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Virgin Coconut Oil Inhibits Leukemic Cell Proliferation Via Apoptosis in K562 and MOLT-4 Cell Lines

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ABSTRACT

OBJECTIVE Leukemia is a blood cancer which is expressed by an uncontrolled increase in abnormal white blood cells. To date, chemotherapy has been regularly used to treat leukemia. However, the major problem with chemotherapy is the side effects for leukemia patients. Nowadays, medicinal plants are of interest as an additional form of cancer treatment. Virgin coconut oil (VCO) is a source of antioxidants, anti-viral, anti-inflammatory, and anti-cancer properties. This study was designed to investigate the potential of VCO to inhibit the growth of leukemic cells by inducing cell cycle arrest and apoptosis.

METHODS The VCO was analyzed using gas chromatography coupled with mass spectrometry (GC-MS). In the experiment, two leukemic cell lines, K562 and MOLT-4, were treated with various concentrations of VCO for 48 h. Cytotoxic effects were measured using the MTT assay and compared to commercial lauric acid. Cell cycle analysis was performed using propidium iodide (PI) staining, and apoptosis was assessed using an Annexin V-FITC kit.

RESULTS The study found that VCO is primarily composed of lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, linoleic acid, oleic acid, and stearic acid. In addition, VCO showed cytotoxic effects in both K562 and MOLT-4 cells, with IC_{50} values of 618.2 µg/mL and between 654 and 760 µg/mL, respectively. Cell cycle analysis demonstrated that VCO induced accumulation of cells in the G2/M phase. Additionally, the apoptosis assay demonstrated that VCO treatment induces apoptosis, particularly in K562 cells, in a cell-dependent manner.

CONCLUSIONS VCO is a promising source of bioactive compounds which exhibit cytotoxic, cell cycle arrest and apoptotic effects on leukemic cells *in-vitro*, suggesting its potential for further investigation as an anti-leukemic agent.

KEYWORDS virgin coconut oil, lauric acid, leukemia, apoptosis, K562, MOLT-4

INTRODUCTION

Cancer is a major cause of morbidity and mortality worldwide, with nearly 20 million new cases and 9.7 million cancer-related deaths in 2022 (1). The number of new cases is anticipated to increase by 70% over the next 2 decades. Approximately one-third of cancer deaths are caused by habit-related factors such as high body mass index, lack of physical activity, low fruit and vegetable consumption, tobacco use, and alcohol use. Leukemia is among the most prevalent cancers worldwide (2, 3). In 2018, the Thai population was close to 65 million. The number of new cancer cases registered that year was 170,500 with 114,200 cancer deaths (4). In Thailand, 21 percent of the population is < 15 years of age; the number of new cancer cases in this age group is approximately 1,000/year (5). During 2007-2008 the cancer related mortality rates in Thai children aged 6-12 and 13-18 were 2.16 and 2.13 per 100,000, respectively. Leukemia is the most common cancer in Thai children (about 30.00%), followed by cancer of the brain and other areas of the nervous system (20.00%), lymphoma (18.00%), renal tumors (10.00%), and others (22.00%) (6).

Leukemia is a type of malignant neoplasm associated with organs that produce blood cells which is identified by an uncontrollable increase of blood cells (7). Development of leukemia is associated with exposure to radiation, toxic chemicals, chemotherapeutic agents, viral and other microorganisms as well as genetic disorders. It is amplified by chromosomal instability, cigarette smoking, and other environmental factors which can lead to genetic changes in leukemic cells that in turn affect the functions of other genes, including tumor suppressor genes and oncogenes. The disease can rapidly proliferate and can be classified as acute or chronic, myeloid or lymphoid (8). The forms of leukemia that impact the myeloid and lymphoid systems include acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL) (9).

Presently, chemotherapy is very often utilized for the treatment of leukemia. It has had a very good outcome in the early stages of treatment (10). However, the main problem of chemotherapy is the side effects on the leukemia patients, e.g., it can result in drug resistance after a long period of treatment. Active compounds from medicinal plants normally included in compilations of traditional Thai drug recipes represent a source of potential additional options for cancer treatment.

Medicinal plants represent local natural resources which long have been associated with Thai culture, e.g., as basic foods and as traditional medicines. Most medicinal plants are relatively inexpensive and are easy to find in many local areas, and most have been demonstrated to be safe and to have few side effects. Coconut oil is widely used worldwide for both food and non-food purposes. It is available in different forms, including virgin coconut oil (VCO), crude coconut oil (CCO), and refined coconut oil (RCO) (11). VCO, commonly consumed in Asia, particularly in Thailand, Malaysia, and the Philippines, is extracted from fresh coconut milk under controlled temperatures to preserve its bioactive compounds (12). These bioactive components include vitamins and polyphenols (e.g., ferulic acid, coumaric acid, gallic acid, catechin, and kaempferol or their sugar derivatives) (13, 14).

Apoptosis, or programmed cell death, is characterized by distinct morphological and biochemical changes, including cell shrinkage, cytoplasmic condensation, chromatin condensation, DNA fragmentation, and plasma membrane blebbing. It plays a crucial role in maintaining tissue homeostasis by regulating cell populations, allowing the removal of aged or damaged cells while facilitating the growth of new ones. However, carcinogens can disrupt apoptotic pathways, enabling uncontrolled cancer cell proliferation (15). Basically, cell division is regulated by cell cycle control mechanisms to ensure the production of identical cells. 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 can normally be divided into 2 major phases, the interphase and the M phase. During the interphase, there will be duplicates of cellular content within the cells and separated into two genetically identical daughter cells in mitosis. DNA replication occurs during the interphase during the S-phase (the synthesis phase). The interphase period, which separates the S-phase from the M-phase, is called the Gap or G1 precede the S phase and G2 phase after the

S phase. According to observational evidence, there is a gap between two main events, the duplication and dissociation of DNA. These phases are important moments for cell cycle regulation and include the decision to enter the cell cycle in the G1 and to initiate the process leading to chromosome segregation in the G2. In unicellular and multicellular eukaryotes, cell division is controlled by a complex network of control, monitoring and balancing mechanisms to ensure that no errors occur before the cells are allowed to enter and progress through the cell cycle and to divide. Checkpoints can delay cell cycle progression, or, in response to irreparable DNA damage, induce cell cycle exit or cell death (16).

