Radioprotective Effect of Spirulina Platensis on Head and Neck Radiation-Induced Xerostomia

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Abstract

Radiotherapy relating to the head and neck is often accompanied by side effects with varying degrees of xerostomia. Spirulina has high nutritional value due its content. The current study aims to establish, the possible effect of Spirulina on salivary dysfunction. A total of 36 male, Wistar Strain Rattus Norvegicus weighing 200-250 grams were divided into six groups: the normal control, subjects only irradiated as the negative control, subjects which received 300 gr/ kgBW of Spirulina (obtained from DXN) and were irradiated, subjects which received 300 mg/ kgBW of Spirulina and were irradiated, subjects which received 600 mg/ kgBW of Spirulina and irradiated, subjects which received Spirulina 900 mg/ kgBW and irradiated. Spirulina was administered for seven days before and during five days of X-ray irradiation. 24 hours after the final fractionation, blood and the submandibular gland were collected and analyzed to determine their levels of F2-isoprostane, caspase-3 expression, and AQP5 expression. Spirulina was able to reduce F2-isoprostane levels and decrease caspase-3 and while also increasing AQP5 expression.

Spirulina with various antioxidant content can prevent xerostomia caused by head and neck radiotherapy. The study showed that 600 mg/kgBW Spirulina was an effective dose.

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Introduction

Radiotherapy is a method of treating cancer using radiation derived from radioactive energy and, therefore, both inhibits the proliferation of tumor cells and results in their destruction of tumor cells[1]. Unfortunately, radiotherapy not only kills tumor cells but also involves risk to the surrounding normal tissue which is exposed to radiation resulting in serious side effects such as xerostomia that can cause to several oral diseases[2, 3, 4]. Radiation therapy applied in cases of head and neck cancer uses a total dose of 50-70 gray (Gy) administered at 1.8-2 Gy per day, five days a week for 5-7 weeks[5]. A massive reduction in saliva production occurs, especially during the first week of radiation administration, where the decrease in saliva production reaches 50-60% subsequent to a total dose of 10 Gy. This constitutes a moderate dose following which the normal function of cells and tissues can still be observed after any exposure to ionizing radiation [6].

Isoprostane represents a series of prostaglandin compounds produced by the reaction of ROS with arachidonic acid which is widely known to be a reliable biomarker of oxidative stress [7]. In the caspase cascade mechanism, caspase proteins can act as caspase initiators or caspase executors. Caspase-3 is the most important caspase executor [8]. Protein damage due to ROS that occurs in acinar membrane cells causes changes in the expression of aquaporin-5 (AQP5) protein which, in turn, initiates AQP5 activity as the water channel decrease, while also reducing its function in the channeling fluid [9].

Spirulina platensis is a blue-green bioactive compound-rich alga widely used as a supplement or natural treatment ingredient. Spirulina platensis is high in protein (60-70%),

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phytopigments (b-carotenes, chlorophyll and phycocyanin), vitamins, minerals, essential fatty acid, essential amino acids and other nutritional elements[10]. *Spirulina platensis* demonstrates multiple biological functions such as antioxidant, anti-inflammatory, immunomodulatory, antidiabetic, neuroprotective, anticancer and hepatoprotective [11].

Materials and methods

Animal preparation and experimental design

Male Wistar rats (*Rattus norvegicus*), 200-250 grams in weight, declared healthy by the veterinary clinic of Dinas Peternakan, East Java, Indonesia were maintained in optimum conditions at the Animal House Unit of the Universitas Airlangga Faculty of Medicine. These subjects were provided with standard rat pellets and water ad libitum and kept in conditions of controlled lighting and temperature. The Animal Care & Ethics Committee of the Airlangga University Faculty of Veterinary Medicine specifically approved this study (2.KE.060.04.2018). After a week of adaptation, a total of 36 male, Wistar Strain Rattus Norvegicus weighing 200-250 grams were divided into six groups: group 1; untreated subjects as the normal control (N group), group 2; subjects only irradiated as the negative control (RT group), group 3; subjects which received 300 mg/ kgBW of *Spirulina* (obtained from DXN Malaysia) and were irradiated as the positive control (RT+Std), group 4; subjects which received 300 mg/ kgBW of *Spirulina* and were irradiated (RT+Sp300), group 5; subjects which received 600 mg/ kgBW of *Spirulina* and irradiated (RT+Sp600), group 6; subjects which received *Spirulina* 900 mg/ kgBW and irradiated (RT+Sp900).

