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Impact of Virgin Coconut Oil (VCO) on Locomotor Speed and Bax Expression in Zebrafish (*Danio rerio*) Larvae Stunting Model

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ABSTRACT

Stunting is a chronic growth disorder caused by prolonged nutritional deficiencies and environmental stress, resulting in reduced height for age. Oxidative stress-induced apoptosis, involving the pro-apoptotic gene B-cell lymphoma 2-associated X protein (Bax), is a key molecular mechanism. Antioxidant-rich virgin coconut oil (VCO) with lauric acid and phenolic compounds has the potential to mitigate oxidative damage. The objective of this study is to evaluate the effect of VCO on locomotor speed and Bax gene expression of zebrafish larvae (*Danio rerio*) in a rotenone-induced stunting model. The larvae were divided into five groups: negative control (NC), positive control given rotenone (PC), and three treatment groups with rotenone and VCO at concentrations of 6.25% (V1), 3.125% (V2), and 1.625% (V3). Locomotor speed was measured at 3, 6, and 9 days post-fertilization (dpf), while Bax expression was analyzed using Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR) at 9 dpf. The results showed that VCO increased larval locomotor speed, with the V1 group showing the best recovery compared to PC. In addition, Bax expression significantly increased in the PC group compared to NC ($p < 0.05$). VCO administration significantly decreased Bax expression in a dose-response manner for V1, V2, and V3. These findings indicate that VCO provides a protective effect against rotenone-induced apoptosis and motor impairment through modulation of oxidative stress. Thus, VCO has the potential as a natural therapeutic agent to reduce cellular damage and functional deficits associated with stunting.

Keywords: Stunting, virgin coconut oil, B-cell lymphoma 2-associated X protein, zebrafish larvae, oxidative stress, apoptosis, locomotor speed.

Introduction

Stunting is a chronic condition characterized by impaired growth and development of children due to prolonged malnutrition and exposure to environmental stress, resulting in a lower height compared to the standard for children of the same age.[1] This condition affects not only physical aspects but also cognitive development, immune function, and long-term health.[2,3] Despite many global efforts, stunting remains a serious public health problem, especially in developing countries such as Indonesia, where the prevalence reached 19.8% in 2024.[4] One of the main mechanisms underlying stunting is oxidative stress that triggers apoptosis (programmed cell death) in growing tissues. The Bax gene plays an important role in apoptosis through mitochondrial membrane permeabilization and caspase cascade activation.[5] Increased Bax expression is associated with increased apoptosis in various models of malnutrition and toxin exposure, contributing to impaired tissue development.[6] In addition, impaired motor function, such as decreased movement speed, is also a characteristic of stunting, which reflects a systemic impact on neuromuscular development.[7]

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Virgin coconut oil (VCO) is a natural product that has high antioxidant and anti-inflammatory content, in particular medium-chain fatty acids (MCFAs) such as lauric acid and phenolic compounds.[8] Previous studies have shown that VCO supplementation can reduce oxidative stress markers and regulate inflammatory cytokine production, which has the potential to reduce cellular damage and to support growth.[9,10] The role of phytochemicals such as flavonoids, tannins, and polyphenols as natural antioxidants has been well-established in various plant extracts. For instance, Okolie *et al*.[11] reported that the root extract of Dennetia tripetala exhibited potent hydrogen peroxide and nitric oxide-scavenging activities, as well as inhibitory effects on lipid peroxidation, comparable to standard antioxidants such as vitamins C and E. These findings further reinforce the concept that plant-derived phenolic compounds play a crucial role in cellular protection against oxidative damage. However, the effects of VCO on apoptosis gene expression and motor function in stunting conditions are still rarely studied. The objective of this study is to examine the effect of VCO on Bax gene expression and motor activity in the form of movement speed of zebrafish larvae (*Danio rerio*) in a rotenone-induced stunting model. Zebrafish larvae were chosen as a model because of their genetic similarity of approximately 70% to humans, transparent embryonic development, rapid growth, and ease of simultaneous behavioral observation and molecular analysis.[12,13] The rotenone-induced stunting model replicates mitochondrial dysfunction and oxidative stress mechanisms, which are closely associated with the pathophysiology of stunting in humans.[14] The novelty of this study lies in the combined approach between molecular analysis of Bax gene expression and functional evaluation of zebrafish larval locomotor speed. This provides a comprehensive picture of the potential of VCO as a natural therapy to

