Combination of Modified Soybean Tempeh M-3 with Carrot Increases the Total Blood Antioxidant Capacity, Decreases 8-Hydroxy-2-Deoxyguanocine, and Skin Texture Damage in Rat Irradiated with Ultraviolet

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Abstract

The combination of tempeh M-3 with carrots is one of the functional foods which contain bioactive compounds in the form of antioxidant that has a function as an agent scavenger to free radical caused oxidative stress following to the exposure of ultraviolet ray. The existence of radicals can increase the level of 8-hydroxy-2-deoksiguanosin and also can damage the skin texture. The aim of the research was to show the effect of combination of tempeh M-3 with carrots supplementation that can increasing blood total antioxidant capacity, reducing level 8-hydroxy-2-deoksiguanosin and skin texture damage caused ultraviolet radiation. This study was designed as the randomized post test only control group design with independent variable are (P_0) 0gram tempeh M-3 and carrot. P_1:1gram Tempeh-3 and carrot. P_2:gram, 2gram, and 3 gram /kg BW/day.
The Result showed that there was an increase of blood total antioxidant capacity in rats in the control group compared with the treatment group $P_1$, $P_2$ and $P_3$. The increase of total antioxidant capacity between the control group compared with the $P_1$, $P_2$ and $P_3$ by 5.12%; 11.26% and 14.20%, respectively and it could be categorized as a significant different ($p<0.05$). The increase between $P_1$ with $P_2$ and $P_1$ with $P_3$ was 5.88% and 8.74% as a significant different ($p>0.005$). In this study, there were decreased levels of 8-hydroxy-2-deoxyguanosine between the control group with $P_1$, $P_3$ and $P_3$ that by 8.25%; 28.95%; 31.70%, respectively, While the decreased levels of 8-hidroxy-2-deoxiguanosin between $P_1$, $P_2$ and $P_3$ were 22.71%, and 25.66% and this decreased significantly ($p<0.05$). The decrease between $P_2$ and $P_3$ was 3.89% which is not significant ($p>0.005$). Variation of tempeh M-3 with carrot supplementation reduced tissue damage that detected by immunohistochemistry technique. The decrease in the skin texture damage between the control group with $P_1$, $P_2$ and $P_3$ were 26.95%; 60.66% and 63.50%, respectively, while between group $P_1$ with $P_2$ and $P_1$ with $P_3$ were 46.09% and 49.64%. The decrease was significant ($p<0.005$) but for the treatments 2 and 3, there was a reduction of 60.60% and was not significant ($p>0.05$).

This research concluded that supplementation with a combination of tempeh M-3 with carrot can increase the blood total antioxidant capacity, and decrease the level of 8-hidroxy-2-deoxiguanosine and skin texture damage. The supplementation of combination of tempeh M-3 with carrot 2 gram/kg BW/day result in the maximal effect by increasing the total antioxidant capacity, decreasing the level of 8-hidroxy-2-deoxiguanosin and decreasing skin texture damage.

**Keywords:** Tempeh M-3 with carrot, Total anti oxidan (TAC), 8 hydroxy-2-deoxiguanosin, and damage to skin texture.

**INTRODUCTION**

Functional food that can be made from soybeans for tempeh made by fermentation using microorganisms roads mold / fungus Rhizopus sp. Tempe Modified soy-3 functions as an antioxidant, because it contains isoflavonoids in soybean tempeh which is a secondary metabolite which consists of nineteen (19) group and six groups of compounds aglycone glycosides. Aglycone and glycoside is a chemical compound alcoholic, phenolic, nitrite sianogenetik antresen derivatives, steroids, and flavonoid glycosides lately widely used as a medicinal plant (King 2002; Soobrattee, et al; 2005).
Medicinal found more than a hundred years ago, a flavonoid compound found in soy tempeh can replace vitamin E as an antioxidant. Antioxidants present in the form of isoflavones are soy tempeh genestein, daidzein, and 8-hydroxy daidzein. Modifications-3Tempe(tempehM-3) made with three (3) boiling process. The first and second stew using water to maximize the amount of isoflavones and then using boiled with water. This process is useful resitend alkaline conditions (atmosphere anaerobes) of soybeans that will be fermented for 3 (three) days with a combination M-3 and carrots that have tedensi folatil increase in the degree of lack of fat, thus, the fatty acid thus, polyunsaturated fatty acids (Polyunsaturated Fatty Acyds / PUFA) increased in number. Where the addition of similar carrot tuber vegetables rich in antioxidants beta-carotene, a precursor of vitamin A and contains a fair amount of thiamine and riboflavin (Muchtadi, D.2009).

