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Virgin Coconut Oil and Folic Acid Improve Insulin Signaling and Cardiac Function in Rotenone Induced Stunted Zebrafish Larvae

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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. Mitochondrial dysfunction and oxidative stress are increasingly recognized as key contributors to impaired growth and cardiometabolic instability during early development. This study investigated the potential protective effects of Virgin Coconut Oil (VCO) and folic acid on insulin signaling and cardiac finction in a rotenone induced stunted zebrafish larval model. A laboratory experimental study with a true experimental post test only controlled group design was conducted among 30 Zebrafish (*Danio rerio*) for Irs gene expression at 9 day post fertilization (dpf) as samples per tube, heart rate was measured at 3, 6, and 9 dpf. The total group treatment was five with inclusion and exclusion criterias, negative control (NC), positive control exposed to rotenone (12.5 ppb), VCO treatment (6.25%), folic acid treatment (70 µM), and a combination of VCO and folic acid. Variables assessed in this study included heart rate and insulin receptor substrate (Irs) were evaluated using real time polymerase chain reaction (RT-qPCR) analysis. Data were analyzed using SPSS version 27 for windows. Rotenone significantly reduced Irs expression compared with negative control (0.272 ± 0.128 vc 1.014 ± 0.187; p<0.001). Treatment with VCO (0.678 ± 0.250), folic acid (0.676 ± 0.191), and their combination (0.695 ± 0.231) increased Irs expression relative to the rotenone group (p<0.05), although no significant differences were observed among the treatment groups. Rotenone also elevated heart rate at 9 dpf (236.9 ± 19.2 bpm) compared with the negative control (163.7 ± 10.1 bpm), whereas treatment groups showed improved cardiac parameters. These findings highlight the potential of nutritional interventions targeting mitochondrial oxidative stress to support recovery of insulin signaling and improve metabolic stability in stunting related conditions.

Keywords: Growth of Children; Rotenone; RT-qPCR; Stunting; Virgin Coconut Oil.

Abbreviations: IRS - Insulin Receptor Substrate; RT-PCR - Real-Time Polymerase Chain Reaction; VCO - Virgin Coconut Oil.

INTRODUCTION

Stunting remains a critical global public health challenge, affecting approximately 22% of children under five worldwide, particularly in low and middle income countries. Beyond linear growth retardation (LGR), stunting is associated with increased morbidity, reduced cognitive capacity, and long term cardiometabolic risk, making it a priority issue in international health and global development agendas (Prendergast et al., 2014; WHO, 2023). In Indonesia, stunting prevalence remains high at 19.8%, exceeding the World Health Organization threshold and underscoring the need for effective, evidence based interventions aligned with global health targets, including the Sustainable Development Goals (SDG 2: Zero Hunger) (Pusdatin, 2022).

Emerging evidence indicates that stunting has been attributed chronic undernutrition is not only a nutritional deficiency disorder but involves complex biological mechanisms, including oxidative stress, mitochondrial dysfunction, and metabolic dysregulation. Growth hormones have a role in stimulating the formation of IGF-1. IGF-1 receptor (IGF-IR) activates tyrosine kinases which will undergo a phosphorylation reaction, so that the Irs (insulin receptor substrate) as effector, perfoms the main function of the receptor as signal transduction, by forming carious complex pathways, through MAPKinase and PI3Kinase, which consequences for differentiation cell, proliferation, pre and postnatal growth, which are related to nutritional intake (Chitnis et al., 2008; Wood A.W., 2007). Disruption of these pathways during early life may contribute not only to growth failure but also to functional impairments in vital organs, including the cardiovascular system (Rains & Jain, 2011).

Rotenone is a natural pesticide from the roots of tuba (*Derris eelliptica (Wellich) Benth*). The mechanism of action rotenone inhibits mitochondrial complex I, has been widely used to induce oxidative stress and metabolic disruption in experimental models (Sherer et al., 2003). In zebrafish (*Danio rerio*), rotenone exposure leads to growth retardation, altered insulin signaling, and impaired organ development, making it a relevant model for investigating stunting related metabolic and functional outcomes (Heo et al., 2022; Woro et al., 2020).

