Targeted Reduction of Lipid Peroxidation in Parotid Gland Cell Membranes Due to Fractionated Dose of X-Ray Radiotherapy by Bioactive Compounds of Blue-Green Algae

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Abstract

This study aims to identify the bioactive compounds contained in blue-green algae (Spirulina platensis), determine the malondialdehyde levels between groups induced by single dose X-ray radiotherapy (single fractionation of 10 Gy) and fractionation dose, and determine the ability of these active compounds to repair the lipid membrane damage of the fractionated dose irradiated group. Thirty-six male Rattus norvegicus were divided into six groups: K0 (control group), K1 (single dose irradiated group), K2 (fractionated dose irradiated group), P1 (group irradiated with fractionated dose and given 300 mg/kg BW blue-green algae), P2 (group irradiated at a fractionated dose and given 600 mg/kg BW blue-green algae), and P3 (group irradiated at a fractionated dose and given 900 mg/kg BW blue-green algae).

The results showed that the group irradiated with a single dose showed a higher increase in malondialdehyde levels than the group induced by fractionated dose radiotherapy. The bioactive content of blue-green algae can reduce malondialdehyde levels.

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Introduction

X-rays used in radiotherapy are electromagnetic radiation types that have relatively large energy and high penetrating power. It can ionize by transferring a certain amount of energy to the target atoms and biomolecules so that their characteristics change. This energy transfer has a necessary consequence in the form of an increase in reactive oxygen species (ROS), which occurs mainly through decomposing cellular water[1,2].

ROS is an unstable molecule with an unpaired electron that has high reactivity with other molecules in a cell. These irregular forms are called free radicals and may cause cell damage, cell function disorders, and cell death. The main molecules that are disrupted are deoxyribonucleic acid (DNA), membrane lipids, and proteins[3,4]. Polyunsaturated Fatty Acids (PUFA) in cell membranes are essential components of phospholipids that compose cell membranes. ROS can oxidize PUFA, which is known as lipid peroxidation. This process affects changes in cell membrane permeability that result in cell death. Lipid peroxidation is an essential component of oxidative stress[5, 6]. Assessment of oxidative stress conditions in biological systems is generally carried out by measuring markers of oxidative damage (towards lipids, proteins, and DNA) and oxidant status.

One indicator to detect oxidative stress conditions – resulting from cell membrane lipid peroxidation in the body due to ROS – is malondialdehyde (MDA) levels. MDA is a good and widely used marker for lipid peroxidation[7]. Previous research has shown that blue-green algae (Spirulina platensis) help to bind radioactive elements and is useful for protecting the human body from exposure to radiation therapy (radioprotective effect). Spirulina platensis was obtained from Jepara, Central Java, Indonesia. Phycocyanin is one of the ingredients found in Spirulina platensis, which

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has the ability as an antioxidant that can inhibit lipid peroxidation in rats[5].

Materials and methods

Animal preparation and experimental design

Thirty-six male rats of Rattus norvegicus Wistar strain obtained from the Laboratory of Biochemistry Laboratory of the Faculty of Medicine, Airlangga University, Indonesia, 200-250 grams in weight were randomly divided into 6 groups: K0 was the control group, K1 was the group irradiated at a single dose, K2 is the group irradiated with fractionated doses, P1 is the group irradiated with a fractionated dose and given 300 mg/kg BW blue-green algae, P2 is the group irradiated with a fractionated dose and given 600 mg/kg BW blue-green algae, and P3 is the group irradiated with a fractionated dose and given 900 mg/kg BW blue-green algae. This study obtained the ethical feasibility of experimental animal research from the Faculty of Veterinary Medicine, Airlangga University.

Blue-green algae powder preparation and secondary metabolite phytochemical screening test

The phytochemical screening test is a quantitative test aiming to determine the content of compounds in a material. A phytochemical screening test is carried out by looking for a change in color or the appearance of a precipitate using the required reagents.

Preparation of suspension given to experimental animals was done by mixing powdered blue-green algae (Spirulina platensis) into 0.5% CMCNa as a solvent which was then given to mice using a feeding tube (TERUMO, Hangzhou, China) 40 cm long. The doses were given at the same time/hour in the morning before the rats were fed. Spirulina platensis was given once a day for seven days before and five days during radiation.

