EFFECTS OF RED DRAGON FRUIT PEEL EXTRACT ADMINISTRATION ON NEUTROPHIL COUNTS FOLLOWING PERiapICAL RADIOPHOROGRAPHY EXPOSURE

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ABSTRACT
Background: Radiation exposure from periapical imaging can cause cell damage in various tissues due to the formation of free radicals. Cell damage in the tissue can be observed from the number of neutrophils which are immune cells. Free radicals can be suppressed by administering exogenous antioxidants. One source of natural antioxidants is red dragon fruit, especially on its peel. Objective: To analyze the effect of periapical imaging x-ray radiation exposure after the administration of red dragon fruit peel extract on absolute neutrophil counts in mice. Method: This research was a true experimental study with a post-test only and control group design using 24 mice that were divided into 6 groups. The control group was not given any treatment while P1 to P5 group were given red dragon fruit peel extract at a dose of 100 mg/kg BW and exposed to radiation with different exposures. P1 group was exposed 1 time (0.63 mGy), P2 group was exposed 4 times (1.66 mGy), P3 group was exposed 8 times (4.37 mGy), P4 group was exposed 12 times (8.19 mGy), and group P5 was given no exposure. Results: There were changes in the number of neutrophils in all groups. The LSD Posthoc test results showed a significant difference between the control group (12.470 / mm³) and the P5 group (12.470 / mm³) and the P5 group (12.470 / mm³) compared to P3 group with 8 times exposure (40.348 / mm³). Conclusion: There is an effect of periapical radiographic x-ray radiation exposure by red dragon fruit peel extract on the absolute neutrophil count in mice.

Keywords: Antioxidants, Neutrophils, Periapical Radiography, Radiation, Red Dragon Fruit Peel.
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INTRODUCTION
Radiographic examination in dentistry has a role to support the diagnosis, prognosis, and treatment plan. However, radiation exposure has negative effects to cause biological changes and causes cell death¹,². One type of radiography that is often used in dentistry is periapical imaging. The reference for dose limits of periapical radiography proposed by the International Atomic Energy Agency (IAEA) is 7 mGy. Damage occurs and worsens along with the increase of doses absorbed by the body. In taking periapical photos, technical and processing errors often occur so that the quality of photo is poor and requires imaging retake. Repeated radiation exposure increases the dose absorbed by the patient thereby increasing the risk of negative effects on the patients³,⁴.

Radiographic examination for diagnostic purposes is not only undertaken by adult patients but also by children. Physically, psychologically, and psychologically, there are differences between children and adults. The function of the organs of the child's body is not yet fully developed. Similarly, the defense function is not perfect and the cells are still in the process of growth so that they are very sensitive to radiation. Also, theoretically, large doses of radiation can increase the risk of radiation-induced cancers in children⁵,⁶.

Radiation can cause DNA damage directly and indirectly. Damage directly occurs due to radiation exposure causing DNA to be ionized or excited due to broken covalent bonds. DNA changes that occur can cause cell death. Meanwhile, indirect damage to DNA occurs due to the formation of free radicals from the radiolysis of water molecules. As much as 70% of DNA damage due to radiation occurs by indirect mechanism as the result of water radiolysis and free radical formation⁷. Radiation exposure can trigger excessive amounts of free radicals and cause the body's defense system to be unable to cope with such condition, thus resulting in a state of oxidative stress. Cell damage in tissue can cause cells to undergo apoptosis if they cannot generate self-repair. Cell damage in tissue can be characterized by an increase in neutrophils which act as immune cells. Oxidative stress illustrates the number of Reactive Oxygen Species (ROS) in the oxidation process, thus causing the body's antioxidant capacity to fail in defending the body from excessive ROS production. Body produces endogenous antioxidants as protectors against oxidative stress. However, the endogenous antioxidant activity can decrease in...
pathological conditions due to the excessive amount of free radicals, so exogenous antioxidants are required to eliminate and neutralize the effects of free radicals. One source of natural antioxidants is red dragon fruit (*Hylocereus costaricensis*). Red dragon fruit is one of the fruits that have antioxidant properties with higher content of antioxidant presented on its skin. Phytochemical test results show red dragon fruit peel extract contains antioxidants in the form of vitamin C, flavonoids, tannins, alkaldoids, steroids, and saponins. Although red dragon fruit, in general, can be found throughout Indonesia, dragon fruit has become a unique commodity in Tanah Laut Regency that has been widely cultivated as a horticulural crop in the Pelaehari sub-district since 2006. Research by Rahman et al (2016) testing the immunomodulatory activity of red dragon fruit extract on the number of mice leukocytes showed the results at a dose of 100 mg/kg BW is the best dose as an immunomodulator so that it can help maintain the immune system.