Thiamine and riboflavin is an organic molecule that is not included in the class of carbohydrates, proteins, and fats. Fat into food for humans, and other animals are triglycerides, sterols, and membrane phospholipids are present in animals and plants. Synthesizing lipid metabolic process and outlines the functional lipid reserves, and produces the characteristic functional lipids and structural lipids in the tissues of individuals (Murray, 2009). Individual tissue damage can occur, through two (2) theory that the first theory of direct impact on the target molecule called the target theory, the second is the theory of action that is not a direct collision / theory poison water, is due to approximately 70% by free radicals. Hydrogen peroxide is an active oxidant. The numbers are important to be considered as symptoms of radiation effects in a system that beraiir. Hydroxyl radicals, hydrogen and water molecules are formed in the water can cause a variety of effects on other molecules found in biological systems. H radical is a very strong reductant, so apsbila reacts with oxygen (O2) generates OH radicals and water radical (· H2O). The main reaction of OH radicals, such as oxidation and this reaction is responsible for DNA damage, and damage to the membrane by ionizing radiation. (Manfred, 2001; Siti Maryam, 2011).

Radiation to the tissue / depends on the radiation dose, dose fractionation, the presence of protective materials / sensitizing (sensitivity) and so on. When presented in a short time in an acute dose would be more dangerous, than compared to chronic doses presented in the monthly / yearly. Increased severity of radiation effects can improve deterministic effects, for example skin erythema or decrease in the number of lymphocytes. Harmful ultraviolet at a wavelength of 300 nm (Muhaimin MT, 2001).
Materials and Method

Materials research is: Wistar strain male rats, the animals coba. Bahan other materials used are M - 3 tempeh and carrots, diethyl ether (narcotic substances), Total Antioxidant Capacity checks (TAC), using the brand BioVision Assay Kit Catalog 274-100; Philip W UV Lamp 15 Watt Kit 8-hydroxy - 2 - deoksiguanosin with Elisa technique by No.589320 and No.589321 for inspection katalag 8-hydroxy - 2 - deoksiguanosin, all serum stored at -200C with a view to the number of samples collected serum are met, and the kit is opened, after the centrifuge for 10 minutes, checked each Total Antioxidant, and 8-OHdG according to the respective procedures.

The method used in this study are: the actual experimental design. The only randomized posttest control group design, only take into account the levels of total antioxidants and allocation of some four different animals in each treatment group randomized treatment. Each treatment 5 times. Research procedures can be explained 40 Wistar rats aged 1 month 70 -75 gram of weight, were fed a standard (P0), P1 fed tempeh M - 3 and carrots 1 gram; P2 fed tempeh M - 3 and carrots 2 grams; P3 fed tempeh M - 3 and 3 grams of carrots. Long treatment Po, P1, P2, and P3 for 3 months of supplementation tempeh given two hours prior to the ultraviolet radiation for 1 hour, then measured total antioxidant, and 8-OHdG. All data were analyzed descriptively. Further analysis of differences in antioxidant capacity and levels of 8-hydroxy - 2 - Deoksiguanosin performed analysis steps: data selection includes editing, coding and tabulation of statistical programs use the file navigator for Windows (Triton, 2006; Premesti 2007); Analysis of data normality Total antioxidant and 8-OHdG Wistar rats, each group was analyzed by Shapiro Wilk test with significance level $\alpha = 0.05$. hypothesis Ho: the frequency of observation frequency $\neq$ expectations, Ha: $\neq$ observation frequency expected frequency. Ho accepted (normal distribution of data) when $p > 0.05$. Ho is rejected (data not normally distributed), if the data obtained are not normally distributed, we used nonparametric statistical test and vice versa. Homogeneity of variance were analyzed by Levent's test to determine whether the variance in each homogeneous group. Hypotheses Ho: variation = variation of the control group treatment group 1 (P1) = variation in treatment group 2 (P2) = variation in treatment group 3 (P3) = variation in treatment group 4 (P4). Ha: $\neq$ variation variation control group treatment group 1 (P1) $\neq$ variations in treatment group 2 (P2) $\neq$ variations in treatment group 3 (P3) $\neq$ variations in treatment group 4 (P4). Ho is accepted.

