotene of tomatoes prevent the expression of AP-1, MMP-1 activity, MDA and the levels of collagen in the skin aging process as a result of UV-B radiation?

Is there any relation among the expression of AP-1, MMP-1 activity, MDA and collagen from the application of lycopene and β-carotene of tomatoes (Lycopersicum pyriforme) to the irradiated skin aging process by UV-B? How is the mechanism of lycopene and β-carotene of tomatoes in preventing the increase in the expression of AP-1, MMP-1 activity, MDA and preventing the degradation of collagen in the skin aging process as a result of UV-B radiation?

MATERIALS AND METHODS

This experimental research employed the design of the "Pre-Posttest control design group" as follows: The population in this research was the population of Wistar rats aged 4 months from inbred that have been treated from birth until they were used as the sample on the age above (5; 6).

The research material was tomato (Lycopersicum pyriform) grown using organic fertilizers obtained from the Laboratory of Agronomy, Faculty of Agriculture, University of Muhammadiyah Malang. Fruits were ripe on the tree; after being washed, its juice was extracted. The given treatments were:

K-(P0): The treatment to the rats fed by water 11 g kg^{-1} and not irradiated by UV-B dose of 150 mJ cm^{-2} (control-).

K+(P1): The treatment of the rats that were given water 11 g kg^{-1} and UV-B irradiated by a dose of 150 mJ cm^{-2} (control+).

L (P2): The provision of lycopene and UV-B radiation at a dose of 150 mJ cm^{-2}.

B (P3): The provision of β-carotene and UV-B irradiated by a dose of 150 mJ cm^{-2}.

The manufacture of tomato fruit extract and the maintenance of the rats were carried out in the laboratory of Chemistry, University of Malang. Standardized plant testing, tomato fruit extracts and plant determination were conducted in the laboratory of Botany-Pharmacognosy Faculty of Pharmacy, University of Malang. Making the preparations was carried out in Anatomical Pathology Laboratory RSDR Sutomo/FK Airlangga University. Testing and the measurement of MMP-1, AP-1, MDA and Collagen were conducted at the Lab. of Biochemistry UB School of Medicine and Biomedical Lab of FK UMM.

This current research observed the rats up to the age of 7 months; the stages were elaborated as follows: to determine the effect of lycopene tomatoes that can prevent the expression of AP-1 activity of MMP-1, MDA and the levels of collagen in the skin aging due to UV-B radiation. To determine the effects of β-carotene of tomatoes that can prevent the expression of AP-1 activity of MMP-1, MDA and the levels of collagen in the skin aging process as a result of UV-B radiation. To determine the relationship of the expression of AP-1 activity of MMP-1, MDA and collagen from the application of lycopene and β-carotene of tomatoes (Lycopersicum pyriform) on the irradiated skin aging process by UV-B. To elucidate the mechanism of lycopene and β-carotene of tomatoes in preventing the increase in the expression of AP-1 activity of MMP-1, MDA and preventing the degradation of collagen in the skin aging process as a result of UV-B radiation.

The level of MDA was measured by using the test of "Method of Lipid Peroxide Abcam with the ELISA technique". The activities of MMP-1 (Unit) were measured by ELISA. AP-1 expression was measured by the technique of "Colorimetric ELISA". The examination of collagen-1 (Unit) was measured by the test of Sircol Solouble collagen Assay.

RESULTS AND DISCUSSION

Test results were obtained from implementing the ELISA technique against MDA levels as an indicator of ROS activity. ELISA technique is one technique that can be used to check the levels of MDA in the skin tissue. In this research, it was aimed to determine MDA levels as an indicator of ROS activity on the back of Wistar rats' skin tissue in all treatment groups.

ANOVA results showed significant differences ($p<0.01$). Similarly, LSD test showed significant differences. There were significant differences between P0 to P1, P2 and P3 but they did not differ significantly between P1 and P2 and between P2 and P3. This suggests that skin cells decreased its number of MDA in the delivery of lycopene and β-carotene (Fig. 1).

