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The Effect of Virgin Coconut Oil on IL-6 Gene Expression and Body Length in Stunted Zebrafish (*Danio rerio*) Larvae

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ABSTRACT

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Stunting poses a major global health concern, impairing physical and cognitive development, particularly in developing nations; it persists despite various nutrition initiatives. Virgin Coconut Oil (VCO) derived from local resources is emerging as an effective option to enhance nutrition and promote brain and motor skills growth in children suffering from stunting. The objective of this study is to evaluate the effect of VCO on the expression of Interleukin-6 (IL-6) and body length in zebrafish larvae induced with stunting by rotenone exposure. Zebrafish larvae 2 hours post-fertilization were treated with rotenone to induce stunting and subsequently administered VCO at various concentrations. IL-6 expression was measured using the Reverse Transcription Polymerase Chain Reaction (RT-PCR) method, and body length was measured at 3, 6, and 9 days post-fertilization. The results of the study showed that the body length of zebrafish larvae exposed to rotenone significantly decreased compared to the negative control group, measuring 3.04 mm on day 3 and 3.72 mm on day 9. Administration of VCO at a dose of 6.25% was able to increase the body length of the larvae to 3.32 mm on day 3 and 4.06 mm on day 9. The expression of the IL-6 gene, statistical analysis using one-way ANOVA revealed a highly significant difference among the groups (p < 0.001). These findings indicate that VCO effectively reduces inflammation and improves growth inhibited by rotenone exposure.

Keywords: Virgin Coconut Oil, stunting, zebrafish model, Interleukin-6, body length, rotenone, growth

Introduction

Stunting is a condition caused by chronic malnutrition that impedes physical growth and development in children. This condition is associated with cognitive development disorders and increased susceptibility to infections. One of the contributing factors to stunting is oxidative stress which is generated by free radicals that damage cells and tissues, inhibit growth, and affect the differentiation of essential cells such as osteoblasts. Furthermore, chronic inflammation triggered by pro-inflammatory cytokines such as Interleukin-6 (IL-6) contributes to the occurrence of stunting by hindering muscle growth and exacerbating tissue damage. Virgin Coconut Oil (VCO) contains Medium Chain Fatty Acids (MCFAs) that possess antioxidant and anti-inflammatory properties. Virgin Coconut Oil has been shown to reduce oxidative stress and decrease the expression of pro-inflammatory cytokines, including Interleukin-6, while improving physical growth parameters that are disrupted due to toxin exposure or environmental stress

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Virgin Coconut Oil has the potential to serve as an effective therapeutic alternative for preventing or addressing stunting through mechanisms of inflammation reduction and enhanced cellular metabolism.^{3,4}
The objective of this study is to evaluate the effect of VCO on IL-6

expression and body length in zebrafish larvae in a stunting model induced by rotenone. Virgin Coconut Oil is expected to mitigate the effects of stunting and serve as a basis for the development of natural therapies for stunting prevention.

Materials and Methods

Virgin Coconut Oil (VCO)

Virgin Coconut Oil (VCO) was used in the treatments for the assessment of its effect on Interleukin-6 (IL-6) expression and body length in zebrafish larvae. The Virgin Coconut Oil used in this study is a product produced by KWT Vigur Asri (VCO Palm 7, Cemoro Kandang, Malang, Product No. P-IRT 2063573011924-29).

Preparation of Virgin Coconut Oil Concentrations

The research team conducted exploratory testing to establish suitable concentrations of VCO to be used in this study. The VCO concentrations were determined by the results of the exploratory testing. Virgin Coconut Oil was prepared by mixing 1 ml of VCO with 20 μL of DMSO and water to reach a volume of 16 ml, resulting in a 6.25% concentration. The 6.25% solution was subsequently diluted to obtain concentrations of 3.125% and 1.5625%. Each treatment used a volume of 54 ml in total, which was distributed into multiple well plates with three replications.

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Stunting Model Induced by Rotenone

Rotenone was used to induce stunting in zebrafish larvae. It was dissolved in dimethylsulfoxide (DMSO) as the solvent, to prepare a solution with the appropriate concentration (12.5 ppb) used in this study. Zebrafish larvae 2 hours post-fertilization were divided into five groups: a negative control group, a positive control group, treatment 1 (T1), treatment 2 (T2), and treatment 3 (T3). The negative control group was not given treatment of rotenone or VCO and was maintained in embryonic medium. The positive control group was treated with 12.5 ppb rotenone, while the treatment groups were given the same dose of rotenone along with three concentrations of VCO. Treatment 1 (T1) was administered rotenone and 6.25% VCO, Treatment 2 (T2) was administered rotenone and 3.125% VCO, and Treatment 3 (T3) was administered rotenone and 1.5625% VCO.