VCO contains a high amount of medium-chain saturated fatty acids (MCFAs), particularly lauric acid which comprises approximately 45.00-52.00% of its total fatty acid content (17). Lauric acid has been reported to exhibit antimicrobial properties (18). For example, lauric acid and its derivative monolaurin have been shown to disrupt the cell membranes of Gram-positive bacteria and lipid-coated viruses. Additionally, they interfere with cellular responses by stimulating transduction cascades and gene transcription. Beyond its antimicrobial activity, lauric acid also plays a role in anti-inflammation by inhibition of the nuclear factor-κB (NF-κB) transcription factor activation and the phosphorylation of MAP kinases (19). Moreover, VCO has demonstrated dose-dependent cytotoxicity in various cancer cell lines, including HCT-15 (human colon cancer), HepG2 (human hepatocellular carcinoma), and RAW 264.7 (murine macrophages), by inducing apoptotic morphological changes (14). Furthermore, consuming VCO during chemotherapy has been associated with improved quality of life in breast cancer patients (20). VCO has also exhibited antiviral, anti-inflammatory (13), and antidiabetic properties (17), as well as therapeutic potential for skin disease (17). Additionally, low-dose VCO treatment has been reported to reverse hepatic structural alterations and certain biochemical changes in obese rats (21). VCO has also been shown to enhance resistance to methotrexate-induced oxidative stress and inflammation in rats (22). However, few studies have explored the anticancer properties of VCO. Calderon et al. reported that VCO inhibited the growth of SKBr-3 breast cancer cells (23), while Verma et al. demonstrated its anticancer effects in liver and oral cancer models (24). Currently, no studies have investigated the effects of VCO on cell proliferation, cell cycle arrest, and apoptosis in K562 (human erythroleukemia) and MOLT-4 (T-cell leukemia) cell lines. Therefore, the objective of this study was to evaluate the antiproliferative effects of VCO in these leukemic cell lines by assessing its ability to induce cell cycle arrest and apoptosis.

METHODS

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 obtained from Cytiva HyClone™ (Logan, UT, USA). MTT dye and commercial lauric acid were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Test materials

Five samples of 100% pure VCO were used in this study. Sample No. 1 was prepared traditionally using indigenous methods by a local villager. Samples No. 2-5 were commercially available and obtained from a pharmacy (Chiang Mai University Pharmacy, Chiang Mai, Thailand).

Cells and cell culture condition

The human CML cell line K562 (ATCC® CCL-243™) and the human ALL cell line MOLT-4 (ATCC® CRL-1582™) were obtained from the Cell Bank of the Division of Immunology, Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University (Chiang Mai, Thailand). K562 and MOLT-4 cells were cultured in RPMI 1640 medium supplemented with 10% FBS, 25 mM HEPES, L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin. The cells were maintained in a humidified incubator at 37°C with 5% CO_2.

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

The VCO samples (No. 1-5) were pre-processed as follows. A 20 mg aliquot of each sample was mixed with 1 mL of methanolic NaOH and heated

at 65°C for 15 min with intermittent shaking. After incubation, the samples were cooled to room temperature for 5 min, followed by the addition of 1 mL of Milli-Q water and 1 mL of distilled hexane. The mixture was vortexed and allowed to undergo phase separation. The upper organic layer was carefully withdrawn using a micropipette without disturbing the lower aqueous phase. The hexane extract was transferred to a fresh GC vial for GC-MS analysis. Fatty acid separation was performed using a VF-5ms column on a Trace GC Ultra gas chromatograph under the following conditions. The injection port was maintained at 250°C, and the samples were introduced through a split injection mode (50 : 1 split ratio). The oven temperature was initially set at 50°C, then increased to 120°C and held for 5 min. The temperature was further ramped up to 170°C at a rate of 5°C/min and then held for 5 min, followed by an increase to 230°C at 5°C/min and then held for 5 min. Finally, the temperature was raised to 250°C at 5°C/min and held for 2 min. The separated compounds were analyzed using a TSQ Quantum XLS mass spectrometer, and the total ion chromatogram was recorded in scan mode. Mass spectra were acquired for ions ranging from m/z 50 to 500.

MTT cytotoxicity assay

The cytotoxic effects of VCO No. 1 (hand-made) and VCO No. 2-5 (commercially manufactured) were evaluated using the MTT assay. The VCO No. 1-5 were dissolved in dimethyl sulfoxide (DMSO) as a stock substance at a concentration of 25 mg/mL. Then the stock substance was diluted by 0.2% DMSO in RPMI 1640 medium to concentrations between 0-3,200 µg/mL. Briefly, K562 and MOLT-4 cells (1.0×10^4 cells/well) were seeded in 96-well plates containing 100 µL of culture medium and incubated for 24 h. Following incubation, 100 µL of fresh medium containing various concentrations of VCO (0-3,200 µg/mL) was added to each well, and the cells were incubated for an additional 48 h. After treatment, 15 µL of MTT dye solution per 100 µL of medium was added to each well, and the plates were incubated at 37°C in a humidified 5% CO_2 atmosphere for 4 h. Subsequently, 200 µL of DMSO was added to dissolve the formazan 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. Higher optical density values corresponded to a greater number of viable cells capable of metabolizing MTT salts.

Cell viability was calculated using 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 rate, obtained from triplicate determinations at each concentration, was plotted as a dose-response curve. The experiment was conducted in three independent trials to ensure reproducibility. The 50.00% inhibitory concentration (IC_{50}) of VCO was determined as the lowest concentration that reduced cell viability by 50.00% compared to the untreated control or the vehicle control (0.2% DMSO in culture medium). The IC_{50} values were expressed as the mean ± standard deviation (SD) and were compared to evaluate cytotoxic activity (25). The IC_{50} values (cytotoxic doses) from the VCOs were compared to lauric acid with regard to their effects on cell cycle analysis and apoptosis assay.