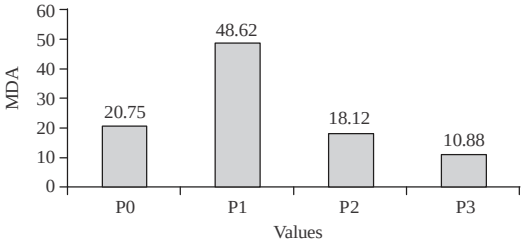

Fig. 1: MDA after various treatments on the rats' skin tissue

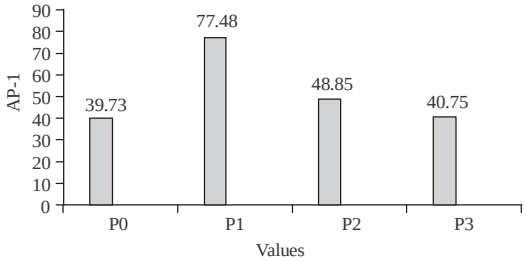

Fig. 2: The expression of AP-1 in the rats' skin tissue after various treatments

Description: P0 = MDA levels in skin cells of the control group (without UV-B radiation and without the administration of antioxidants); P1 = MDA levels in skin cells of the control group (administration of UV-B radiation without giving antioxidants), for 6 weeks; P2 = MDA on β-carotene administration to rats irradiated by UV-B, for 6 weeks and P3 = MDA in rats by the administration of lycopene against UV-B irradiated for 6 weeks. The same letters on the graph show that between the treatments there was no significant difference.

Figure 1 has shown that the control group (P1) obtained MDA the most (0.5948±0.0468 nmol) and the least was found in the group (P3) as many as (0.1760±0.0274 nmol). However, there was no significant differences between P2 and P3. This showed that the activity of ROS in the skin tissue decreased after the provision of lycopene and β-carotene.

Immunohistochemical test results ELISA AP-1: Immunohistochemical technique is one technique that can be used to examine the expression of AP-1 in the skin tissue. The identification of AP-1 in this research aimed to determine the presence of AP-1 on the back skin tissue of Wistar rats in all treatment groups.

ANOVA results showed significant differences ($p<0.01$). LSD test showed no significant difference between P1 and P0, P2 and P3 while between P0 and P2 and between P2 and P3 there showed no significant difference. This suggests that skin cells decreased the expression of AP-1 after the provision of lycopene and β-carotene on rats irradiated by UV-B.

Description: P0 = AP-1 expression in skin cells of the control group (without UV-B radiation and without the administration of antioxidants), for 6 weeks; P1 = AP-1 expression in skin cells of the control group (administration of UV-B radiation without giving antioxidants), for 6 weeks; P2 = the expression of AP-1 on β-carotene administration to rats irradiated by UV-B, for 6 weeks and P3 = AP-1 expression on the treatment of lycopene on rats irradiated by UV-B, for 6 weeks. The same letters on the graph show that between the treatments there was no significant difference.

Figure 2 has shown that the control group (P1) obtained an average expression at most (0.703±0.149) and the least was found in the group (P0) as many as (0.197±0.0955). However, there was no significant differences between P2 and P3. This suggests that the expression of AP-1 in the skin tissue decreased after the provision of β-carotene and lycopene.

ELISA Test Results of the Activity of MMP-1: ELISA technique is one technique that can be used to examine the activity of MMP-1 in the skin tissue. In this research, it was aimed to examine the activity of MMP-1 in the skin tissue of Wistar rats' back in all treatment groups.

ANOVA results showed significant differences ($p<0.01$). LSD test showed no significant difference between P1 and P0, P1 and P3 while between P0 and P2 and between P2 and P3 there showed no significant difference. This suggests that skin cells decreased the activity of MMP-1 after the administration of β-carotene and lycopene.

