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Antioxidant Effects of Temulawak and Black Cumin Honey in Stunted Children: A Study in Cirebon

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ABSTRACT

Introduction: The imbalance between the production of free radicals and the antioxidant defense system in the body will cause the body to experience oxidative stress. This can be caused by chronic malnutrition. In children, this condition causes growth and development failure called stunting. Honey, temulawak and black cumin are herbs with high antioxidant content, formulated in one preparation to test its effectiveness. The purpose of the study was to determine specific descriptions experienced by stunted children and to test the antioxidant effectiveness of preparation temulawak-black cumin honey.

Methods: The research method used was quasi-experimental, pre-post design, the research time starts from January-May 2023. The population of Stunting children in the Tegalwangi locus area, Cirebon Regency, the selected samples or subjects are children aged 24-60 months who meet the inclusion criteria. The subjects were divided into 2 groups, namely the treatment group that received the test preparation for 4 months and the control group without the provision of the test preparation. Blood sampling was carried out simultaneously or simultaneously at the village hall with the help of trained analysts. The antioxidant parameters superoxide dismutase, catalase, glutathione peroxidase and free radical parameters Nitric oxide and malondialdehyde. The data obtained will be analysed using an independent t-test.

Results: The results of the study on 50 stunted children showed low levels of Superoxide Dismutase, Catalase, Glutathione peroxidase, and free radical parameters Nitric oxide and Malondialdehyde were obtained high. This means that stunted children experience oxidative stress. There was significant antioxidant effectiveness with increased levels of SOD and Catalase in the treatment group ($P<0.05$), a decrease in NO and MDA in the treatment group ($P<0.05$) compared to the control group.

Conclusion: Temulawak-black cumin honey preparation has activity to reduce oxidative stress in stunted children in Cirebon.

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INTRODUCTION

Oxidative stress is a biological consequence resulting from the inability of the body's detoxification machinery to combat the excessive formation of reactive oxygen species (ROS) (1). Its causes damage to biological systems due to free radicals, where free radicals are reactive chemical species with unpaired electrons in their outermost orbitals. This unstable configuration produces energy that is released through reactions with surrounding molecules, such as proteins, lipids, carbohydrates, and nucleic acids (2). Free radicals are atoms of the hydrogen element with one proton and one electron. Free radicals can also be nitrogen or carbon, and oxygen, where oxygen is a form of free radical that plays an important role in aerobic organisms (3). This is related to various molecular pathways in cells, closely related to the onset and severity of a number of degenerative diseases, which trigger macromolecular damage.

Antioxidants are substances that can neutralize free radicals, their deficiency can increase the risk of damage to the body from degenerative diseases. However, to protect the body from free radicals, the amount of antioxidants must be maintained and increased. Otherwise, decreased body resistance will occur which will result in various infectious diseases (4). High intake of antioxidants can help prevent various diseases and reduce health problems. Food can increase antioxidants in the body. The main sources of antioxidants are fruits and vegetables, which contain vitamins A, C, E, beta-carotene, and 4 important minerals, such as zinc and selenium (5).

Honey is rich in macronutrients and micronutrients that have effectiveness as neuroprotective, antioxidant, and anti-inflammatory (6). Temulawak has many chemical compounds, where curcumin and starch are one of the contents found in the temulawak plant (7). Temulawak is generally used by Javanese people as the main ingredient of traditional medicine that is useful for maintaining body health, treating diseases and improving health (8). Plants with the Zingiberaceae family are effective as antioxidants that can ward off free radicals, so they can be used to prevent the onset of chronic diseases

Black cumin (Nigella sativa L.) is a plant that has immunomodulatory activity. The content of black cumin seeds is essential oil, alkaloids, saponins, tannins, polyphenols and fatty oils (9). Black cumin contains thymoquinone compounds that act as immunostimulants, increase body resistance that can protect the body from free radicals and as a weight gainer. Efforts made to gain weight, namely carbohydrates and fats are the main sources of energy. Protein, vitamins, minerals and trace elements are essential for the growth and development of water tissue, protein and vitamins can be needed to regulate metabolism (10).