Cell cycle analysis

K562 and MOLT-4 cells (5×10^5 cells/well) were seeded into 6-well plates and treated with vehicle control, VCO (No. 1 represents hand-made VCO and No. 3 represents commercial VCO with bottle identification "100% VCO, high lauric acid, premium grade, and chemical free", or lauric acid for 24 h. Following treatment, the cells were harvested, washed twice with PBS, and fixed in cold 70% ethanol at -20°C overnight. The ethanol was carefully removed by centrifugation at 1,500 x g for 3 minutes, and the cells were washed with PBS to minimize cell loss. The pelleted cells were then resuspended in 1 mL of staining solution containing 25 µg/mL RNase (United States Biological, Salem, MA, USA) and 50 µg/mL propidium iodide (PI) (Merck Millipore, Darmstadt, Germany), and incubated in the dark at room temperature for 30 minutes. Cell cycle distribution was analyzed using flow cytometry (Beckman Coulter DxFLEX, Brea, CA, USA).

Apoptosis detection via Annexin-V-FITC/PI assay

K562 and MOLT-4 cells (3×10^5 cells/well) were seeded into 6-well plates and treated with vehicle control, VCO (No. 1 and No. 3), or lauric acid at the indicated concentrations for 48 h. Following treatment, the cells were harvested by centrifugation at 1,500 × g for 3 minutes at room temperature and washed twice with ice-cold PBS. The cells were then stained with Annexin V-FITC/PI (Elabscience, Houston, TX, USA) following the manufacturer's instructions and analyzed using flow cytometry.

Statistical analysis

All data are presented as mean ± SD from the three independent experiments. The comparison between the treated and control group was performed using Student's *t*-test (for comparisons of two treatment group) (26, 27). Differences were considered statistically significant if $p < 0.05$ (*), highly significant if $p < 0.01$ (**), and extremely significant if $p < 0.001$ (***).

RESULTS

Characterization of VCO fatty acid composition

The GC-MS analysis of VCO No. 1-5 revealed an identical fatty acid composition across all samples (Figure 1A-1E), indicating consistency in their chemical profiles. The identified fatty acids included lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, linoleic acid, oleic acid, and stearic acid. These findings confirm the uniformity of the VCO samples, ensuring that any observed biological effects are attributable to the intrinsic properties of VCO rather than compositional variability.

Cytotoxic effects of VCO on K562 and MOLT-4 cell lines

As shown in Table 1, the IC_{50} value of VCO samples (No. 1-5) in K562 cells was 618.2 µg/mL, whereas lauric acid exhibited significantly higher cytotoxicity, with an IC_{50} of 42.3 µg/mL. Similarly, in MOLT-4 cells, the IC_{50} values for VCO samples ranged from 654.5 to 760.0 µg/mL (No. 1-5: 690.9, 654.5, 654.5, 690.9, and 760.0 µg/mL, respectively). In contrast, lauric acid displayed a markedly lower IC_{50} of 42.2 µg/mL, indicating its greater potency in inhibiting cell viability. The dose-dependent cytotoxic effects of VCO and lauric acid on K562 and MOLT-4 cells are illustrated in Figures 2 and 3. A progressive decline in cell viability was observed with increasing concentrations of both treatments. Notably, lauric acid induced a more pronounced reduction in cell viability at lower concentrations, further supporting its stronger cytotoxic potential compared to VCO.

VCO induces cell cycle arrest in K562 and MOLT-4 cells

The effect of VCO on cell cycle progression in K562 and MOLT-4 cells were determined. K562 cells was treated with varying concentrations (close to the IC_{50} values) of VCO No. 1 and VCO No. 3 compared to (close to the IC_{50} values) lauric acid for 24 h to evaluate their impact on cell cycle progression. As shown in Figure 4A, treatment with VCO No. 1 at 150, 300, and 500 µg/mL resulted in a dose-dependent decrease in S-phase DNA content from 29.11% to 24.91%, 25.06%, and 23.20%, respectively. Concurrently, the G2/M-phase DNA content significantly increased from 23.75% in the control to 28.06%, 32.43%, and 32.16%, respectively. Similarly, treatment with VCO No. 3 at 150, 300, and 500 µg/mL (Figure 4B) led to a decrease in S-phase DNA content from 29.11% to 25.00%, 23.30%, and 21.79%, respectively, while the G2/M-phase DNA content significantly increased from 23.75% to 28.66%, 31.13%, and 30.14%, respectively. These findings indicate that both VCO No. 1 and VCO No. 3 induce G2/M-phase cell cycle arrest in K562 cells in a dose-dependent manner. In contrast, treatment with 40 µg/mL lauric acid for 24 h reduced the G2/M-phase DNA content from 23.75% to 18.97%, while significantly increasing the G1-phase DNA content from 41.80% to 51.51% compared to the control. In MOLT-4 cells, treatment with 300 µg/mL VCO No. 1 significantly increased the G2/M-phase DNA content from 26.57% to 30.19%, while the S-phase population slightly decreased from 29.59% to 29.06% compared to the control (Figure 5A). Similarly, treatment with 300 µg/mL VCO No. 3 significantly increased the G2/M-phase DNA content from 26.57% to 30.31%, while the S-phase population decreased from 29.59% to 27.68% compared to the control (Figure 5B). Treatment with 40 µg/mL lauric acid induced a significant increase in the G1-phase population from 38.10% to 55.00%, while reducing the