Description: P0 = the activity of MMP-1 in the skin cells of the control group (without UV-B radiation and without the administration of antioxidants); P1 = the activities of MMP-1 in the skin cells of the control group (administration of UV-B radiation without giving

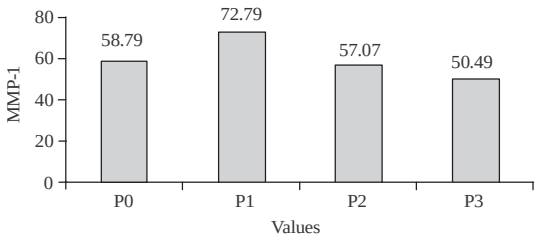

Fig. 3: MMP-1 activity after the provision of various treatments on rats' skin tissue

antioxidants), for 6 weeks; P2 = the activity of MMP-1 after the administration of β-carotene on rats irradiated by UV-B, for 6 weeks and P3 = the activity of MMP-1 after the provision of lycopene on rats irradiated by UV-B, for 6 weeks. The same letters on the graph show that between the treatments there was no significant difference.

Figure 3 has shown that the control group (P1) discovered the activity of MMP-1 at most (0.596±0.015 U) and the least was found in the group (P0) as many as (0.195±0.066 U). However, there was no significant differences in P2. This suggests that skin cells decreased the activity of MMP-1 after the administration of β-carotene and lycopene.

UV radiation on the skin is absorbed by the chromophore which is beginning photochemical reaction resulting in premature skin aging. Photochemistry reaction can cause changes in the DNA, including nucleic acid oxidation. The oxidation reaction can also modify proteins and lipids that lead to impaired cell function. Accumulation of both causes aging tissues (tissue aging)[1]. The body is already equipped to deal with oxidative stress that is naturally in the form of antioxidants to reduce the bad influence but UV rays and the formation of free radicals ultimately lead to oxidative damage.

Free radicals are one of the reactive oxygen species, which are generally known as compounds that have unpaired electrons. These compounds are formed in the body, triggered by various factors. These free radicals can be formed, for example when the food component is converted into a form of energy through metabolic processes. At this metabolic process, there are often leak electrons. In such conditions, it is easy to form free radicals such as superoxide anion, hydroxyl and others. Radicals can also be formed from other compounds that are actually not free radicals, for example, hydrogen peroxide (H_2O_2), ozone and others. Both groups are often called Reactive Oxygen Species (ROS)[2]. ROS can cause damage to DNA, proteins and lipids in the cell. On aging, the increased peroxidation generally can damage the membrane which may affect the function of the membrane and the cell physiological functions including functions of trans-membrane receptors[3]. Thus, the high levels of MDA can be used as an indicator of ROS in the high activity of the living body.

The results showed that the levels of MDA after the administration of β-carotene or lycopene differed significantly (p = 0.000) compared to the positive control treatment (P1) but no difference from the negative control (P0). The mean level of lycopene MDA in the treatment group was (0.1760±0.0274 n mol) followed by the lowest average β-carotene level (0.2565±0.071 n mol) and significantly different (p = 0.000) compared to the average level of the positive control (0.703±0.048 n mol) and significantly different from the mean of the negative control level (0.5948±0.059 mol mL^{-1}). These results provide an indication that the treatment of lycopene and β-carotene in rats irradiated by UV-B rays of 150 mJ cm^{-2} could prevent an increase in ROS activities measured by MDA.

In the administration of the combination treatments of lycopene, the obtained MDA levels were the lowest compared to the positive control treatment but no difference from the negative control and the administration of β-carotene. This shows that the administration of the combination treatments of β-carotene and lycopene was the quite effective in lowering the levels of MDA in rats irradiated by UV-B rays of 150 mJ cm^{-2}.

The presence of lycopene or β-carotene in the body will be able to bind free radicals such as hydroxyl and superoxide anion ion. Radical (OH ●) and (O ● -) resulted from the formation of ROS have been tied up in advance by lycopene, β-carotene and vitamin C before damaging the cell components like DNA that result in the decreased levels of MDA. Thus, the administration of lycopene or β-carotene can prevent the increase in MDA as a result of UV-B radiation dose of 150 mJ cm^{-2}. In line with Black etc., the administration of Vitamin C can decrease MDA, given to groups of seniors. Lycopene is a carotenoid that is efficient in converting singlet oxygen and ROS. From the results, it has been shown that carotenoids can significantly lower MDA and also consuming carotenoids can prevent lipid peroxidation in the cell[4]. In other words, the administration of β-carotene or lycopene would reduce ROS characterized by the decreasing levels of MDA.

