

Inhibitory Effect of Virgin Coconut Oil (VCO) on Leukemic Cell Proliferation in Human Promyelocytic Leukemia HL-60 Cells

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ABSTRACT

OBJECTIVE Leukemia is characterized by the uncontrolled proliferation of abnormal white blood cells, and although chemotherapy remains a standard treatment, toxicity, relapse, and drug resistance continue to pose major clinical challenges. These limitations draw attention to the need for safer and more effective therapeutic alternatives. Natural products have attracted considerable interest due to their diverse bioactive constituents and promising anti-cancer effects. Virgin coconut oil (VCO) has been reported to offer several health benefits, including antioxidant, anti-stress, anti-inflammatory, weight-reducing, and anti-cancer properties. This study was designed to investigate the capability of VCO to inhibit HL-60 (human promyelocytic leukemia) cell growth by inducing cell cycle arrest and apoptosis.

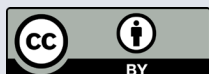
METHODS VCO was analyzed using gas chromatography-mass spectrometry (GC-MS) analysis. Cell viability was assessed using the MTT assay; HL-60 cells were treated with various VCO concentrations for 48 hours. Additionally, cell cycle analysis was conducted by means of staining with propidium iodide, and an apoptosis assay was performed with Annexin V-FITC.

RESULTS GC-MS analysis revealed that VCO contains lauric acid (LA), caprylic acid, capric acid, myristic acid, palmitic acid, linoleic acid, oleic acid, and stearic acid. After treatment, VCO exhibited cytotoxic effects in HL-60 cells with IC₅₀ values between 269 and 300 µg/mL, and its activity was compared to commercial lauric acid (C-LA). Additionally, VCO was found to inhibit cell proliferation in a dose-dependent manner. Cell cycle analysis confirmed that VCO induced cell cycle arrest at the G1-phase. Furthermore, an apoptosis assay demonstrated that treatment with VCO induced apoptotic cell death in HL-60 cells.

CONCLUSION These findings suggest that the anti-leukemic effect of VCO is associated with cell cycle arrest and reduced cell viability through apoptosis.

KEYWORDS virgin coconut oil, human promyelocytic leukemia, HL-60 cells, anti-leukemic activity, cell cycle arrest, apoptosis

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INTRODUCTION

Cancer remains one of the leading causes of death among both males and females. In 2022, there were approximately 20 million new cases and 9.7 million cancer-associated deaths worldwide (1). Over the next two decades, the number of individuals diagnosed with cancer is expected to rise significantly. Leukemia is a type of cancerous neoplasm that is associated with the organs that produce blood cells and is characterized by an increase in the number of abnormal white blood cells (2). Leukemia has been linked to exposure to radiation, toxic chemicals, chemotherapy, viruses and other microorganisms, genetic disorders associated with chromosomal instability, cigarette smoking, and other environmental factors. These factors lead to changes in the genetic structure of leukemia cells, which, in turn, affect the function of other genes, including tumor suppressors and oncogenes. The disease spreads rapidly and can be classified as acute or chronic, myeloid or lymphoid (3). The forms of leukemia are those that impact the myeloid and lymphoid systems, including acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL), and chronic lymphocytic leukemia (CLL) (4).

This situation highlights the ongoing challenges in cancer management, particularly the lack of effective new drugs to reduce cancer incidence. Recently, plant-derived natural products have attracted considerable interest as potential agents for cancer treatment. Medicinal plants have been used therapeutically for thousands of years and represent a fundamental source of pharmacologically active compounds. The appeal of plant-derived compounds lies in their ability to enhance cancer treatment, often exhibiting synergistic effects when used together with conventional therapies. For example, green tea has been shown to modulate molecular pathways involved in inflammation, oxidative stress, and apoptosis in cancer cells (5). Similarly, curcumin, a bioactive compound from turmeric, has been widely reported to have diverse biological activities, including anti-inflammatory, antioxidant, and anti-cancer properties (6). In this study, we investigated virgin coconut oil (VCO), which is obtained from fresh coconut by a controlled temperature process without the use of chemical additives. This

method retains the bioactive compounds such as polyphenols, fatty acids, sterols, and tocopherols (7). VCO has been reported to exhibit antioxidant, anti-stress (8), anti-inflammatory (9), weight-reducing (10), anti-hypercholesterolemic (11), anti-dermatitic (12) and anti-cancer properties. In addition, VCO consumption during chemotherapy has been shown to improve the quality of life of breast cancer patients (13). However, few studies have examined the anti-cancer effects of VCO. Interestingly, Sheela et al. reported that lauric acid (LA), a major component of VCO, exhibited dose-dependent cytotoxicity in HCT-15 (human colon cancer), HepG2 (human hepatocellular carcinoma), and RAW 264.7 (murine macrophage) cells by inducing apoptotic morphological changes (14). Additionally, VCO has demonstrated anti-liver and oral cancer activity (15).

LA, a saturated medium-chain fatty acid (MCFA) which contains 12 carbon atoms and is the predominant fatty acid in coconut oil, has been linked to several reported health benefits associated with coconut oil consumption (16, 17). Fatty acids are acyclic carboxylic acids with aliphatic chains of varying lengths. Based on carbon chain length, they are categorized into three groups: short-chain fatty acids (approximately 6 carbon atoms), MCFAs (6-12 carbon atoms), and long-chain fatty acids (more than 12 carbon atoms) (18). Fatty acids serve as fundamental components of triacylglycerols, phospholipids, and other complex lipids, and constitute major contributors to dietary fat in humans (19). Previous studies have shown that fatty acids participate in multiple cellular processes, including signal transduction, regulation of gene transcription, metabolism, inflammation, apoptosis, and the synthesis of bioactive lipid mediators (20).