reduce apoptosis and motor deficits due to stunting. The method for the study, composed of a combination of RT-qPCR and locomotor speed measurements, is very suitable for assessing molecular and functional effects in an efficient biological model. The results of this study are expected to provide new contributions to the development of natural nutrition-based interventions for the prevention and treatment of stunting, as well as to expand the application of the zebrafish model as a translational tool in the study of growth disorders and therapy.

Materials and Methods

Place and Time of Research
This study was conducted at the Pharmacology Laboratory and Integrated Biomedical Laboratory, Faculty of Medicine, Brawijaya University, Malang, Indonesia (GPS coordinates: 7°57'13.7"S 112°36'47.6"E) from December 2024 to March 2025. All procedures were approved by the Health Research Ethics Committee of the Faculty of Medicine, Brawijaya University, with ethics ID number 463/EC/KEPK-S2/12/2024.

Animal Models
The subjects of the study are zebrafish (*Danio rerio*) embryos of wild-type strain aged 2 hours post-fertilization (hpf) to larvae aged 9 dpf. The embryos were divided into five groups: Positive Control (PC), Negative Control (NC), Treatment 1 (V1), Treatment 2 (V2), and Treatment 3 (V3). Each group consisted of 30 larvae with three repetitions (n = 450). Stunting induction was carried out using 12.5 parts per billion (ppb) rotenone (Product No. R8875-1G).[14,15]

Virgin Coconut Oil
The virgin coconut oil used for the study was obtained from KWT Vigur Asri (VCO Palm 7, Cemoro Kandang, Malang, Indonesia; P-IRT 2063573011924-29), and contains 99.73% fat. For the preparation of the stock solution, 1 mL of VCO was added to 20 µL of Dimethyl sulfoxide (DMSO) and topped with distilled water to a final volume of 16 mL to produce a solution with a concentration of 6.25%. The solution was then serially diluted into 3.125% and 1.5625% concentrations for treatments V2 and V3, while the 6.25% concentration was used for V1.

Locomotor Speed Observation
Measurements of larval locomotor speed were carried out at 3, 6, and 9 dpf using an OptiLab light microscope connected to OptiLab Viewer 2.2 software (Miconoss, DI Yogyakarta). Observations of larval movement speed were carried out using the EthoVision XT 15 application (Noldus Information Technology, Wageningen, Netherlands) with a calibrated camera system and constant lighting. Measurements were carried out in a 6-well plate with 1 larva in each well. Observations of movement speed were carried out for 10 minutes.[16]

Ribonucleic Acid (RNA) Isolation and Complementary Deoxyribonucleic Acid (cDNA) Synthesis
Ribonucleic acid (RNA) was isolated from the whole body of zebrafish larvae that had been stored in RNAlater solution and frozen at -80°C. Total RNA extraction using TRIzol reagent (Sigma, St. Louis, MO, USA) followed a standard protocol that included the stages of cell lysis followed by RNA binding, washing, and elution. The cell lysis process was carried out by adding 400 µL RB Buffer and 4 µL β-mercaptoethanol; the tissue was then crushed with a micro pestle until homogeneous. The lysate was filtered through a 20G needle 10 times and left for 3 minutes at room temperature. The filtrate was obtained after centrifugation at 1,000×g for 30 seconds and mixed with 70% ethanol before being inserted into the RNA binding column. After washing with W1 Buffer and Wash Buffer containing ethanol, the filter matrix was dried by centrifugation at 14,000-16,000 × g for 3 minutes. Purified RNA was eluted with 50 µL RNase-Free Water and extracted by centrifugation at 14,000-16,000×g for 1 min. RNA concentration was measured using a BioDrop spectrophotometer (PT. Biotek Prima Indoplus, Sidoarjo, Indonesia).[17] cDNA synthesis was performed using the ReverTra Ace™ qPCR RT Master Mix with gDNA Remover kit (Toyobo, FSQ-3010) according to the manufacturer's protocol. The isolated RNA template was used for cDNA synthesis via reverse transcription reaction for further amplification preparation.[17]