Spirulina platensis

Members of Group RT+Std, as the positive control, were fed *Spirulina platensis* purchased from DXN Pharmaceutical Sdn. Bhd. (401692-K) Lot 1169, Mukim Malau, Daerah Kubang Pasu, 06000 Jitra, Kedah Darul Aman, Malaysia. The members of Group RT+Sp300, Group RT+Sp600 and Group RT+Sp900 were fed *Spirulina platensis* from Balai Besar Perikanan Budi Daya Air Payau, Jepara, Central Java, Indonesia. It was sieved (400 mesh), suspended in CMCNa 0.5% and administered once a day orally using a 22-gauge oral feeding needle (Popper and Sons Inc., New York, U.S.A.) for seven consecutive days prior to and during five days of being subjected to X-ray radiation. *Spirulina platensis* was administered to the subjects every morning prior to their being fed.

Radiation exposure

The subjects were immobilized on a board without the aid of anesthesia. The *ventral* surface area of the neck was exposed to an X-ray radiation source, namely; a Linear Accelerator (CLINAC 2100 Ex Model), SSD (*Source to Skin Distance*) = 100 cm, field size 7 cm x 40 cm, 6MV [12]. Each subject was exposed to a 10 Gy fractionation dose. X-ray fractionation dose was given 2 Gray (Gy) for 5 days, and sacrificed 24 hours after final irradiation.

Plasma collection and ELISA analysis

Cardiac puncture directly into the organ and slowly aspirating it [13]. Up to 3ml of blood can be taken by a sterile vacutainer tube containing Heparin. An 8-iso-PGF2α level examination was performed using a Rat Macrophage Migration Inhibitory Factor (MIF) ELISA Kit (Wuhan Fine Biotech Co., Ltd. China). F2-isoprostane in the blood plasma of Wistar rats was measured using ELISA.

Immunohistochemical detection of Caspase-3 and AQP5

Caspase-3 expression and AQP5 expression using immunohistochemistry with primary polyclonal rat anti-caspase-3 (cleaved Asp175) GTX86952 (Gene Tex, Irvine, USA) and polyclonal anti-aquaporin-5 primary mouse antibody GTX11586 (Gene Tex, Irvine, USA), conjugated secondary anti-mouse biotin antibody. Expressions were seen microscopically using a Nikon Eclipse E200 light microscope connected to a monitor with NIS-Elements D5 software. 11. 00 64 bits. The calculation was performed on stains that were stated to be positive, at 5 fields of view with a magnification of 400x [9].

Data processing and analysis

All data and results were analyzed statistically using SPSS version 21.0. A Shapiro-Wilk test was used to test the assumption of normality and a Levene's test was completed to test the assumption of variance equality. The Brown Forsythe test was used for statistical analysis of F2-isoprostanes and caspase-3. The comparison tests for F2-isoprostanes and caspase-3 consisted of Brown Forsythe, and the comparison tests for AQP5 was One Way ANOVA.

Results

Regulating radiation - induced xerostomia indicators

Observations were conducted at 24 hours (point of acute damage) after the administration of a fractionation dose of 10 Gy (2 Gy x 5 days). The results of F2-isoprostanes levels can be seen in Table 1 and Figure 1.

Groups	F2 IsoPs (ng/mL)	Caspase 3 expression	AQP5 expression
N	83.71^{ab}±4.46	3.33^{a}±0.82	8.25^{ab}±1.92
RT	90.94^{b}±7.02	11.67^{d}±1.63	6.42^{a}±2.11
RT + Std	78.70^{a}±2.76	9.83^{bcd}±2.97	16.25^{c}±2.43
RT+Sp300	86.86^{ab}±7.17	8.33^{c}±1.63	10.83^{ab}±2.35
RT+Sp600	77.79^{a}±1.13	6.50^{bc}±1.05	12.33^{bc}±2.80
RT+Sp900	77.91^{a}±1.05	5.17^{b}±0.75	16.25^{c}±3.00

Table 1. Effects of Spirulina platensis on biomolecular indicators.

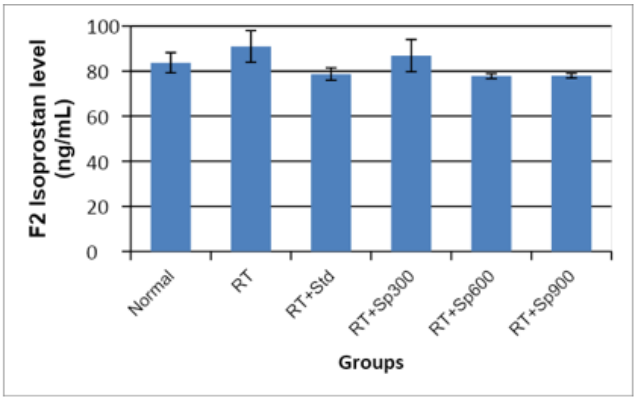

Figure 1. The effect of treatment with Spirulina platensis on F2-isoprostane levels.