Stunting is one of the early effects of oxidative stress on child growth and development (11). An imbalance between the production of free radicals and the antioxidant defense system in the body will cause the body to experience oxidative stress (12). Environment, nutrition, and biological events have a direct influence on human metabolism. Oxidative stress is divided into early oxidative stress (growth and development) and chronic oxidative stress (outside of growth and development). Early oxidative stress during growth and development requires more energy and nutrients, as well as vitamins and minerals, which affect child development. Chronic oxidative stress occurs when the early growth and development process is disrupted for a long period of time.

Stunting can be caused etiologically by one or more diseases or injuries that directly result in nutritional imbalance or as a result of environmental or behavioral factors related to reduced nutrient intake or as a result of both (13). The national prevalence of stunting in children in 2021 was 24.4%. The stunting rate in Cirebon Regency was 30.6%, above the national prevalence (14). According to the Decree of the Minister of National Development Planning No. 42 of 2020, Cirebon Regency is one of the cities/regencies that is the focus of interventions to reduce stunting (10).

This study will reveal the oxidative stress experienced by stunted children in Tegalwangi village, oxidative stress with parameters of nitric oxide, malondialdehyde and antioxidant enzyme activity with parameters of superoxide dismutase, catalase, glutathione peroxidase. Honey, temulawak and black cumin are herbs with high antioxidant content, formulated in one preparation to test its effectiveness. The study was continued by testing the effectiveness of black cumin-temulawak honey antioxidants in stunted children.

METHOD

Research Design

This study was a quasi-experimental study with a pre-post design. The research has been registered with the Health Development Policy Agency through the Indonesian Disease Registry with No. INA-Y6CT9L0. We conducted the research ethics licensing process to the Ethics Committee of YPIB Majalengka University with permit number 003/KEPK/EC/III/2023. Majalengka, Cirebon Regency Health Office, Karangsari Health Center and Tegalwangi Village Head

Subject

The population of the study was stunted toddlers in the health monitoring area of the Karangsari Health Center, Cirebon Regency. The subjects of the study were 67 children who met the selection criteria for prospective subjects and stated their willingness to be volunteers. Samples with inclusion criteria were 2-5 years old, not sick, and not disabled, while the exclusion criteria were parents who did not fill in the informed consent and moved domicile, the sample obtained was 50 stunted children. Quasi-experimental research, where researchers do not control the diet and economic status of stunted children. The research location was in Tegalwangi Village, Weru District, Cirebon Regency, the research period started from January to May 2023.

Steps

Subject Recruitment

Subject data was obtained from the Karangsari Health Center where Tegalwangi is a health monitoring area under the Health Center. Based on this data, we invited parents and their children to the Tegalwangi Village Hall. At the Village Hall, we provided an explanation regarding this study. Parents who agree to their children as research subjects will be given a consent form to be signed.

Anthropometric measurements

Measurements of height, weight, arm circumference, and head circumference were carried out on children who were the subjects of the study. Anthropometric measurements used tools according to the standards of the Ministry of Health and were carried out by health center officers or trained posyandu cadres.

Treatment of administration of black cumin-temulawak honey

After recording the child's identity and anthropometric measurements, groups were divided. The treatment group will receive black cumin-temulawak honey with a dose of 5 ml once a day for children aged 5 years, 4 ml for children aged 4 years, 3 ml for children aged 3 years, and 2 ml for children aged 2 years. Administration was carried out for 4 months from January-May 2023. Meanwhile, the control group did not receive black cumin turmeric honey.