Analysis of B-cell Lymphoma 2 Associated X Protein (Bax) Gene Expression by Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR)
Bax gene expression analysis using the RT-qPCR method was performed at 9 dpf, with 30 larvae from each group. The PCR reaction was prepared with a total volume of 20 µL, consisting of 10 µL of 2× SensiFAST™ SYBR® No-ROX One-Step Mix, 0.8 µL of forward primer, 0.8 µL of reverse primer, and 5 µL of cDNA template, with the remaining volume consisting of RNase-free water. The PCR protocol for Bax analysis involved enzyme activation at 95℃ for 2 minutes, denaturation at 95℃ for 5 seconds, and annealing/extension at 56.2℃ for 20 seconds for 40 cycles. Relative expression was calculated using the 2^–ΔΔCt method with the β-actin gene as a reference. The following primer sequences were used: Bax forward (5'GAGCTGCACTTCTCAACAACT-3'), Bax reverse (5'CTGGTTGAAATAGCCTT GATGAC-3'), β-actin forward (5'CGAGCAGGAGATGGGAACC-3'), and β-actin reverse (5'CAACGGAAACGCTCATTGC-3').[18]

Statistical Analysis
Data from locomotor speed and gene expression are presented as mean ± standard deviation (mean ± SD). The Shapiro-Wilk test was performed to check normality; this was followed by one-way ANOVA for Bax expression and Welch's ANOVA for locomotor speed. A post-hoc Least Significant Differences (LSD) test was conducted for Bax expression, and a post-hoc Games-Howell test was conducted for locomotor speed to determine differences between groups. All statistical analyses comparing group means were performed using IBM SPSS Statistics for Windows software, version 26.0 (IBM Corp., Armonk, NY, USA; 2019 release).[19)]

Results and Discussion

Locomotor Speed of Zebrafish Larvae as Stunting Model
Table 1 shows that at 3 dpf, the NC group had the highest locomotor speed (0.17 ± 0.05 cm/s), while the PC group had the lowest (0.07 ± 0.03 cm/s). At 6 dpf, treatment with VCO (V1, V2, and V3) showed an increase in locomotor speed compared to PC. At 9 dpf, the locomotor speed of larvae in the V1 treatment group reached the highest value (1.66 ± 0.21 cm/s), while the PC group still showed a lower locomotor speed compared to other treatment groups. This indicates the effect of VCO dose on the increase in locomotion activity of zebrafish larvae induced by rotenone.
In Table 2, at the age of 3 dpf, the results of the post-hoc test showed a significant difference between the PC group and the NC group, as well as all treatment groups of V1, V2, and V3 ($p < 0.05$). The PC group that was given rotenone induction had a significantly lower locomotor speed in comparison to the NC group and the treatment groups receiving VCO with various doses. This indicates that rotenone induction causes a significant decrease in locomotion activity, while VCO administration is able to increase the movement speed of larvae significantly.
In Table 3, at 6 dpf, a similar pattern was observed, where the PC group still showed significantly lower locomotor speed compared to the NC group and all three treatment groups of V1, V2, and V3 ($p < 0.05$). This difference confirms that the toxic effects of rotenone are still ongoing, but VCO treatment provides a significant recovery effect toward motor activity.
In Table 4, at the age of 9 dpf, significant differences between the PC group and the NC as well as the V1 treatment groups were still visible ($p < 0.05$), while the differences between PC and the V2 and V3 groups were not significant ($p > 0.05$). This indicates that a higher dose of VCO (V1 with a concentration of 6.25%) is still effective in restoring impaired locomotion activity, while the lower doses of V2 and V3 have effects that are less than optimal at this stage.