Virgin Coconut Oil (VCO) is a natural product that has high antioxidant and anti inflammatory content, contains medium-chain fatty acids (MCFAs) such as lauric acid and phenolic compounds (Gouda et al., 2024). While folic acid is essential for plays a crucial role in one-carbon metabolism and redox balance, and cellular proliferation, and has been implicated in supporting cardiovascular and metabolic homeostasis (Asbaghi et al., 2021; Menezo et al., 2022). Although both nutrients have demonstrated beneficial effects individually, their combined effects on insulin signaling and cardiac function in stunting conditions has not been adequately explored.

The Zebrafish *(Danio rerio)* emerges as a valuable model for investigating metabolic disease like stunting due to its genetic similarity to humans, sharing approximately 70% orthologous genes, as well as possessing analogous metabolic processes in major organs (Choi TY., 2021). Additionally, zebrafish offer practical advantages such as high fecundity and rapid embryonic development, facilitating efficient experimental protocols (Jeevanandam et al., 2019).

This study aimed to evaluate the effects of Virgin Coconut Oil and folic acid supplementation on insulin signaling, assessed by IRS expression, and cardiac function, assessed by heart rate, in rotenone induced stunted zebrafish larvae, contributing to nutrition based strategies for improving metabolic and functional outcomes associated with stunting.

MATERIALS AND METHODS

Experimental design

Zebrafish *(Danio rerio)* embryos of wild type strain aged 0-2 hours post fertilization (hpf) to larva aged 9 days post fertilization (dpf) was used for study. The type of embryo used is transparent, not moldy, round in shape has been identified at the Laboratory of Fish Reproduction, Faculty of Fisheries ang Marine Sciences, Brawijaya University. Three hundred (450) embryos were divided into five group of 30 embryos each with three repetitions and were distributed into 6-well plates. The groups were delineated as follows, Negative Control group (NC) received 5 mL of embryonic medium per well, Positive Control group (PC) stunting group administered 12,5 parts per billion (ppb) of rotenone, Treatment groups Treatment 1 (T1) received VCO 6,25% and rotenone 12,5 ppb, Treatment 2 (T2) received Folic acid 70µM and rotenone 12,5 ppb, Treatment 3 (T3) combination of VCO and Folic acid and rotenone 12,5 ppb.

Exposure to rotenone and the VCO-AF commenced at 2 hpf and continued until 72 hpf, covering the zebrafish larval stage (Nurdiana, Sartika, et al., 2025).

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 coordinate: 7°57'13.7"S 112°36'47.6"E) from November 2025 to February 2026. All procedures were approved by the Health Research Ethics Committee of the Faculty of Medicine, Brawijaya University, with ethics ID number 523/EC/KEPK-S2/12/2025.

Procedures

Preparation of Embryonic medium

A 10-fold stock solution of 0,25 g Cacl, 0,15 g KCl, 5 g NaCl, and 0,815 g MgSO4 was made by dissolving in distilled water and made up to 500 mL. A 10-fold dilution of the stock solution was made by mixing one part of stock solution with nine parts of distilled water (Choi TY, Choi TI, Lee YR, Choe SK, 2021).

Zebrafish model of Stunting

The zebrafish model of stunting wa induced by exposure of zebrafish embryo (2 hpf to 72 hpf) to a 12,5 ppb of rotenone (Product No. R8875-IG) and it was dissolved in dimethylsulfoxide (DMSO) as the solvent. This stunting model aligns with the developmental equivalence of zebrafish embryo age 0-72 hpf to the fetal stage in utero. This temporal adaptation underscores the sutability of zebrafish as a model organism for studying stunting conditions (Primihastuti et al., 2022; Sumiyati, 2024).