Based on the background above, this study aimed to determine the effect of periapical radiographic x-ray radiation exposure after the administration of red dragon fruit peel extract on the absolute neutrophil count in mice.

**MATERIALS AND METHODS**

This research was initiated by collecting research permit and ethical clearance issued by the Ethics Committee of the Dentistry Faculty Lambung Mangkurat University No. 053 / KEPKG-FKGULM / EC / I / 2020. This study was a true experiment with a post-test only and control group design using 24 mice as samples. The inclusion criteria in this study were male mice, 20-25 grams weight, 3-4 months old, active, and agile. Mice were divided into 6 groups with each group consisted of 4 mice. The treatment group in this study included the control group without treatment, P1 group given 100 mg/kg BW red dragon fruit extract and exposed to radiation once, P2 group given 100 mg/kg BW red dragon fruit extract and exposed to 4 times radiation, P3 group red dragon fruit peel extract given 100 mg/kg BW and exposed to radiation 8 times, group P4 given red dragon fruit peel extract 100 mg/kg BW and exposed to radiation 12 times, group P5 given red dragon fruit peel extract 100 mg/kg BW without being exposed.

The equipments used in this research were ASAHI brand dental radiography with a voltage of 60 kV and a current of 10 mA, dosimeter, mouse cage, food container, extracting equipment (funnel, blender, measuring flask, volume pipette, water bath, cuvette, and laboratory glassware), fixation cages, gastric tube for extracts, 1 ml syringe, vaculab + EDTA tubes, masks, and handscoons. The ingredients used in this research were red dragon fruit peel, 70% ethanol solvent, distilled water, comfeed feed, and diethyl ether solution. The raw material used was first determined in the Basic Laboratory Faculty of Mathematics and Science Lambung Mangkurat University and the results were obtained that the plants used were red dragon fruit (*Hylocereus costaricensis*).

**Red Dragon Fruit Skin Extraction**

As much as 3 kg of red dragon fruit skin were obtained from the dragon fruit plantation in Jilatan, Tanah Laut Regency. Fresh red dragon fruit peel samples were taken and wet-sorted. Next, the chopping and the cutting of dragon fruit skin into thin and small pieces were performed. The pieces were then dried for 3-4 days under sunlight and dried further in the dryer (oven) at 55 °C temperature for 24 hours. The process of extracting red dragon fruit peel using maceration method included the weighting of dragon fruit skin and the maceration process using 70% ethanol solvent. The ethanol solvent was poured slowly into a maceration container that has filled with the sample while stirring until it was evenly distributed. Extraction was carried out for 3 x 24 hours and every 24 hours the liquid was replaced while stirring the filtrate once, the extracted filtrate was evaporated until a thick extract was obtained and then evaporated using a rotary evaporator at a temperature of 50°C - 70°C, then the extract was thickened using water baths until thick red dragon fruit peel extract was obtained.

**Giving Extract Solution in Mice**

Red dragon fruit extract was thickened then dissolved into 100 mg/kgBW solution and administered to experimental animals with a bodyweight of 20-25 grams. The dose of extract in the required solution was 2-2.5 mg which was then dissolved in 0.5 ml distilled water to adjust the volume of experimental animal gastric. Extract was administrated using gastric tube once daily for 6 days.

**Periapical Radiography Radiation**

Periapical radiographic irradiation was carried out on the sixth day at the radiology unit of RSGM Gusti Hasan Aman Banjarmasin. Mice were fixed with wire cage and styrofoam to hold the mice during the exposure process. The time interval for each repetition was 1 minute. After 24 hours after radiation, blood samples were taken to be examined.

**Examination of Neutrophils**

Mice were first anesthetized by the inhalation of dietyl ether. Mice are put in a jar filled with cotton that had been covered with diethyl ether and kept for a few minutes until the mice was observed with no movement. Mice then disected using tweezers and surgical scissors to ease blood aspiration from the heart. Blood was drawn from the heart using a 1 ml syringe and then placed in a vaculab containing EDTA. Blood samples taken to the laboratory for examination of neutrophils by Giemsa staining.