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Table 1: Interpretation of Locomotor Speed in Zebrafish Larvae for Each Group

Age	3 dpf					6 dpf					9 dpf				
Group	NC	PC	V1	V2	V3	NC	PC	V1	V2	V3	NC	PC	V1	V2	V3
***Mean* (cm/s) ± SD**	0.17± 0.05	0.07± 0.03	0.18± 0.03	0.14± 0.02	0.11± 0.02	1.15± 0.06	0.34± 0.17	1.23± 0.16	1.02± 0.1	0.89± 0.11	1.48± 0.23	0.81± 0.51	1.66± 0.21	1.19± 0.12	0.95± 0.12
p-value			0.000					0.000					0.000		

Overall, the results of the inter-group difference test showed that rotenone induction in the PC group significantly decreased the locomotor speed of zebrafish larvae compared to the NC group and the VCO treatment group, and the most optimal recovery effect was seen with a higher VCO dose and at a more mature larval age.

Table 2: Post-Hoc Test for Locomotor Speed at 3 dpf

Group	NC	PC	V1	V2	V3
NC		0.000*	1.000	0.188	0.002*
PC	0.000*		0.000*	0.000*	0.002*
V1	1.000	0.000		0.009	0.000
V2	0.188	0.000	0.009		0.005
V3	0.002	0.002	0.000	0.005	

Notes: An asterisk (*) denotes a statistically significant difference between groups, defined as a p-value less than 0.05.

Table 3: Post-Hoc Test for Locomotor Speed at 6 dpf

Group	NC	PC	V1	V2	V3
NC		0.000	0.304	0.003	0.000
PC	0.000*		0.000*	0.000*	0.000*
V1	0.304	0.000		0.001	0.000
V2	0.003	0.000	0.001		0.019
V3	0.000	0.000	0.000	0.019	

Notes: An asterisk (*) denotes a statistically significant difference between groups, defined as a p-value less than 0.05.

Table 4: Post-Hoc Test for Locomotor Speed at 9 dpf

Group	NC	PC	V1	V2	V3
NC		0.002	0.159	0.003	0.000
PC	0.002*		0.000*	0.085	0.840
V1	0.159	0.000		0.000	0.000
V2	0.003	0.085	0.000		0.000
V3	0.000	0.840	0.000	0.000	

Notes: An asterisk (*) denotes a statistically significant difference between groups, defined as a p-value less than 0.05.

This study showed that the administration of VCO significantly increased the movement speed of zebrafish larvae as a stunting model by rotenone induction. At 3 dpf, the NC group showed the highest movement speed (0.17 ± 0.05 cm/s), while the PC group given rotenone experienced a significant decrease in locomotor speed (0.07 ± 0.03 cm/s). Administration of VCO at the highest dose (V1, 6.25%) successfully increased the locomotor speed of larvae by 0.18 ± 0.03 cm/s at 3 dpf and significantly at 6 dpf and 9 dpf (1.23 ± 0.16 cm/s and 1.66 ± 0.21 cm/s, respectively). These improvements in locomotor performance across time points are illustrated in Figure 1, which shows the mean locomotor speed and corresponding standard deviations for each treatment group at 3, 6, and 9 dpf.