Preparation of VCO

The Virgin coconut oil (VCO) used for the study was obtained from VCO Palm 7 (Cemoro Kandang, Malang, Indonesia; P-IRT 2063573011924-29) and contain 99,73% fat was food grade VCO produced by the cold press method from fresh coconut meat (*Cocos Nucifera L.*). The oil was stored at room temperature (25 °C) in tightly closed container until use. For the preparation of stock solution, 4 mL of VCO was added 80 µL of Dimethyl sulfoxide (DMSO) as the solvent and 8 µL rotenone, followed by addition of distilled water to a final volume of 64 mL to produce a solution with concentration of 6,25%. The solution was mixed throughly before use. The selected concentration (6,25%) was based on the optimal dose identified in the preliminary exploratory study (Nurdiana, Sartika, et al., 2025).

Preparation of Folic Acid

Folic Acid solution was prepared using folic acid powder (Sigma F7876, Sigma Aldrich, USA). Sodium Carbonate

(Na_2CO_3) was used as a solvent to enhance solubility, and distilled water was used for dilution. The equipment included 64 mL Falcon Tubes, a digital analytical balance (Mattler Toledo), and standart laboratory mixing tools. A stock solution of folic acid ($5x10^3$ µM) was prepared prior to dilution. The working solution was prepare to obtain a final concentration of 70 µM in a total volume of 64 mL. The appropriate volume of stock solution was transferred into a Falcon tube and diluted with distilled water to reach the final volume. Based on previous literature, 70 µM was selected as the optimal concentration for this study (Wati, 2018).

Preparation of VCO and Folic Acid Combination

The combination treatment was prepared based on the optimal doses identified in preliminary study. The selected concentrations were 6,25% for vigin coconut oil (VCO) and 70 µM for folic acid. The mixture consisted of 4 ml VCO, 0,896 mL folic acid solution (70 µM), 8 µL rotenone, and 80 µL of 1% dimethyl sulfoxide (DMSO). Distilled water was added to reach a final volume of 64 mL. The solution was mixed throughly prior to adminnistration. The total volume used for each zebrafish treatment group wa 64 mL.

Measurement of heart rate larvae zebrafish

The heart rate of zebrafish larvae (age 3,6,9 dpf) was measured on a stereoptical microscope (Olympus SZ61) with 40x magnification connected to a Panasonic Lumix GF8 camera, and a recording device. The heart rate was recorded for 15 second, using a tally counter, the reading was multiplied by 4 to measure the number of heartbeats per minute. Measurement of larval heart beat were carried out using DanioVision application (Figure 1).

Figure 1. Measurements of the heart rate by using Danio Scope.

Measurement of insulin receptor substrate expression by Reverse Transcriptase Polymerase Chain Reaction (RT-qPCR)

The IRS expression was measured by reverse transcriptase polymerase chain reaction (RT-qPCR) method to assess the inflammatory response in zebrafish larvae tissue. Zebrafish larvae (30 per group) aged 9 days post ferfertilization (dpf) were euthanized by rapid coolid, and then frozen in a -80C freezer. The total RNA of the zebrafish larvae was extracted according to the protocol of the Total RNA Mini Kit (Geneaid, catalog number RT100, New Taipei City, Taiwan). Reverse transcription to convert RNA into cDNA template, Isolated RNA following the protocol of the ReverTra Ace qPCR RT Mastermix with gDNA Remover kit (TOYOBO catalog number FSQ-301, Osaka, Japan). The reaction process was carried out using a Real Time PCR instrument (CFX Opus 96) with 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 1 µL of cDNA template, with the remaining volume consisting of RNase-free water (Nurdiana, Sartika, et al., 2025). The base sequence of the primers is listed in Table 1 and and the RT-PCR programs used is shown in Table 2, respectively (Khotimah et al., 2025).

Table 1. Primer Base Sequence of Insulin receptor substrate (IRS).