Apoptosis, or programmed cell death, is a specific set of morphological and biochemical changes including cell shrinkage, cytoplasmic condensation, chromatin condensation, DNA fragmentation, and plasma membrane blebbing, that are involved in maintaining tissue homeostasis by controlling cell populations, eliminating aged or damaged cells, and allowing the proliferation of new cells (21). However, carcinogens can interfere with apoptotic pathways, allowing uncontrolled cancer cell proliferation. In general, cell division is regulated by cell cycle control

mechanisms to produce an identical cell. The cell cycle contains 4 stages: gap 1 (G1) phase, synthesis (S) phase, gap 2 (G2) phase, and mitosis (M) phase, during which the cell grows, replicates its DNA, and prepares for mitosis, respectively. The mitotic cell cycle is typically divided into two major phases: the interphase and the M-phase. During the interphase, there will be duplicates of cellular content within the cells which are separated into two genetically identical daughter cells during mitosis. DNA replication occurs during the interphase, specifically in the S-phase. The interphase period separating the S-phase from the M-phase is called the gap phase, with the G1-phase preceding the S-phase and the G2-phase following the S-phase. Evidence suggests that there is a gap between the two main events, duplication and DNA dissociation. However, the steps occurring in the gap are important in the regulation of the cell cycle and involve important decisions, e.g., to enter the cell cycle in G1 and to initiate the process of chromosome division in G2. In unicellular and multicellular eukaryotes, cell division is regulated by a complex network of control, monitoring, and compensation mechanisms that ensure that no errors occur before cells can enter and complete the cell cycle for division. Check-points may delay the progression of the cell cycle or induce the end of the cell cycle or cell death in response to irreversible DNA damage (22). To date, no studies have investigated the antiproliferative, cell cycle arrest, or pro-apoptotic effects of VCO in HL-60 (human promyelocytic leukemia) cells. The present study is designed to evaluate the inhibitory effects of VCO on HL-60 leukemic cell proliferation through the induction of cell cycle arrest and apoptosis.

METHOD

Chemical materials

RPMI 1640 medium supplemented with 25 mM HEPES and L-glutamine, fetal bovine serum (FBS), penicillin/streptomycin, phosphate-buffered saline (PBS), and trypan blue dye solution were purchased from Cytiva HyClone™ (Logan, UT, USA). MTT dye and commercial lauric acid (C-LA) ($\geq 99\%$, GC/titration) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Test materials

Five 100% pure VCO samples were used. Sample No. 1 was a handmade VCO produced using traditional methods by local people. Mature coconuts, aged more than 12 months, were obtained from a coconut farm in Mae Taeng District, Chiang Mai Province, Thailand. Only coconuts that had not sprouted were selected, and their milk was removed from the coconut fruits. The traditional extraction process began with grating mature coconuts, followed by pressing the grated flesh in warm water to obtain coconut milk. The coconut milk was then placed in a clean bag and subsequently frozen at -29°C for 48 hours. After freezing, the solidified coconut milk was melted in sunlight at an ambient temperature of approximately 50°C for 1 hour and 30 minutes to facilitate phase separation into three layers: a water layer, a cream layer, and an oil layer. The oil layer was carefully collected in a clean container and subjected to mild steaming for 5 minutes to remove residual moisture, a step intended to prevent rancidity and improve storage stability. The VCO was then allowed to cool at room temperature before being transferred into sterilized bottles for storage (23). Samples No. 2-5 were obtained from a drugstore (Chiang Mai University Pharmacy, Chiang Mai, Thailand).

Cells and cell culture conditions

The human promyelocytic leukemia (HL-60) cell line (ATCC® CCL-240™) was purchased from ATCC. HL-60 cells were cultured in RPMI 1640 medium supplemented with 10% FBS, 25 mM HEPES, L-glutamine, 100 U/mL penicillin, and 100 $\mu\text{g}/\text{mL}$ streptomycin. The cells were grown in a 5% CO_2 humidified atmosphere in incubators maintained at 37°C .

Chemical fingerprinting and fatty acid analysis using gas chromatography-mass spectrometry (GC-MS)

The VCO samples (No. 1-5) were pre-processed as described below. Twenty milligrams of the VCO samples were mixed with 1 mL of methanolic NaOH. The samples were heated at 65°C for 15 minutes with intermediate shaking. After heating and shaking, the samples were cooled to room temperature for 5 minutes. Then, 1 mL of MilliQ water was added to the mixture, followed by 1 mL

of distilled hexane. The mixture was vortexed for phase separation. The clear upper hexane layer was gently withdrawn using a micropipette without disturbing the bottom layer. The hexane layer was transferred to a fresh GC vial and subjected to GC-MS analysis. The fatty acids were separated in a VF-5ms column using a Trace GC Ultra gas chromatograph under the following conditions. The samples were injected at an injection port maintained at 250°C. The samples were then transferred to the column through a split injection having a 50:1 split ratio. The initial oven temperature was set at 50°C, ramped up to 120°C, and then held for 5 minutes after which the temperature was increased to 170°C at a rate of 5°C/min and held for an additional 5 minutes. Subsequently, the temperature was ramped up to 230°C at the same rate and held there for 5 minutes. Finally, the oven temperature was raised to 250°C at 5°C/min and held for 2 minutes. The separated compounds were then injected into a TSQ Quantum XLS Mass spectrometer. The total ion chromatogram was recorded in a scan mode. Ions ranging from m/z 50 to 500 were recorded.