**RESULT**

In this study, the average absorption dosage data obtained from the dosimeter reading in each experimental group is tabulated as follows:
Table 1. Average doses of periapical radiographic radiation absorbed by mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>X-Ray Radiation Exposure of Periapical Radiography</th>
<th>Average exposure dose (mGy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>1 times</td>
<td>0.63</td>
</tr>
<tr>
<td>P2</td>
<td>4 times</td>
<td>1.66</td>
</tr>
<tr>
<td>P3</td>
<td>8 times</td>
<td>4.37</td>
</tr>
<tr>
<td>P4</td>
<td>12 times</td>
<td>8.19</td>
</tr>
</tbody>
</table>

Table 1 shows the average radiation dose absorbed by mice at 1, 4, 8, and 12 times of periapical radiographic x-ray radiation exposure. P1 group with 1 time exposure showed an average absorbed dose of 0.63 mGy. P2 group with 4 times the amount of exposure showed an average absorbed dose of 1.66 mGy. P3 group with 8 times the amount of exposure showed an average absorbed dose of 4.37 mGy. P4 group with 12 times the amount of exposure showed an average absorbed dose of 8.19 mGy. Radiation at 1, 4, and 8 times exposure shows that the dose of radiation absorbed is still below the reference IAEA limit which is 7 mGy, while the radiation dose absorbed at the twelfth exposure was above 7 mGy that is beyond the recommendation of IAEA.

Table 2. Mean and Standard Deviation of absolute neutrophil count (ANC) in mice exposed to periapical radiographic radiation and given a red dragon fruit peel extract

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Mean ± SD</th>
<th>scoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>4</td>
<td>17.668 ± 11.9825</td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>4</td>
<td>15.910 ± 3.3336</td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>4</td>
<td>5.040 ± 4.3132</td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>4</td>
<td>40.348 ± 19.3874</td>
<td></td>
</tr>
<tr>
<td>P4</td>
<td>4</td>
<td>33.820 ± 27.3288</td>
<td></td>
</tr>
<tr>
<td>P5</td>
<td>4</td>
<td>12.470 ± 9.1327</td>
<td></td>
</tr>
</tbody>
</table>

Note: K: Control; P1: Extract + 1 exposure; P2: extract + 4 times exposure; P3: Extract + 8 times exposure; P4: extract + 12 times exposure; P5: extract without radiation.

The numerical data obtained from the study are then presented in the form of bar charts as follows:

Figure 1. Diagram of the Average Amount of Neutrophils in Mice

Table 2 and Figure 1 show the average number of neutrophils in the study group. The control group had an average number of neutrophils of 17,668 cells / mm3.

P1 group had an average neutrophil count of 15,910 cells / mm3. P2 group has an average number of neutrophils of 5,040 cells / mm3. The P3 group had an average neutrophil count of 40,348 cells / mm3. The P4 group had an average number of neutrophils of 33,820 cells / mm3. The P5 group had an average neutrophil count of 12,470 cells / mm3.

Before testing the hypothesis, Shapiro Wilk normality test and Levene’s test of homogeneity test were performed using SPSS. The results of the normality test showed that the data were normally distributed because all groups had p > 0.05. The homogeneity test was followed and resulted in a significance value of 0.084 (p > 0.05) denoting homogeneous sampling of the data. Data analysis ANOVA showed a significant difference with a value of 0.033 (p < 0.05) so that the LSD Post-Hoc test could be performed to find out which groups that had significant differences.

Table 3. Post-Hoc Least Significance Difference (LSD) Test Results

<table>
<thead>
<tr>
<th>Groups</th>
<th>K</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>-</td>
<td>.872</td>
<td>.254</td>
<td>.049</td>
<td>.149</td>
<td>.634</td>
</tr>
<tr>
<td>P2</td>
<td>.872</td>
<td>-</td>
<td>.324</td>
<td>.035</td>
<td>.112</td>
<td>.752</td>
</tr>
<tr>
<td>P3</td>
<td>.254</td>
<td>.32</td>
<td>-</td>
<td>.004</td>
<td>.015</td>
<td>.497</td>
</tr>
<tr>
<td>P4</td>
<td>.049</td>
<td>.03</td>
<td>.004</td>
<td>-</td>
<td>.550</td>
<td>.018</td>
</tr>
<tr>
<td>P5</td>
<td>.149</td>
<td>.11</td>
<td>.015</td>
<td>.550</td>
<td>-</td>
<td>.062</td>
</tr>
<tr>
<td></td>
<td>.634</td>
<td>.75</td>
<td>.497</td>
<td>.018</td>
<td>.062</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: blue = there is a significant difference