	Forward Primer
Irs	5’-GGTGTCTTTTCAACACCGCC-3’
β-Actin	5’-CGA GCA GGA GAT GGG AAC-3’
	Reverse Primer
Irs	5’-TCAAAACAAGCGCAGTCAGC-3’
β-Actin	5’-CAA CGG AAA CGC TCA TTG C-3’

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

Steps	Irs
Pre Denaturation (time/temperature)	2’/95°C
Denaturation (time/temperature)	5”/95°C
Annealing (time/temperature)	30”/55°C
Cycles	40 cycles

Statistical analysis

IBM SPSS software (Version 27) was used for statistical analysis. Normality test used one-sample Shapiro Wilk test, the homogeneity test used Levene test followed by Tukey HSD. Data were subjected to one-way analysis of variance (ANOVA) with a p-value of less than 0,05 used to establish the significant difference among means.

RESULTS AND DISCUSSION

Rotenone exposure induce a stunting in zebrafish larvae, characterized in physiological parameters associated with growth and metabolic function. In this study, cardiac activity and insulin signaling were assessed as functional indicators of metabolic status in the stunting model (Nurdiana, Sartika, et al., 2025). The effects of Virgin Coconut Oil (VCO) and folic acid supplementation were evaluated to determine their effects on these stunting related physiological alterations.

Heart rate of Zebrafish Larvae as Stunting Model

The study results of the average heart rate of zebrafish larvae using the stunting model and administration of VCO and folic acid at various concentrations at 3, 6, 9 dpf (Fig. 2). The results showed that there was a significant difference in heart rate between positive control group and the treatment group at 3,6, and 9 dpf ($p < 0.05$) (Table 3). Table 3 shows that at 3 dpf, the NC group had the lowest heart rate speed (103.2 ± 5.5 bpm), while the PC group had the highest (145.9 ± 3.7 bpm). At 6 dpf, treatment with VCO and folic acid (T1, T2, T3) showed a decrease in heart rate compared to PC. At 9 dpf, the heart rate of larvae in T1 treatment group reached the highest value (206.6 ± 35.6 bpm), while PC group still showed a lower heart rate compared to other treatment groups.

In table 4, 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 T1, T2, and T3 ($p<0.05$). In table 5, at 6 dpf, a similar pattern was observed, where the PC group still showed significantly highest heart rate in comparison to the NC group and all the treatment groups of T1, T2, and T3 ($p<0.05$). In table 6, at the age of 9 dpf, significant differences between PC group and the NC as well as the T1 treatment groups were still visible ($p<0.05$), while the differences between PC and the T2 and T3 groups were not significant ($p>0.05$).

Table 3. Mean ± SD of heart rate (BPM) in zebrafish larvae at 3, 6, and 9 dpf.

Age	**3 dpf**					**6 dpf**					**9 dpf**				
Group	NC	PC	T1	T2	T3	NC	PC	T1	T2	T3	NC	PC	T1	T2	T3
Mean (BPM) ± SD	103.2 ± 5.6	145.9 ± 3.7	109.6 ± 0.7	110.8 ± 0.5	139.9 ± 0.1	125.5 ± 5.2	169.6 ± 4.8	126.5 ± 3.08	131.2 ± 3.4	156.9 ± 0.64	163.7 ± 10.06	236.9 ± 19.2	206.6 ± 35.6	218.9 ± 42.1	231.9 ± 34.5
p-value			0,000					0.000					0.003		

Table 4. Post-Hoc Test for Heart Rate at 3 dpf.

Group	**NC**	**PC**	**T1**	**T2**	**T3**
NC		0.000*	0,743	0.517	0.14*
PC	0.000*		0.000*	0.000*	0.000*
T1	0.743	0.000		0.993	0.092
T2	0.517	0.000	0.993		0.168
T3	0.014	0.000	0.092	0.168	

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

Table 5. Post-Hoc Test for Heart Rate at 6 dpf.

Group	**NC**	**PC**	**T1**	**T2**	**T3**
NC		0.000*	0,998	0.532	0.058
PC	0.000*		0.000*	0.000*	0.000*
T1	0.998	0.000		0.693	0.090
T2	0.532	0.000	0.693		0.529
T3	0.058	0.000	0.090	0.529	

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

Table 6. Post-Hoc Test for Heart Rate at 9 dpf.