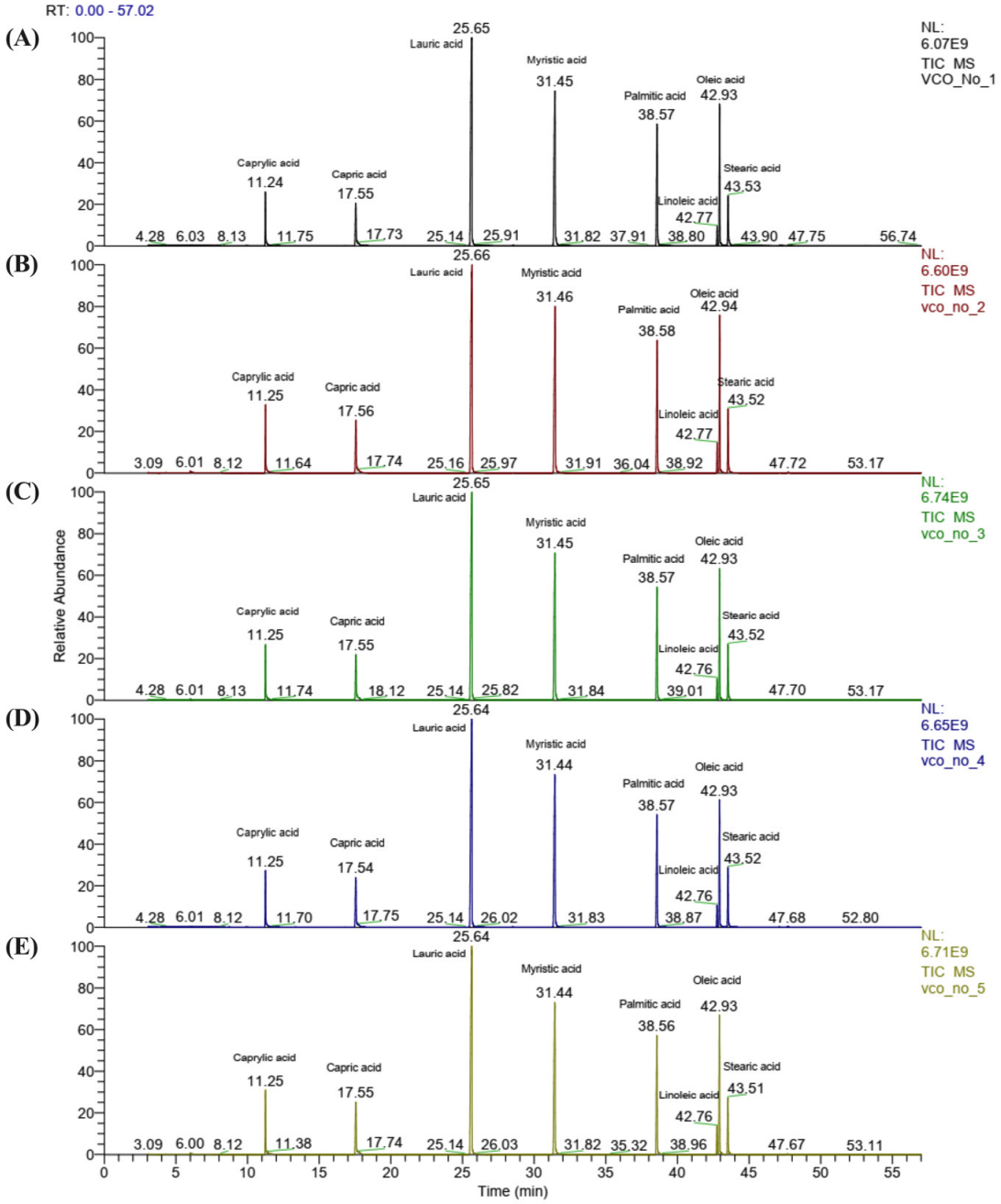

Figure 1. GC-MS characterization of the fatty acid profile in VCO No. 1-5. Total ion chromatograms of (A) VCO No. 1, (B) VCO No. 2, (C) VCO No. 3, (D) VCO No. 4, and (E) VCO No. 5, with retention times labeled at each peak.
VCO, virgin coconut oil

Table 1. IC_{50} values (mean±SD) after VCO No. 1-5 and lauric acid treatments in K562 and MOLT-4 cell lines

Compounds	IC_{50} (μg/mL)	
	K562	MOLT-4
VCO No. 1	618.2±4.94	690.9±1.28
VCO No. 2	618.2±2.94	654.5±4.20
VCO No. 3	618.2±3.15	654.5±4.02
VCO No. 4	618.2±1.37	690.9±1.29
VCO No. 5	618.2±1.98	760.0±1.85
Lauric acid	42.3±6.90	42.2±0.41

VCO, virgin coconut oil

S-phase from 29.60% to 24.30%, and the G2/M-phase from 26.57% to 14.24% in MOLT-4 cells (Figure 5A-5B).

These findings indicate that VCO No. 1 and VCO No. 3 promote G2/M-phase cell cycle arrest in a dose-dependent manner in both K562 and MOLT-4 cells. In contrast, lauric acid induces G1-phase accumulation, suggesting distinct mechanisms of action. The observed alterations in cell cycle distribution imply that VCO may exert cytostatic effects by delaying G2/M-phase