Animal Model

This study has been approved by the Health Research Ethics Committee of the Faculty of Medicine Brawijaya University with approval number 492/EC/KEPK/12/2024. This study utilized zebrafish larvae (*Danio rerio*) as an animal model. Zebrafish embryos 2 hours post-fertilization were used as the research model. Larvae were studied from 0 to 9 days post-fertilization. Zebrafish were selected due to their similarity to human physiology and their ability to develop rapidly within a short period. The developmental stage of zebrafish larvae at 3 days post-fertilization (dpf) is comparable to that of a human newborn, while larvae at 6 dpf are equivalent to a 2-year-old human infant, and larvae at 9 dpf are comparable to an 8-year-old human child.⁶

Embryonic Medium

Calcium chloride (CaCl₂), potassium chloride (KCl), sodium chloride (NaCl), and magnesium sulfate (MgSO₄) were used to prepare the zebrafish embryonic medium.⁷

Measurement of Zebrafish Larvae Body Length

After treatment, zebrafish larvae were maintained until 9 days post-fertilization. Body length measurements of zebrafish larvae were performed at 3, 6, and 9 days post-fertilization using a microscope and an image raster application.⁸

Measurement of Interleukin-6 (IL-6) Expression by Reverse Transcription Polymerase Chain Reaction (RT-PCR)

The expression of Interleukin-6 (IL-6) was measured using the Reverse Transcription Polymerase Chain Reaction (RT-PCR) method to assess the inflammatory response in zebrafish larvae tissue. Zebrafish larvae were extracted and DNA was isolated using the RNA Mini Kit Tissue (Genoid) followed by reverse transcription to convert RNA into cDNA using ReverTra Ace qPCR RT Master Mix with gDNA Remover (Toyobo). The base sequence of the primers used is listed in Table 1 and the RT-PCR protocol is provided in Table 2. The reaction process was carried out using a Real-time PCR instrument (CFX Opus 96) with the following composition: 5 μ L of cDNA template, 10 μ L of SensiFAST SYBR, 3.4 μ L of Nuclease-Free Water, 0.8 μ L of reverse primer, and 0.8 μ L of forward primer.

Table 1: Primer Base Sequence of Interleukin-6 (IL-6)

Forward Primer				
β-Actin 5'-CGAGCAGGAGATGGGAACC-3' Reverse Primer IL-6 5'-GCTGTAGATTCGCGTTAGACATC-3'				

Data Analysis

Statistical analysis for One Way ANOVA and Post-Hoc Least Significant Difference (LSD) test was performed using SPSS statistical software version 26.

Table 2: Reverse Transcription Polymerase Chain Reaction (RT-PCR) Protocol

Steps	IL-6
Pre Denaturation(time/temperature)	2'/95°C
Denaturation(time/temperature)	5"/95°C
Extension(time/temperature)	20"/52.3°C

Results and Discussion

Body Length of Zebrafish Larvae in the Positive Control, Negative Control, and Virgin Coconut Oil (VCO) Treatment Groups

The objective of this study is to analyze the effect of VCO on the body length growth of zebrafish larvae (Danio rerio) induced by rotenone as a stunting model. Measurements were taken at 3, 6, and 9 days postfertilization (dpf) with various treatments involving different doses of VCO. In Table 3, at 3 dpf, the negative control group that was not exposed to rotenone or VCO showed the greatest body length of the larvae (3.33 mm), indicating normal larval growth. In contrast, the positive control group exposed to rotenone showed the smallest body length (3.04 mm), indicating stunting. The treatment group that was given 6.25% VCO (T1) showed a significant increase in body length, reaching 3.32 mm, which was better than the positive control group. The other treatment groups of T2 (3.125% dose) and T3 (1.5625% dose) also showed improvements, although the differences were smaller. The analysis using One-Way ANOVA yielded a p-value of 0.000 for the body length of zebrafish larvae at 3 days post-fertilization (dpf), indicating that p < 0.05. This result suggests significant differences in the body length of stunted zebrafish larvae among the negative control group, positive control group, and the T1, T2, and T3 treatment groups. In Table 4, at 6 dpf, the increase in body length of the larvae is more evident. The positive control group still showed the smallest growth (3.41 mm), while the VCO treatment groups demonstrated progressive improvements. The T1 group that received the highest VCO dose showed the greatest body length (3.63 mm), followed by the T2 group (3.62 mm) and the T3 group (3.57 mm). This indicates that VCO can support growth improvement even when the larvae are exposed to rotenone. The analysis using One-Way ANOVA yielded a p-value of 0.000 for the body length of zebrafish larvae at 6 days post-fertilization (dpf), indicating that p < 0.05. This result suggests significant differences in the body length of stunted zebrafish larvae among the negative control group, positive control group, and the T1, T2, and T3 treatment groups. In Table 5, at 9 dpf, the negative control group still exhibited the greatest body length (4.15 mm), while the groups treated with VCO showed significant improvements compared to the positive control group. The T1 group that received a 6.25% VCO dose exhibited a body length of 4.06 mm, which is greater than the positive control group (3.72 mm), indicating that VCO is effective in mitigating the negative effects of rotenone that inhibit growth. The analysis using One-Way ANOVA yielded a p-value of 0.000 for the body length of zebrafish larvae at 9 days post-fertilization (dpf), indicating that p < 0.05. This result suggests significant differences in the body length of stunted zebrafish larvae among the negative control group, positive control group, and the T1, T2, and T3 treatment groups. Overall, the results of this study suggest that VCO administration can improve the body length growth of zebrafish larvae that are hindered by rotenoneinduced stunting. The 6.25% VCO dose (T1) yielded the best results with a significant increase in body length, highlighting the potential of VCO as an agent to address stunting in the zebrafish larvae model. This study demonstrates that the administration of VCO can improve

