Impact of artificial rice from *Gracilaria* sp. seaweed on Superoxide Dismutase (SOD) levels in prediabetic and diabetic patients

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Abstract. Analog rice made from local flour and seaweed flour underwent proximate and fiber content tests, followed by clinical trials involving prediabetic and diabetic sufferers over 42 days. The aim of this study was to determine the effect of consuming seaweed-based rice analogues on Superoxide Dismutase (SOD) levels in prediabetic and diabetic sufferers. Initial assessments were conducted on days 21 and 42 as the final evaluation. The study involved 40 respondents (both prediabetic and diabetic patients), with 20 consuming regular rice and 20 consuming analog rice. Both groups had the same vegetables and side dishes. Additionally, the chemical content of the product was tested. The SOD activity in this study was measured using a colorimetric assay method. The results showed that consumption of analog rice significantly reduced blood SOD levels in prediabetic and diabetic sufferers (initial value 0.59 ± 0.12 and final value 0.41 ± 0.16), whereas regular rice consumption significantly increased blood SOD levels (initial value 0.71 ± 0.20 and final value 0.87 ± 0.32). The chemical composition of the seaweed-based rice analog included 61.57 ± 0.49% water, 13.22 ± 0.04% protein, 0.11 ± 0.01% ash, 0.74 ± 0.01% fat, 34.38 ± 0.40% carbohydrates, and 10.17 ± 0.15% fiber. Ultimately, this research opens a new avenue, suggesting that artificial rice can serve not only as a primary energy source but also as a functional food with potential antidiabetic properties

1 Introduction

Indonesia is the second largest seaweed producer after China. The Indonesian Central Statistics Agency (BPS) recorded that in 2022, exports of seaweed and other algae reached 232,081.2 tons [1]. Purwaningsih and Deskawati [2] studied that *Gracilaria* sp. seaweed containing bioactive components such as alkaloids, phenols, saponins, triterpenoids, and

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flavonoids. *Gracilaria* sp. also contains high dietary fiber, which is 64.74%, and strong antioxidant activity with an IC_{50} value of 22.15 ± 1.63 µg / mL [3]. According to Neto [4], *Gracilaria* sp. is one of the seaweeds with high fiber content (40.6 g/100 g DW). Seaweed offers an alternative natural and sustainable fiber for health because of its low lignin content and high water-soluble fiber content [5, 6]. The *Gracilaria* sp. seaweed has the potential as a functional food for diabetes because it has strong antioxidant activity, has anti-α-glucosidase activity, contains active components, and has high fiber.

The IDF Diabetes Atlas [7] reported that the global prevalence of diabetes in people aged 20–79 years in 2021 is estimated at 10.5% (536.6 million people), increasing to 12.2% (783.2 million) in 2045. Indonesia is the country with the fifth highest number of diabetes sufferers, at 19.5 million, and is estimated to increase to 28.5 million in 2045. The Indonesian Pediatrician Association reported that the prevalence of children with diabetes increased significantly by 70 times compared to 2010, which was only 0.028/100000 population. Diabetes has been treated in various ways, one of which is oral antidiabetic drugs and insulin therapy. The use of these drugs has side effects, such as metformin, which can cause anemia [8], sitagliptin, which can cause nasopharyngitis and headaches [9], and sulfonylurea and insulin therapy, which can cause pancreatitis, inflammation, and the risk of hypoglycemia [10]. Interestingly, increasing natural fiber intake can be an alternative for preventing diabetes by reducing glucose absorption from food into the blood [11]. In many cases, the target population for evaluating potential natural sources as antidiabetic agents includes both prediabetic and diabetic patients, as treatment and prevention are essential within the context of functional food applications. The prediabetic group comprises individuals with a family history or genetic predisposition to diabetes, while the diabetic group consists of patients with a confirmed diagnosis.