MTT cytotoxicity assay

The cytotoxicity of VCO No. 1 (handmade) and VCO No. 2-5 (commercially manufactured) was evaluated using the MTT assay. Briefly, HL-60 cells in the exponential phase of growth (5.0×10^4 cells/well) were cultured in 96-well plates containing 100 μ L of medium for 24 hours prior to treatment. After that, 100 μ L of fresh medium containing various concentrations (0-3,200 μ g/mL) of the VCO were added to each well and incubated for 48 hours at 37°C. The MTT dye solution was added (15 μ L/100 μ L medium), and the plates were then incubated at 37°C for 4 hours in a humidified 5% CO₂ atmosphere. Following that, 200 μ L of DMSO was added to each well and mixed thoroughly to dissolve the dye crystals. The absorbance was measured using an ELISA plate reader (Thermo Scientific, Multiskan SkyHigh, Singapore) at 540 nm with a reference wavelength of 630 nm. Untreated cells under similar conditions were used as a negative control. High optical density readings corresponded to a high intensity of dye color, indicating a high number of viable cells able to metabolize MTT salts. The fractional absorbance was calculated by the following formula:

$$\% \text{ Cell survival} = \frac{\text{Mean absorbance in test wells}}{\text{Mean absorbance in control wells}} \times 100$$

The average cell survival obtained from triplicate determinations at each concentration was plotted as a dose-response curve. The experiment was performed in three independent replicates. The 50% inhibitory concentration (IC₅₀) of VCO was defined as the lowest concentration that reduced cell growth by 50% compared to untreated or vehicle control cultures (0.2% DMSO in culture medium). IC₅₀ values are presented as the mean $\pm$ standard deviation (SD) and were compared to assess their activities (24, 25). The IC₅₀ values (cytotoxic doses) of the VCOs were compared with those of C-LA in relation to their effects on cell cycle analysis and apoptosis assay.

Cell cycle analysis

HL-60 cells (7.5×10^5 cells/well) were seeded into 6-well plates and then treated with a vehicle control or varying concentrations (close to the IC₅₀ values) of VCO No. 1 (representing handmade VCO) and VCO No. 3 (representing commercial VCO, as indicated on the bottle label: "100% virgin coconut oil, high lauric acid, premium grade, and chemical-free"), compared to (close to the IC₅₀ value) C-LA for 24 hours (26). Following treatment, the cells were collected, washed twice with PBS, and fixed in cold (-20°C) 70% ethanol overnight. Ethanol was then carefully removed by centrifugation at $1,500 \times g$ for 3 minutes. The cells were resuspended in 1 mL of staining reagent containing 25 μ g/mL RNase (United States Biological, Salem, MA, USA) and 50 μ g/mL propidium iodide (PI) (Merck Millipore, Darmstadt, Germany) and incubated in the dark at room temperature for 30 minutes. Cell cycle distribution was subsequently analyzed by flow cytometry (Beckman Coulter DxFLEX, Brea, CA, USA).

Apoptosis detection via Annexin V-FITC/PI assay

Phosphatidylserine exposure on the outer membrane of apoptotic cells was assessed using an Annexin V-FITC apoptosis detection kit (Elabscience, Houston, TX, USA). Briefly, HL-60 cells (5×10^5 cells/well) were plated in 6-well plates and treated with vehicle control, VCO (No. 1 and No. 3), or C-LA at the indicated concentrations for 48 hours (27). The treated cells were harvested

by centrifugation at $1,500 \times g$ for 3 minutes at room temperature and washed twice with ice-cold PBS. Then, the cells were stained with Annexin V-FITC/PI according to the manufacturer's instructions and analyzed using flow cytometry.

Statistical analysis

All data are expressed as the mean $\pm$ SD from at least three independent experiments. The comparison between the treated and control groups was performed using Student's t-test. Differences were considered significant when the probability value obtained was found to be less than 0.05 ($p < 0.05$), highly significant if $^{**}p < 0.01$, and extremely significant if $^{***}p < 0.001$.

RESULTS

Chemical parameters of VCO fatty acid profiling of VCO samples

The GC-MS analysis revealed that VCO No. 1-5 contained several saturated fatty acids, along with detectable amounts of unsaturated fatty acids. Notably, all samples shared an identical composition, consisting of LA, caprylic acid, capric acid, myristic acid, palmitic acid, linoleic acid, oleic acid, and stearic acid. The corresponding total ion chromatograms (TICs) for each sample are presented in a previous study (28).

Cytotoxicity of VCO in HL-60 cell line

HL-60 cells were treated with various concentrations of VCO No. 1-5 for 48 hours, resulting

in a pronounced, dose-dependent reduction in cell viability, as shown in Figure 1A-E. The corresponding IC_{50} values for VCO No. 1-5 were 300.0, 285.7, 281.8, 269.6, and 276.2 $\mu g/mL$, respectively. In contrast, C-LA demonstrated substantially greater cytotoxicity at 48 hours, with an IC_{50} value of 20.8 $\mu g/mL$, as shown in Figure 1F. Collectively, these results indicate that although all VCO samples decreased HL-60 cell viability in a concentration-dependent manner, C-LA exhibited markedly higher potency under the experimental conditions.

