

Kelakai Extract Protects Skin from UV-Induced Oxidative Damage

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Kelakai Extract Protects Skin From UV-Induced Oxidative Damage

M Mashuri¹, L D M Sihombing², S Alfaqihah², E Edyson³ and E Suhartono^{3*}

¹Department of Radiology, Faculty of Medicine, Lambung Mangkurat University, Veteran Street No. 128, Banjarmasin 70232, South Kalimantan, Indonesia

²Faculty of Medicine, Lambung Mangkurat University, Veteran Street No. 128, Banjarmasin 70232, South Kalimantan, Indonesia

³Department of Medical Chemistry/Biochemistry, Faculty of Medicine, Lambung Mangkurat University, Ahmad Yani Street Km. 36, Banjarbaru 70712, South Kalimantan, Indonesia

*Corresponding author's email: esuhartono@ulm.ac.id

Abstract. Oxidative stress is an unbalance situation between oxidant and antioxidant. Oxidative stress can be caused by UV exposure until it can result skin damage. This damage caused by the production of reactive oxygen compound (ROC), such as excessive superoxide anion. Thus, natural exogen antioxidant is needed, that is Kelakai. This research is pure experimental research with rats (*Rattus norvegicus*) as its subject. There were 24 samples were divided into 4 treatment groups; samples were chosen used simple random sampling method and repetition value used Federe method. Kelakai leaves were extracted using ethanol with maseration method. Superoxide anion level and superoxide dismutase enzyme activities were measured with Misra and Fridovich method; carbonyl level was measured using modified DNPH (*dinitro phenilhidrazin*) method; and conjugated diena level was measured with Kwiat Kowska method. The data was analysed statistically, and resulted significant differences between the group that only exposed to UV and the group that was given Kelakai extract and exposed to UV. Based on that result, it can be concluded that Kelakai extract can influence superoxide dismutase activities, superoxide anion level, carbonyl level, and conjugated diena in rats skin exposed to UV.

1. Introduction

Oxidative stress is an unbalance situation between oxidant and antioxidant, which leads to oxidative damage (lipid peroxidation, DNA oxidation, protein oxidation, etc). Those oxidative damage can be caused by many factors, one of them is ultra violet (UV) radiation [1,2].

UV is an electromagnetic wave with a wavelength at 100-400 nm. UV that comes from sun rays can cause skin disorders, such as erythema, dry skin, wrinkles, hyperpigmentation, and also can leads to cancer [3]. Those disorders were started by the absorbance of UV by oxygen, which influence its electronical structure. The changed structure causes the forming of singlet oxygen and reactive superoxide anion [4]. Both oxygen types are reactive and can oxidate biomolecule compound, such as lipid and protein. One of indicators from protein damages is the forming of carbonyl compound [5], while lipid damage is marked by the forming of conjugated diena compound [6].



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To prevent oxidative damage due to UV exposure, need natural ingredients that has function as antioxidant. The natural ingredients for example is Kelakai. Kelakai (*Stenochlaena palustris*) is one of typical plants for South Kalimantan that has been used beneficially as functional food, dismenore medication, reducing fever, etc. Adenan's research (2010) stated that Kelakai extract has been proven in reducing liver prooxidative stress in rats induced fever [7]. Kelakai has flavonoid compound 14,5 QE/ $\mu\text{g}/\text{mL}$ which has role as peroxide [8]. Kelakai also capable to inhibit glycation and fructation reactions; the reaction between glucose/fructose and protein [9].

Antioxidant in extract Kelakai allegedly capable to be a protector to skin that has been induced acute UV. However, no one has revealed this. Because of that, in this research Kelakai extract role will be analyzed in inhibiting oxidative stress for skin exposure with UV, by measuring superoxide anioncarbonyl, and conjugated diena level, also the activity of superoxide dismutase (SOD) enzyme [10].

2. Research methodology

2.1. Research subject

Research subject is male rat aged 8-10 weeks with weight 200-300 grms. Rats were acclimated for one week and given the same meals and drink. Next, rats were divided into four groups: control (P0); rats given UV exposure for 24 hours (P1), rats given Kelakai extract in dosage 4 mg/cm² (P2), rats given Kelakai extract in dosage 4 mg/cm² and exposed with UV for 24 hours (P3).

2.2. Treatment of Experimental Animals

Rats were shaved its back hair in width 3 x 3 cm² then rubbed with Kelakai extract in dosage 4 mg/cm². Next, they were put in box with 106x34x53 cm³ in width where there was Philips UV lamp with wavelength of 253,7 nm. Radiation was given for 24 hours. After that, rats were dissected in order to extarct its skin.

2.3. Making of Rats Skin Homogenat

Rats skin was extracted by dissection, then it as cleaned up and smoothed using 1 ml of TCA (trichloroasetar acid) 20% and 3 ml of buffer phosphate pH 7,4. After that, the skin was put into tubes and centrifugated in 2000 rpm of rate for 10 minutes for its supernatant to be used. The supernatant was used to measured superoxide anion level, carbonyl compound, conjugated diena, and dismutase enzyme activity.