This study demonstrates that the administration of VCO can improve the body length growth of zebrafish larvae exposed to rotenone, the model of stunting in this research. Rotenone exposure resulted in significant reduction in body length, reflecting the occurrence of stunting. This finding is consistent with previous research that indicated that rotenone is a pesticide that specifically inhibits mitochondrial complex I of the electron transport chain as the initial point in oxidative phosphorylation for ATP production. When complex I is inhibited, the production of ATP, the primary energy source for cells, decreases. The resulting reduction in energy significantly affects energy-demanding cells, such as muscle cells, nerve cells, and osteoblasts (bone-forming

cells). The disruption of ATP production caused by rotenone also triggers an increase in Reactive Oxygen Species (ROS), which are reactive oxygen molecules that can damage cellular structures, DNA, proteins, and membranes. This reduces the ability of cells to proliferate and differentiate, including in the processes of bone elongation and overall body growth. Rotenone effectively induces stunting conditions through disruptions in energy metabolism oxidative stress and cell apoptosis, which affects body growth and in particular bone elongation.

Table 3: Average Body Length of Zebrafish Larvae at 3 dpf

Age	3 days post fertilization					
Group	Negative	Positive	T1	T2	Т3	
	Control	Control	(Rotenone	(Rotenone	(Rotenone	
		(Rotenone	12.5 ppb +	12.5 ppb +	12.5 ppb +	
		12.5 ppb)	VCO	VCO	VCO	
			6.25%)	3.13%)	1.5625%)	
Picture		1				
Mean	3.33	3.04	3.32	3.29	3.24	
(mm)	±	±	±	±	±	
±SD	0.07	0.15	0.09	0.07	0.09	
P			0.000			
Value						

Rotenone causes a decrease in body length (stunting) in zebrafish larvae (Danio rerio) through mechanisms involving bioenergetic disruption and oxidative stress. As an endocrine-disrupting chemical (EDC), rotenone acts as an inhibitor of mitochondrial complex I of the electron transport chain. The inhibition of complex I disrupts oxidative phosphorylation, leading to a decrease in Adenosine Triphosphate (ATP) production, the primary energy molecule in cells. 12 Virgin Coconut Oil has been proven effective in reducing the negative effects of rotenone, which is shown by an increase in body length in zebrafish larvae across all treatment groups given VCO. Virgin Coconut Oil contains medium-chain fatty acids, which have strong antioxidant properties that enable the repair of cell and tissue damage caused by oxidative stress. The medium-chain fatty acids in VCO function to reduce the toxic effects of harmful chemicals such as rotenone. These medium-chain fatty acids, particularly lauric acid (C12:0), capric acid (C10:0), and caprylic acid (C8:0), each possess biologically significant properties beneficial for human health. The production process of VCO is carried out without high heat or chemicals, which preserves its natural bioactive compound content. Lauric acid as the primary component of VCO has antimicrobial, antiviral, anti-inflammatory, and immunomodulatory effects. Virgin Coconut Oil exhibits strong antioxidant activity due to its total phenolic content. Studies have shown that VCO can suppress the formation of free radicals and reduce oxidative stress. 13,14 The effects and benefits of VCO on stunted toddlers, with an emphasis on the metabolic and immunological roles of the MCFAs in VCO, are significant. VCO is a plant-based oil containing approximately 90% saturated fats, mostly in the form of MCFAs such as lauric acid (approximately 47-53%). These fatty acids have different properties from long-chain fatty acids; they are more rapidly digested and directly transported to the liver to be metabolized into energy, without the need for complex enzymatic processes such as bile or pancreatic enzymes. This makes VCO an efficient energy source that is quickly utilized by the body rather than being stored as fat. This process also increases calorie expenditure and induces satiety more quickly, which has the potential of reducing the risk of obesity and improving metabolic efficiency in children with growth disorders. Another benefit of VCO is its ability to enhance nutrient absorption, including vitamins, minerals, and amino acids, which are essential in supporting child growth and development. The increase in body length