$p > \alpha$, Ho is rejected when $p < 0.005$.
RESULTS AND DISCUSSION

Blood levels of total antioxidants in Wistar Rats Ultraviolet Radiated

Data total blood antioxidant levels 40 Wistar rats were exposed to UV light with UV light previously supplemented soy tempeh respectively 0 grams tempehM-3 and carrots / kg body weight / day (P0); 1 gram tempeh M-3 and carrots / kg body weight / day (P1), 2 M-3 gram1 gram tempeh and carrots / kg body weight / day (P2); 3 gram tempeh M-3 and carrots / kg body weight / day (P3) can be observed in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>P0</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1464.99</td>
<td>1526.99</td>
<td>1626.99</td>
<td>1676.99</td>
</tr>
<tr>
<td>2</td>
<td>1569.98</td>
<td>1602.99</td>
<td>1677.99</td>
<td>1894.99</td>
</tr>
<tr>
<td>3</td>
<td>1477.52</td>
<td>1576.99</td>
<td>1719.99</td>
<td>1806.99</td>
</tr>
<tr>
<td>4</td>
<td>1512.60</td>
<td>1591.99</td>
<td>1627.51</td>
<td>1666.99</td>
</tr>
<tr>
<td>5</td>
<td>1499.99</td>
<td>1591.86</td>
<td>1714.99</td>
<td>1762.50</td>
</tr>
<tr>
<td>6</td>
<td>1476.99</td>
<td>1578.99</td>
<td>1626.99</td>
<td>1649.99</td>
</tr>
<tr>
<td>Σ</td>
<td>8922.05</td>
<td>9444.82</td>
<td>9994.46</td>
<td>10253.99</td>
</tr>
<tr>
<td>Rata-rata (mM)</td>
<td>1478.75 ± 34.59</td>
<td>1573.96 ± 26.07</td>
<td>1665.70 ± 44.54</td>
<td>1707.30 ± 61.56</td>
</tr>
</tbody>
</table>

Table 1 above shows the treatment P2 (1665.70 mM) with treatment P3 (1707.30 mM) when compared to control (without Tempeh M-3 and carrots), no significant differences appear. While P1 (1573.96 mM) compared to P3 (1707.30 mM) appear significantly increased (p > 0.05). In the P2 treatment (1665.70 mM) compared to P3 (1707.30 mM) seen no difference. Thus the total antioxidant levels in the blood of Wistar rats is the total antioxidant capacity levels in the blood of mice after getting radiation for 60 days, every day 5 hours using a UV lamp Philips TL 15 watts.

By looking at the results of Table 1 above description can be explained, that supplementation tempeh M-3 and carrots in P2 than P3 treatment was not significantly different. Thus can be explained the 3 treatment does not increase the total antioxidant capacity in the blood of UV irradiated Wistar rats. Because the composition of components in the form of antioxidant isoflavones genestain of 67.21
mg, and 8-hydroxy of 494.4 mg daidzein become saturated, so that the total antioxidant effect in the blood can not be increased, this finding is consistent (Kooter.2004). Kooter say that a anioksidan will work effectively in a certain concentration.