Data is presented as mean ± SD. The mean difference is significant at the level of 0.05. The results shown that the highest F2-isoprostane levels to occur in the RT group (90.94 ng/mL). Caspase-3 expression is an indicator of apoptosis induction.

The results indicated a significant increase in caspase-3 expression in the RT group (11.67) compared to the normal group. The expression of AQP5 is an indicator of radiation damage to the gland transmembrane water channel. The lowest AQP5 expression results were found in the RT group (6.42) compared to the normal group.

Effect of Spirulina platensis on F2-isoprostanes

As shown in Table 1 and Figure 1, *Spirulina platensis* treatment of groups RT+Sp600 and RT+Sp900 significantly ($p < 0.05$) reduced the F2 isoprostanes level to that of the nearly normal group.

Effect of Spirulina platensis on the expression of caspase-3 submandibular gland acini cells

In Table 1, the caspase-3 expression of the submandibular gland decreased significantly ($p < 0.05$) as a result of administering the appropriate dose of *Spirulina platensis* compared to the X-ray irradiated group (RT).

The lowest caspase-3 expression was found in the RT+Sp900 group, there was no significant difference in caspase-3 expression between this group and the RT+Sp600 group. Administering 900 mg/kgBW of *Spirulina platensis* was able to reduce caspase-3 expression to a level close to normal.

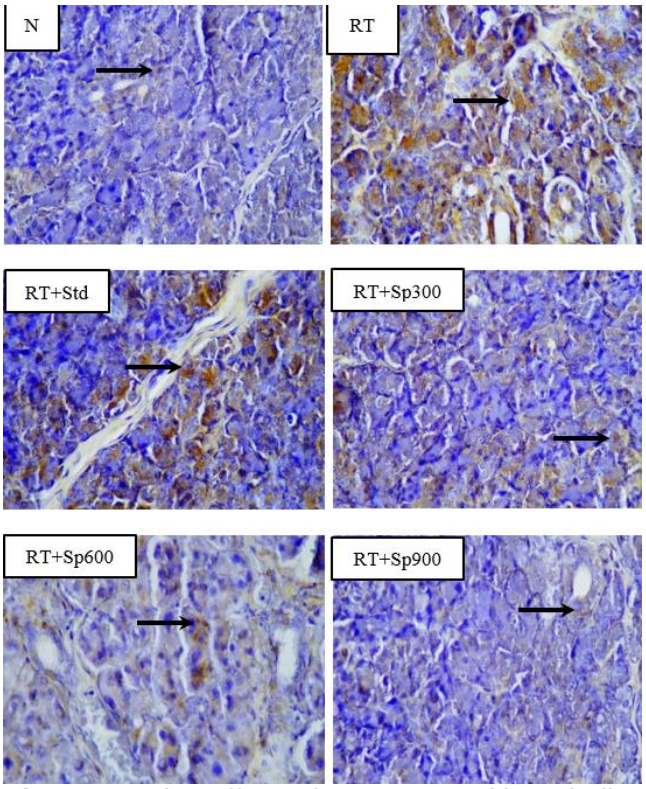

Figure 2. The effect of treatment with Spirulina platensis on the caspase-3 expression in the submandibular gland acini cell.

Effect of Spirulina platensis on the expression of AQP5 submandibular gland acini cells

The results showed that *Spirulina platensis* can increase the expression of AQP5 reach to the normal conditions. This can be seen from the results produced by the groups given *Spirulina platensis*, the RT+Sp300, RT+Sp600, and RT+Sp900 groups following their respective administered dose. Table 1 shows that the AQP5 expression of the submandibular gland significantly increased ($p < 0.05$), depending on the dose of *Spirulina platensis* administered, compared to the X-ray irradiated (RT) group. The highest increase in expression of AQP5 was found in the RT+Sp900 group where there was no difference between the RT+Sp600 group and the RT+Std group. The RT+Sp300 group experienced a decline culminating in the same conditions as the normal group.