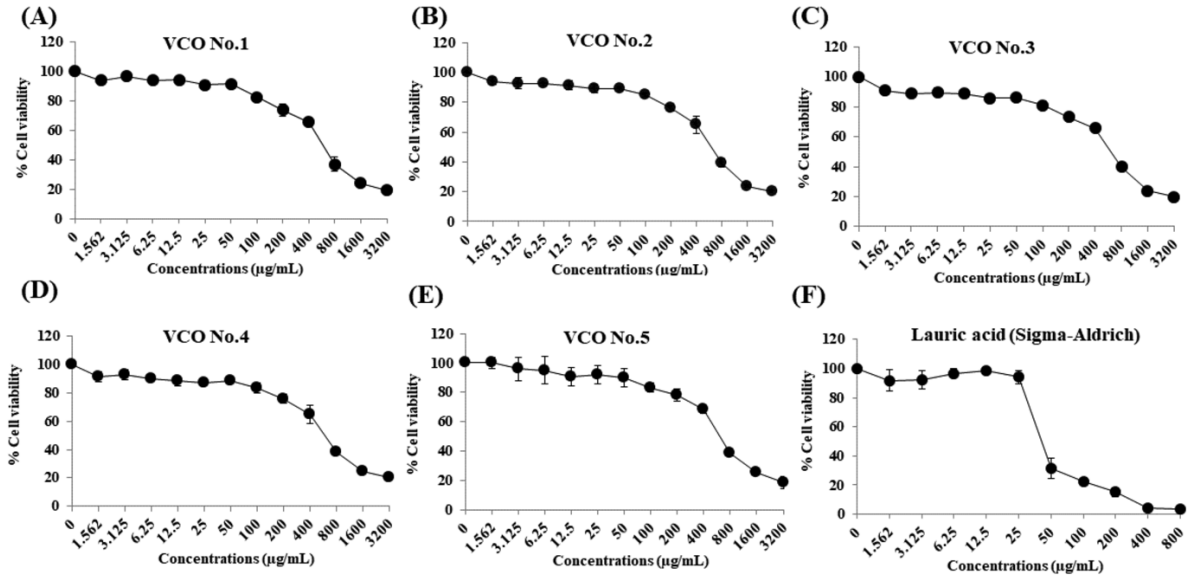

Figure 2. Cytotoxic effects of VCO No. 1-5 (A-E) and lauric acid (F) on K562 cells. Data are presented as mean ± SD from three independent experiments. VCO, virgin coconut oil.

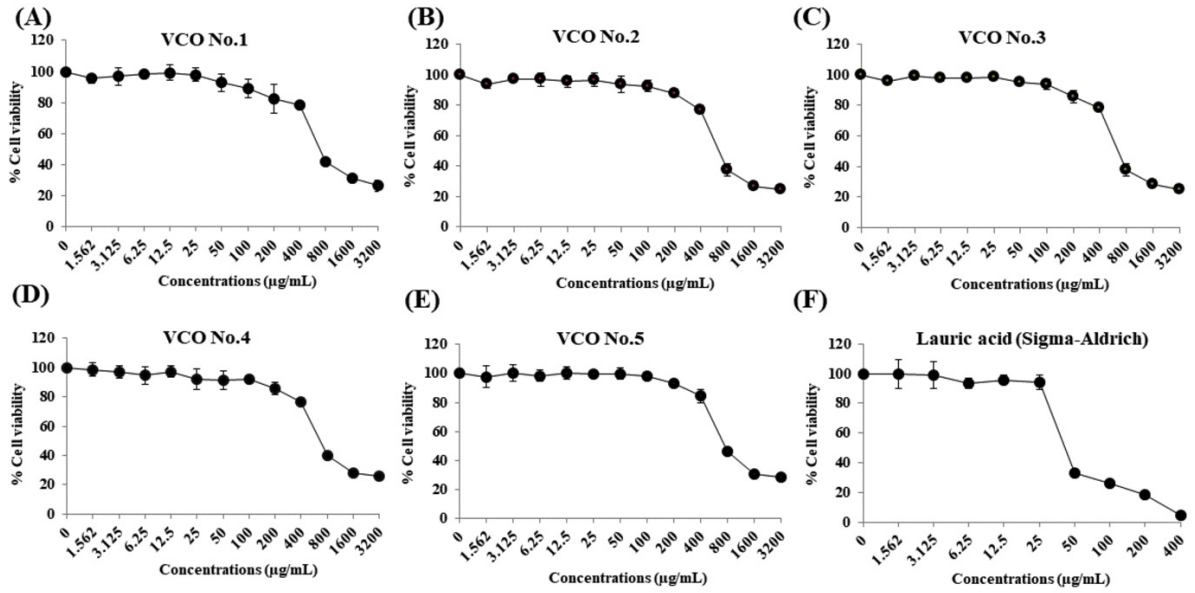

Figure 3. Cytotoxic effects of VCO No. 1-5 (A-E) and lauric acid (F) on MOLT-4 cells. Data are presented as mean ± SD from three independent experiments. VCO, virgin coconut oil

progression, whereas lauric acid facilitates G1-phase arrest. The results of chemical fingerprinting and fatty acid analysis using gas chromatography-mass spectrometry (GC-MS) showed that VCO contains a variety of fatty acids, including lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, linoleic acid, oleic acid, and stearic acid. Fatty acids other than lauric acid may cause changes in the arrest of cells in the cell cycle after treatment with VCO, which is different from treating only lauric acid in both cell lines.

Induction of apoptosis by VCO in K562 and MOLT-4 Cells

To examine VCO's role in apoptosis, we assessed the effects of VCO No. 1 and VCO No. 3 in K562 cells. Our results indicate that treatment with 300 and 500 µg/mL of VCO No. 1 for 48 h significantly increased apoptosis in a dose-dependent manner, from 4.69% in the control to 8.23% and 10.01%, respectively (Figure 6A). Likewise, treatment with 300 and 500 µg/mL VCO No. 3 resulted in significant increases in apoptosis, from 4.69% to 8.66% and 10.50%, respectively (Figure