X-ray radiotherapy

The group with 10 Gy single fraction X-ray radiation and the group with a total dose of 10 Gy given by fractionation of 2 Gray (Gy) were given radiations for 5 days on the ventral surface of the rat neck. This process used a linear accelerator (CLINAC 2100 Ex Model), Source to Skin Distance (SSD) = 100 cm, field size 7 cm x 40 cm, 6MV[8].

Measurement of MDA levels in the salivary glands of Wistar rats

Spectrophotometer were based on reaction with Thiobarbituric acid (TBA) using a commercial kit. The parotid gland was weighed 100 mg, put in a cold mortar, crushed, and added 200 µL of NaCl fis 0.9%. Add back 550 µL aquadest, 100 µL TCA, 250 µL HCl 1N, and 100 µL Na-thio to it at each addition of these reagents, as the solution is homogenized with a vortex. Incubated in a water bath with a temperature of 100^{o}C for 10 minutes, it was removed and left at room temperature before centrifuged at 500 rpm for 10 minutes at 4^{o}C. The supernatant was taken at 300µl and transferred to a new test tube. The Thiobarbituric acid (TBA) test was based on a condensation reaction between 2 moles of TBA and 1 mole of MDA. The absorbance of the sample was measured using a spectrophotometer at the maximum wavelength of the TBA test (532 nm), and the absorbance value was then plotted on the MDA standard curve that had been made to calculate the sample concentration[9].

Statistical analysis used the normality Shapiro-Wilk test to determine the observed levels of malondialdehyde ($p > 0.05$). Oneway Anova test was utilized to find the differences between the groups, and multiple comparison LSD tests were employed to see differences in detail.

Results

The results showed the presence of alkaloid compounds using Mayer and Dragendorff reagents. The Spirulina platensis changed to green, indicating the extract contained steroids. Analysis using the Froth test showed a stable foam for 10 minutes, indicating the presence of saponins. Spirulina platensis color changes to yellow, so the extract contains flavonoids, as shown in Table 1.

In Table 2, the malondialdehyde level of the parotid gland decreased significantly ($p < 0.05$) due to administering Spirulina platensis compared to the irradiated x-ray group. The lowest malondialdehyde level was found in the P3 group. There was no significant difference in malondialdehyde level between this group and the P2 group. Administering 900 mg/kg BW of Spirulina platensis reduced malondialdehyde level close to normal.

Metabolites	Results
Steroid	+
Saponin	+
Alkaloid	+
Flavonoid	+
Tanin	-
Triterpenoid	-
Note: (+) presence, (-) absence	

Table 1. Screening Test Results. Secondary Metabolite Phytochemical Spirulina platensis from Jepara, Central Java, Indonesia.

Groups	N	Malondialdehyde (µg/mL)	P
K0	6	18.93^{a} ± 3.11	0.000*
K1	6	32.79^{b} ±5.07	
K2	6	31.94^{b} ±7.07	
P1	6	28.86^{b} ±2.00	
P2	6	26.17^{ab} ±1.64	
P3	6	25.67^{ab} ±4.43	

Table 2. Shows the mean levels of malondialdehyde in control and treatment groups.

Discussion

The results showed that X-ray radiation caused a significant increase in malondialdehyde levels in the group that was only given radiation, both single and fractionated dose. A single dose caused an increase in malondialdehyde levels, which was higher, but not significant compared to the malondialdehyde levels in the fractional dose irradiated group. This study has not been able to prove the theory which states that fractionated doses provide an opportunity for cells to repair themselves while single doses cause more severe damage than fractionated doses. This is due to the administration of a total radiation dose of 10 Gy, which allows normal cells to be observed, in addition to paying attention to the dose per fraction and the time between fractions [10].Free radicals are highly reactive and unstable in the human body cells and can cause cellular, tissue, and genetic damage (mutations)[11]. Free radicals have a very short half-life because, once formed, these components will immediately react with other molecules[12]. Wistar rats induced by X-ray radiotherapy went through absorption during the transmission process in the material. The penetrating power of X-rays depends on the type of material and its energy. X-rays are ionizing radiation to produce free electrons in the acini cells. X-rays can change body cells[13]. Indirect ionization occurs as water-contained acini cells of the parotid gland was affected. The ionization process that occurs causes a hydrolysis reaction of water. This ionization process can lead to the formation of hydrogen peroxide free radicals and hydroperoxyl radicals[14].