2.4. Measurement of Superoxide Anion Level

Superoxide anion level was measured by using Misra and Fridovich method. Supernatant was added into 500 μl of solution that contained 800 μl of buffer carbonate 0,1 M pH 10,2. Next, in that solution, was also added 100 μl of adrenaline (epinephrine 3 mM) and then was measured its initial uptake (A_0) using spectrophotometer at wavelength 480 nm [11,12].

2.5. Measurement of Superoxide Dismutase Enzyme Activity

Superoxide dismutase enzyme activity in supernatant was measured by Misra and Fridovich method. The 500 μl of supernatant were added to 800 μl of buffer carbonate (100 nM pH 10,2) and 100 ml of epinephrine (3 mM). The absorbance changes in each sample were recorded at spectrophotometer with wavelength 480 nm in interval 15 seconds [11,12].

2.6. Measurement of Carbonyl Level

Carbonyl level was measured using modified DNPH (dinitro phenilhidrazin) method. Each solutions were taken for 0,5 ml and divided into two tubes, tube A and tube B. tube A was added with DPNH for 1 ml; and Tube B was added 1 ml of HCl 2,5 M. those solutions were incubated and shaken with vortex. The next step was adding 1 ml of TCA 20% then incubated in ice for 5 minutes and centrifugated for 5 minutes in rate 1400 rpm, and the supernatant was discarded. The further step was the cleaning by adding

1 ml of *metanol-etil asetat*. It was then added by 1 ml of urea to be incubated and centrifugated. The resulted color was measured its absorbance at wavelength 390 nm. Protein damage was calculated at equation $C=A/(eb)$ (A =absorbance, e =extinction coefficient $22.000 \text{ mM}^{-1} \text{ cm}^{-1}$, and $b=1 \text{ cm}$) [13].

2.7. Measurement of Conjugated Diena Level

Conjugated diena level was measured with *Kwiat Kowska* method. Supernatant for 0,5 ml was mixed with 7 ml of *choloroform/methanol* (1:1 u/u). Next, it was shaken for 2 minutes (1500 gr for 5 minutes). The 5 ml of the solution at the lower layer mixed with 2 ml of aquadest which acidified with HCl pH 2,5. Then the solution was shaken again for 2 minutes in the same way at the previous step. The residue was dissolved with 2 ml of heptane (cyclohexane) and measured its absorbance 233 nm with heptane solution as its blank (cyclohexane). The result was stated as absorbance unit or resulted hydroperoxide [14].

3. Result and discussion

3.1. Superoxide Anion

Superoxide anion is a reactive oxygen derivative, resulted form UV absorbance by oxygen which caused the changes in its electronic structure. Superoxide anion level in this research is shown in figure 1.

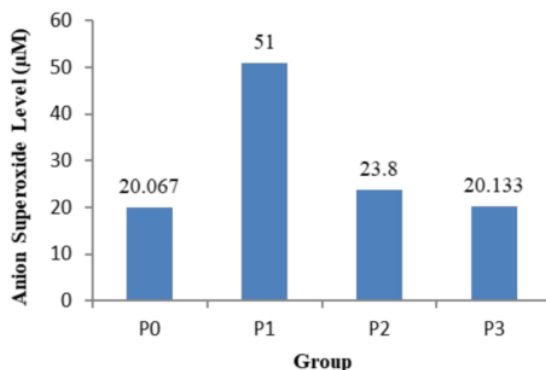


Figure 1. Superoxide anion level in rats skin homogenat (*= significantly different with P0).

One way ANOVA test result concluded that there was significant differences ($p=0,000$; $p< 0.05$). Skin contains of melanine that has chromophor group, which has ability to absorb UV rays. UV rays absorbance causes melanine changes into a more active compound. The melanine which more active will react to oxygen and forms superoxide anion.[15] The excess of UV exposure can stimulate the forming of ROC that leads to the increase of the formed superoxide anion level.[16] Kelakai extract contains flavonoid, which is able to capture superoxide anion resulted due to UV exposure.[10]

3.2. Activity of Superoxide Dismutase Enzyme

Superoxide anion can be neutralized using dismutase superoxide enzyme by forming H_2O_2 . The activity of superoxide dismutase enzyme in this research can be seen in figure 2. One way ANOVA test result concluded that there was signifacant differences ($p=0,000$; $p< 0.05$).