in the treatment groups suggests that VCO can help improve growth development that has been hindered by exposure to toxic substances.⁴ The increase in body length in the treatment groups indicates that VCO can help restore growth development that was hindered by exposure to the toxic substance. Furthermore, the effect of different VCO doses on body length growth in larvae showed significant differences, with the 6.25% dose (T1) having the most pronounced effect in increasing larval body length. This indicates that VCO can dose-dependently repair damage caused by rotenone, with higher doses yielding greater improvements. These results are consistent with previous findings that suggest that higher doses of natural substances such as VCO can produce stronger therapeutic effects.¹⁵

Table 4: Average Body Length of Zebrafish Larvae at 6 dpf

Tabl	Table 4: Average Body Length of Zebrafish Larvae at 6 dpf				
Age	6 days post fertilization				
Group	Negative	Positive	T1	T2	Т3
	Control	Control	(Rotenone	(Rotenone	(Rotenone
		(Rotenone	12.5 ppb +	12.5 ppb +	12.5 ppb +
		12.5 ppb)	VCO	VCO	VCO
			6.25%)	3.13%)	1.5625%)
Picture					
Mean	3.74	3.41	3.63	3.62	3.57
(mm)	±	±	±	±	±
±SD	0.17	0.19	0.10	0.10	0.12
P Value		•	0.000		

The increase in body length in the larvae groups treated with VCO also reflects the potential of VCO to repair damage caused by oxidative stress. Oxidative stress induced by exposure to harmful chemicals such as rotenone can disrupt normal physiological processes in organisms. With its antioxidant properties, VCO can reduce damage caused by free radicals, allowing for the restoration of body cells and ultimately supporting faster growth recovery in zebrafish larvae. ^{16,17} Overall, the results of this study indicate that VCO has the potential to mitigate the negative effects of rotenone exposure on zebrafish larvae development. Virgin Coconut Oil administration not only improved body length that was impaired due to rotenone exposure but also provided protection against tissue damage caused by oxidative stress, enhancing recovery and supporting normal growth. Therefore, VCO can be considered as an effective therapeutic agent for addressing growth disturbances caused by exposure to harmful chemicals in a stunting model.

The Effect of Virgin Coconut Oil (VCO) on IL-6 Expression in Zebrafish Larvae Using RT-PCR Method

The objective of this study is to measure the effect of VCO administration on IL-6 expression in zebrafish larvae induced with rotenone. Measurements were taken at 9 days post-fertilization (dpf) using the RT-qPCR method. Normality testing showed that IL-6 expression data were normally distributed, with a p-value of 0.061, indicating that the data met the assumption of normal distribution. Homogeneity testing also indicated that the IL-6 expression data were homogeneous, with a p-value of 0.092. Virgin Coconut Oil is an oil obtained from fresh coconut meat without high heat processing, preserving the natural compounds present in coconut oil. This oil is rich in MCFAs, including lauric acid, and contains phenolic compounds, tocopherols, and tocotrienols that have strong antioxidant effects. Virgin Coconut Oil is known to have various pharmacological benefits such as reducing lipid peroxidation, enhancing the body's antioxidant defense system, and decreasing oxidative stress, which plays a role in various medical conditions. ^{18,19}

Table 5: Average Body Length of Zebrafish Larvae at 9 dpf

Table 5. Average Body Length of Zeoransii Larvae at 9 upi					
Age	9 days post fertilization				
Group	Negativ	Positive	T1	T2	Т3
	е	Control	(Rotenon	(Rotenon	(Rotenon
	Control	(Rotenon	e 12.5	e 12.5	e 12.5
		e 12.5	ppb +	ppb +	ppb +
		ppb)	VCO	VCO	VCO
			6.25%)	3.13%)	1.5625%)
Pictur	_			•	
е	- 1	93	100	100	•
	W	6	7	•	*
		1)	I
		- 1		l l	-
	1	- 1	1		
	1	1	- 1	-	1
	1	1	1	¥	1
	,				
Mean	4.15	3.72	4.06	4.00	3.80
(mm)	±	±	±	±	±
±SD	0.11	0.14	0.11	0.20	0.18
Р			0.000		
value					