This increase in locomotor speed indicates that VCO is able to improve the motor function of larvae that is disrupted by oxidative stress through rotenone, which is known to cause damage to the nervous and muscular systems.[20,21] The content of MCFA in VCO, especially lauric acid, provides antioxidant and anti-inflammatory effects that support the recovery of energy metabolism and improve neuromuscular function in zebrafish larvae.[22,23]

Stunting in humans is generally associated with impaired motor development due to nutritional deficiencies that affect muscle growth and nerve maturation, especially in the cerebellum, which affects locomotor speed and other motor skills.[24,25] The zebrafish larval model used in this study reflects this condition, where larvae with stunting have low locomotor speed due to metabolic and oxidative disorders, resulting in decreased adenosine triphosphate (ATP) levels and increased reactive oxygen species (ROS). This oxidative stress contributes to growth retardation and can affect the speed of larval movement.[26]

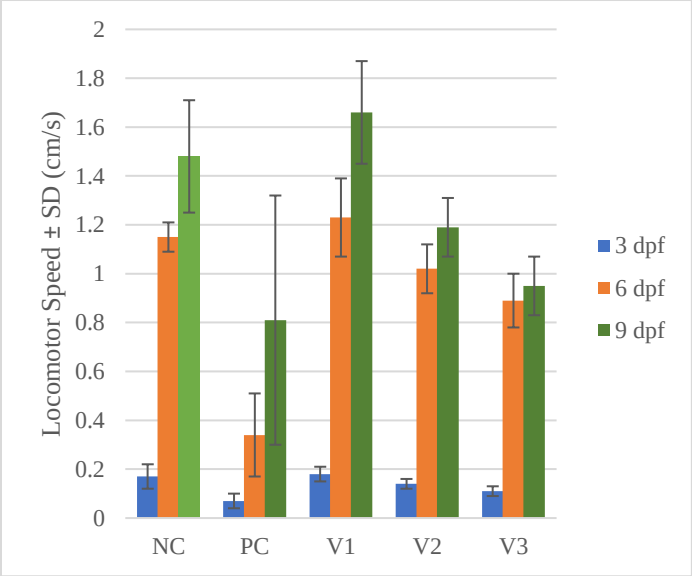

Figure 1: Mean Locomotor Speed of Zebrafish Larvae at 3, 6, and 9 dpf Across Treatment Groups (Mean ± SD)

In addition, the biological activity of VCO, which includes immunomodulatory and antimicrobial effects, can also help stabilize the physiological conditions of larvae by suppressing inflammation and improving the balance of microflora, which is important for the recovery of the motor function.[10,27] Previous research by Ariani *et al*.[28] showed that administration of asiatic acid from *Centella asiatica* to zebrafish larvae exposed to intrauterine hypoxia could significantly increase locomotor activity and head width. This is accompanied by increased expression of the brain neurotrophic factor (BDNF), which acts as a neuroprotector through antioxidant and anti-inflammatory mechanisms. These findings indicate that treatment with natural compounds that have neuroprotective properties can improve the motor function and brain growth in conditions of oxidative stress due to hypoxia. Thus, VCO has the potential to be an effective additional nutrient in overcoming motor disorders associated with stunting through metabolic and neuromuscular improvement mechanisms.

Overall, the results of this study support the role of VCO as a therapeutic agent that can increase locomotor speed and improve motor function in stunting models, which can be a reference for the development of nutritional interventions for children with stunting in the future.

The Effect of Virgin Coconut Oil on Bax Expression in Zebrafish Larvae Stunting Model

Measurement of Bax gene expression in zebrafish larvae age of 9 dpf was carried out using the RT-qPCR method for the five treatment groups of NC, PC, and three VCO treatment groups with different doses (V1 = 6.25%, V2 = 3.125%, and V3 = 1.625%). The data for the average fold change of Bax expression from each group are presented in Table 5. The results of statistical analysis using the LSD post-hoc test showed significant differences in Bax expression among all groups ($p = 0.000$). The PC group exposed to rotenone showed a significant increase in Bax expression compared to the NC group, with a fold change of 3.63 ± 0.29, which indicates an increase in apoptosis activity due to rotenone toxicity. In contrast, treatment with VCO at all three doses (V1, V2, and V3) significantly decreased Bax expression compared to PC group, although it was still higher than the NC group (Table 5).