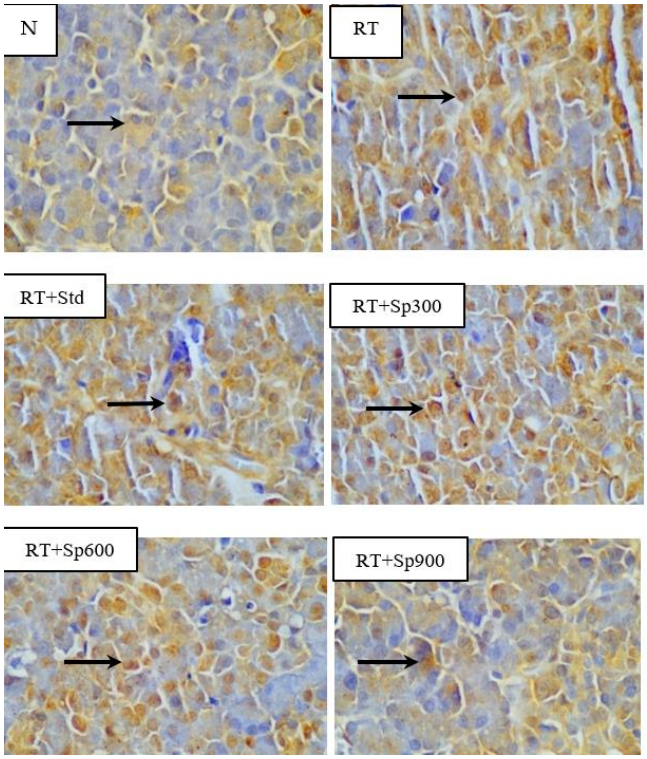

Figure 3. Observation AQP5 expression on submandibular acini cells using immunohistochemical methods.

Discussion

Head and neck radiation can cause side effects such as xerostomia by producing reactive oxygen species (ROS) in the submandibular salivary gland. The tissue response to radiotherapy is important because radiotherapy can be progressive and irreversible. The mechanism of late post-irradiation phase damage has been associated with a lack of proper cell renewal due to DNA damage[14]. Increased levels of F2-isoprostane, caspase-3 expression, and decreased AQP5 expression in the RT group showed an increase in ROS due to 10 Gy fractionation radiotherapy. This study, observations were made 24 hours after the final fractionation irradiation. Reactive oxigen species following head and neck radiation in the current research is in agreement with the prior studies[15,16].

Spirulina platensis in several studies can overcome ROS. Phenolic acids, vitamin E, phycocyanin, β-carotene and, chlorophyll - provide antioxidant abilities in *Spirulina platensis*. As an antioxidant, Phenolic acids have the ability to donate hydrogen atoms. *Spirulina platensis* has phycocyanin, a powerful antioxidant, and blue-green pigments that are easily soluble in water. Vitamin E is an antioxidant that works by searching, reacting and damaging the ROS chain so that it can function to protect important cell structures, especially cell membranes from damage due to ROS. Carotenoids have the ability to quench singlets of oxygen, besides being a potent scavenger because they can deactivate free radicals. *Chlorophyll-a* is a polar green pigment that has the ability to react with peroxyl radicals. *Spirulina platensis* also has selenium which can eliminate superoxide radicals and hydrogen peroxide[10,11].

Research data from the current work show the ability of *Spirulina platensis* to prevent xerostomia as indicated by changes in the indicators F2-isoprostane, caspase-3, and AQP5. F2-isoprostane is a reliable method of assessing the presence of lipid peroxidation in cell membranes *in vivo*[17]. *Spirulina platensis* treatment of groups RT+Sp600 and RT+Sp900 reduced the F2 isoprostanes level to that of the nearly normal group. This shows indicates that *Spirulina platensis* was able to significantly reduce the level of F2-isoprostanes, thereby confirming the effectiveness of *Spirulina platensis* to maintain balanced oxidant and antioxidant levels depending on the dose administered[18]. This study is supported by the research of Lee et al. which states that the effectiveness of *Spirulina platensis* to maintain oxidant and antioxidant levels depends on the route of administration and

duration of treatment [18]. Activated caspase-3 will eventually cause apoptosis. Caspase-3 is the most important executor caspase in the apoptosis process[19]. Administration of *Spirulina platensis* in this study shows can reduce caspase-3 expression adjusting to the dose administration. AQP5 is a transmembrane water channel protein that allows selective water transfer through the transcellular lipid bilayer in the salivary gland [20]. The expression of AQP5 is an indicator of radiation damage to the gland transmembrane water channel. The results showed that *Spirulina platensis* had been able to increase the expression of AQP5 to normal conditions suggesting the antioxidant potential of *Spirulina platensis*. Our findings reinforce the findings of previous authors, regarding the antioxidant effects of *Spirulina platensis*[10,11].

Conclusions

In this study, *Spirulina platensis* exhibited scavenging activities of free radicals in vivo. The animal experiment indicated that *Spirulina platensis* could prevent xerostomia by reducing levels of F2-isoprostanes, and caspase-3 expression, while increasing AQP5 expression. The study showed that 600 mg/kgBW *Spirulina platensis* was the efective dose. These findings are promising for further clinical research to prevent the occurrence of xerostomia due to head and neck radiotherapy.

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Declaration of Interest

The authors declare no conflict of interest resulting from the conduct of the research reported here.

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