VCO induces G1 phase arrest in HL-60 cells

To determine whether the anti-proliferative effect of VCO is associated with cell cycle regulation, HL-60 cells were treated with VCO No. 1 (handmade VCO), VCO No. 3 (commercial VCO), C-LA, or a control vehicle. Cell cycle distribution was analyzed by flow cytometry following PI staining. As shown in Figure 2A, treatment with 75, 150, and 250 $\mu g/mL$ VCO No. 1 for 24 hours resulted in a dose-dependent accumulation of cells in the G1 phase, increasing the G1 population from 46.07% in the control group to 47.88%, 49.15%, and 49.86%, respectively. These findings are supported by the quantification in Figure 2B. Similarly, VCO No. 3 enhanced G1-phase accumulation (Figure 3A), with G1 content increasing to 50.33%, 50.12%, and 50.57% at the corresponding concentrations, as shown in Figure 3B. In contrast, treatment with 20 $\mu g/mL$ C-LA significantly

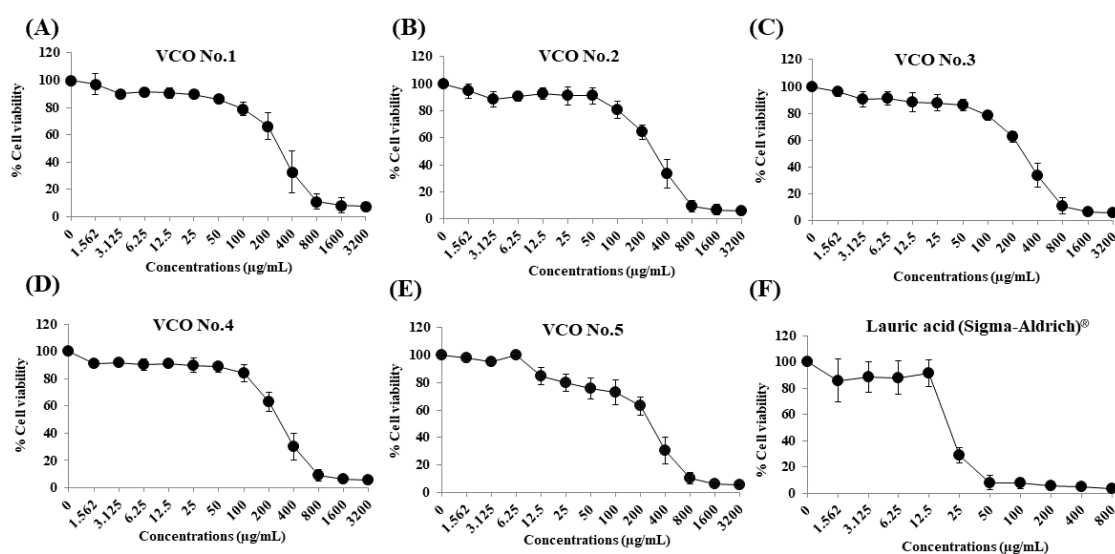


Figure 1. Cytotoxic effects of VCO No. 1-5 (A-E) and C-LA (F) on HL-60 cells. Cells were treated with various concentrations of each sample for 48 hours (VCO) or (C-LA), and cell viability was assessed using the MTT assay. Data are expressed as mean $\pm$ SD from three independent experiments.