Group	**NC**	**PC**	**T1**	**T2**	**T3**
NC		0.005*	0,907	0.043*	0.025*
PC	0.005*		0.020*	0.667	0.849
T1	0.907	0.020		0.157	0.092
T2	0.043	0.667	0.157		0.996
T3	0.025	0.849	0.092	0.996	

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 decrease the heart rate of zebrafish larvae as a stunting model by rotenone induction rather than treatment of folic acid and the combination of VCO and folic acid. At 3 dpf, NC group showed the lowest heart rate (103.2 ± 5.6 BPM), while PC group given rotenone experienced a significant increasing heart rate (145.9 ± 3.7 BPM). Administration of VCO at the highest dose (T1 6,25%) successfully decrease the heart rate of larvae by 109.6 ± 0.7 BPM at 3 dpf and significantly at 6 dpf and 9 dpf (1126.5 ± 3.08 bpm and 206.6 ± 35.6 bpm, respectively). These improvements in heart rate performance across time points are illustrated in Figure 2, which shows the mean heart rate and corresponding standard deviations for each treatment group at 3, 6, and 9 dpf.

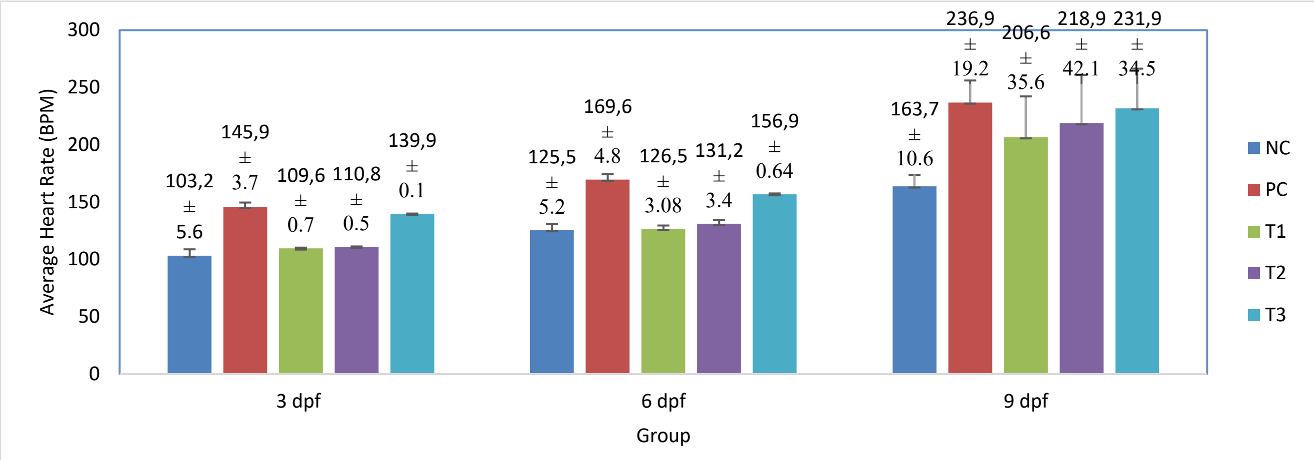

Figure 2. Average heart rate (BPM) of zebrafish larvae at 3, 6, and 9 dpf across experimental groups. Values are presented as mean ± SD.