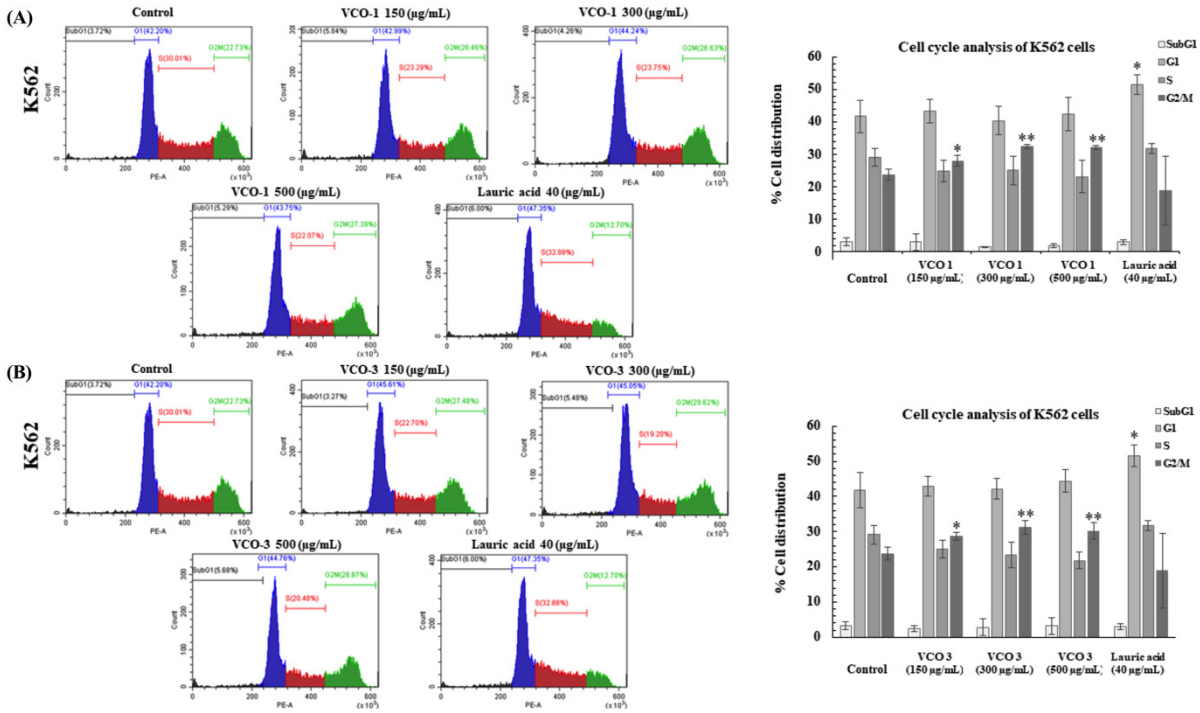

Figure 4. VCO inhibits cell cycle progression. (A) VCO No. 1 and (B) VCO No. 3. Data are presented as the mean ± SD of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$. VCO, virgin coconut oil.

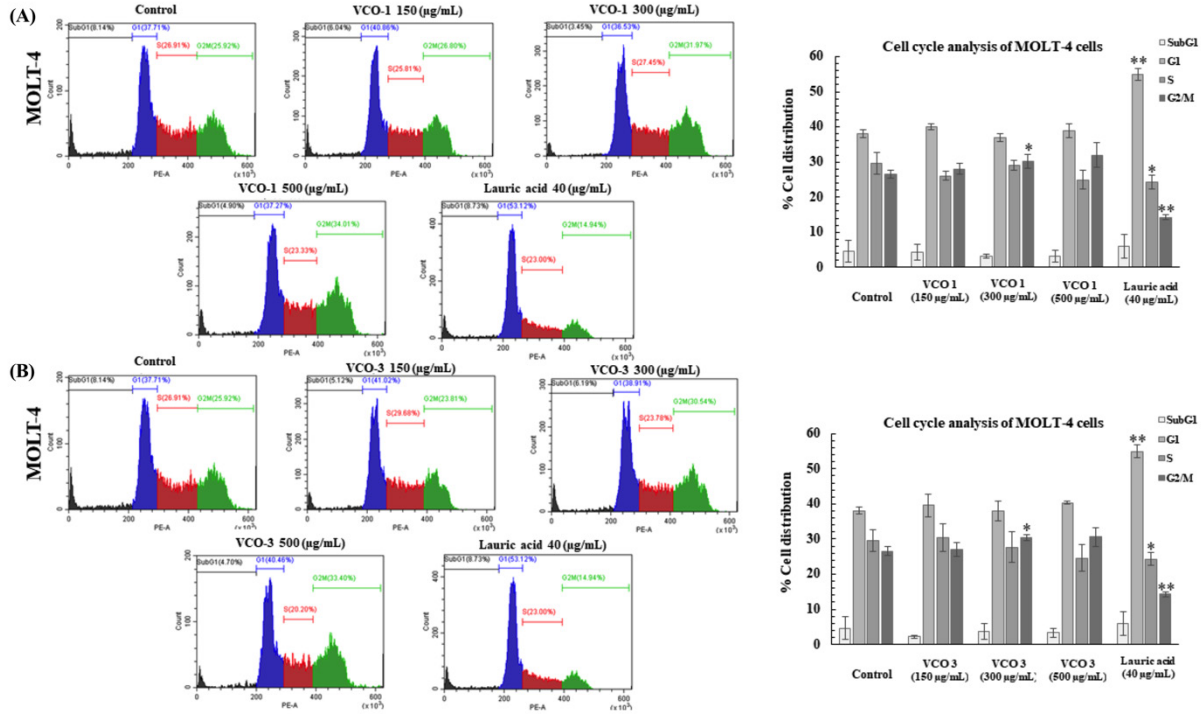

Figure 5. VCO inhibits cell cycle progression. (A) VCO No. 1 and (B) VCO No. 3. Data are presented as mean ± SD from three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$. VCO, virgin coconut oil.

6B). Our findings further demonstrate that treatment with 40 μg/mL lauric acid markedly increased apoptotic cell death to 26.57%, while 1 μM doxorubicin induced apoptosis at a rate of 21.82%, both significantly higher than the control (Figure 6B). For the MOLT-4 cells, treatment with 300 and 500 μg/mL VCO No. 1 and VCO No. 3 for 48 h did not result in a significant increase in

apoptosis compared to the control (Figure 7A-7B). In contrast, treatment with 40 µg/mL lauric acid significantly increased apoptotic cell death from 5.53% to 9.38% (Figure 7B). Additionally, treatment with 1 µM doxorubicin induced apoptosis at a rate of 15.85%, which was significantly higher than the control (Figure 7B).

These findings indicate that VCO No. 1 and VCO No. 3 promote apoptosis in K562 cells in a dose-dependent manner, whereas lauric acid and doxorubicin significantly increased apoptotic cell death compared to control. In contrast, VCO treatment did not significantly enhance apoptosis in MOLT-4 cells, while lauric acid and doxorubicin significantly increased apoptotic cell death, suggesting a differential apoptotic response between the two cell lines.