In Figure 1, the results of analysis for IL-6 expression showed significant differences among the treatment groups. The negative control group that was not given treatment showed an average fold change in IL-6 expression, with a value of 1.0067 ± 0.13204 . The positive control group exposed to rotenone (12.5 ppb) showed a significant increase in IL-6 expression, with an average value of 3.5733 ± 0.15503 .

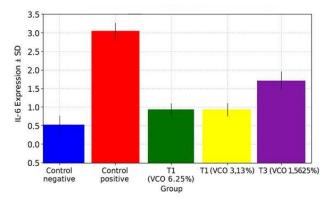


Figure 1: The Effect of Virgin Coconut Oil on IL-6 Expression in Zebrafish Larvae Stunting Model

The treatment groups that received VCO showed a reduction in IL-6 expression compared to the positive control group, with significant differences between the groups with administered VCO doses. The T1 group (VCO 6.25%) and T2 group (VCO 3.13%) showed similar results, with average fold changes of 1.5167 ± 0.31565 and 1.5533 ± 0.06658 respectively and no significant difference between them. However, the T3 group (VCO 1.5625%) showed an increase in IL-6 expression with an average fold change of 2.3567 ± 0.18930 , which was higher than the T1 and T2 groups but still lower than the positive control group.

Further analysis using One-Way ANOVA showed significant differences in IL-6 expression between groups with a p-value of 0.000, indicating that VCO treatment can alter IL-6 expression in zebrafish larvae with rotenone-induced stunting. Post Hoc LSD testing identified significant differences across all treatment groups except between the T1 and T2 groups, which did not show significant differences from each other.

Overall, the results of this study indicate that VCO administration can reduce IL-6 expression, which is increased due to rotenone exposure in

zebrafish larvae with the 6.25% (T1) and 3.13% (T2) VCO doses providing better effects compared to lower doses.

This study demonstrates that the administration of VCO can reduce the increased expression of IL-6 caused by rotenone exposure in zebrafish larvae. Rotenone, which is a neurotoxic agent, induces oxidative stress and inflammation in zebrafish larvae, as evidenced by the elevated IL-6 expression in the positive control group. The increased expression of IL-6 indicates that rotenone can stimulate the body's inflammatory response; this is consistent with previous findings showing that IL-6 is one of the pro-inflammatory cytokines produced in response to oxidative stress and tissue damage caused by toxin exposure. IL-6 is a cytokine that serves as a key mediator in chronic inflammation. Inflammation triggered by IL-6 leads to the release of nitric oxide (NO). IL-6 can exacerbate neurodegenerative damage by increasing the expression of other inflammatory mediators, such as TNF-α and IL-1β, which further disrupt metabolic processes and elevate oxidative stress in the brain. ^{20,21,22}

Interleukin-6 (IL-6) is a pro-inflammatory cytokine produced during the inflammatory process. IL-6 serves as a key signal in modulating the body's immune response by regulating the activation of immune cells such as macrophages and microglia (immune cells in the brain). When IL-6 production is excessive and prolonged, this cytokine exacerbates the inflammatory process, increases oxidative stress, and causes tissue damage including neuronal damage in the brain. ²³

Interleukin-6 plays a crucial role in the inflammatory process that affects child growth. An increase in IL-6 contributes to detrimental changes in the gut microbiome, mucosal damage, and systemic inflammation, which ultimately leads to stunting. IL-6 can be used as an indicator to identify children at risk of stunting, and managing systemic inflammation may be an integral part of interventions to prevent or correct stunting in children.²⁴

The administration of VCO in the treatment groups resulted in a significant reduction in IL-6 expression. The groups treated with 6.25% VCO (T1) and 3.13% VCO (T2) showed similar results in reducing IL-6 expression and there were no significant differences between the two groups. This indicates that both doses have similar anti-inflammatory effects. This reduction is highly relevant to the anti-inflammatory properties of VCO, which are likely due to the presence of active compounds such as lauric acid, polyphenols, and flavonoids that can inhibit inflammatory signaling pathways, including the NF- κ B pathway, which plays a crucial role in IL-6 production. Therefore, VCO may provide protection against inflammation induced by rotenone exposure.