Previously, our team successfully developed artificial or analogue rice from *Gracillaria* seaweed to meet the increasing demand for rice and introduce seaweed-based analogue rice as a functional food. This product offers convenient cooking methods, catering to the growing need for quick and efficient meal preparation in today's fast-paced lifestyles [12]. Furthermore, this innovative product has been designed specifically to explore its potential as a functional food. According to Purwaningsih [13], this analogue rice is classified as a food ingredient rich in dietary fiber, exhibiting strong antioxidant and anti-alpha-glucosidase activity. Results from in vivo tests on analogue rice made from *Gracilaria* sp. indicate that it can help lower blood sugar levels in animal models [14]. Additionally, Purwaningsih further reported that clinical trials where analogue rice from Gracilaria sp. was consumed for 42 days demonstrated a significant reduction in both fasting blood sugar and HbA1c levels in human respondents [15].

While research on seaweed's potential as a functional food has gained traction, few studies have specifically targeted seaweed-based analogue rice for diabetes management. Existing studies on seaweed components, such as dietary fibers and bioactive compounds, have shown their general health benefits, particularly in promoting gut health and enhancing metabolic function [16]. However, these studies primarily focus on seaweed extracts rather than whole analogue rice products. In contrast, this study is among the first to incorporate *Gracilaria* sp. in a rice form that can serve as a staple food substitute, offering a practical dietary approach for managing blood sugar levels.

Previous research has established that *Gracilaria* and other red algae possess significant antioxidant capacities, largely attributed to their polysaccharides and phenolic compounds [17]. Yet, most studies have concentrated on the biochemical properties and antioxidant capacity of *Gracilaria* extracts in vitro rather than assessing the effects of whole-food applications, such as analogue rice, in clinical research. This study bridges this gap by providing in vivo and clinical evidence of *Gracilaria*-based analogue rice's effects on glycemic markers in both animal models and human participants. Ultimately, the development of seaweed-based food products shows promise for preventing or managing diabetes. This innovation has been patented, specifically revealed the potent of artificial rice from seaweeds to lower blood sugar levels [18].

Oxidative stress significantly contributes to diabetes progression and complications, highlighting the importance of antioxidant markers in assessing patient health. Superoxide dismutase (SOD) is vital in the body's defense, converting harmful superoxide radicals into hydrogen peroxide, thereby preventing cellular damage at its source. Complementing SOD, catalase (CAT) breaks down hydrogen peroxide, reducing oxidative harm; both enzymes are often found in lower levels in diabetic patients, indicating weakened defenses against free radicals [19]. Glutathione Peroxidase (GPx) reduces lipid hydroperoxides, and glutathione (GSH) neutralizes free radicals and repairs oxidative damage, but their levels are also frequently diminished in diabetes [20]. Another key marker, malondialdehyde (MDA), indicates lipid peroxidation, with elevated levels showing extensive oxidative stress associated with diabetic complications [21]. In fact, SOD is particularly powerful as it acts at the start of the antioxidant chain, neutralizing superoxide radicals before they escalate into more reactive species [22]. SOD plays a crucial role as a sensitive indicator for assessing interventions, such as seaweed-based analog rice, which could boost antioxidant defenses and alleviate oxidative stress in diabetes.

As the next stage in the claim of functional food, this report presents the results of clinical trials on seaweed-based analog rice and its effect on SOD levels in prediabetic and diabetic patients, used as an indicator of oxidative stress in patients with type 2 diabetes mellitus. Since Superoxide Dismutase (SOD) is the first line of defense against free radical compounds, the aim of this study was to determine the effect of seaweed-based rice on the SOD levels of respondents.

2 Methods

2.1 Materials and tools

The tools and materials for this study included a 5 ml Terumo syringe (Tokyo, Japan), microtubes (Eppendorf, Germany), micropipettes and microtips (Gilson, France), and a centrifuge (Thermo Fisher Scientific, USA) to support accurate sample preparation and handling. A cooler box was utilized to maintain sample integrity, along with cuvettes and a spectrophotometer (Agilent Technologies, USA) for spectrophotometric analysis. Samples were stored in non-EDTA tubes and vacutainers (BD, USA), and prepared using needle holders, winged needles, alcohol swabs, and plaster.