DISCUSSION

Natural products have been widely utilized in traditional medicine because of their diverse bioactive compounds, which play a crucial role in drug discovery and development (28). Among them, VCO has garnered significant attention for its potential health benefits, including hypocholesterolemic, antidiabetic, hepatoprotective,

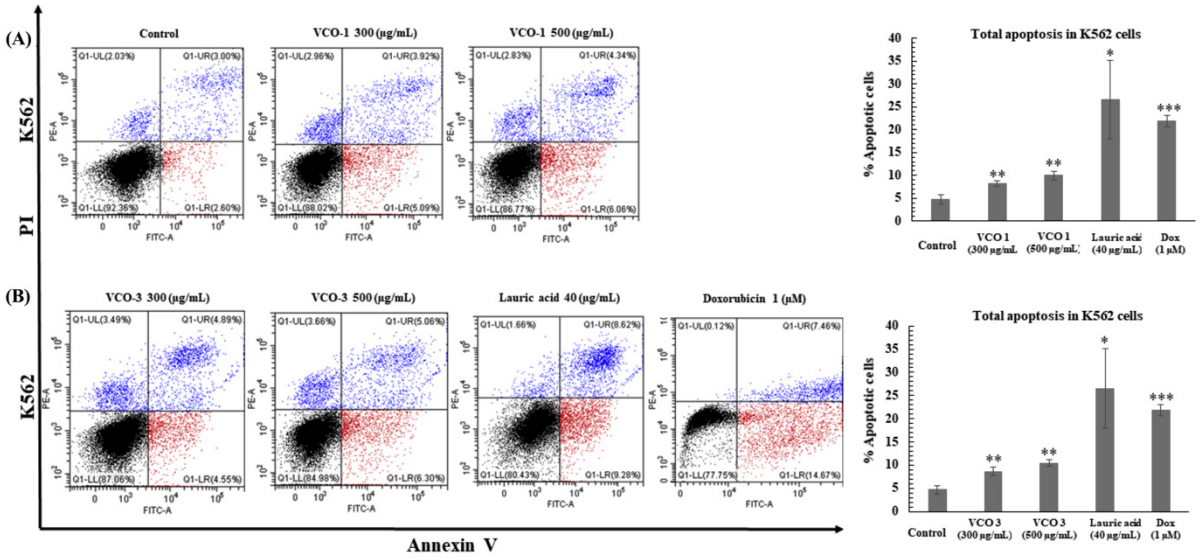

Figure 6. VCO induces apoptosis in K562 cells. (A) Effects of VCO No. 1 and (B) VCO No. 3 on apoptosis induction. Data are presented as the mean ± SD of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.
VCO, virgin coconut oil

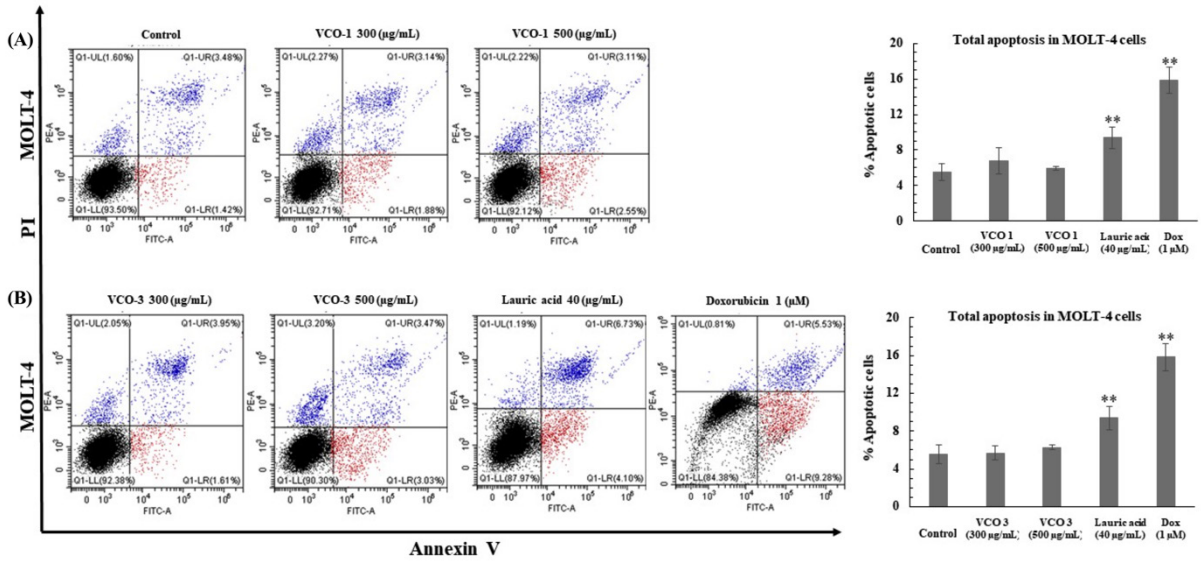

Figure 7. VCO induces apoptosis in MOLT-4 cells. (A) Effects of VCO No. 1 and (B) VCO No. 3 on apoptosis induction. Data are presented as the mean ± SD of three independent experiments. $^{*}p < 0.05$ and $^{**}p < 0.01$. VCO, virgin coconut oil.
VCO, virgin coconut oil

antioxidant, anti-inflammatory, antimicrobial, skin-moisturizing, and wound-healing properties. These effects are primarily attributed to its medium-chain fatty acids (MCFAs), particularly lauric acid (C12), which constitutes approximately 50% of VCO (17, 29-35). Despite its well-documented bioactivities, the effects of VCO on leukemia cells remain largely unexplored. To address this gap, this study investigate the effects of VCO on K562 and MOLT-4 leukemic cells, the first study to do so. Five VCO samples, including one hand-made (VCO No. 1) and four commercially manufactured (VCO No. 2-5), were analyzed. Their anti-leukemic potential was assessed by evaluating cell cycle arrest and apoptosis induction, with comparisons to lauric acid. The findings revealed that VCO exposure led to a marked decrease in leukemic cell survival, suggesting its cytotoxic potential. This effect may be attributed to the high lauric acid content in VCO, as previous studies have shown that MCFAs can disrupt cancer cell metabolism and induce apoptosis. Similarly, lauric acid exhibited strong cytotoxicity, further supporting the role of lauric acid in mediating these effects. Consistent with these findings, Tima et al. reported that *Houttuynia cordata* extract exerted cytotoxic effects on leukemic cells at comparable concentrations (36). Additionally, Sheela et al. demonstrated that lauric acid induced dose-dependent cytotoxicity in various cancer cell lines, including colorectal and liver cancer, as well as macrophages (14). These findings further highlight the potential of lauric acid as a key bioactive component contributing to the cytotoxic activity of VCO.