Energy deposition occurs unevenly in cells and through a series of biochemical processes, which includes the formation of free radicals at the molecular level, and the release of biological mediators at the cellular and tissue levels [14]. Free radical chain reactions often happen during the formation of organic peroxides – indicating a very large increase in the number of molecules affected. Peroxyl radicals can cause lipid peroxidation chain reactions in cell membranes. This resulted in the breakdown of fatty acids into various compounds that are toxic to cell walls, including various aldehydes such as malondialdehyde[15].

The target of ROS in the early stages of malondialdehyde formation is unsaturated fatty acid bonds[16]. Malondialdehyde parameters done in the parotid glands of Wistar rats irradiated by X-ray indicate the occurrence of lipid peroxidation due to the reaction between hydroxyl radicals and PUFAs on cell membranes. This occurs due to an imbalance where oxidation exceeds the antioxidant system in the body. Malondialdehyde assay is a reliable approach for in vivo assessment of oxidative stress status [17,18]. Some of the most important membrane constituent structures, such as phospholipids and glycolipids, contain unsaturated fatty acids and PUFAs which are very susceptible to radicals, especially hydroxyl radicals. Hydroxyl radicals can cause toxic compounds, including various aldehydes such as malondialdehyde, 9-hydroxyl-neonatal, and various hydrocarbons such as ethene and pentene. The main toxic compound formed is malondialdehyde[16].

This study showed that there was a significant difference ($p < 0.05$) between groups that were not given Spirulina platensis, and the group that was given Spirulina platensis before and during irradiation. The administration of Spirulina at a dose of 300 mg/kg BW showed a decrease in malondialdehyde levels but still did not show a significant difference compared to the group given radiation. Whilst the administration of Spirulina platensis at a dose of 600 mg/kg BW and 900 mg/kg BW could reduce MDA levels

compared to the group irradiated without Spirulina platensis.

The theory was proven in this study by the decrease in malondialdehyde in groups P2 and P3. ROS in several studies can be overcome by Spirulina platensis. Phenolic acid, vitamin E, phycocyanin, -carotenes and, chlorophyll-a give Spirulina platensis antioxidant abilities. Phenolic acid can act as an antioxidant because it has the ability to donate hydrogen atoms. Spirulina platensis has a blue-green pigment, phycocyanin – a strong antioxidant [19] and is easily soluble in water. Selenium-containing phycocyanins have been shown to scavenge strong superoxide radicals and hydrogen peroxide[19].

The results of this study show that Spirulina platensis was able to reduce malondialdehyde levels. This indicates the effectiveness of Spirulina platensis to maintain balanced levels of oxidants and antioxidants depending on the dose. The decrease in malondialdehyde levels was proportional to the dose of Spirulina platensis. The higher the dose of Spirulina platensis, the greater the decrease in malondialdehyde levels. Group P2 was given Spirulina platensis at a dose of 600 mg/kg BW and Group P3 was given Spirulina platensis at a dose of 900 mg/kg BW given for 7 days before radiation and for 5 days given radiation. Both results show that more antioxidants play a role in neutralizing free radicals. This study is supported by research by Lee et al. which states that the effectiveness of Spirulina platensis to maintain oxidant and antioxidant levels depends on the duration of treatment[20]. The administration of Spirulina platensis 600 mg/kg BW and the administration of 900 mg/kg BW showed the same effectiveness, meaning that the administration of 600/kg BW was sufficient to neutralize free radicals with the same ability in the group given 900/kg BW. Spirulina platensis is known as the best source of nutrients that are 100% derived from nature, namely the coast[21]. Ancient residents such as the Mexican Aztecs have consumed it as their food ingredient.

Conclusions

Blue green algae powder (Spirulina platensis) originating from Jepara, Central Java, Indonesia, contains secondary metabolites such as alkaloids, flavonoids, steroids, and saponins. The higher the dose of Spirulina platensis, the greater the decrease in malondialdehyde levels that occurred.

Declaration of Interest

There are no conflicts of interest reported by the author.

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PENELITIAN BERJUDUL	: Proteksi Xerostomia Dengan Algae Biru Hijau (*Spirulina plantesis*) Melalui Analisis Kadar F2 Isoprostan, Ekspresi p53, Bcl-2, Caspase-3, AQP-5, dan Sekresi Saliva
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