Virgin Coconut Oil is a potential anti-inflammatory agent due to its primary content of medium-chain fatty acids (MCFAs) and their derivatives, such as monolaurin. Medium-chain fatty acids, in particular lauric acid, capric acid, and caprylic acid, are known to have various biological benefits, including anti-inflammatory properties. The anti-inflammatory effects of VCO are primarily attributed to the ability of its active compounds to inhibit the activity of enzymes involved in the inflammatory process, one of which is cyclooxygenase-2 (COX-2). After being metabolized into monolaurin, lauric acid exerts a stronger effect in inhibiting COX-2 and reducing the production of prostaglandins that cause inflammation. These anti-inflammatory effects are also associated with the ability of VCO to reduce oxidative stress, which plays a crucial role in various chronic inflammatory conditions. ^{26,25}

One of the mechanisms of action of VCO is through the reduction of oxidative stress. As a strong antioxidant, VCO contains compounds that can reduce the formation of free radicals generated by rotenone exposure, thereby preventing the activation of inflammatory pathways. The presence of tocopherols and phenolic compounds in VCO also plays a role in suppressing the activation of NF-kB, which is a key pathway involved in the production of pro-inflammatory cytokines such as IL-6. This explains how VCO can reduce IL-6 expression in zebrafish larvae exposed to rotenone.^{27,28} Phenolic compounds, recognized for their antioxidant properties, play a crucial role in regulating inflammatory responses by inhibiting pro-inflammatory cytokines such as interleukin-6 (IL-6), scavenging free radicals, reducing oxidative stress, preventing oxidative damage, and preserving

cellular and tissue integrity. In this way, they contribute to the reduction of chronic inflammation and protection against diseases related to oxidative stress.^{29,30} Oxidative stress occurs when the production of free radicals exceeds the body's defense system capacity to neutralize them, resulting in damage to lipids, proteins, and DNA within cells, including neurons. Excessive free radicals can trigger inflammation and cell death. ³¹ Oxidative stress occurs due to an imbalance between the production of free radicals and the body's antioxidant defense system, which can lead to cell damage and various health issues. The ability of VCO to reduce oxidative stress is particularly beneficial when the body is exposed to intense physical activity, which increases the formation of free radicals. ^{32,33}

Another relevant mechanism is the effect of rotenone as a neurotoxic agent, which inhibits complex I in the electron transport chain in the mitochondria, resulting in an increase in ROS and oxidative stress. This condition activates inflammatory signaling pathways such as MAPK and NF-kB and subsequently stimulates the production of inflammatory cytokines, including IL-6. The administration of antioxidant-rich VCO can reduce this oxidative stress, inhibit the activation of inflammatory pathways, and ultimately decrease IL-6 expression.34 Overall, the results of this study demonstrate that VCO has potential as an antiinflammatory agent that can reduce the inflammatory impact during the early life stages of zebrafish larvae exposed to rotenone. The administration of VCO can decrease IL-6 expression induced by oxidative stress, provide protection against tissue damage, and improve the disrupted inflammatory response caused by toxin exposure. These anti-inflammatory effects support the potential of VCO as a therapeutic candidate for addressing the inflammatory impacts caused by exposure to harmful chemicals in organisms, particularly during vulnerable developmental stages.

Conclusion

This study demonstrates that the administration of VCO significantly reduces Interleukin-6 (IL-6) expression and increases body length in zebrafish larvae induced with stunting due to rotenone exposure. These findings support the hypothesis that VCO can reduce IL-6-mediated inflammation and improve physical growth impaired by oxidative stress. These results highlight the potential of VCO as an effective intervention for addressing stunting by modulating inflammation and supporting body growth.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

- Siagian FE. Role of Intestinal Parasitic Infection in Stunting. South Asian J Res Microbiol. 2023;15(2):41–50.
- Pelosi L, Berardinelli MG, Forcina L, Ascenzi F, Rizzuto E, Sandri M, Benedetti FD, Scicchitano BM and Musaro A. Sustained systemic levels of IL-6 impinge early muscle growth and induce muscle atrophy and wasting in adulthood. Cells. 2021;10(7):1–15.
- Cansiz D, Unal I, Ustundag UV, Alturfan AA, A.Altinoz M, Elmacr I, Alturfan EE. Caprylic acid ameliorates rotenone induced inflammation and oxidative stress in the gut-brain axis in Zebrafish. Mol Biol Rep. 2021;48(6):5259–5273.
- Iswati RS, Indria Nuraini. Effectiveness of Virgin Coconut Oil (VCO) on Changes in Weight and Height among Under-five