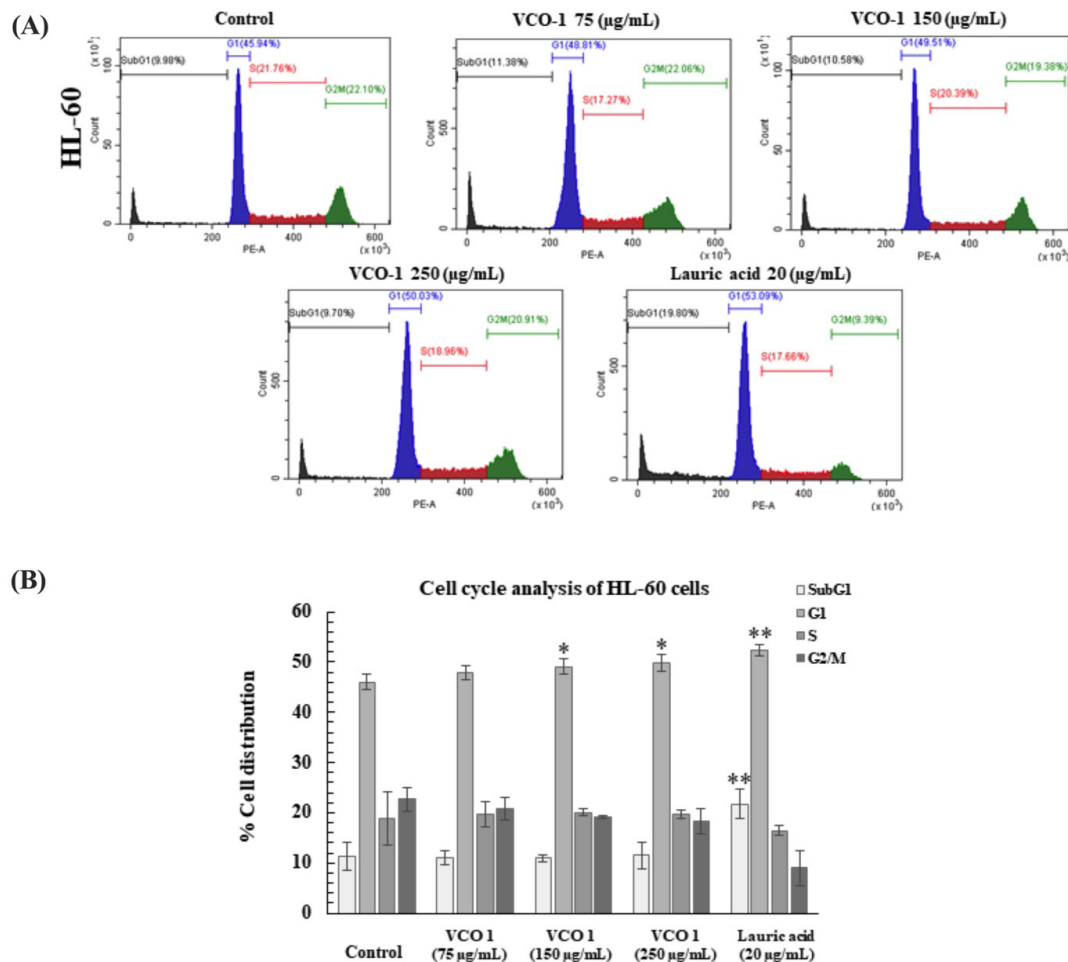


Figure 2. VCO No. 1 inhibits cell cycle progression in HL-60 cells. (A) Representative histograms of cell cycle distribution following 24 hours of treatment with VCO No. 1 or C-LA, stained with PI and analyzed by flow cytometry. (B) Quantitative analysis of cell cycle phases. Data are presented as mean $\pm$ SD from three independent experiments. * $p < 0.05$, ** $p < 0.01$ vs. control.

increased the Sub-G1 population from 11.30% to 21.70% and the G1-phase content from 46.07% to 52.37%, suggesting apoptotic DNA fragmentation. Collectively, these findings demonstrate that both handmade (VCO No. 1) and commercial (VCO No. 3) VCO samples similarly induce G1-phase arrest in HL-60 cells, suggesting comparable cell cycle-modulating effects despite differences in preparation.

VCO induces apoptosis in HL-60 cells

To evaluate whether the anti-proliferative activity of VCO is associated with the induction of apoptosis, HL-60 cells were treated with VCO No. 1 and VCO No. 3 at concentrations of 150 and 250 $\mu\text{g/mL}$ for 48 hours. C-LA (20 $\mu\text{g/mL}$) and doxorubicin (0.5 μM), a chemotherapeutic agent known to induce apoptosis, were included as positive controls. Apoptotic cells were quantified

using Annexin V-FITC/PI staining followed by flow cytometric analysis. As shown in Figures 4A and 5A, treatment with both VCO No. 1 and No. 3 resulted in a modest, dose-dependent increase in total apoptotic cells, reaching 7.55% and 7.50%, respectively, at 250 $\mu\text{g/mL}$. In contrast, C-LA and doxorubicin markedly induced apoptosis, increasing the apoptotic population to 20.44% and 19.26%, respectively (Figures 4B and 5B). Taken together, these findings indicate that VCO No. 1 and VCO No. 3 can induce apoptosis in HL-60 cells, although to a lesser extent than established pro-apoptotic agents.

DISCUSSION

The purpose of this study was to investigate the effects of VCO on the human promyelocytic leukemia cell line HL-60. VCO is known to contain a high proportion of medium-chain saturated

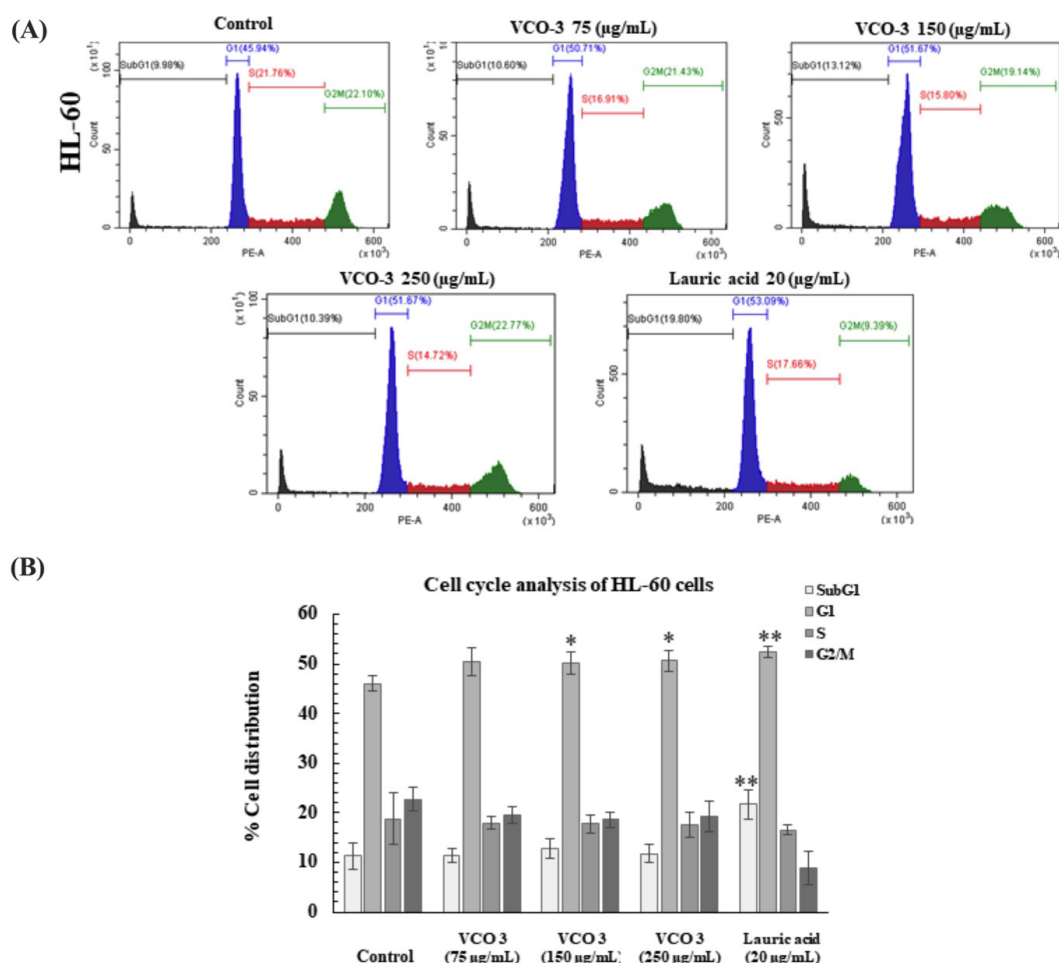


Figure 3. VCO No. 3 inhibits cell cycle progression in HL-60 cells. (A) Representative histograms of cell cycle distribution following 24 hours treatment with VCO No. 3 or C-LA, stained with PI and analyzed by flow cytometry. (B) Quantitative analysis of cell cycle phases. Data are presented as mean $\pm$ SD from three independent experiments. * $p < 0.05$, ** $p < 0.01$ vs. control.

