

Effect of Tender Coconut Water Powder Enriched with Vitamin E in Preventing Oxidative Stress of Diabetes Male Wistar Rats

Ratnawati Ratnawati¹, Siti Thomas Zulaikhah^{1*}, Atina Husaana², Arrizki Azka Pratama³

Ratnawati Ratnawati¹, Siti Thomas Zulaikhah^{1*}, Atina Husaana², Arrizki Azka Pratama³

¹Department of Public Health, Faculty of Medicine, Universitas Islam Sultan Agung Semarang, INDONESIA.

²Department of Pharmacology, Faculty of Medicine, Universitas Islam Sultan Agung Semarang, INDONESIA.

³Neurology Resident, Faculty of Medicine, Universitas Diponegoro Semarang, INDONESIA

Correspondence

Siti Thomas Zulaikhah

Department of Public Health, Faculty of Medicine, Universitas Islam Sultan Agung (UNISSULA), Kaligawe Street Km 4, 50112, Semarang, Central Java, INDONESIA.

Tel: +62246582455

E-mail: sitithomas@unissula.ac.id

History

- Submission Date: 10-09-2024;
- Review completed: 06-10-2024;
- Accepted Date: 15-10-2024.

DOI : 10.5530/pj.2024.16.172

Article Available online

<http://www.phcogj.com/v16/i5>

Copyright

© 2024 Phcogj.Com. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.



ABSTRACT

Background: Diabetes mellitus (DM) is associated with increased oxidative stress and higher mortality rates. Analysis is needed to identify compounds in natural foods that can prevent oxidative stress. According to previous studies, tender coconut water with added vitamin E is more effective in preventing oxidative stress caused by DM compared to plain tender coconut water. This development is necessary to provide added value and practicality. **Objective:** To evaluate the potential of tender coconut water powder enriched with vitamin E on male rats diabetes by measuring the oxidative stress (SOD, GPx, MDA, IL-1, IL-6, TNF- α , and CRP levels). **Methods:** An experimental method was used with a posttest control group design. A total of 36 male Wistar rats used were randomly divided into 6 groups namely normal; DM; P1 (type 2 DM + Spray Drying tender coconut water powder); P2 (type 2 DM + Spray Drying tender coconut water powder + vitamin E); P3 (type 2 DM + Freeze Drying tender coconut water powder); and P4 (type 2 DM + Freeze Drying tender coconut water powder + vitamin E). Type 2 DM induction was carried out using Streptozotocin (STZ) 65 mg/kgBW and Nicotinamide 230 mg/kgBW. Tender coconut water powder was administered daily for 4 weeks beginning on the 3rd day after inducing DM. The data obtained were analyzed using the *One Way Analysis of Variance test* (ANOVA). **Result:** The results showed that the average levels of SOD and GPx in P4 were higher compared to P2, while MDA, IL-1, IL-6, TNF α , and CRP in P4 were lower compared to P2. **Conclusion:** Tender coconut water powder enriched with vitamin E effectively prevented oxidative stress, as indicated by increased SOD and GPx, along with decreased MDA, IL-1, IL-6, TNF α , and CRP levels.

Keywords: Tender coconut water powder; oxidative stress; Diabetes mellitus; spray drying; freeze drying.

INTRODUCTION

Diabetes mellitus (DM) is a significant public health concern causing suffering, death, and economic losses. The prevalence is increasing worldwide due to population growth, aging, urbanization, and the rise in obesity resulting from a lack of physical activity.¹ In Indonesia, DM ranks sixth, and it is estimated that more than 25% of patients diagnosed with type 2 DM already have systemic inflammation at the time of diagnosis. This chronic condition significantly impacts life quality of patients and leads to complications in other organs. Type 2 DM is a hyperglycemic disease caused by insulin insensitivity. Patients with this condition have abnormalities in insulin binding to receptors. The risk factors are often associated with lifestyle, such as obesity, but the number of deaths from cardiovascular disease and DM tends to be higher in developing countries than in developed. Furthermore, low levels of magnesium (Mg) can also be found, which is related to inflammation and increased ROS production leading to damaged tyrosine kinase activity, reduced post-receptor insulin action, and insulin resistance.²

Foods containing natural antioxidants can be used as a strategy to reduce morbidity and mortality, specifically for diseases caused by oxidative stress, such as DM, hypertension, hyperlipidemia, and others.³ Amarowicz (2000) stated that the long-term use of synthetic antioxidants had side effects, including inflammation, liver damage, and an increased risk of carcinogenesis in animal experiments.⁴

Indonesia is rich in plants containing antioxidant compounds that have been traditionally consumed for generations. For example, tender coconut water is a natural beverage with antioxidants such as vitamin C, L-arginine amino acids, polyphenols, selenium, and minerals.⁵ It can prevent oxidative stress⁶, anemia, lipid peroxidation, lower lipid profiles, reduce blood pressure, enhance antioxidant enzyme status, and prevent lipid peroxidation.⁷ The benefits have been used by the community for various health purposes, for instance, during World War II, tender coconut water was commonly used as an alternative treatment for cholera cases. Regardless of myths or facts, many people still use this substance to maintain their fitness and immune systems. In the early stages of type 2 DM, β cells show disturbances in the first-phase of insulin secretion, failing to compensate for resistance. When not managed properly, this can result in further damage to pancreatic β cells and eventual insulin deficiency, requiring exogenous supply in the end. Both factors, insulin resistance, and deficiency, are commonly found in type 2 DM patients.⁸