Measurement of SOD, Catalase, Glutathione Peroxidase, NO, and MDA Levels

Blood sampling of stunted toddlers as much as 1 cc by analysts from the Health Center and Muhammadiyah Hospital Cirebon. This blood sampling was carried out before treatment (January 2023) and after 4 months of treatment (May 2023). The blood taken was stored in a red EDTA tube which would then be centrifuged to collect the plasma. The blood plasma that had been taken was sent to the Physiology Laboratory of the Faculty of Medicine, Brawijaya University for further analysis of SOD Assay Kit levels, G-Bioscience #BAQ077 with an iMark 21225 spectrophotometric reader at a wavelength of 450 nm. Catalase level analysis using Catalase Assay Kit #E-BC-K031-S-prt with Reader Type RLx800 at a wavelength of 405 nm. NO level analysis using NO Assay Kit prt with Reader Type RLx800 at a wavelength of 405 nm. Malondialdehyde level analysis using MDA Assay Kit prt with Reader Type RLx800 at a wavelength of 535 nm.

Data analysis

Univariate analysis includes frequency and percentage. Bivariate analysis using Chi square test at 95% Confident interval, relating between risk factors of gender, age, weight/age, and height/age to the activity of endogenous antioxidant enzymes SOD, Catalase, and to free radicals Malondialdehyde and Nitric oxide. Data analysis for effectiveness test using independent t-test between treatment and control groups and between pre and post treatment (CI=95%, α=5%).

RESULTS

The list of children at risk of stunting was obtained from the Karangsari Health Center, whose work area covers Tegalwangi village. After filling in the willingness to be subjects by parents, 50 stunted children were obtained whose parents were willing for their children to be research subjects.

Demographics of Stunting Children

After the parents filled in the informed consent, the child's height, weight, and personal data were measured to ensure that the subjects used were stunted children. Respondent characteristics are presented in Table 1.

Table 1. Demographic and Clinical Data Overview of Stunting Children (n=50)

Characteristics	Parameter	Frequency (%)
Age	24 – 41 Month	21 (42)
	42 – 60 Month	29 (58)
Gender	Boys	29 (58)
	Girls	21 (42)
Birth Weight	Low	6 (12)
	Normal	44 (88)
Birth Length	Low	27 (54)
	Normal	23 (46)
Gestational age at birth	Prematur	3 (6%)
	Aterm	47 (94%)
Z score Height for Age	Very short	5 (10%)
	Short	45 (90%)
Z score Weight for Age	Malnutrition	2 (4%)
	Under nutrition	18 (36%)
	Adequate nutrition	30 (60%)

Overview of Oxidative Stress and Antioxidant Activity

Oxidative stress parameters are indicated by the levels of Nitric Oxide and Malondialdehyde, while antioxidant activity parameters are represented by the enzymes Superoxide Dismutase, Catalase, and Glutathione Peroxidase. The following are the results of measurements and data analysis of these parameters:

Table 2. Results and Data Analysis of Superoxide Dismutase Enzyme Antioxidant Activity Pre-test

Category		%		Superoxide Dismutase Enzyme		
				Mean ±SD	**OR (95% CI lower-upper)**	**P-value**
Gender	Boys	29	58	1,34 ± 0,88	A) 1,20	0,793
	Girls	21	42	1,25 ± 0,92	B) (0,31-4,61)	
Age (month)	24 – 41	21	42	1,36 ± 0,92	C) 0,53	0,287
	42 – 60	29	58	1,09 ± 0,66	D) (0,17-2,10)	
Z-score Weight for Age	Adequate nutrition	20	40	1,41 ± 1,01	E) 0,88 F) (0,09-8,74)	G) 0,912
	Under nutrition	30	60	1,90 ± 1,45		
Z-score Height for Age	Very short	44	88	1,57 ± 0,79	H) 1,46	0,482
	Short	6	12	1,21 ± 0,76	I) (0,90-5,91)	

Table 3. Results and Data Analysis of Antioxidant Activity of Catalase Enzyme Pre-test

Category		n	%	Catalase Enzym Mean ±SD	OR (95% CI lower-upper)	P-value
Gender	Boys	29	58	3,26 ± 2,01	J) 0,53 (0,41-0,70)	0,046
	Girls	21	42	2,46 ± 2,53		
Age (month)	24 – 41	21	42	3,41 ± 2,02	K) 0,301 (0,05-2,00)	0,203
	42 – 60	29	58	2,82 ± 1,84		
Z-score Weight for Age	Adequate nutrition	20	40	3,08 ± 2,01	L) 2.56 (0,23-28,81)	0,442
	Under nutrition	30	60	2,97 ± 2,25		
Z-score Height for Age	Very short	44	88	2,50 ± 2,45	M) 0,14 (0,06-4,90)	0,323
	Short	6	12	3,48 ± 1,96		