Table 2: Levels of 8-hidroksu-2-deoksiguanosin Wistar Rat Urine Teradiation UltravioletTempeh supplemented with M-3 and carrots

<table>
<thead>
<tr>
<th>No.</th>
<th>P0</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.99</td>
<td>16.73</td>
<td>13.89</td>
<td>12.99</td>
</tr>
<tr>
<td>2</td>
<td>18.93</td>
<td>16.55</td>
<td>12.61</td>
<td>12.19</td>
</tr>
<tr>
<td>3</td>
<td>19.15</td>
<td>16.33</td>
<td>12.99</td>
<td>12.20</td>
</tr>
<tr>
<td>4</td>
<td>17.65</td>
<td>17.15</td>
<td>12.60</td>
<td>12.71</td>
</tr>
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<td>5</td>
<td>17.15</td>
<td>17.21</td>
<td>12.73</td>
<td>12.46</td>
</tr>
<tr>
<td>6</td>
<td>18.59</td>
<td>16.34</td>
<td>12.99</td>
<td>12.23</td>
</tr>
<tr>
<td>Σ</td>
<td>109.47</td>
<td>100.32</td>
<td>77.80</td>
<td>74.78</td>
</tr>
<tr>
<td>Ratarata</td>
<td>18.24 ±0,79</td>
<td>16.77 ±0,39</td>
<td>12.97 ±0,49</td>
<td>12.46 ±0,79</td>
</tr>
</tbody>
</table>

In Table 2 above on P1 (16.77 ng / mL), P2 (12.97 ng / mL), and P3 (12.46 ng / mL) compared to controls without tempeh M-3 and carrots (P1 = 18.24 ng / mL) decreased levels of DNA damage is significant, this is due to ultraviolet radiation. But at P2 and P3 did not decrease as more and more tempeh supplementation given in experimental animals will cause more and more antioxidant content there. Hence the ability to capture free radicals will also be stronger and the impact that can be seen is a decline in the levels of 8-OHdG as a sign of DNA damage by hydroxyl free radical 22.61%, so the required daily intake of vitamin E in 450 UI (Astawan., 2009). Damage is damage to skin tissues lining the body wrapping constantly changing as a result of the influence of the outside environment. In general, the skin is divided menjai 3 parts: layers of the epidermis, dermis, and lapiasan subkutan. Apabila denan irradiated UV light from fluorescent lamp of 15 watts for 5 hours per day for six days will cause tens skin changes; Because Oxygen Radical species can be oxidized to form melatonin clinically as pimentation immediate darkening (IPD) (Steeghs MS., et.al. 2006).

Percentage of core damage to the epidermal layer of cells from radiation for 5 hours per day in the next 2 months can be seen in Table 3 below.
Tabel 3: Prosentase Damaging of Nuclear cell in skin *Wistarrat* Teradiation

<table>
<thead>
<tr>
<th>No</th>
<th>$P_0$</th>
<th>$P_1$</th>
<th>$P_2$</th>
<th>$P_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>78,27</td>
<td>57,13</td>
<td>31,99</td>
<td>27,58</td>
</tr>
<tr>
<td>2</td>
<td>73,92</td>
<td>54,36</td>
<td>31,35</td>
<td>28,26</td>
</tr>
<tr>
<td>3</td>
<td>72,71</td>
<td>57,99</td>
<td>27,60</td>
<td>28,99</td>
</tr>
<tr>
<td>4</td>
<td>76,93</td>
<td>55,55</td>
<td>28,99</td>
<td>27,39</td>
</tr>
<tr>
<td>5</td>
<td>77,77</td>
<td>56,64</td>
<td>26,99</td>
<td>25,99</td>
</tr>
<tr>
<td>6</td>
<td>76,01</td>
<td>52,18</td>
<td>33,73</td>
<td>39,01</td>
</tr>
<tr>
<td>Σ</td>
<td>75,85 ± 2,33</td>
<td>55,42 ± 2,24</td>
<td>29,86 ±2,79</td>
<td>29,92 ±1,13</td>
</tr>
</tbody>
</table>

Table 3 is significantly above the 2 treatment, because tempehM-3 supplementation and carrots in 2 treatment decreased tissue damage by 60.60%.

**CONCLUSION**

The study found some biological effect of supplementation with isoflavones on Tempeh, M-3 and carrots for the decrease in total antioxidants, reduced levels of 8-OHdG, and a decrease in epidermal cell damage that UV irradiated for 2 months jamper exposed 5 days. All these suplentasi given to the treatment of animals try tempeh M-3 and carrots in P2 will lead to increased damping process of the M-3 antioxidant tempeh and carrots to inhibit the mice skin tissue damage.

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