Tender coconut water has been shown to reduce TNF- α , IL-1, and IL-6 in type 2 DM rats², lower glucose, reduce MDA, and increase plasma insulin levels in pregnant rats with gestational DM (GDM) (Nova et al., 2020). It can also prevent oxidative stress due to DM, heavy metal exposure, and cigarette smoke. The nutritional content is highly beneficial for human health but the processing into powder and its reevaluation for preventing oxidative stress has not been achieved. Efforts are needed

Cite this article: Ratnawati R, Zulaikhah ST, Husaana A, Pratama AA. Effect of Tender Coconut Water Powder Enriched with Vitamin E in Preventing Oxidative Stress of Diabetes Male Wistar Rats. *Pharmacogn J.* 2024;16(5): 1062-1068.

to develop tender coconut water into a powdered product enriched with vitamin E which has added value and greater practicality. This powder can serve as an exogenous antioxidant source from natural ingredients which are more convenient and easy to consume without altering its nutritional content. According to Zulaikhah et al. (2023), active compounds in freeze-dried tender coconut water powder such as L-arginine, Mg, and potassium (K) were higher compared to powder processed by spray drying.⁹ This research aims to reveal the potential of tender coconut water powder enriched with vitamin E on male rats Diabetes by measuring the oxidative stress (SOD, GPx, MDA, IL-1, IL-6, TNF- α , and CRP levels).

METHODS

Tender coconut water powder

Tender coconut water powder used was obtained from a green variety of *Cocos nucifera* L. var. *viridis*, aged 5-7 months, and sourced from the vicinity of the Yogyakarta. The material was transformed into powder using spray and freeze drying, followed by enrichment with vitamin E. The powder dosage used was 0.4 g/200 g of the body weight, and the vitamin E dosage was 1.8 IU/200 g.⁹

Experimental Animal

This study obtained ethical clearance from the Medical/Health Research Bioethics Commission of the Faculty of Medicine, Unissula Semarang, with reference number 326/VIII/2023/Komisi-Bioetik. The treatment of experimental animals and the examination of SOD, GPx, MDA, IL-1, IL-6, TNF α , and CRP levels were conducted at Center for Food & Nutrition Studies (PSPG) Gadjah Mada University Yogyakarta.

Induction of Streptozotocin (STZ) and Nicotinamide (NA)

Rats were initially acclimatized to the environment for 7 days, and then intraperitoneally induced with streptozotocin (STZ) at a dosage of 65 mg/kg and nicotinamide at 230 mg/kg body weight. After waiting for three days, their glucose levels were measured with a glucometer. When the glucose levels exceeded 250 mg/dL, rats were considered suitable for this study.

Treatment Administration

The subjects used were male Wistar rats that fulfilled the criteria of being two months old, weighing approximately 180-220 g, appearing healthy, displaying active movement, eating and drinking normally, having no injuries, and not being handicapped. A total of 36 rats were randomly divided into 6 groups, each consisting of 6 members (Table 1).

Blood Collection Method

The equipment used included sterile microhematocrit tubes, blood collection bottles, and sterile cotton. Blood was collected by inserting a microhematocrit tube into the ophthalmic vein at the corner of the eye, periorbitally, and then slowly rotating it. The collected blood was transferred to an Eppendorf tube, about 2 mL. The microhematocrit tube was removed when the required amount was collected, and sterile cotton was used to clean any remaining blood at the corner of the eye.¹⁰

Measurement of SOD, GPx, MDA, IL-1, IL-6, TNF α , and CRP Levels

The examination of oxidative stress by measuring SOD, GPx, MDA, IL-1, IL-6, TNF α , and CRP levels was performed using the Enzyme-Linked Immunosorbent Assay (Elisa) method.

Statistical Analysis

The statistical data analysis was performed utilizing SPSS software version 26.0. Data obtained from the measurements of SOD, GPx, MDA, IL-1, IL-6, TNF α , and CRP levels were tested for normality and homogeneity using the Shapiro-Wilk test and Levene tests, respectively. Since the data were normally distributed and homogeneous, analysis was performed with One-way ANOVA. Subsequently, the Post Hoc LSD test was used to determine differences between groups. Decisions to accept or reject hypotheses were based on an alpha level of 5%. P-values < 0.05 were statistically significant.

RESULTS & DISCUSSION

The effects of tender coconut water powder enriched with vitamin E on oxidative stress are shown in Table 2.

Table 2 shows that type 2 DM rats had the lowest average SOD levels (30.88 \pm 3.38%), while the normal demonstrated the highest (84.06 \pm 3.65%). The average SOD levels in rats treated with tender coconut water powder processed using spray drying and enriched with vitamin E (P2) were better or higher compared to those without vitamin E (P1) (69.61 \pm 4.52% > 59.55 \pm 4.03%). Similarly, the average SOD levels in rats administered with tender coconut water powder processed using freeze drying with added vitamin E (P4) was greater compared to the method without vitamin E (P3) (79.41 \pm 3.47% > 71.81 \pm 4.68%). The P4 treatment had the most optimal result in increasing SOD levels (p-value:0,0001).