The effect of VCO and Folic Acid on Irs Expression

Measurement of Irs Expression in zebrafish larvae age of 9 days post fertilization (9 dpf) was perfomed using the RT-qPCR method for the five treatment groups of negative control (NC), positive control (PC) exposed to rotenone (12,5 ppb) and three treatment groups of VCO (T1 = 6.25%), folic acid (T2 = 70 µM), and a of VCO and folic acid (T3 = 6,25% + 70 µM). Normality testing showed that Irs expression data were normally distributed, with p-value > 0.05, indicating that the data met the assumption of normal distribution. Homogeneity testing also indicated that the variance among groups was homogeneous with a p-value of 0.451, indicating that the assumptions for parametric analysis were met. The data for the average fold change and standart deviations of Irs expression from each group are presented in Figure 3. The negative control group showed the highest expression level with value 1.014 ± 0.187, representing normal physiological conditions. In contrast, the positive control group exposed to rotenone (12,5 ppb) showed a marked decrease in Irs expression, with average value 0.272 ± 0.128, suggesting that rotenone exposure suppressed the expression of the Irs gene (Table 7).

Table 7. Effect of Combination of VCO and Folic Acid on Irs Expression in Each Group Measured by RT-qPCR.

Group	**NC**	**PC**	**T1**	**T2**	**T3**
n (larva)	30	30	30	30	30
Mean Fold Change ± SD	$1.014^{a} \pm 0.187$	$0.272^{b} \pm 0.128$	$0.678^{a} \pm 0.250$	$0.676^{a} \pm 0.191$	$0.695^{a} \pm 0.231$
p-value			0,005		

Notes: Value with different superscript letters indicate statistically significant differences.

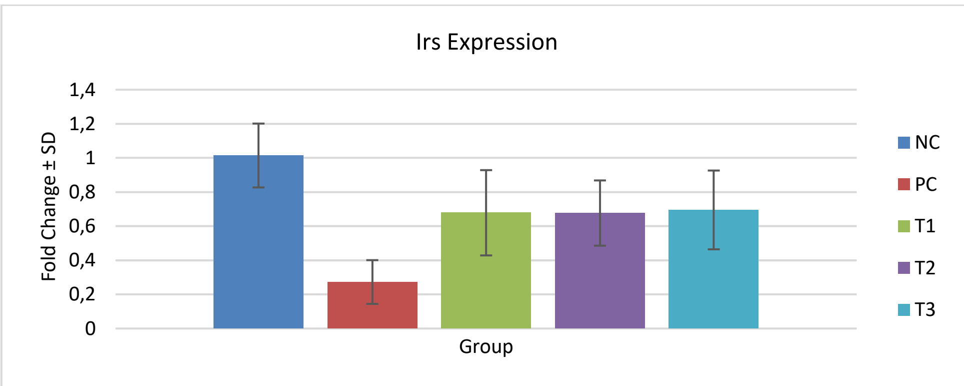

Figure 3. Insulin receptor substrate (Irs) expression by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). Values are presented as mean fold change ± SD.

The treatment groups that received VCO, folic acid and their combination increase Irs expression compared to the positive control group. The T1 group (VCO 6,25%) showed an average fold change of 0.678 ± 0.250, the T2 group (folic acid 70 µM) showed 0.676 ± 0.191, and a T3 group (VCO 6,25% + folic acid 70 µM) showed 0.695 ± 0.231. Statistical analysis using One-Way ANOVA showed a significant differences in Irs expression between groups (p-value of <0.001). Post hoc analysis using Tukey HSD testing identified that the positive control group (PC) significant differences from the negative control group and all treatment groups ($p<0.05$). however, no significant differences were observed among the treatment groups (T1, T2, T3). The combination treatment (T3) showed the highest mean expression among treatment groups, although the difference was not statistically significant.

Overall, these findings indicate that administration of VCO, folic acid, and their combination was able to iincrease irs expression compared to the rotenone exposed group, although the difference among the treatment groups were not statistically significant.

Discussion

This study demonstrated that rotenone induced stunting in mitochondrial stress significantly impaired insulin signaling and altered cardiac function in zebrafish larvae, as indicated by the marked reduction in Irs expression in the positive control group and significant changes in heart rate parameters. Mitochondrial dysfunction was also accompanied by alterations in cardiac parameters, suggesting that metabolic disturbances during early development affect both molecular signaling pathways and physiological function. Treatment with Virgin Coconut Oil (VCO), folic acid, and their combination increased Irs expression compared with the rotenone exposed group. Among the treatment groups, the combination of VCO and folic acid (T3) showed the highest mean Irs expression, however the differences among treatment groups were not statistically significant. These findis suggest that nutritional interventions targeting mitochondrial oxidative stress may contribute to the restoration of insulin signaling under conditions of mitochondrial dysfuntion.