Table 4. Results and Data Analysis of Antioxidant Activity of Glutathione Peroxidase Enzyme Pre-test

Category		n	%	Enzim Glutation Peroksidase Mean ±SD	OR (95% CI lower-upper)	P-value
Gender	Boys	29	58	3,77±1,94	2,25	0,390
	Girls	21	42	4,58±3,63	(0,34±14,83)	
Age (month)	24 – 41	21	42	3,18±1,59	2,21	0,486
	42 – 60	29	58	4,59±3,134	(0,23±21,46)	
Z-score Weight for Age	Adequate nutrition	20	40	3,73±2,07	0,41 (0,06±2,67)	0,336
	Under nutrition	30	60	4,68±3,57		
Z-score Height for Age	Very short	44	88	4,06±2,75	2,00	0,562
	Short	**6**	**12**	**4,51±3,18**	(0,189±21,62)	

Table 5. Research Results and Data Analysis of Pre-test Nitric Oxide Levels

Category		n	%	Nitric Oxide Mean ±SD	OR (95% CI lower-upper)	P-value
Gender	Boys	29	58	42,20 ± 51,70	N) 0,478 (0,152-1,509)	0,214
	Girls	21	42	26,82 ± 13,38		
Age (month)	24 – 41	21	42	39,08 ± 40,68	O) 0,950 (0,290-3,114)	0,588
	42 – 60	29	58	24,73 ± 11,30		
Z-score Weight for Age	Adequate nutrition	20	40	40,87 ± 53,11	P) 0,896 Q) (0,813-0,987)	R) 0,808
	Under nutrition	30	60	37,03 ± 42,74		
Z-score Height for Age	Very short	44	88	24,15 ± 6,53	S) 2,445 (1,904-3,990)	0,217
	Short	6	12	31,60 ± 31,68		

Table 6. Results and Data Analysis of Pre-test Malondialdehyde Levels

Category		n	%	Malondialdehyde		
				Mean ±SD	OR (95% CI lower-upper)	P-value
Gender	Boys	29	58	15,12 ± 8,99	T) 0,563 (0,438-0,722)	0,228
	Girls	21	42	13,57 ± 8,48		
Age (month)	24 – 41	21	42	16,13 ± 7,47	U) 0,646 (0,524-0,796)	0,310
	42 – 60	29	58	16,20 ± 12,52		
Z-score Weight for Age	Adequate nutrition	20	40	11,71 ± 5,61	V) 0,152 W) (0,016-1,473)	X) 0,092
	Under nutrition	30	60	13,14 ± 6,66		
Z-score Height for Age	Very short	44	88	26,46 ± 15,65	Y) 1,389 (0,905-2,658)	0,283
	Short	6	12	14,41 ± 9,85		

Antioxidant Effectiveness Test of Black Cumin-Temulawak Honey

The effectiveness test was conducted experimentally on a group of children with stunting, where they were divided into 2 groups. The sample or treatment group received black cumin-temulawak honey for 4 months. Honey was consumed daily. Meanwhile, the control group did not receive honey. The division of the control and treatment groups is in table 7.

Table 7. Description of the Homogeneity of the Distribution of Stunting Children in the Treatment and Control Groups

Category	Group	Mean ± SD		P1
		Pre test	Post test	value
	Treatment	91,177±8,280	93,062±8,599	0,000
Body Height (cm)	Control	87,963±5,606	88,383±5,524	0,000
	P2 value			0,028*
Weight (kg)	Treatment	13,3538±3,753	15,200±2,777	0,000*
	Control	11,8208±2,208	12,767±2,714	0,007*
	P2 value			0,003*
	Treatment	15,62±1,26	15,90±1,90	0,000*
Arm circumference (cm)	Control	15,02±1,08	15,24±1,06	0,327
	P2 value			0,045*
Head circumference (cm)	Treatment	47,29±3,23	47,77±2,02	0,000*
	Control	46,43±1,30	46,78±1,30	0,412
	P2 value			0,048*
	Treatment	-2,095±1,496	-1,860±1,304	0,000*
Height for Age	Control	-2,610±0,428	-2,459±0,414	0,233
	P2 value			0,003*
	Treatment	-1,424 ±1,309	0,066±1,599	0,000*
Weight for Age	Control	-1,563 ±1,244	-1,305±1,449	0,259
	P2 value			0,036*