The lowest average GPx levels in type 2 DM rats were (22.76 \pm 1.44 u/mg), while in the normal, the highest was (73.44 \pm 2.64 u/mg). The average GPx levels in P2 were higher P1 (52.21 \pm 2.16 u/mg vs. 40.77 \pm 1.78 u/mg). Similarly, the average GPx levels in P4 were greater

Table 1. Division of Treatment Groups. After 4 weeks, blood samples were collected to measure the levels of SOD, GPx, MDA, IL-1, IL-6, TNF α , and CRP.

Group 1 (Normal):	Male Wistar rats received a standard diet + <i>ad libitum</i> drinking.
Group 2 (DM):	Male Wistar white rats received a standard diet + <i>ad libitum</i> drinking and were induced with STZ at a dosage of 65 mg/kg and nicotinamide at a dosage of 230 mg/kg body weight (type 2 DM).
Group 3 (P1):	Male Wistar white rats received a standard diet + <i>ad libitum</i> drinking and were induced with STZ at a dosage of 65 mg/kg and nicotinamide at a dosage of 230 mg/kg body weight (type 2 DM) together with spray-dried tender coconut water powder (0.4 gr/200 g).
Group 4 (P2):	Male Wistar white rats received a standard diet + <i>ad libitum</i> drinking and were induced with STZ at a dosage of 65 mg/kg and nicotinamide at a dosage of 230 mg/kg body weight (type 2 DM) together with spray-dried tender coconut water powder + vitamin E (0.4 g/200 g + Vit E 1.8 IU/200 g).
Group 5 (P3):	Male Wistar white rats received a standard diet + <i>ad libitum</i> drinking and were induced with STZ at a dosage of 65 mg/kg and nicotinamide at a dosage of 230 mg/kg body weight (type 2 DM) together with freeze-dried tender coconut water powder (0.4 g/200 g).
Group 6 (P4):	Male Wistar white rats received a standard diet + <i>ad libitum</i> drinking and were induced with STZ at a dosage of 65 mg/kg and nicotinamide at a dosage of 230 mg/kg body weight (type 2 DM) together with freeze-dried tender coconut water powder + vitamin E (0.4 g/200 g + Vit E 1.8 IU/200 g).

Table 2. Average, Normality Test, Homogeneity Test, and Anova Test for SOD, GPx, MDA, IL-1, IL-6, TNF α , and CRP levels in 6 groups.

VARIABLE (OXIDATIVE STRESS)	GROUP						p-value
	NORMAL	DM	P1	P2	P3	P4	
	Average \pm SD	Average \pm SD	Average \pm SD	Average \pm SD	Average \pm SD	Average \pm SD	
Levels of SOD (%)							
Shapiro wilk	84,06 \pm 3,65	30,88 \pm 3,38	59,55 \pm 4,03	69,61 \pm 4,52	71,81 \pm 4,68	79,41 \pm 3,47	>0,05*
Levene test	0,753	0,871	0,801	0,582	0,667	0,739	>0,05**
One way ANOVA							0,0001***
Level of GPx (u/mg))							
Shapiro wilk	73,44 \pm 2,64	22,76 \pm 1,44	40,77 \pm 1,78	52,21 \pm 2,16	58,26 \pm 3,04	69,58 \pm 1,92	>0,05*
Levene test	0,695	0,961	0,802	0,658	0,713	0,795	>0,05**
One way ANOVA							0,0001***
Levels of MDA (nmol/ mL)							
Shapiro wilk	1,02 \pm 0,15	10,09 \pm 0,37	5,03 \pm 0,28	3,46 \pm 0,35	2,24 \pm 0,29	1,90 \pm 0,90	>0,05*
Levene test	0,779	0,781	0,834	0,852	0,783	0,970	>0,05**
One way ANOVA							0,0001***
Levels of TNF α (pg/ mL)							
Shapiro wilk	5,76 \pm 0,28	17,89 \pm 0,39	11,30 \pm 0,31	8,47 \pm 0,41	7,73 \pm 0,33	6,59 \pm 0,22	>0,05*
Levene test	0,506	0,883	0,574	0,923	0,495	0,786	>0,05**
One way ANOVA							0,0001***
Levels of IL-1 (pg/mL)							
Shapiro wilk	318,69 \pm 3,79	395,17 \pm 5,24	358,56 \pm 3,39	351,60 \pm 7,54	339,34 \pm 3,51	328,66 \pm 4,4	>0,05*
Levene test	0,806	0,615	0,977	0,275	0,501	0,859	>0,05**
One way ANOVA							0,0001***
Levels of IL-6 (pg/mL)							
Shapiro wilk	28,31 \pm 0,62	69,24 \pm 0,77	47,62 \pm 0,82	41,88 \pm 1,18	38,74 \pm 0,92	31,13 \pm 0,83	>0,05*
Levene test	0,383	0,936	0,943	0,991	0,242	0,783	>0,05**
One way ANOVA							0,0001***
Levels of hs-CRP (ng/ mL)							
Shapiro wilk	0,76 \pm 0,01	2,28 \pm 0,01	1,43 \pm 0,02	1,00 \pm 0,01	0,90 \pm 0,01	0,84 \pm 0,03	>0,05*
Levene test	0,212	0,191	0,863	0,212	0,167	0,955	>0,05**
One way ANOVA							0,0001***

Description: Significant: * $p > 0,05$; ** $p > 0,05$; *** $p < 0,05$.

compared to P3 (69.58 \pm 1.92 u/mg vs. 58.26 \pm 3.04 u/mg). P4 had the most optimal result increasing GPx levels ((p-value:0,0001).).