Activator Protein-1 (AP-1) is a transcription factor that inhibits the production of collagen and increases the regulation of the breakdown of collagen[5]. Collagen is one of the main constituents of the skin of rats which provides most of the power on the skin. Part fibroblasts produce precursor molecules called as pro-collagen which would then be converted into collagen. There are two main controllers in the manufacture of collagen, namely: Transforming Growth Factor (TGF-β) and Activator Protein (AP-1). TGF-β is a cytokine that promotes the

production of collagen, the reverse AP-1 inhibits the production of collagen. Collagen in the skin to change the shape and structure as well as TGF-β and AP-1, plays an important role in the process. TGF-β encourages the formation of collagen while the AP-1 encourages the breakdown of collagen by raising the controlling enzyme called Matrix Metalloproteinase (MMP)[3]. Therefore, the AP-1 has an important role in the breakdown of collagen in the skin.

The results showed that the expression of AP-1 on the administration of lycopene and β-carotene decreased significantly (p = 0.000) compared to the positive control treatment (P1) and the negative control. The mean of expression of AP-1 in the treatment group lycopene was (0.314±0.10) and the administration of β-carotene treatment showed that the mean of expression (0.3995±0.067) was significantly lower (p = 0.000) compared to the positive control mean expression (0.7035±0.045) and compared to the negative control of which mean of expression was (0.197±0.082). These results indicate that the administration of β-carotene or lycopene in rats irradiated by UV-B rays of 150 mJ cm^{-2} could prevent an increase in AP-1.

The lower expression of AP-1 was shown in the treatment group of lycopene, compared with the treatment of positive and negative controls. It could happen because lycopene and β-carotene are quite effective in reducing the expression of AP-1. In fact, the positive control treatment showed the highest expression of AP-1, since, the provision of UV-B radiation produced free radicals. Free radicals are atoms or molecules that have unpaired electrons, unpaired in the outer orbit. These free radical molecules can attract electrons from other molecules, causing abnormal new free radicals that eventually lead to a domino effect (self-perpetuating process). These free radicals would then raise the expression of AP-1.

Reactive Oxygen Species (ROS) plays an important role in the metabolism of the cells because it can form the hydroxyl ion (OH ●) which is a compounds that is highly reactive and dangerous[2]. Similar to the research of Kang *et al*.[5], the radiation on the skin of a human buttock using 2 MED (twice the dose of UV-B that causes redness of the skin) will also lead to the increased formation of hydrogen peroxide and ROS within 15 min. At the same time, it turns out that AP-1 also increased at least 24 h after UV-B radiation.

The presence of β-carotene or lycopene is able to bind free radicals such as hydroxyl and superoxide anion ion. Radical (OH ●) and (O ● -) resulted from the formation of ROS have been tied up in advance by lycopene or β-carotene before damaging the cell components like DNA that resulted in the increased AP-1. Thus, the administration of lycopene or β-carotene could effectively prevent the increase in AP-1 as a result of UV-B radiation dose of 150 mJ cm^{-2}.

MMP-1 collagen fibers break the skin, especially during the photo damage. Skin fibroblasts produce MMP-1 in response to UV-B radiation. MMP-1 is active to initiate the damage of collagen fibers. Collagen in the skin which experiences change and reshapes the skin continuously and which plays an important role here are TGF-b and AP-1. TGF-b encourages the formation of collagen while the AP-1 leads to a breakdown of collagen by regulating the return of enzymes called Matrix Metalloproteinases (MMPs)[3]. MMP-breaking collagen regulated by AP-1 also significantly increased within 24 h of UV radiation. Within a period of 24 h with a single dose of UV radiation, it also appears to increase the breakdown of collagen. Thus, there is the apparent link between the provision of UV-B radiation and AP-1 and MMP-1.

The results showed that the activity of MMP-1 in the treatment of lycopene or lycopene administration decreased significantly (p = 0.000) compared to the positive control treatment (P1). The mean of the activity of MMP-1 in the treatment group administration of lycopene was (0.492±0.066 U) and the treatment that was the administration of β-carotene (0.4795±0.047 U) was significantly lower (p = 0.000) than the mean of the positive control activity (0.5960±0,015 U) and differed significantly when compared to the negative control average of expression (0.195±0.06 U). These results indicate that the administration of lycopene and β-carotene before being irradiated by UV-B 150 mJ cm^{-2} could prevent an increase in the activity of MPP-1.