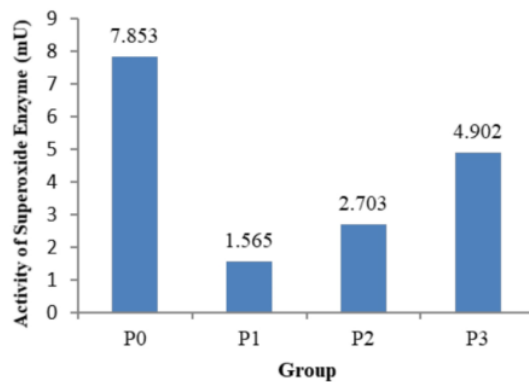


Figure 2. The activity of Superoxide Dismutase Enzyme in rats skin homogenate. (*= significantly different to P0)

UV rays that reached skin will create the forming of ROC which is able to cause molecular and cellular damage. Skin has endogen antioxidant in order to againts that ROC that is superoxide dismutase enzyme. UV exposure will cause the use of superperoxide dismutase enzyme increased, therefore the enzym activity is decreaed.[17] Flavonoid contents in Kelakai extract capable to capture free radicals (*free radical scavenging*), so that the use of superoxide dismutase enzyme decreased.[10] Decreased using of superperoxide dismutase enzyme will increase superperoxide dismutase enzyme activitiy. But, flavonoid also can be prooxidant if aroxyl radical resulted from the work mechanism of flavonoid reacted to oxygen. That reaction will produce superoxide anion, so that will cause the decreased of superoxide dismutase enzyme activity.[18]

3.3. Conjugated Carbonyl and Diena

Carbonyl is compound formed due to protein damage. The level of carbonyl compound can be seen in figure 3.

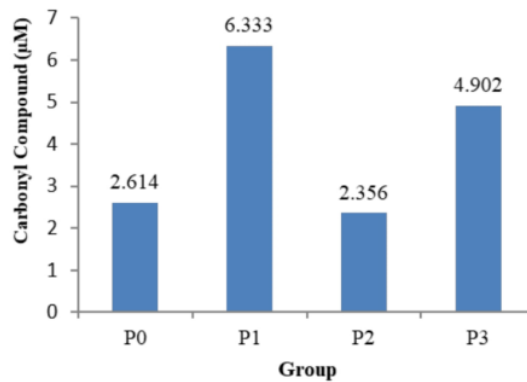


Figure 3. The average of carbonyl compound in rats skin homogenate. (*= significantly different to P0)

Kruskal-Wallis result test concluded that there was singnificant differences ($p=0,000$; $p< 0.05$).

Conjugated diena is compound that formed due to lipid damage. The level of conjugated diena in this research can be seen in figure 4.

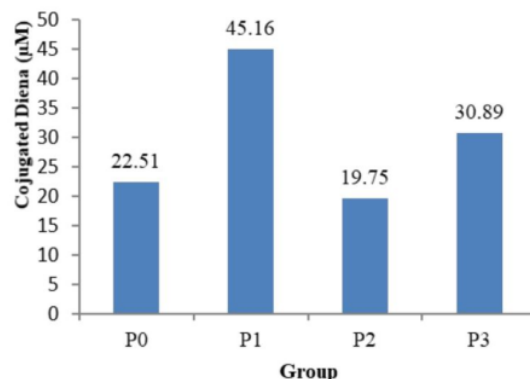


Figure 4. The average of conjugate diena compound in rats skin homogenate.
(* = significantly different to P0)

Kruskal-Wallis result test concluded that there was significant differences ($p=0,000$; $p<0.05$).

Oxidative stress happened in UV exposed rats skin valued from carbonyl compound level. UV compound can be absorbed by O_2 in biosphere, until forms 1O_2 , which for the next can transform into $\bullet O_2$. Both oxygen molecules will oxidize protein side chain, especially proline, arginine, lysine and threonine, even carbonyl compound.[5] UV radiation can oxidize epithelial keratinocyte protein in normal human. That research also reported that protein GRP 78 radiated by UV caused the increasing of carbonyl level.[19]

Furthermore, oxidative stress happened in UV exposed rats skin can be valued from conjugated diena compound level. UV compound absorbed by O_2 transforms into 1O_2 , which for the next can transform into $\bullet O_2$. Both oxygen molecules are named reactive oxygen compound (ROC). The formed ROC does not only damage to protein, but also damage to lipid. Lipid damage will form a compound which will be detected as lipid damage marker that is conjugated diena.[19] Bignon research said UV exposed on skin for 15-30 seconds, measured with wavelength 230 and 270 nm resulted the decrease of cellular metabolic activity and increase cell death in lipid and protein at maximal UV exposure for 230 nm. PARP marker formed as the result from cell apoptosis process.[20]

In order to decrease the damage due to oxidative stress, an additional antioxidant is needed. In this research, the additional antioxidant used was Kelakai extract. Kelakai extract contains a compound that is able to capture free radical, flavonoid. The mechanism of capturing free radical by flavonoid is started from electron release, forming reactive flavonoid radicals which at the end bind free radical, superoxide anion.[7] Superoxide anion binding by reactive flavonoid radicals will reduce damage happened in protein and lipid, which finally decrease carbonyl compound and conjugated diena levels.

4. Conclusion

Based on the research that has been done, it can be concluded that there is an influence from the administration of Kelakai extract to rats' skin exposed to UV rays, reviewed from superoxide dismutase enzyme activity, superoxide anion level, carbonyl level, and conjugated diena level.

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