The highest average MDA levels were found in type 2 DM rats (10.09 \pm 0.37 nmol/mL), while the lowest was recorded in the normal (1.02 \pm 0.15 nmol/mL). The average MDA levels in P2 were lower compared to P1 (3.46 \pm 0.35 nmol/mL vs. 5.03 \pm 0.28 nmol/mL). Similarly, the average MDA levels in P4 were lower compared to P3 (1.90 \pm 0.90 nmol/mL vs. 2.24 \pm 0.29 nmol/mL). P4 showed the best result in reducing MDA levels ((p-value:0,0001).).

Regarding the TNF- α , the highest levels were found in type 2 DM rats (17.89 \pm 0.39 pg/mL), while the lowest was recorded in the normal (5.76 \pm 0.28 pg/mL). The average TNF- α levels in P2 were lower compared to P1 (8.47 \pm 0.41 pg/mL vs. 11.30 \pm 0.31 pg/mL). Similarly, the average TNF- α levels in P4 were lower compared to P3 (6.59 \pm 0.22 pg/mL vs. 7.73 \pm 0.33 pg/mL). P4 had the most optimal result in reducing TNF- α levels ((p-value:0,0001).).

The highest average IL-1 levels were recorded in diabetic (DM) rats (395.17 \pm 5.24 pg/mL), while the lowest was found in the normal (318.69 \pm 3.79 pg/mL). The average IL-1 levels in P2 were lower compared to P1 (351.60 \pm 7.54 pg/mL vs. 358.56 \pm 3.39 pg/mL). Furthermore, the average IL-1 levels in P4 were lower compared to P3 (328.66 \pm 4.4 pg/mL vs. 339.34 \pm 3.51 pg/mL). P4 had the most optimal result in reducing IL-1 levels ((p-value:0,0001).).

Regarding IL-6, the highest levels were found in type 2 DM rats (69.24 \pm 0.77 Pg/mL), while the lowest was recorded in the normal

(28.31 \pm 0.62 Pg/mL). The average IL-6 levels in P2 were lower compared to P1 (41.88 \pm 1.18 Pg/mL vs. 47.62 \pm 0.82 Pg/mL). Similarly, the average IL-6 levels in P4 were lower compared to P3 (31.13 \pm 0.83 Pg/mL vs. 38.74 \pm 0.92 Pg/mL). P4 showed the most optimal result in reducing IL-6 levels ((p-value:0,0001).).

The highest average CRP levels were found in type 2 DM rats (2.28 \pm 0.01 ng/mL), while the lowest was in normal (0.76 \pm 0.01 ng/mL). The average CRP levels in P2 were lower compared to P1 (1.00 \pm 0.01 ng/mL vs. 1.43 \pm 0.02 ng/mL). Similarly, the average CRP levels in P4 were lower compared to P3 (0.84 \pm 0.03 ng/mL vs. 0.90 \pm 0.01 ng/mL). P4 showed the most optimal result in reducing CRP levels ((p-value:0,0001).).

The ANOVA test showed that the administration of tender coconut water powder, processed using either spray or freeze drying effectively prevented oxidative stress as indicated by an increase in SOD and GPx along with a decrease in MDA, TNF α , IL-1, IL-6 and CRP levels in type 2 DM rats but the best results were observed in P4 ((p-value:0,0001).).

The results of Post Hoc LSD test analysis conducted to determine differences between groups for the SOD, GPx, MDA, IL-1, IL-6, TNF α , and CRP levels are presented in the Figure 1.

This study indicated that the group of type 2 DM rats experienced oxidative stress, marked by increased levels of SOD and GPx, as well as decreased MDA, IL-1, IL-6, TNF- α , and CRP compared to the normal group. Oxidative stress refers to an imbalance between free radicals and the endogenous antioxidants in the body. This condition can lead to cell damage, resulting in cancer, heart disease, cataracts, DM, premature