This study showed that rotenone exposure significantly increased the expression of the Bax pro-apoptotic gene in zebrafish larvae as a stunting model, reflecting increased apoptosis in response to oxidative stress induced by the toxin. The 3.63-fold increase in Bax expression in the PC group compared to the NC group ($p < 0.05$) confirmed the role of rotenone in triggering mitochondrial damage and the intrinsic apoptotic pathway.[6,29]

Table 5: Effect of Virgin Coconut Oil on Bax Expression in Each Group Measured by RT-qPCR

Group	NC	PC	V1	V2	V3
n (larva)	30	30	30	30	30
Mean Fold Change ± SD	1.00 ± 0.11[a]	3.63 ± 0.29[b]	1.41 ± 0.16[c]	1.80 ± 0.09[d]	2.26 ± 0.06[e]
p-value			0.000		

Note: Values with different superscript letters indicate statistically significant differences.

A significant decrease in Bax expression in the VCO treatment group at a dose of 6.25% (V1) indicates that VCO has a protective effect against the apoptosis process induced by rotenone. The decrease in Bax expression from 3.63 to 1.41 indicates that VCO can effectively inhibit the pro-apoptotic pathway and reduce cell death. This is supported by the antioxidant and anti-inflammatory properties of VCO, which is rich in lauric acid and phenolic compounds that can eliminate free radicals and reduce oxidative stress.[30,31] In addition, studies have shown that VCO can increase the bioavailability of these antioxidants, further strengthening their protective effects against oxidative stress caused by environmental toxins such as sodium fluoride.[9]

The mechanism of decreasing Bax expression by VCO is also thought to involve the modulation of mitochondrial signaling pathways and improvement of cell metabolic function. VCO can activate the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, which plays a role in increasing the expression of endogenous antioxidant enzymes and decreasing ROS levels, thereby suppressing the apoptosis pathway.[32–35] This is in line with the research of Ariani *et al*.[36], in which it was found that asiatic acid from *Centella asiatica* can increase the growth and locomotor activity of zebrafish through activation of the insulin-like growth factor 1 receptor (IGF-1R) pathway. Similarly, another study demonstrated that the ethanol extract of *Centella asiatica* protects zebrafish larvae from aluminum-induced oxidative stress and apoptosis, which leads to the improvement of developmental and neuromuscular outcomes such as hatching rate, growth, heart rate, and locomotor activity.[37] These two natural compounds have the potential to improve growth disorders and motor function with antioxidant and anti-apoptotic mechanisms. Thus, VCO not only acts as a direct scavenger of free radicals but also regulates the cellular defense response to oxidative stress.

Clinically, these results have important implications in the context of the prevention and treatment of stunting, especially stunting that is triggered by environmental factors such as toxin exposure. Excessive apoptosis mediated by Bax can lead to impaired growth and tissue development; thus, inhibition of this pathway by VCO can improve cellular and physiological conditions. This supports the view that nutritional management with supplementation of natural antioxidants such as VCO can be an effective strategy in the management of stunting.

Conclusion

This study proves that administration of VCO can significantly reduce the expression of the pro-apoptotic gene Bax, which is increased due to exposure to rotenone in zebrafish larvae of stunting models. The resulting decrease in Bax expression indicates the protective effect of VCO against the apoptosis process triggered by oxidative stress. In addition, VCO also significantly increased the motor activity of larvae, especially at a dose of 6.25%, which indicates an improvement in neuromuscular function that is disrupted due to rotenone induction. Thus, VCO has the potential as an effective natural therapeutic agent to reduce cellular damage and motor function impairment associated with stunting. These findings support the use of VCO as a nutritional intervention for the prevention and treatment of stunting; they also open up opportunities for the development of natural antioxidant-based therapies.

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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