- Children with Stunting. Embrio J Kebidanan. 2023;15(1):112–118.
- Khotimah H, Alfred T, Wangta C, Chandra K, Tjan B, Christianto A, Ali M, Kalsum U. Miracle tree (Moringa oleifera) extract prevents rotenone-induced stunting in zebrafish (Danio rerio) through decreasing caspase-3. Int Conf Life Sci Technol. 2021:030003.
- Sorribes A, Porsteinsson H, Arnardóttir H, Jóhannesdóttir IP, Sigurgeirsson B, de Polavieja GG, Karlsson KAE. The ontogeny of sleep-wake cycles in zebrafish: A comparison to humans. Front Neural Circuits. 2013;7(NOV):1–13.
- Cory'ah FAN, Primaditya V, Ariati LIP, Zakiah, Wardani DWKK, Primihastuti D, Yuningsih, Khotimah H, Ali MM, Nurdiana. Ethanolic extract of centella asiatica increased IGF-1 and IRS expression in zebrafish (*Danio rerio*) rotenoneinduced. AIP Conf Proc. 2020;2231(April).
- 8. Christianto A, Khotimah H, Kalsum U. The Effect of Moringa oleifera Leaf Extract on Body Length, Malondialdehyde Levels, and Interleukin-6 in Zebrafish (*Danio rerio*) Larvae as a Stunting Model Induced by Rotenone.2018;1–114. Available from: https://repository.ub.ac.id/id/eprint/195285/
- 9. Vanhauwaert S, Lefever S, Coucke P, Speleman F, De Paepe A, Vandesompele J, Willaert A. RT-qPCR gene expression analysis in zebrafish: Preanalytical precautions and use of expressed repetitive elements for normalization. Methods Cell Biol. 2016;135:329–342.
- Yuningsih, Wardani DWKK, Ali M, Khotimah H, Nurdiana, Riawan W, Primihastuti D, Cory'ah FAN, Ariati LIP, Primaditya V, Zakiyah. The effect of Centella asiatica ethanolic extract on expression of glucose transporter 1 and osteocalcin on stunting larvae of Zebrafish (*Danio rerio*). GSC Biol Pharm Sci. 2022;19(3):178–189.
- 11. Wijayanti AR, Ridlayanti A, Ali HKM. Protection of Pegagan (*Centella asiatica*) Extract Through Hsp60 and Bax Expression on Stunting Model Zebrafish Larvae (*Danio rerio*) By Rotenone Inducted.pdf. IOP Science; 2021.
- Primaditya V, Cory'ah FAN, Ariati LIP, Zakiah, Wardani DWKK, Yuningsih, Primihastuti D, Khotimah H, Ali MM, Riawan W. Effect of centella asiatica to the glucose transporter 4 and osteocalcin on the rotenonee-induced zebrafish larvae (*Danio rerio*) stunting model. AIP Conf Proc. 2020;2231.
- Selvaraj KV, Bharathi A, Sivakumar V, Karthikeyan A, Maheswarappa H. Virgin coconut oil: A nutraceutical and therapeutic food. Int J Chem Stud. 2020;8(3):662–663.
- Kusuma MA, Putri NA. Review: Fatty Acids of Virgin Coconut Oil (VCO) and Their Health Benefits. J Agrinika: Journal of Agrotechnology and Agribusiness.2020;4(1):93.
- Rohman A, Irnawati, Erwanto Y, Lukitaningsih E, Rafi M, Fadzilah NA, Windarsih A, Sulaiman A, Zakaria Z. Virgin Coconut Oil: Extraction, Physicochemical Properties, Biological Activities and Its Authentication Analysis. Food Rev Int [Internet]. 2021;37(1):46–66. Available from: https://doi.org/10.1080/87559129.2019.1687515
- Álvarez-Martínez FJ, Barrajón-Catalán E, Herranz-López M, Micol V. Antibacterial plant compounds, extracts and essential oils: An updated review on their effects and putative mechanisms of action. Phytomedicine. 2021;90.
- 17. Roopashree PG, Shetty SS, Suchetha Kumari N. Effect of medium chain fatty acid in human health and disease. J Funct Foods [Internet]. 2021;87:104724. Available from: https://doi.org/10.1016/j.jff.2021.104724
- Kamri AM. Review: Virgin Coconut Oil and The Pharmacology Effect. J Pharmascience. 2024;11(1):115.
- Asish A, Rajan S, Jose SP, Sheethal S, Ratheesh M, Sandya S.
 Extracellular Modulatory and Anti-Inflammatory Effects of a Combiation of Curcumin, Piperine, and Virgin Coconut Oil in Animal Model. Asian J Pharm Clin Res. 2024;17(12):73–79.
- Chen A Di, Cao JX, Chen HC, Du HL, Xi XX, Sun J, Yin J, Jing YH, Gao LP. Rotenone aggravates PD-like pathology in A53T mutant human α-synuclein transgenic mice in an age-