The determination of Superoxide Dismutase (SOD) activity was conducted with a colorimetric assay kit provided by Zellbio GmbH (Germany, CAT No. ZX-44108-96). This study used a Konelab 20xt analyzer (Thermo Scientific, USA), SOD reagent kit, and phosphate-buffered saline (PBS) solution (Gibco, Thermo Fisher Scientific, USA). All chemical reagents were sourced from Merck (Darmstadt, Germany), and samples included blood serum diluted in distilled water and kept on ice to prevent degradation during processing.

2.2 Procedures of research

The research was conducted in Prodia Laboratory, Jakarta, Indonesia, from December 2020 to January 2021. With Ethical Clearance No: 909/TT3.KEPMSM-IPB/SK/2021. The research method applied in this study is the Design Randomized clinical trial with pre-mid-post with control method referred to the [23].

Subject criteria were male/female 40-60 years old, have normal blood pressure, no liver or kidney disorders, were not pregnant, and were willing to voluntarily become respondents. The other criteria for respondents are a BMI of more than 18.5, fasting blood glucose of 100-200 mg/dL, not currently undergoing treatment from a doctor, not currently receiving seaweed rice intervention or something similar programs, will not be out of town when the intervention takes place. The exclusion criteria are: pregnant, breastfeeding/lactacy, indigestion, allergy, kidney complication, alcohol consumption, and herbal consumption. Total selected subjects were 445 people, and N = 22 subjects per group, than total subjects analyzed were 44 people. The respondent criteria were presented in Table 1. The SOD activity in this study was determined using colorimetric assay kits provided by Zellbio GmbH (Germany)(CAT No. ZX-44108-96). SOD activity was analyzed by measuring the colorimetric reaction that indicates the conversion of superoxide radicals, which is quantified to assess antioxidant enzymatic activity. This method aligns with standard biochemical techniques used to evaluate SOD's impact in oxidative stress studies [24].

First, the samples were diluted in an Assay Buffer, which contains a detergent to stabilize the reaction. Ten microliters of each diluted sample or standard were carefully pipetted into individual wells, ensuring no bubbles formed to maintain assay accuracy. Assay Buffer was also added to designated wells to serve as a 0 U/mL baseline standard. A 1:10 dilution of the Substrate Concentrate in Substrate Diluent was prepared to create the Substrate Working Solution, which was added at 50 µL per well using a multichannel pipette. In the presence of oxygen, 25 µL of Xanthine Oxidase Solution, diluted 1:25 in Xanthine Oxidase Buffer, was introduced to each well to catalyze superoxide generation. The plate was incubated at room temperature for 20 minutes to allow the reaction to proceed, during which superoxide radicals converted the colorless substrate in the Detection Reagent to a yellow compound. The absorbance was then measured at 450 nm to determine the SOD activity indirectly, as higher SOD activity reduces superoxide levels, thus diminishing the yellow product's intensity. A standard curve was prepared using a bovine erythrocyte SOD standard provided in the kit, enabling quantification of SOD activity, which was expressed in units per milliliter (U/mL).

The blood sampling of prediabetic and diabetic patients, and measurement of superoxide dismutase (SOD) activity levels were conducted at the Prodia Laboratory, Jakarta, Indonesia. Blood sampling was carried out at the beginning of the study on day zero, in the middle (day 21), and at the end of the study on day 42.

Table 1. Subject characteristics of research respondents

Characteristics	Control	Intervention	Total	*p-value*
BMI (kg/m^2)				
Before intervention	29.0±4.6	29.0±4.5	28.9±4.9	0.744
Body Weight (kg)				
Before intervention	73.7±13.4	73.4±17.6	72.6±15.1	0.605
Fasting Blood Glucose (mg/dL)				
Before intervention	135±31	136±15	135.6±24.3	0.711

Information: All of the p-value is more than 0.05, which means there is no significant difference between control and intervention group.

The statistical processing of SOD data in this study involved a structured approach using Microsoft Excel 2016 and SPSS version 21.0 for Windows. Initially, data entry, coding, editing, cleaning, and analysis were performed. Subject characteristics were then evaluated using Mann-Whitney tests for ordinal or numeric data. A Shapiro-Wilk test was conducted to assess the normality of the data. For normally distributed data, independent t-tests were applied to compare groups, while the Mann-Whitney test was used for non-normally distributed data. To assess changes within the same group, paired t-tests were employed for normally distributed data, and the Wilcoxon test was used for data that did not meet normality assumptions. This comprehensive approach ensured accurate analysis and interpretation of the SOD levels in both the intervention and control groups, with a p-value of 0.05.