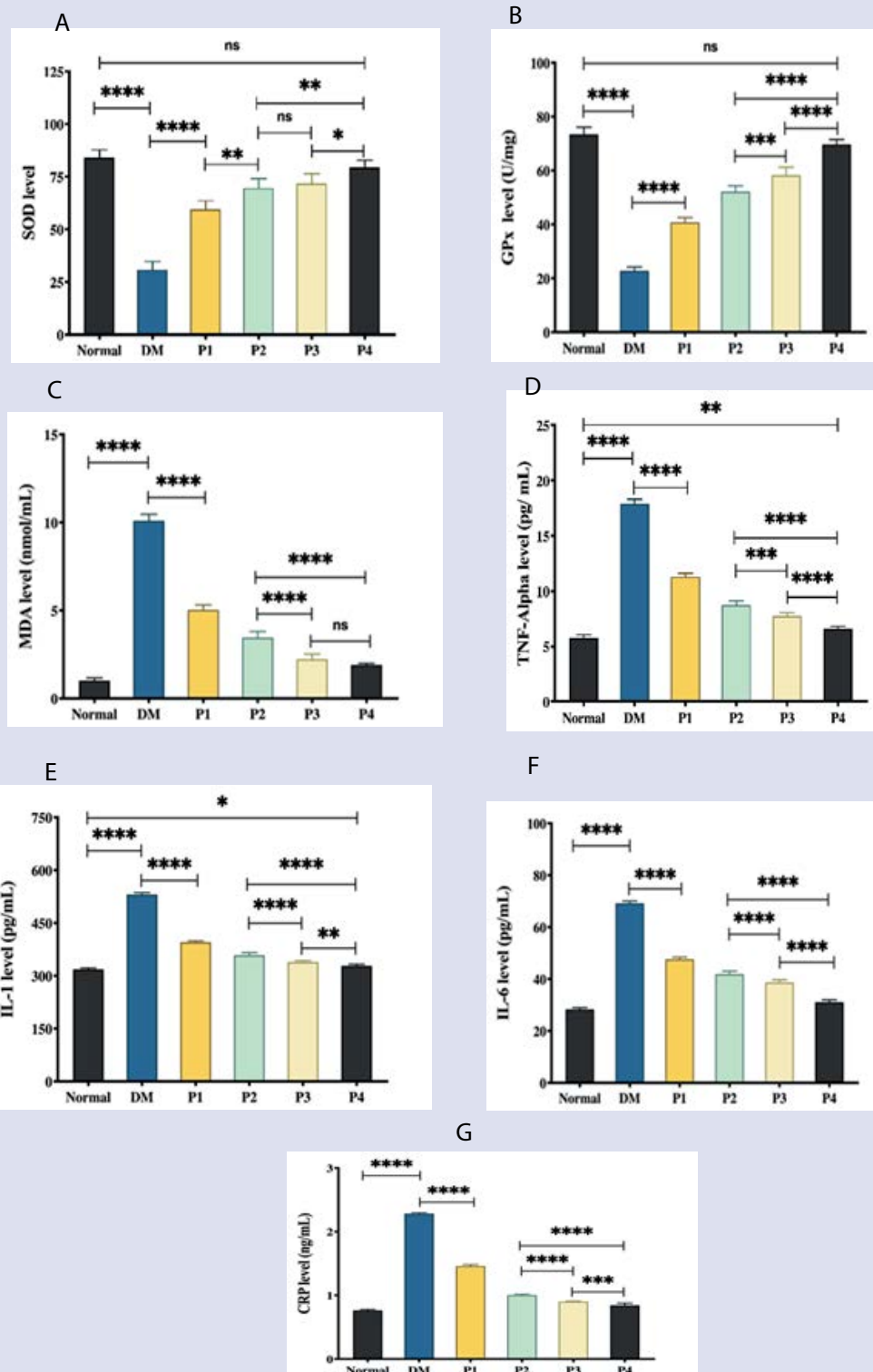


Figure 1. The results of Post Hoc LSD test analysis conducted to determine differences between groups for the SOD, GPx, MDA, IL-1, IL-6, TNF α , and CRP levels. **(A)** Differences between groups in SOD levels; **(B)** Differences between groups in GPx levels; **(C)** Differences between groups in MDA levels; **(D)** Differences between groups in TNF α levels; **(E)** Differences between groups in IL-1 levels; **(F)** Differences between groups in IL-6 levels; **(G)** Differences between groups in CRP levels

Results are expressed as mean \pm standard deviation (n = 36). Based on Figure A, SOD levels were not significantly different between the normal group and P4 as well as the P2 and P3. However, there were significant differences among other groups. As shown in Figure B, there was no significant difference in the GPx levels between the normal rat group and P4. However, significant differences were observed among other groups. Figure C shows that MDA levels were not significantly different in P3 and P4, while other groups indicated significant differences. Based on Figure D, TNF- α levels differed significantly among all groups. Figure E showed that IL-1 levels had significant differences among all groups. Based on Figure F, IL-6 levels showed significant differences among all groups. As shown in Figure G, CRP levels showed significant differences among all groups.

aging, and other degenerative diseases. In diabetic patients, an increase in ROS (Reactive Oxygen Species) occurs in the mitochondria. Meanwhile, parameters commonly used to indicate oxidative stress include levels of endogenous antioxidants (SOD, catalase, and GPx), as well as lipid peroxidation (MDA), and proinflammatory cytokines (TNF α , IL-1, IL-6).

Oxidative stress in DM stems from the disruption of the redox balance due to changes in carbohydrate and lipid metabolism, leading to increased ROS production through glycation reactions and lipid oxidation, thereby reducing the antioxidant defense system. ROS is produced through various pathways, such as increased polyol, advanced glycation end-products (AGEs) formation, and activation of protein kinase C (PKC). Glucose can be oxidized before or after binding to proteins (glycated protein), resulting in ROS production. The combination of glycation and glucose oxidation leads to the formation of AGEs. Glycated proteins and AGEs-modified proteins potentially cause oxidative stress by releasing superoxide anions ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and toxic carbonyls that can damage proteins. In DM, the levels of methylglyoxal, formed from glycolysis intermediates play a role in AGEs formation.