Rotenone induced mitocondrial stress and Irs suppression

Rotenone is a well establish mitocondrial complex I inhibitor that disrupts electron transport and enhances electron leakage, resulting in increasing reactive oxygen species (ROS) production (Heo et al., 2022). In our model, rotenone exposure led to significant suppression of IRS expression compared with the negative control group, indicating that mitochondrial stress interferes with insulin signaling pathways. The findings are consistent with previous study demonstrating that excessive ROS production disrupts metabolic signaling and impairs cellular growth processes (Toyoshima et al., 2025). In developing organism, excessive ROS impairs ATP production and disrupts insulin receptor substrate (IRS) mediated signaling pathways (Almansa-Ordonez et al., 2020; Moh, 2022).

Inhibition of complex I reduces oxidative phosphorylation efficiency and limits ATP production while simultaneously increasing ROS (Davila et al., 2015). Elevated ROS activates stress sensitive kinase such as c-Jun N-terminal kinase (JNK) and p38 MAPK, which phosphorylate IRS at inhibitory serine residues. This modification reduces IRS stability and impairs downstream PI3K-Akt signaling, leading to impaired glucose uptake and reduced anabolic activity required for normal growth (Meijles et al., 2020; Son et al., 2011). Therefore, the reduce IRS expression observed in the rotenone group likely reflects oxidative stress mediated disruption of insulin receptor signaling.

Insulin signaling plays a fundamental role in somatic growth and metabolic regulation during early development. The IRS–PI3K–Akt axis regulates protein synthesis, cell survival, and energy metabolism. In developing organism such as zebrafish larvae, mitochondrial dysfunction can severely disrupt these processes because energy demand is high during rapid growth and organ formation (Mccluskey & Braasch, 2020). Consequently, suppression of IRS signaling may contribute to growth retardation and metabolic instability in rotenone exposed larvae.

Effects of VCO ad Folic acid on Mitochondrial function and Insulin Signaling

In the present study, treatment with VCO, folic acid, and their combination increased Irs expression compared with rotenone exposed group, suggesting a protective efect against mitochondrial oxidative stress. Although all treatment groups showed improvement relative to the positive control group, the combination treatment (T3) demonstrated the highest mean Irs expression among the treatments. However, statistical analysis indicated no significant differences among the treatment groups, suggesting that each intervention may comparable biological effects in restoring insulin signaling.

Virgin Coconut Oil (VCO) contains high levels of medium-chain fatty acids (MCFAs), particularly lauric acid, which are rapidly transported into mitochondria and oxidized independently of the carnitine transport system (Nurdiana, Claudia, et al., 2025). This metabolic property enhances mitochondrial β-oxidation efficiency and ATP production while reducing overload of the electron transport chain (Arunima & Rajamohan, 2014). Improved mitochondrial metabolic flux can decrease electron leakage and limit ROS generation. In addition, VCO contains polyphenolic compounds with antioxidant properties that can scavenge free radicals and reduce lipid peroxidation. Through these mechanism, VCO may attenuate oxidative stress induced IRS degradation and support recovery of PI3K-Akt signaling (Moh, 2022; Zhao et al., 2022).

Folic acid contributes through complementary metabolic pathways. As a key component of one carbon metabolism, folate supports nucleotida syntehesis, methylation reactions, and mitochondrial DNA stability (Menezo et al., 2022). Folate deficiency has been associated with elevated homocystein levels and increase oxidative stress, both of which can impair mitochondrial function (Asbaghi et al., 2021). Adequate folate availability may therefore help stabilize cellular redox balance and maintain gene expression involved in metabolic regulation . Additionally, folate has been reported to enhance mitochondrial biogenesis and reduce ROS accumulation, indirectly preserving insulin signaling integrity (Hsu et al., 2013; Kranenburg et al., 2025).