3 Results and discussion

Free radicals were molecules or atoms that contain one or more unpaired electrons in the outermost orbit, making them unstable. Reactive Oxygen Species (ROS) consists of free radicals (superoxide, hydroxyl radicals, alkoxyl, and peroxyl) and non-radicals (hydrogen peroxide and hypochlorite). Free radicals tried to stabilize themselves by taking electrons from other molecules. Under normal circumstances, there was a balance between ROS formation and antioxidant activity in cells. If this balance was disturbed, it will caused oxidative stress which could caused damage to cell components which will reduced the body's defenses. One of the diseases related to ROS compounds is type 2 of diabetic mellitus. The pathogenesis of type 2 diabetic mellitus was characterized by metabolic disorders, namely a decrease in the response of peripheral tissues in responding to insulin (insulin resistance) [25]. Damage to peripheral tissues is thought to be due to increased free radicals in the body, which damage insulin receptors or glucose transporters found in cell membranes. Free radicals in the body are produced by normal cell metabolism [26]. Without realizing it, free radicals are continuously formed in the body, both through normal cell metabolism processes and from external factors.

Excessive amounts of free radicals would oxidize and attack the lipid components of cell membranes, causing lipid peroxidation. As free radicals increase, cell membrane lipid peroxidation also increases, producing the final product Malondialdehyde (MDA), and to reduce the damage caused by free radicals, antioxidants are needed [27]. Naturally, in the body there was an antioxidant defense system in the form of enzymes, namely Superoxide Dismutase (SOD), catalase, and glutathione peroxidase. Superoxide Dismutase (SOD) was the front line of defense against free radical compounds [28]. This antioxidant plays a role in changing superoxide anions (O_2^-) which were strong initiators of various chain reactions into oxygen (O_2) and hydrogen peroxide (H_2O_2) which are more stable than superoxide.

The study was structured with two distinct groups: an intervention group and a control group. The intervention group received analog rice made from *Gracilaria sp.* seaweed, consumed daily for a period of 42 days. This analog rice is known for its bioactive properties, which are hypothesized to influence oxidative stress markers, such as Superoxide Dismutase (SOD). The control group, on the other hand, continued with their regular dietary intake of standard rice for the same duration. This setup allowed for a comparative analysis of SOD activity between those consuming bioactive-enriched analog rice and those consuming regular rice.

The results of research on patients with type 2 prediabetic and diabetic mellitus in consuming analog rice from *Gracillaria* sp. seaweed show that at the beginning of the studied there was no difference between the intervention group and the control group, but after the intervention it showed that there was a significant difference between the treatment group and the control group, where the treatment group experienced a decreased and the control group experienced an increased in SOD. The complete on SOD values were presented in Figure 1.