- dependent manner. Front Aging Neurosci. 2022;14:1-15.
- Azimullah S, Jayaraj RL, Meeran MFN, Jalal FY, Adem A, Ojha S, Beiram R. Myrcene Salvages Rotenone-Induced Loss of Dopaminergic Neurons by Inhibiting Oxidative Stress, Inflammation, Apoptosis, and Autophagy. Molecules. 2023;28(2):1–14.
- Widjaja NA, Hamidah A, Purnomo MT, Ardianah E. Effect of lactoferrin in oral nutrition supplement (ONS) towards IL-6 and IL-10 in failure to thrive children with infection. F1000Research. 2023;12:1–40.
- 23. Eddin LB, Azimullah S, Jha NK, Nagoor Meeran MF, Beiram R, Ojha S. Limonene, a Monoterpene, Mitigates Rotenone-Induced Dopaminergic Neurodegeneration by Modulating Neuroinflammation, Hippo Signaling and Apoptosis in Rats. Int J Mol Sci. 2023;24(6).
- Zambruni M, Ochoa TJ, Somasunderam A, Cabada MM, Morales ML, Mitreva M, Rosa BA, Acosta AJ, Riveros M, Arango S, Durand D, Berends MN, Melby P, Utay NS. Stunting is preceded by intestinal mucosal damage and microbiome changes and is associated with systemic inflammation in a cohort of Peruvian infants. Am J Trop Med Hyg. 2019;101(5):1009–1017.
- Anusha G, Sunayana R, Ponnam M, Kumar BA. Asian Journal of Pharmaceutical Research and Development. Asian J Pharm Res Dev. 2020;8(6):77–80.
- 26. Jack KS, Asaruddin MR Bin, Bhawani SA. Pharmacophore study, molecular docking and molecular dynamic simulation of virgin coconut oil derivatives as anti-inflammatory agent against COX-2. Chem Biol Technol Agric [Internet]. 2022;9(1):1–26. Available from: https://doi.org/10.1186/s40538-022-00340-0
- Zeng YQ, He JT, Hu BY, Li W, Deng J, Lin QL. Virgin coconut oil: A comprehensive review of antioxidant activity

- and mechanisms contributed by phenolic compounds. Crit Rev Food Sci Nutr. 2024;64(4):1052–1075.
- 28. Olędzka AJ, Czerwińska ME. Role of Plant-Derived Compounds in the Molecular Pathways Related to Inflammation. Int J Mol Sci. 2023;24(5).
- Ariani A, Ghofar IF, Khotimah H, Nurdiana N, Rahayu M. Asiatic acid in Centella asiatica extract towards morphological development in an intermittent hypoxia intrauterine embryo model and molecular prediction pathway of insulin-like growth factor-1 receptor signalling. Open Vet J. 2023;13(5):629-637.
- Okolie NP, Falodun A, Davids O. Evaluation of the Antioxidant Activity of Root Extract of Pepper Fruit (Dennetia Tripetala), and It'S Potential for the Inhibition of Lipid Peroxidation. African J Tradit Complement Altern Med. 2014;11(3):221–227.
- 31. Ariani A, Khotimah H, Sulistyarini A, Daniaridevi AS. Centella asiatica extract ameliorates deoxygenation-induced neurological dysfunction in zebrafish larvae. Open Vet J. 2024;14(5):1154–1160.
- 32. Nikmah UK, Pertiwi D, Hussaana A. The Effect of Virgin Coconut Oil (VCO) on Malondialdehyde, Glutathione Peroxidase, and Cortisol Hormone Levels. Int J Multidiscip Res Anal. 2023;06(03):897–903.
- 33. Kandiyil SP, Jose A, Mohanan C, Illam SP, Raghavamenon AC. Virgin coconut oil mitigates ageing-associated oxidative stress and dyslipidaemia in male Wistar rats. Nutr Metab Cardiovasc Dis [Internet]. 2024;34(12):2834–2841. Available from: https://doi.org/10.1016/j.numecd.2024.08.012
- Fadlalla EA, Seddik A. Anti-Inflammatory and Nephroprotective Influence of Virgin Coconut Oil on Gentamicin-Induced Nephrotoxicity in Nondiabetic and Streptozotocin-Induced Diabetic Rats. African J Food, Agric Nutr Dev. 2023;23(2):22492–22512.