Based on the viability assay results, all VCO samples exhibited comparable cytotoxicity. Therefore, VCO No. 1 (hand-made) and No. 3 (manufactured) were selected to represent traditional and commercial preparations in further analyses of cell cycle arrest and apoptosis. Both induced G2/M phase arrest in K562 and MOLT-4 cells, whereas lauric acid treatment led to G1 phase accumulation. In addition, it has been reported that lauric acid induced apoptosis, reduced colon cancer cells (Caco2) and IEC-6 cells in the G0/G1 phase, resulting in S and M phase cell cycle arrest, increased reactive oxygen species (ROS), and depleted intracellular glutathione levels (37, 38). Similarly, Li et al. reported that EM23, a sesquiterpene lactone from *Elephantopus mollis*, induced apoptosis and G2/M or S phase arrest in K562 and HL-60 cells (27). Collectively, these results suggest that VCO suppresses cell proliferation and induces cell cycle arrest, leading to apoptosis in leukemic cells.

Apoptosis is crucial for tissue homeostasis, eliminating damaged cells while preserving healthy proliferation (15). Disruptions in this process can lead to mutations, malignant transformation, metastasis, and therapy resistance (39). The apoptotic effect of VCO in K562 and MOLT-4 cells was evidenced by G2/M phase arrest. Lappano et al. demonstrated that 100 μM (20 μg/mL) lauric acid induces apoptosis in breast cancer cells (40); however, the present study is the first to report the apoptotic effects of VCO on leukemic cell lines. Apoptosis assays revealed a significant increase in apoptotic K562 cells following treatment with VCO No. 1 and No. 3, whereas no significant change was observed in MOLT-4 cells. The results of cell cycle analysis showed a higher concentration of VCO in MOLT-4 cells (300 μg/mL) significantly induced greater cell accumulation in the G2/M phase than in K562 cells (150 μg/mL). Thus, K562 cells are much more sensitive to VCO than MOLT-4 cells. This differential response may be attributed to the distinct cellular origins of K562 (CML-derived lymphoblasts) and MOLT-4 (ALL-derived T-lymphoblasts), suggesting that VCO exerts its apoptotic effects in a cell type-dependent manner. Furthermore, treatment with 40 μg/mL lauric acid and 1 μM doxorubicin significantly increased apoptosis in both cell lines. These findings align with Kamalaldin et al., which reported that VCO induced apoptosis in A549 lung cancer cells but not in NCI-H1299 cells, highlighting its selective cytotoxicity based on cancer cell characteristics (41). However, its selective apoptotic activity suggests a cell type-dependent mechanism.

These findings underscore the cytotoxic effects of VCO on leukemic cells through cell cycle arrest and apoptosis, potentially mediated by its lauric acid content as the effective concentrations of VCO (e.g., 300-500 ug/mL) are quite high compared to lauric acid (40 ug/mL). Thus, in *in-vivo* and clinical studies, it is necessary to administer VCO, which mainly consists of MCTs (medium chain triglycerides; C6-C12), at a high

concentration as shown in previous studies. Regarding the potential feasibility of *in-vivo* studies, it has been reported that targeting energy metabolism with a modified diet supplemented with 25% 8- and 10-carbon MCTs may be considered as part of a multimodal treatment regimen to improve the efficacy of neuroblastoma anticancer therapy in the CD-1 Nu mouse model (42). In addition, an antiproliferative action of MCTs was evidenced in a study by Otto et al. (43). The authors of that study showed that a ketogenic diet supplemented with omega-3 fatty acids and 21.45% MCTs (6-12 carbons) inhibited tumor growth in a xenograft model of human gastric adenocarcinoma cells and increased the mean survival time of animals. The clinical application of MCTs in relation to their potent anticancer and therapeutic effects has been reported in several clinical studies. Extensive research on the ketogenic diet containing MCTs has been carried out in a pilot study conducted by Schmidt et al. in patients with advanced metastatic tumors. That study demonstrated notable improvements in patients who followed a ketogenic diet for three months (containing MCTs in a form of an oil-protein mixture: 21 g of fat with MCTs, 5 g of carbohydrate, and 14 g of proteins) in terms of physical wellbeing and observed tumor shrinkage without adverse side effects or changes in cholesterol or blood lipids (44). Nevertheless, further investigations are warranted to elucidate the underlying molecular pathways and to assess the therapeutic potential in leukemia treatment.

CONCLUSIONS

This study is preliminary to reporting the cytotoxicity of VCO in K562 and MOLT-4 leukemic cell lines. Our findings indicate that VCO inhibits leukemic cell proliferation, induces cell cycle arrest, and promotes apoptosis in both types of cells. These results suggest that VCO may inhibit the growth of leukemic cells by regulating cell cycle progression and apoptotic pathways. However, the precise molecular mechanisms underlying these effects remain unclear. Further investigations are needed to elucidate the signaling pathways involved and to determine whether the bioactive components of VCO act independently or synergistically in exerting their anticancer effects.

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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 CONTRIBUTION

S.S.: project administration, conceptualization, methodology, formal analysis, software, original draft preparation, review, and editing; S.Y.: conceptualization, methodology, 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 corresponding author upon reasonable request

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable, because of the studies not involving human

INFORMED CONSENT STATEMENT

Not applicable, because of the studies not involving human

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