fatty acids (MCFAs), particularly LA, which typically accounts for a substantial portion of its fatty acid content (17). In the present study, GC-MS analysis revealed that all five VCO samples (VCO No. 1, handmade; VCO No. 2-5, commercial) contained LA, caprylic acid, capric acid, myristic acid, palmitic acid, linoleic acid, oleic acid, and stearic acid. To evaluate their anti-leukemic potential, HL-60 cells were treated with the VCO samples and subsequently assessed for changes in cell viability, cell cycle distribution, and apoptosis. Treatment with VCO (No. 1-5) significantly reduced cell viability compared with the untreated control, indicating cytotoxic activity against leukemic cells. These results are consistent with previous studies reporting that crude *Houttuynia cordata* (Plu-Kao) extract exhibits cytotoxic effects in HL-60 cells (29), and that LA induces dose-dependent cytotoxicity in various cancer cell lines, including colorectal (HCT-15), hepatocellular (HepG2), and

murine macrophage (RAW 264.7) cells (14). Based on the identical fatty acid profiles determined by chemical fingerprinting and gas chromatography-mass spectrometry, together with the comparable cytotoxic concentrations observed in the MTT assay, VCO No. 1 was selected as the representative handmade VCO. In contrast, VCO No. 3 was chosen as the representative commercial VCO, as indicated by its product labeling stating “100% virgin coconut oil, high lauric acid, premium grade, and chemical-free”. When comparing VCO No. 1 and VCO No. 3, the viability assay demonstrated that VCO No. 3 exhibited slightly higher cytotoxic activity than VCO No. 1. Collectively, these findings support the idea that the bioactive components of VCO, particularly LA, may contribute to its observed anti-proliferative effects in leukemia cells.

Leukemia treatment continues to face major challenges due to the adverse side effects, relapse,