The parameters for this test are divided into endogenous antioxidant parameters Superoxide Dismutase (SOD), Catalase, and free radical parameters Nitric Oxide and Malondialdehyde. The following are the research results presented in table 8.

Table 8. Results of the Effectiveness Test of Antioxidant Temulawak-Black Cumin Honey in the Treatment and Control Groups

Category	Groups	Mean ± SD Pre test	Post test	P-value
SOD (μ/mL)	Treatment	0,936 ±0,58	1,440±0,95	0,043
	Conrol	1,270±0,86	0,775±0,37	0,021
	P value			0,307
Catalase (μ/mL)	Treatment	2,121±0,38	3,160±2,17	0,019
	Conrol	3,070±1,73	2,423±0,71	0,068
	P value			0,063
Nitric Oxide (μmol/L)	Treatment	41,227±25,66	39,495±46,19	0,843
	Conrol	31,689±34,40	37,652±22,02	0,346
	P value			0,601
Malondialdehyde (μM)	Perlakuan	18,143±11,51	15,980±15,55	0,575
	Konrol	12,410±7,26	15,698±7,27	0,148
	P value			0,934

DISCUSSION

The results of the study showed that children aged ≤36 months were at 1.826x risk of stunting compared to those aged 36-60 months, but this value was not significant (P value>0.5, 95% CI). This is in line with previous research conducted in Nepal stating that children aged 0-23 months had a significantly lower risk of stunting, compared to children age > 23 months. Children at this age can still get the protection of breast milk that they previously received (16).

Boys are at 1x greater risk than girls, this value is not significant because P value>0.5. Boys tend to be more physically active so they spend more energy on activities and not on their growth. In addition, in general boys have faster growth after going through puberty while girls generally experience faster growth than boys before and during puberty (17). During their growth period, boys require more energy and protein and are at risk of experiencing malnutrition if these nutritional needs are not met (18).

The results of the body length data at birth were divided into ≤48 cm and >48 cm, the reason 48 cm was used as the standard was the normal body length when a child was born for Indonesian children. (19). Birth Length (BL) ≤48 cm is at risk of 4.162 times the risk of becoming stunted compared to BL >48 cm, this value is significantly different (P value <0.05). Birth length below the standard for children in West Java is at risk of becoming stunted during adolescence (20) In contrast to previous research by Della, there was no relationship between birth length and the incidence of stunting in Tasikmalaya(21). Meanwhile, for birth weight, the OR obtained that low birth weight is 2.135 x at risk of stunting, but this value is not significant because the P value> 0.05. Previous research was conducted by Purwanto, et al. that there is a relationship between birth weight and birth length with the incidence of stunting (22,23). In addition, babies born with a low birth weight tend to experience stunting as much as 83%, because toddlers who have poor nutrition are more susceptible to infectious diseases, such as diarrhea and lower respiratory tract infections (URTIs) compared to children with normal body weight, so that physical growth is not optimal (24).

Based on nutritional status, data obtained shows that stunted children with nutritional status of less than 40%, this value is at risk of 2.776x stunting compared to normal nutritional status. Stunting in children reflects a condition of growth failure in toddlers (under 5 years) due to chronic malnutrition, so that children become too short for their age. Malnutrition occurs since the baby is in the womb and in the early period after the baby is born, however, stunting conditions only appear after the baby is 2 years old (25). Household food security has an impact on providing nutritional intake to children to support growth and development. Low food security will put children at risk of stunting in the future (26).