DM is a syndrome characterized by chronic high blood sugar levels (hyperglycemia) due to disturbances in insulin production, secretion, or resistance. In type 2 DM, apart from insulin deficiency, there is resistance preventing optimal regulation of blood sugar, and contributing to elevated levels. Furthermore, hyperglycemia worsens the formation of ROS through several mechanisms. ROS increases the expression of Tumor Necrosis Factor- α (TNF- α) and worsens oxidative stress. TNF- α may cause resistance by decreasing insulin receptor auto-phosphorylation, changing insulin receptor substrate 1 into an inhibitor of tyrosine kinase activity, reducing glucose transporter (GLUT-4), increasing the circulation of fatty acids, altering the function of beta cells, elevating triglyceride levels, and decreasing HDL levels. A previous study showed that injecting TNF into healthy test animals minimized insulin sensitivity due to hyperglycemia without a decrease in plasma insulin levels.

DM causes hyperglycemia and catalyzes the formation of superoxide anion radicals ($O_2^{\cdot-}$), sourced from both mitochondria and the cytoplasm. This leads to lipid oxidation and the production of glyoxal, a precursor to carboxymethyllysine (CML). In DM patients, the levels of methylglyoxal formed from glycolysis intermediates increase, subsequently forming carboxyethyllysine (CEL). Both CML and CEL can bind with Cu or Fe, forming CML/CEL-metal-protein complexes that trigger the formation of hydroxyl radicals ($\cdot OH$). The relationship between DM and hyperlipidemia in free radical production is depicted in Figure 2.

ROS consists of superoxide ($O_2^{\cdot-}$), hydroxyl ($\cdot OH$), peroxy ($ROO\cdot$), hydrogen peroxide (H_2O_2), singlet oxygen (1O_2), nitric oxide (NO), peroxyne (ONOO \cdot), hypochlorous acid (HOCl), and oxidized lipids in food. These compounds stem from normal cell metabolism or cells exposed to substances that cause inflammation. ROS can also be generated during the inflammatory process, such as the conversion of NADPH to NADP catalyzed by NADPH oxidase. This results in the leakage of $O_2^{\cdot-}$, which subsequently becomes superoxide radicals ($\cdot O_2$), capable of stimulating the formation of proinflammatory cytokines such as TNF- α and IL-6.

Elevated levels of TNF- α and IL-6 are associated with insulin resistance and type 2 DM. In this study, type 2 DM patients had elevated levels of IL-6 and TNF- α . The relationship between type 2 DM, oxidative stress, and inflammation is depicted in Figure 3.

In biological systems, the body can usually produce its antioxidants in the form of enzymes such as SOD, catalase, and GPx (endogenous

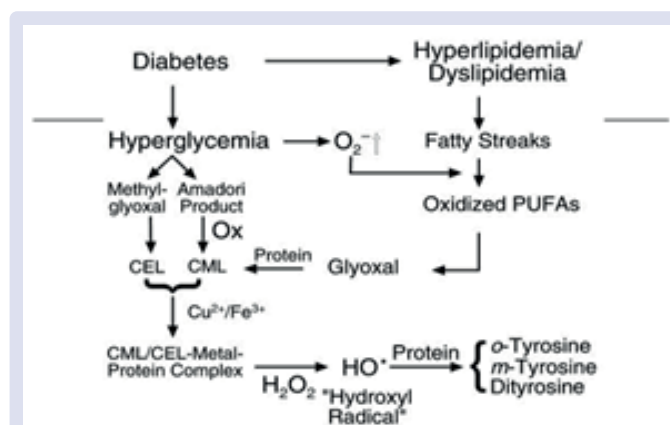


Figure 2. The Relationship Between DM and Hyperlipidemia in Free Radical Production ($\cdot OH$).¹¹

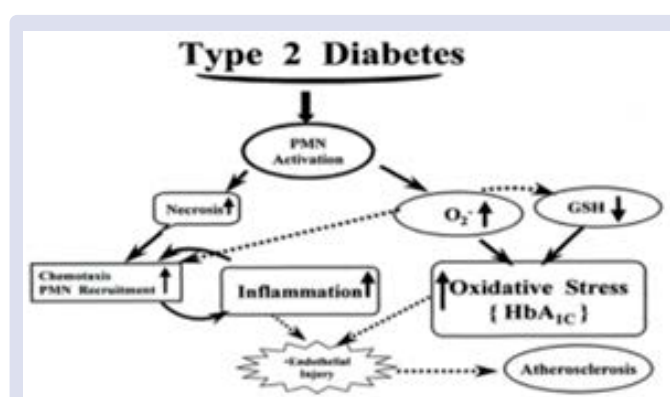


Figure 3. The Relationship Between type 2 DM, Oxidative Stress, and Inflammation.¹

antioxidants). However, in conditions of excessive ROS production due to oxidative stress, these endogenous antioxidants may require additional support from external sources (exogenous). This might stem from the daily intake of food and beverages. Antioxidants play a crucial role in human health by inhibiting and neutralizing oxidative reactions associated with free radicals. Administering antioxidants in the form of vitamins can reduce oxidative stress in individuals with DM. The majority of antioxidants in plasma tend to decrease type 2 DM patients due to related complications, including atherosclerosis and coronary heart disease.³

Antioxidants and vitamins are beneficial in reducing oxidative damage in DM patients. The results showed that in 30 type 2 DM patients, there was an imbalance between oxidants and antioxidants in the plasma compared to the control group. According to the Centers for Disease Control and Prevention (CDC), the levels of vitamin A and E are lower in DM patients compared to non-diabetics. Vitamin C helps prevent complications by inhibiting the production of sorbitol, a byproduct of sugar metabolism that accumulates in cells and contributes to the development of neuropathy and cataracts.