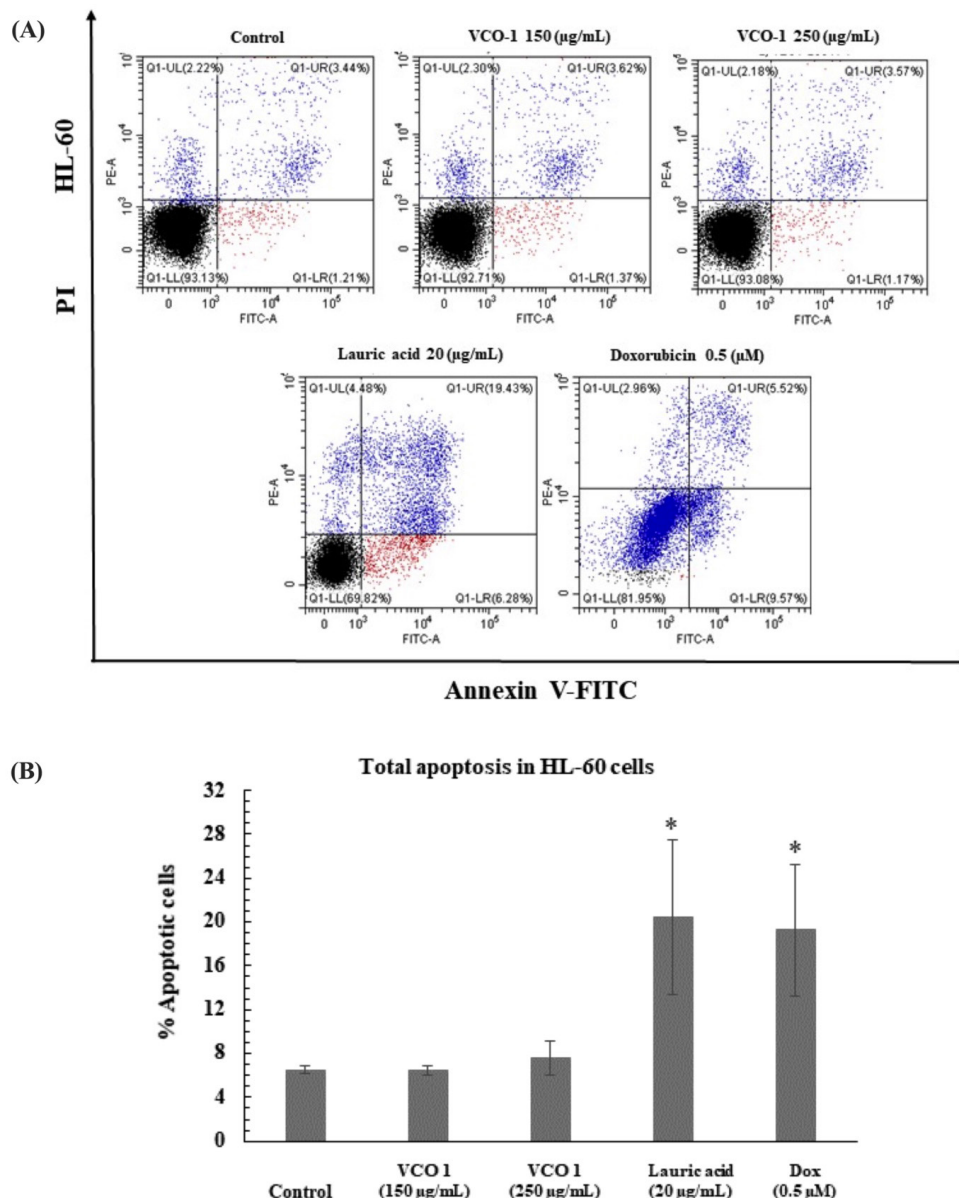


Figure 4. VCO No. 1 induces apoptosis in HL-60 cells. (A) Flow cytometric analysis of apoptosis in HL-60 cells treated with VCO No. 1 (150 and 250 µg/mL) for 48 hours, assessed using Annexin V-FITC/PI staining. Cells treated with 20 µg/mL C-LA and 0.5 µM doxorubicin were served as positive controls. (B) Quantification of total apoptotic cells. Data are presented as mean $\pm$ SD from at least three independent experiments. * $p < 0.05$ vs. control.

and drug resistance associated with conventional chemotherapeutic agents. Therefore, identification of alternative compounds with anti-leukemic potential remains an important objective in cancer therapy. In drug development, cell growth and viability assays are essential for determining the minimum effective concentration required to suppress cancer cell proliferation. In the present study, VCO demonstrated growth-inhibitory effects against HL-60 cells, as revealed by viability assays. Based on these findings, VCO No. 1 and No. 3 were selected for further analysis of cell cycle arrest and apoptosis. Both VCO No. 1 and

No. 3 induced G1-phase accumulation in HL-60 cells, with VCO No. 3 producing a slightly greater effect than VCO No. 1. C-LA also increased cell accumulation in both the Sub-G1 and G1-phases, indicative of apoptotic initiation. These results suggest that VCO inhibits leukemia cell proliferation, at least in part, by inducing G1-phase arrest and promoting apoptotic cell death. This conclusion is consistent with previous studies in colon cancer cells (Caco-2), where LA was reported to induce apoptosis and cell cycle arrest at multiple checkpoints, including G0/G1 and G2/M phases. Moreover, LA has been shown to elevate intra-