Superoxide Dismutase (SOD) is a metalloenzyme that catalyzes the dismutation of superoxide into hydrogen peroxide and oxygen. This occurs because the mitochondrial matrix contains manganese and copper, zinc, or iron atoms that are formed in the cytosol (27). Next, the enzymes catalase and glutathione peroxidase convert hydrogen peroxide into water molecules. The body uses SOD as its first line of defense against free radicals, a very powerful antioxidant enzyme (28). In this study, the average SOD value obtained from various criteria was below the SOD value of normal children, namely 2.11 ± 0.08 U/mL. This low value indicates a decrease in SOD antioxidant activity which can have an impact on the child's body's ability to ward off free radicals (29). The low SOD value is caused because the research subjects are children with poor nutritional status indicated by stunting and low nutritional status. This has an effect because the nutritional intake that will be used as raw material for the formation of cells, tissues or organs is insufficient, causing cells to experience oxidative stress. Previous research showed that the SOD enzyme activity value ranged from 1-3 U/mL serum, age, gender, and body mass index factors did not affect SOD enzyme activity, low nutritional intake, especially zinc, affected the activity of this enzyme. (30). There was a significant difference between pre and post in the treatment group, and the SOD value increased after honey administration. While in the control group there was a significant decrease in SOD. However, after data analysis using independent T-test, a value of 0.307 was obtained, meaning it was not significant.

Catalase (CAT) is not present in human endothelial cells or smooth muscle wall cells of blood vessels, but is present in peroxisomes, mitochondria, and the cytosol of mature erythrocytes. CAT activity is lowest in heart and brain tissue, but highest in liver, erythrocytes, kidneys, and adipose tissue in mammalian organs (31). To control the concentration of H2O2 and other cytotoxic oxygen derivatives, catalase primarily detoxifies ROS. ROS molecules are generated in many cellular locations, especially in mitochondria, due to the reduction of electrons to oxygen in the respiratory chain that produces superoxide anion. SOD converts H_2O_2 to H_2O, which can then combine with metal ions to form hydroxyls that are highly reactive to lipids, proteins, and nitric acid (32). In this study, the average Catalase value obtained various from criteria was below the Catalase value of normal children, namely 5.49±0.49 (27). Catalase in normal children in other studies is 6 U/mL, this difference in value may be due to differences in population and measurement methods (34). This low value has the same effect as the previous decrease in SOD, because Catalase, although not the only enzyme that acts as an endogenous antioxidant, Catalase plays a role in counteracting free radicals in the body. Long-term malnutrition in stunted children will affect the enzymatic processes in the body. Including the formation of the catalase enzyme which the body will use as an antioxidant. There was a significant increase in the treatment group after giving black cumin temulawak honey to the levels of the catalase enzyme. While in the control group there was a decrease although not significant.

Nutritional status does not significantly affect Glutathione peroxidase levels. Of all the antioxidant activity criteria, the glutathione peroxidase enzyme is below the normal value for children, which is 6.5-6.9 µg/mL (28). This is because other more important factors that affect the activity of this enzyme are physical activity. Excessive physical activity will reduce oxidative stress and increase the production of the enzyme Glutathione peroxidase. Nutritional status and physical activity are very dominant in their influence on antioxidant production. With more physical activity, it will reduce oxidative stress and increase the production of the enzyme Glutathione peroxidase (29). Other studies show that oxidative stress experienced by children with a history of chronic kidney failure shows low Glutathione Peroxidase activity from the normal value of 4-10 U/mL(37).

Stunting causes a decrease in protein and amino acids, one of which is cysteine, an amino acid that contains sulfur. Because cysteine is a component that forms glutathione, which is an antioxidant, cysteine deficiency is associated with a general decrease in antioxidants (38).

Nitric oxide, a compound that functions as an intracellular signal molecule in mammals including humans with modulation in the form of blood flow, thrombosis and neural activity. NO molecules are often also produced by pollutants from coal combustion in factories, cigarette smoke, vehicles and others, so they are often considered toxic and highly reactive. Accumulation of NO is also one of the biomarkers of oxidative stress (39). The results of the average measurement of stunted children as a whole were above the NO value in previous studies on normal children, namely 2.2 ± 11.5. The high value illustrates the high free radicals from the nitrogen group in the body. The results of the effectiveness test of black cumin temulawak honey showed a decrease in NO in the sample group, while there was an increase in the control group. However, the decrease and increase values in both groups were not significant.