The results showed that the administration of tender coconut water powder enriched with vitamin E effectively prevented oxidative stress, as evidenced by an increase in SOD and GPx along with a decrease in MDA,¹² IL-1, IL-6, TNF- α , and CRP levels. A previous study found similar results, where tender coconut water increased SOD and GPx, and reduced MDA, TNF- α , IL-1, as well as IL-6 levels in type 2 DM rats.² Furthermore, the treatment decreased glucose, and increased plasma insulin levels in pregnant DM rats (GDS) ultimately preventing oxidative stress.¹³

In this study, tender coconut water powder processed using spray and freeze drying was used, each enriched with vitamin E. Subsequently, the sample was tested on experimentally conditioned type 2 DM rats. This method was adopted to meet the demand for a powdered product enriched with vitamin E. The greater diversity of products derived from tender coconut water can add value and provide more convenience. The derived powder may be used as a natural exogenous source of antioxidants which are more practical and easier to consume without compromising nutritional content.

This study indicated that tender coconut water processed using freeze drying and enriched with vitamin E yielded the most optimal results in increasing SOD and GPx, as well as reducing MDA, IL-1, IL-6, TNF- α , and CRP levels. Zulaikhah et al. 2023 stated that the active compounds in tender coconut water powder processed by freeze drying such as L-arginine, Mg, and K were higher compared to spray drying.⁹

Freeze drying is a specialized method that produces dry materials while preserving their characteristic properties. Zulaikhah et al. 2023 showed that tender coconut water powder processed by freeze drying and enriched with vitamin E yielded the highest content of L-arginine (231.1 $\mu\text{g/g}$), Mg (377.31 mg/kg), and K (7260.42 mg/kg). These contents were higher compared to those produced by spray drying.⁹

High L-arginine content can be used to reduce the generation of free radicals, enhance antioxidant activity, and inhibit lipid peroxidation processes. It is also a source of nitric oxide (NO), which potentially inhibits Xanthine Oxidase (XO), as well as increases SOD, total thiol (T-SH), vitamin C, and total antioxidant capacity (TAC).^{14,15}

Epidemiological studies indicated that most DM patients had low Mg levels. Insulin and glucose are essential compounds in Mg metabolism. Low intracellular levels can lead to damaged tyrosine kinase activity, decreased post-receptor insulin function, and worsened resistance in diabetic patients. Additionally, low Mg intake is associated with type 2 DM development. Mg deficiency can trigger proinflammatory processes, leading to excessive production and release of interleukins.¹⁶ Compounds high Mg in tender coconut water powder processed by freeze drying and enriched with vitamin E can be used as a strategy to prevent oxidative stress caused by type 2 DM

The high nutritional content in tender coconut water powder can be used as a source of important vitamins and minerals for the body. One significant role is acting as a cofactor for the natural antioxidant enzyme SOD. SOD is an endogenous antioxidant that plays a role in resisting free radicals by changing superoxide ions to stable hydrogen peroxide.¹⁷ Deficiency of minerals such as copper (Cu), zinc (Zn), and manganese (Mn) can reduce the activity of Cu-Zn SOD and Mn-SOD, thereby triggering oxidative stress.¹²

This study indicated that type 2 DM rats given tender coconut water powder processed with freeze drying and vitamin E showed the most optimal results in preventing oxidative stress compared to those without the addition of vitamin E or spray drying. Vitamin E potentially improved DM complications, enhanced kidney function, and normalized hypertension in test animals suffering from type 2 DM. Other studies reported that the administration prevented DM and protected against kidney disorders in rats. A diet rich in vitamin E lowered blood glucose levels compared to the control group in experimental animals. As an important exogenous antioxidant, vitamin E plays a critical role in inhibiting increased production of proinflammatory cytokines including interleukin-6 (IL-6) or Tumor Necrosis Factor (TNF- α).

Vitamin C can reduce superoxide radicals, hydrogen peroxide, and reactive oxygen species from activated neutrophils and monocytes. Oxidized vitamin E affected by free radicals reacts with vitamin C, receiving a hydrogen ion and transforming into tocopherol.¹²

CONCLUSION

In conclusion, tender coconut water powder processed using spray and freeze drying effectively prevented oxidative stress, indicated by increased levels of SOD and GPx as well as reduced levels of MDA, TNF- α , IL-1, IL-6, and CRP in type 2 DM rats. The most optimal results were achieved with tender coconut water powder processed using freeze drying and enriched with vitamin E.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENT

The authors are grateful to:

1. The Directorate of Research, Technology, and Community Engagement (DRTPM) of the Ministry of Education, Culture, Research, and Technology for funding the PDUPT (Research funding for the Decentralization program) grant in the second year (2023) under agreement/contract number 76a/B.1/SA/LPPM/V/2023.
2. The Research and Community Engagement Institute (LPPM) of the Sultan Agung Islamic University (UNISSULA) in Semarang, Central Java, Indonesia.
3. Center for Food & Nutrition Studies (PSPG) of Gadjah Mada University (UGM) in Yogyakarta.