The combination of VCO and folic acid may provide complementary mitochondrial protection. VCO primarily improves mitochondrial energy metabolism and reduces ROS formation, whereas folic acid supports mitochondrial genomic stability and redox regulation (Hsu et al., 2013; Wang et al., 2015). This complementary mechanism may explain the slightly higher mean Irs expression observed in combination group. However, the absence of significant differences among treatments suggest that restoration of mitochondrial redox balance may reach a functional threshold beyond which additional stimulation does not substantially increase IRS signaling (Toyoshima et al., 2025).

Cardiac Function of Insulin Signaling Disruption

Insulin receptor substrate (IRS) is a central adaptor protein linking insulin receptor activation to downstream PI3K-Akt signaling, which governs cellular metabolism, survival, and growth (Martínez Báez et al., 2024; Toyoshima et al., 2025). In the cardiac tissue, the IRS-PI3K-Akt axis plays an essential role in maintaining cardiomyocyte metabolic homeostasis and contractile performance (Díaz Del Moral et al., 2021; Liao et al., 2020; Meijles et al., 2020).

Under conditions of mitochondrial stress, excessive ROS activates stress sensitive kinases such as JNK and IKKβ, which phosphorylate Irs at inhibitory residues and promote its degradation (Ayer et al., 2022; Masenga et al., 2023). Reduced Irs availability attenuates PI3K activation and downstream Akt phosporylation, impairing glucose uptake and mitochondrial metabloic flexibility (Toyoshima et al., 2025). In cardi0myocytes, such metabolic disturbances compromise ATP production and disrupt excitation contraction coupling (Li et al., 2022; Luptak et al., 2018).

In this study, rotenone exposure was associated with altered heart rate dynamics at 9 days post fertilization. The elevated heart rate observed in the positive control group likely represents a compensatory physiological response to reduced myocardial energetic efficiency (Gonchar et al., 2021). When ATP production declines, increasing heart rate may transiently preserve cardiac output (Lin et al., 2022). However, sustained oxidative stress can impair calcium handling and sarcomeric coordination, indicating that this tachycardis response reflects physiological stress rather than improved cardiac function (Dhalla et al., 2022; Shah et al., 2021).

Treatment groups that demonstrated improved Irs expression also showed more stable cardiac parameters compared with the rotenone exposed group. These findings suggest that restoration of mitochondrial redox balance and insulin signaling may contribute to improved cardiac metabolic stability during early development (Guo & Guo, 2017; Schraps et al., 2024).

Overall, the results indicate that nutritional interventions targeting mitochondrial oxidative stress may help restore insulin signaling pathways disrupted by toxic mitochondrial inhibition. Alhtough all treatments improved Irs expression relative to the rotenone group, the combination of VCO and folic acid demonstrated the highest mean expression level, suggesting a potential complementary protective effect on mitochondrial metabolism and insulin signaling.

CONCLUSIONS

This study demonstrates that rotenone-induced mitochondrial dysfunction suppresses IRS expression and disrupts cardiac function in zebrafish larvae, highlighting a mechanistic link between oxidative stress, impaired insulin signaling, and cardiometabolic instability during early development. Inhibition of mitochondrial complex I increased oxidative burden, which likely promoted stress mediated IRS suppression and downstream attenuation of PI3K-Akt signaling, contributing to growth impairment and altered heart rate dynamics.

Importantly, supplementation with virgin coconut oil (VCO) and folic acid restored IRS expression and improved cardiac parameters, suggesting that mitochondrial-targeted nutritional interventions can reverse toxin induced insulin signaling disruption. The combined therapy appeared to exert its protective effects through redox modulation and bioenergetic stabilization, thereby reestablishing metabolic homeostasis.

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Competing Interests: The authors declare that there are no competing interests.

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