Post-hoc Least Significance Difference (LSD) test results showed that there was an effect of periapical radiographic x-ray exposure by giving red dragon fruit peel extract to the absolute neutrophil count in mice. The control group had a significant difference compared to the P3 group with a value of p equal to 0.049 indicating p<0.05. The P1 group had a significant difference compared to the P3 group with a p-value of 0.035. P2 group had a significant difference compared to P3 group with a value of p = 0.004 and group P4 with a value of p = 0.015. The P3 group had a significant difference compared to almost all groups except for P4 group which is distinguished by the p-value (p < 0.05). The P5 group had a significant difference compared to the P3 group with a p-value of 0.018. The control group and the P5 group did not show any significant differences with the P1, P2, and P4 groups so that it could be interpreted for having equivalent effect.

DISCUSSION

The results of this study shows the influence of periapical radiographic radiation exposure to the absolute neutrophil count in mice given red dragon fruit
peel extract. The group given extract without exposure to radiation showed a slight decrease in absolute neutrophil count compared to the group with no exposure. The decrease in absolute neutrophil count of non-exposed group given red dragon fruit peel extract occurs because the antioxidant content prevent cell damage in tissues and reduce free radicals in the body so that the neutrophil function is preserved.

Significant increase was observed in P3 group that was given extracts and exposed to periapical radiation 8 times and also P4 group that was given extracts and exposed to periapical radiation 12 times. This is caused by the absorbed radiation dose that is getting higher that the damage caused in various tissues increase. Radiation exposure releases x-ray photons that attack cells and alter biological molecules. The affected molecule then undergoes a change in structure and function that is different from the original molecule. Radiation exposure also causes the formation of free radicals that trigger cell damage. In cells that cannot repair themselves, apoptosis, or cell death occurs.

Cellular damage due to radiation, that can cause cell death both in circulation and in tissues, triggers the production of inflammatory mediators by host cells which trigger the release of Granulocytes Colony Stimulating Factor (G-CSF). G-CSF is a hematopoietic cytokine or hematopoietic growth factor that stimulates the formation of neutrophils and induces the release of neutrophils from bone marrow into the blood circulation. The release of circulated neutrophils can increase tenfold in a matter of hours. The P3 group with 8 times radiation exposure had the highest neutrophil number among all groups. This can be caused by the radiation dose in P3 group that was approaching the IAEA reference dose limit of 7 mGy so that the occurring damage triggers the release of more neutrophils into the circulation. Whereas in the P4 group that had a neutrophil count slightly lower than P3 group, the result might be prompted from the radiation doses that had exceeded the reference dose. This causes circulated neutrophils to perish so the number will deplete.

The number of neutrophils in the circulating blood is regulated by the CXCL12 / CXCR4 axis. Bone marrow stromal cells express chemokine CXCL12, a ligand for CXCR4 that regulates neutrophils in the bone marrow or mobilizes blood circulation. Radiation exposure cause cellular injury to tissues, causing the release of various signals, including damage-associated molecular patterns (DAMPs) from damaged cells. Cells in tissue including macrophages, dendritic cells, and endothelium detect these signals and begin the recruitment of neutrophils to the site of tissue injury. The amount of damage is increased and cannot be prevented by the administration of red dragon fruit peel extract as seen from the significant difference of neutrophils in the P3 and P2 groups where the radiation dose is lower. The effectiveness in the use of herbs as antioxidants is influenced by the use of appropriate dosages and the length of time.

In this study, some weaknesses are not absolutely the same with the actual condition. The prior number of neutrophils in mice before treatment was unknown because it was only measured after the treatment so that the effect cannot be known with certainty. Sampling was examined 24 hours after radiation exposure so that the effects known were merely on the mature neutrophils number. The effect on neutrophil formation was unknown since the formation of neutrophils takes 7-8 days. Also, the blood sample that was examined is not absolute according to the condition in the body. This is because the addition of anticoagulants in blood samples can cause lysis of cells so that the number of cells in a sample can be lower than the actual.

Based on the results of research that has been done, it can be concluded that there is an effect of periapical imaging x-ray radiation exposure after administration red dragon fruit peel extract to the absolute neutrophil count in mice. Further research can be done by increasing the extract dose or by increasing the time of administration of the extract to experimental animals exposed to radiation with repeated exposure.

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