Accumulation of malondialdehyde (MDA), a highly reactive compound, indicates a mechanism of cell and tissue damage (40). Previous research on normal children showed that MDA levels in their bodies were 0.33 ± 0.04. While this study obtained high average MDA results. Reduced activity of antioxidant enzymes such as SOD and Catalase in stunted children causes an imbalance so that free radical parameters increase. Overall, this causes stunted children to experience oxidative stress. he limitation of this study is that it focuses on stunted children living in the Tegalwangi area, where Tegalwangi is one of the stunting loci in Cirebon Regency. Reduced activity of antioxidant enzymes such as SOD and Catalase in stunted children causes an imbalance so that free radical parameters increase.

Overall, this causes stunted children to experience oxidative stress. The MDA value in the sample group decreased, but was not significant. While in the control group, there was an increase in MDA from pre-test to post-test, this value was also not significant. The accumulation of malondialdehyde (MDA), a highly reactive compound, indicates a mechanism of cell and tissue damage. Oxidative stress occurs when the production of free radicals, such as reactive oxygen species (ROS), exceeds the ability of the body's antioxidant system to neutralize them. Malondialdehyde (MDA) is an end product of lipid peroxidation, often used as a biomarker to assess the level of oxidative stress in the body. Increased levels of MDA indicate oxidative damage to cell membrane lipids. Nitric Oxide (NO) is a molecule that plays an important role in various physiological processes, including vasodilation and neurotransmission. However, under oxidative stress conditions, NO can react with superoxide to form peroxynitrite, a highly reactive and dangerous molecule, which can cause oxidative damage to cells and tissues (41).

The administration of black cumin turmeric honey is able to provide effectiveness in increasing SOD enzymes, Catalase and decreasing NO and MDA due to the phenolic and flavonoid components contained therein. Phenolic and flavonoid compounds in honey can capture and neutralize free radicals, inhibit lipid (fat) oxidation chain reactions in the body, which if uncontrolled can cause cell and tissue damage. This is done through interaction with peroxyl radicals and preventing the formation of harmful oxidation products, preventing oxidative damage to cells (42). The content of xanthorizol in temulawak also supports the preparation as an antioxidant by repairing cell damage in mitochondria. The dominant curcumin content in temulawak can increase the activity of SOD and Catalase enzymes so that the body can ward off free radicals from both the ROS and RNS groups. Curcumin and xanthorizol, other compound components in temulawak increase endogenous antioxidant activity (43) Black cumin in the honey preparation given to the treatment group can also capture superoxide free radicals which are important in its activity as an antioxidant. Other studies support that the content of thymoquinone and thymohydroquinone in cumin in vitro is able to ward off superoxide ions which are called free radicals (44). This effectiveness is due to honey, ginger and black cumin which have the activity to reduce free radicals in the bodies of stunted children, so that it is hoped that in the future these children will be able to catch up on their growth and development.

CONCLUSION

The combination of temulawak-black cumin honey showed effectiveness in increasing the activity of SOD and Catalase enzymes and reducing the oxidant metabolite Malondialdehyde and reactive oxidant NO in stunted children. It is hoped that the results of this study can be used as a reference by the government for stunted children to consume black cumin turmeric honey to catch up on their delayed growth and development.

AUTHOR’S CONTRIBUTION STATEMENT

This research was conducted by a research team with the following special contributions: 1) Coordination with the health office and health centers for data collection: Siti Pandanwangi TW; Ahmad Azrul Zuniarto: 2) Development of questionnaires and anthropometric measurement training: Akrom; 3) Data collection and analysis: Nurkhasanah

CONFLICTS OF INTEREST

The author declare no conflics of interest

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

In the research and preparation of this article/journal we do not use AI.

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