The lower level of active MMP-1 was in the treatment group of β-carotene and lycopene, compared with the treatment of positive and negative controls. This happens because the lycopene and β-carotene are quite effective in preventing the increase in the activity of MMP-1 caused by UV-B radiation that will produce free radicals. Thus, the presence of free radicals caused by UV-B radiation administration could be prevented by lycopene or β-carotene.

The research results of Brenneisen *et al*.[6, 7, 8, 9], showed that the UV-B radiation can increase the secretion of MMP-1. It was also the same as the results of measurements of AP-1 obtaining the highest in the control treatment and the lowest in the negative treatment of lycopene. Fisher and Voorhees explain that the UV radiation activates receptors of growth factors which stimulate the activity of protein kinase cascade such as the Mitogen-Activated Protein Kinase (MAPK). These activities are then managed with an increase in the expression of c-jun and c-fos which is a form of Activator Protein-1 (AP-1) complex. The transcription of MMP-1 is controlled by AP-1. AP-1 complex formation, Jun-proteins, form homodimers and heterodimers of Fos protein. AP-1 transcriptional activity also depends on the equal levels of phosporilation of c-Jun and c-Fos expression. Moreover, the increased activity of AP-1

caused damage to the extracellular matrix proteins such as collagen, through induction of MMPs[10]. Likewise, some other researchers[11] reported the UV ray effects on MMP-1 which play an important role in the breakdown of extracellular matrix during photo aging.

In the treatment of the provision of lycopene and β-carotene prior to UV-B radiation of 150 mJ cm^{-2}, it was obtained that the activity of MMP-1 was lower than the treatment of negative and positive controls. This shows that the administration of the combination of lycopene is quite effective in preventing the activity of MMP 1. Likewise, the treatment of the provision of β-carotene is also quite effective in lowering the activity of MMP-1 in rats irradiated by UV-B rays of 150 mJ cm^{-2}. This is due to lycopene and β-carotene optimal activity to bind free radicals of which presence is as a result of UV-B radiation. Besides, it is also because there may still be interactions between antioxidant activities in reducing free radicals that are still in a natural state.

The presence of β-carotene or lycopene is able to bind free radicals such as hydroxyl and superoxide anion ion. Radical (OH ●) and (O ● -) resulted from the formation of ROS have been tied up in advance by lycopene or β-carotene before damaging the cell components like DNA that result in the increased AP-1. Accordingly, the tomato fruit juice can prevent the increase in MMP-1 activity as a result of UV-B radiation dose of 150 mJ cm^{-2}. This is supported by the results showing that the increase in AP-1 in this treatment could also be prevented.

CONCLUSION

The provision of β-carotene 0.4257 mg kg BB^{-1} could prevent the increase in MDA levels (as an indicator of ROS activity) and the expression of AP-1, also caused a decrease in the activity of MMP-1 in the skin irradiated by UV-B rays of 150 mJ cm^{-2}.

The provision of lycopene 0.3949 mg kg WB^{-1} could prevent the increase in MDA levels (as an indicator of ROS activity) and the expression of AP-1, also caused a decrease in the activity of MMP-1 in the irradiated skin by UV-B rays of 150 mJ cm^{-2}.

Providing a combination of β-carotene did not give a different effect from that of lycopene, in preventing the increase in MDA levels (as an indicator of ROS activity) and the expression of AP-1, also causing a decrease in MMP-1 activity in the irradiated skin by UV-B rays of 150 mJ cm^{-2}.

This research resulted in a new theory that explains the mechanism of prevention of damage to the collagen in the skin mediated by the prevention of the increase in MDA levels (as an indicator of ROS) and AP-1 expression, also by the decrease in the activity of MMP-1. This is a new contribution to science and provides a scientific basis on the use of lycopene and β-carotene contained in tomato fruit juice in slowing the photo aging due to UV-B irradiation.

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