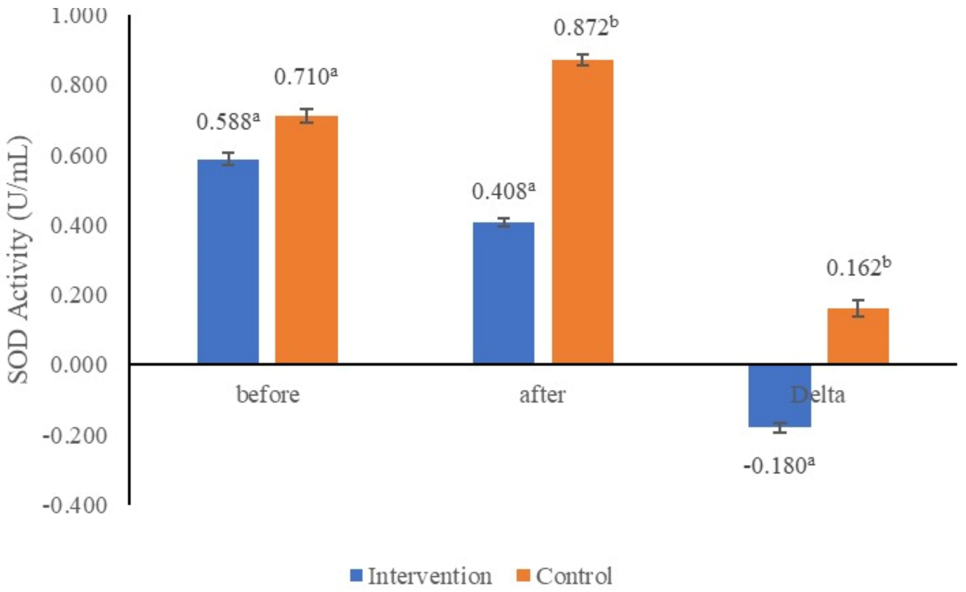

Fig 1. Effect of analog rice intervention from *Gracilaria* sp. seaweed on SOD activity

The initial test data confirmed that SOD activity was homogeneous across both groups, ensuring comparable baseline conditions. Figure 1 illustrates that, at the beginning of the observation period, there was no significant difference in SOD activity between the control and intervention groups. However, by the end of the 42-day observation period, a significant difference in SOD activity emerged: the intervention group, which consumed the analog rice, exhibited a lower mean SOD activity (mean ± SD: 0.41 ± 0.12) compared to the control group, which showed an increase in SOD activity (mean ± SD: 0.87 ± 0.32). These results highlight the potential impact of Gracilaria sp. analog rice on reducing oxidative stress in individuals with type 2 prediabetes and diabetes mellitus.

The results of the SOD activity change test between the beginning and the end of the respondents who were given treatment (eating analog rice from *Gracilaria* sp seaweed for 42 days) experienced a significant decrease in SOD activity with a change in the mean value (0.1795) and standard deviation (0.18±0.13). Meanwhile, control respondents (eating regular rice for 42 days) experienced a significant increase in SOD activity between the beginning and the end of observation with a changed in the average value (0.16216) and a standard deviation of 0.16±0.18. This showed that the intervention of analog rice from *Gracilaria* sp seaweed was able to reduced SOD activity, meaning that the intervention of analog rice from *Gracilaria* sp seaweed is able to suppress ROS in the body. The results showed that SOD activity tended to be higher in individuals with type 2 diabetes mellitus (1.01 ± 2.29 U/mL) compared to normal individuals (0.79 ± 1.01 U/mL). These findings aligned with Moussa's study [30], which reported that the average SOD activity in diabetes mellitus patients was significantly higher (3820 ± 770 U/gHb) than in normal individuals (1321 ± 250 U/gHb). Likewise, research conducted by Likidlilid [31] also observed an increase in SOD activity among diabetes mellitus patients, although this increase was not statistically significant when compared to healthy individuals.

Several studies have highlighted the role of seaweed in reducing oxidative stress, especially due to its bioactive compounds like phenols, flavonoids, and fibers, which act as potent antioxidants. For example, Amaro [32] found that seaweed's phenolic content plays a critical role in neutralizing free radicals such as superoxide anions, which are precursors of oxidative stress and are known to elevate SOD levels in diabetic patients. This aligns with the results of the current study, where *Gracilaria* sp. analog rice consumption resulted in reduced SOD activity, indicating a reduction in oxidative stress.

Furthermore, studies by Long [33] reported that the high dietary fiber in seaweeds, especially *Gracilaria* sp., slows glucose absorption in the digestive system, reduces postprandial blood sugar spikes, and lowers oxidative damage caused by hyperglycemia. This reduction in glucose levels also diminishes reactive oxygen species (ROS) production, subsequently leading to lower SOD activity, as seen in diabetic and prediabetic respondents.

4 Conclusions

The results showed that consumption of analog rice from *Gracilaria* sp seaweed significantly reduced blood SOD levels in prediabetic and diabetic sufferers (initial value 0.59 ± 0.12 and final value 0.41 ± 0.16), whereas regular rice consumption significantly increased blood SOD levels (initial value 0.71 ± 0.20 and final value 0.87 ± 0.32).

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