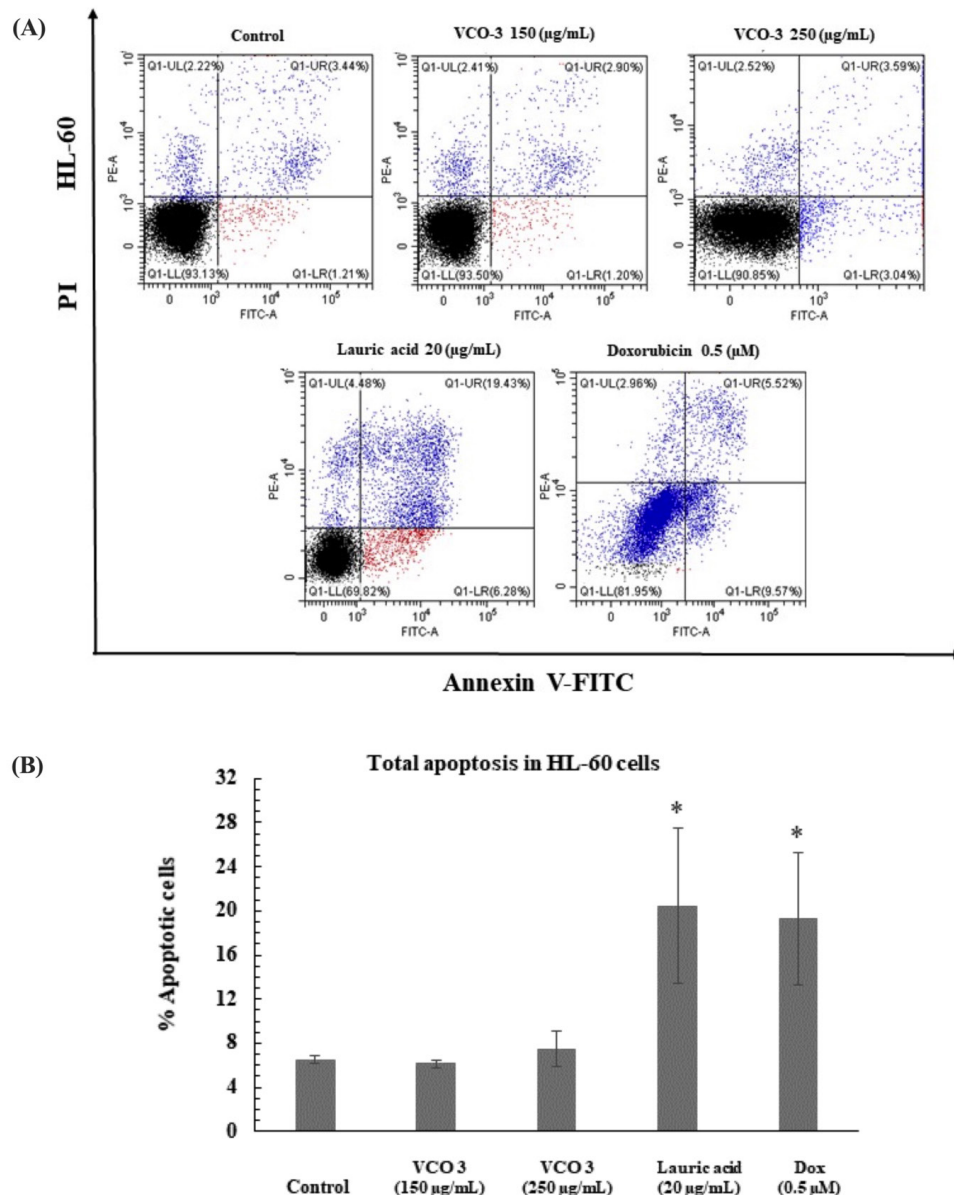


Figure 5. VCO No. 3 induces apoptosis in HL-60 cells. (A) Flow cytometric analysis of apoptosis in HL-60 cells treated with VCO No. 3 (150 and 250 µg/mL) for 48 hours, assessed using Annexin V-FITC/PI staining. Cells treated with 20 µg/mL C-LA and 0.5 µM doxorubicin were served as positive controls. (B) Quantification of total apoptotic cells. Data are presented as mean ± SD from at least three independent experiments. * $p < 0.05$ vs. control

cellular reactive oxygen species (ROS) and reduce glutathione levels, further contributing to its cytotoxic mechanisms (30, 31).

Apoptosis is a programmed cell death mechanism that plays a crucial role in maintaining tissue homeostasis. Through this process, damaged or abnormal cells are eliminated, allowing healthy cells to develop and maintain proper tissue architecture (21). Dysregulation of apoptotic pathways can lead to the persistence of defective cells, thereby contributing to malignant transformation, metastasis, and resistance to anti-cancer therapies (32). In the present study, the ability of VCO to induce

apoptosis in HL-60 cells is supported by its induction of G1-phase cell cycle arrest. Although Lappano et al. previously reported that LA can induce apoptosis in breast cancer cells, this study is the first to demonstrate the pro-apoptotic effects of VCO in HL-60 cells. In the apoptosis assay, treatment with VCO No. 1 and No. 3 led to a modest, dose-dependent increase in apoptotic cells. Both samples induced comparable levels of apoptosis, with VCO No. 1 exhibiting only a slightly higher apoptotic effect than VCO No. 3. In contrast, C-LA and doxorubicin significantly increased apoptotic cell populations in HL-60 cells. Taken together,

these findings indicate that VCO may function as an apoptosis inducer, as evidenced by the elevated apoptotic ratios observed following treatment in HL-60 cells.

These findings highlight the cytotoxic effects of VCO on HL-60 cells through the induction of cell cycle arrest and apoptosis, potentially attributable to their LA content. However, the effective concentrations of VCO (269–300 µg/mL) are substantially higher than in C-LA (20 µg/mL). Consequently, in vivo and clinical applications would necessitate administering VCO predominantly composed of medium-chain triglycerides (MCTs; C6–C12) at relatively high doses. This requirement is consistent with a previous study demonstrating the feasibility of high-dose MCT supplementation. For example, a modified diet containing 25% 8- and 10-carbon MCTs was shown to enhance the efficacy of anti-cancer therapy in a CD-1 Nu mouse model of neuroblastoma (33). Moreover, the clinical application of MCTs for their anti-cancer and therapeutic potential has been documented in several clinical studies. Notably, Schmidt et al. conducted a pilot clinical trial in patients with advanced metastatic tumors to evaluate a ketogenic diet enriched with MCTs. Patients who adhered to the three-month dietary regimen, consisting of an oil-protein mixture containing 21 g of MCT-rich fat, 5 g of carbohydrates, and 14 g of protein, exhibited marked improvements in physical well-being and evidence of tumor reduction, without adverse effects on cholesterol or blood lipid profiles (34).

The present study, together with previous evidence, indicates that VCO exerts inhibitory effects across the three major leukemia subtypes prevalent in Thailand, including chronic myeloid leukemia (CML; represented by K562), acute lymphoblastic leukemia (ALL; represented by MOLT-4) (28), and acute myeloid leukemia (AML; represented by HL-60). The consistent growth suppression observed among these cell lines suggests that VCO may target fundamental cellular processes shared across both myeloid and lymphoid leukemias. Mechanistically, the anti-proliferative effects appear to be mediated, at least in part, through the induction of cell cycle arrest and the activation of apoptotic pathways. Moreover, based on the viability assay, VCO exhibited the greatest cytotoxic potency against the

AML cell line (HL-60), followed by the CML cell line (K562) and the ALL cell line (MOLT-4), respectively (28). These results collectively support the potential of VCO or its bioactive constituents as a complementary therapeutic candidate for leukemia. Nonetheless, further comprehensive investigations are needed to elucidate the specific molecular mechanisms underlying these effects.

CONCLUSIONS

VCO contains both saturated and unsaturated fatty acids, including LA, caprylic, capric, myristic, palmitic, linoleic, oleic, and stearic acids. This study is the first to demonstrate that VCO exerts inhibitory effects on human promyelocytic leukemia HL-60 cells through the induction of G1-phase arrest and apoptosis. Altogether, these results indicate that VCO may possess anti-leukemic potential by modulating cell cycle progression and triggering apoptotic cell death. Moreover, due to its natural origin, VCO holds promise for integration into complementary or alternative therapeutic strategies. However, further in vivo studies and mechanistic investigations are required to confirm its efficacy and therapeutic potential.

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CONFLICTS OF INTEREST

There are no conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

AUTHOR CONTRIBUTIONS

S.S.: project administration, conceptualization, methodology, formal analysis, software, writing — original draft preparation, writing — review and editing; A.W.: formal analysis; N.D.: investigation; N.P.: funding acquisition.

DATA AVAILABILITY STATEMENT

The data that support the finding of this study are available from the corresponding author upon reasonable request.

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable, as this study did not involve human participants.

INFORMED CONSENT STATEMENT

Not applicable, as this study did not involve human participants.

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