REFERENCES

1. Oguntibeju, O. O. 'Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links.', *International journal of physiology, pathophysiology and pharmacology*. 2019; 11(3), pp. 45–63. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/31333808> <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC6628012>.
2. Zulaikhah ST., Wahyuwibowo J, Suharto MN, Enggartiasto BH, Ortanto MIR, Pratama AA. Effect of tender coconut water (TCW) on TNF- α , IL-1 and IL-6 in streptozotocin (STZ) and nicotinamid (NA) induced diabetic rats. *Pharmacogn J*. 2021;13(2):500-5. doi: 10.5530/pj.2021.13.63.
3. Asmat, U., Abad, K. and Ismail, K. 'Diabetes mellitus and oxidative stress—A concise review', *Saudi Pharmaceutical Journal*. 2016; 24(5), pp. 547–553. doi: 10.1016/j.jsps.2015.03.013.
4. Amarowicz, R., Naczki, M. and Shahidi, F. 'Antioxidant activity of crude tannins of canola and rapeseed hulls', *JAOCS, Journal of the American Oil Chemists' Society*. 2000; 77(9), pp. 957–961. doi: 10.1007/s11746-000-0151-0.
5. Kementerian Kesehatan RI. Infodatin Lanjut Usia (lansia). In Pusat Data dan Informasi Kementerian Kesehatan RI (p. 12). 2016
6. Zulaikhah, ST. and Sampurna, S. 'Tender Coconut Water To Prevent Oxidative Stress Due To Mercury Exposure', *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*. 2016; 10(6), pp. 35–38. doi: 10.9790/2402-1006023538.
7. Zulaikhah, ST. 'Health Benefits Of Tender Coconut Water (TCW)', *International Journal of Pharmaceutical Sciences and Research (IJPSR)*. 2019; 10(2), pp. 474–480. doi: 10.13040/IJPSR.0975-8232.10(2).474-80
8. Fatimah, R. N. 'Diabetes Melitus Tipe 2', *Fakultas Kedokteran Universitas Lampung*. 2015; 4, Pp. 93–101. Doi: 10.2337/Dc12-0698.
9. Zulaikhah ST, Ratnawati R, Hussaana A, Muhandri, T. 'Comparison of Powdered Active Compounds Made from Tender Coconut Water Fortified with Vitamin E, Processed by Spray Drying and Freeze Drying', *Pharmacognosy Journal*. 2023; 14(6), pp. 682–686. doi: 10.5530/pj.2022.14.154.

10. Zulaikhah ST, Pertiwi D, Bagus SA, Nuri S, Alfiza NS. Effect of Tender Coconut Water on Blood Lipid Levels in Hight Fat Diet Fed Male Rats. *J Krishna Inst Med Sci Univ.* 2017;6(2):63-8.
11. Widowati, W. 'Potensi Antioksidan Sebagai Antidiabetes', *JKM.* 2008; 7(2), pp. 1–10. doi: 10.52574/syiahkualauniversitypress.350.
12. Zulaikhah ST., Anies, Ari S., S. 'Effects of Tender Coconut Water on Antioxidant Enzymatic Superoxida Dismutase (SOD), CATALASE (CAT), Glutathione Peroxidase (GPx) and Lipid Peroxidation In Mercury Exposure Workers', *International Journal of Science and Research (IJSR).* 2015; 4(12), pp. 517–524
13. Nova FS, Chasani S, Hussanna A, Zulaikhah ST. Tender coconut water Inhibits the process of lipid peroxidation, reduce glucose levels, and increase plasma insulin in pregnant diabetic rats. *Pharmacogn J.* 2020;12(1):24. doi: 10.5530/pj.2020.12.24.
14. Tripathi P, M.Chandra, Misra MK. Oral Administration of L-Arginine in Patients with Angina or following Myocardial Infarction may be Protective by Increasing Plasma Superoxida Dismutase and Total Thiol with Reduction in Serum Cholesterol and Xanthine oxidase. *Oxid Med Cell Longev.* 2009; 2(4):231-7
15. Fazelian S, Hoseini M, Namazi N, Heshmati J, Kish MS, Mirfatahi M, et al. Effects of L-Arginine Supplementation on Antioxidant Status and Body Composition in Obese Patients with Pre-diabetes : A Randomized Controlled Clinical Trial. *Adv Pharm Bull.* 2014; 4(1):449-54.
16. Barbagallo, M., Dominguez, L.J. Diabetes and Clinical Research Magnesium and Type 2 Diabetes: An Update *ClinMed. Int J Diabetes Clin Res.* 2015; 2, 1–5.
17. Umarudin et al. 'Effect of Lissachatina fulica chitosan on the antioxidant and lipid profile of hypercholesterolemic male Wistar rats', *Journal of Pharmacy and Pharmacognosy Research.* 2022; 10(6), pp. 995–1005. doi: 10.56499/jppres22.1468_10.6.995.

Cite this article: Ratnawati R, Zulaikhah ST, Hussaana A, Pratama AA. Effect of Tender Coconut Water Powder Enriched with Vitamin E in Preventing Oxidative Stress of Diabetes Male Wistar Rats. *Pharmacogn J.* 2024;16(5): 1062-1068.