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Original Research Article

Anticancer Activity of Mackerel Scad (*Decapterus macarellus*) Fish Oil on Colorectal Cancer Cell Lines

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ABSTRACT

The rising global burden of colorectal cancer (CRC) and adverse side effects of chemotherapy necessitate safer therapies leveraging marine bioactive compounds. Mackerel scad (*Decapterus macarellus*) is a marine fish that contains fatty acids, which can be potentially used as an anticancer agents. This study aims to assess the cytotoxicity of Mackerel scad's skin oil and its effect on the cell cycle and apoptosis of the human colon carcinoma cell line (WiDr). The sample concentrations for cytotoxicity test were ranged from 4,000-31.25 µg/mL and doxorubicin (from 25 to 0.78 µg/mL) as a positive control. The cytotoxicity test was carried out using the 3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay. Gas chromatography-mass spectrometry (GC-MS) was used to analyze the composition of fatty acids. Flow cytometry was used to test the ability to stop the cell cycle and induce apoptosis. Results showed a significant ($p<0.05$) cytotoxic effect of mackerel scad skin oil against WiDr cells (IC_{50}=958.04 µg/mL) and low toxicity against normal Vero cells. The fish oil-induced cell cycle termination in the G0/G1 phase triggered early apoptosis in WiDr cells. GC-MS analysis detected polyunsaturated fatty acids, including eicosapentaenoic acid (9.59%), docosahexaenoic acid (10.70%), and 9-octadecenoic acid (9.29%), including beneficial saturated fatty acids such as hexadecenoic acid (20.10%). These fatty acids play a role in cell cycle cessation and apoptosis through specific cellular mechanisms. This study demonstrated mackerel scad skin oil as a potential anticancer candidate that can be further developed as a supplement inhibiting cancer growth.

Keywords: Apoptosis, Cell cycle, Mackerel scad, Fish oil, WiDr

Introduction

Cancer is a group of diseases caused by abnormal cell growth, resulting in a drastic decline in life expectancy and death worldwide. Colorectal cancer ranks as the second type of cancer that causes cancer deaths worldwide after breast cancer.[1] This cancer starts as abnormal cell growth of epithelial tissue of the colon or rectum.[2] In 2020, there were more than 1.9 million new cases of colorectal cancer and more than 930 thousand deaths worldwide. Without proper mitigation efforts, by 2040, this disease will annually increase to 3.2 million new cases (63%) and cause 1.6 million deaths (73%) per year.[3] Half of all cases and deaths of patients with colorectal cancer were caused by unhealthy lifestyles as well as the influence of genetic factors.[4] Colorectal cancer treatment with chemotherapy is most commonly used to shrink tumors before surgery.[5] However, the use of chemotherapeutic drugs such as doxorubicin, 5-fluorouracil, and oxaliplatin often fails due to the development of drug resistance and tumor growth, leading to a poor prognosis.[6] Another downside is that patients can suffer from post-treatment side effects, e.g., arrhythmia, neutropenia, hair loss (alopecia), nausea, and vomiting.[6] The effectiveness of chemotherapy depends on the exact dose administered to specific target cancer cells.[7]

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A search for new alternative drugs and therapies for curing cancer is crucial, particularly with high specificity, lower side effects, and affordable cost .Marine resources can be a valuable source of bioactive compounds with anticancer properties. For instance, fish oil extracted from marine fish is rich in polyunsaturated fatty acids (PUFAs) of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).[8] Fish oil intake has been shown to prevent the development of several types of cancer, e.g., breast, colorectal, colon, and prostate. The side effects of chemotherapy also can be reduced by consuming EPA and DHA from fish oil.[9] More importantly, these fatty acids can induce targeted tumor cells' death without intervening with the growth of normal cells.[10] Mackerel scad (*Decapterus macarellus*) is a popular marine fish in the tropics. This fish is abundant in Indonesia and widely consumed, with an average annual production of 50,649 tons.[11] The skin of this fish has a high nutritional content of omega-3 (414.7 mg/g EPA and 956.0 mg/g DHA).[12] Unfortunately, the skin is often discarded as waste despite its potential as a source of valuable bioactive lipids. EPA and DHA can act as bioactive lipids and have been reported to inhibit the progression of several types of cancer, including colorectal cancer.[13] Although the nutritional value and fatty acids of mackerel scad oil are well known, the role of its fatty acid composition as an anticancer candidate remains understudied. This study evaluated the oil extracted from mackerel scad skin using the wet rendering method for its cytotoxicity value on colorectal cancer cell lines and its effect on inducing cell cycle termination and apoptosis. Anticancer tests selected were related to the fatty acid function found in mackerel scad fish oil. The fatty acid composition can shed light on the search for potential anticancer treatment and minimize the use of chemotherapeutic drugs.

Materials and Methods

Mackerel scad's skin oil (MSSO) extraction

The extraction method to obtain mackerel scad skin oil (MSSO) was via the wet rendering method with modifications.[14] Mackerel scad fish was obtained from the local market in Solo, Central Java, Indonesia. Scales were cleaned from the fish, and the skin was separated from the meat. The wet skin was weighed and mixed with a 1:1 (w/v) aquadest. The mixture was subjected to the fat rendering process at 80°C for 2 hours to remove moisture and obtain filtrates. The filtrate was centrifuged at 7000 rpm, 25°C for 20 minutes. Supernatants were added with 3% bentonite (1:1 v/v). The solution was homogenized at 29°C for 20 minutes, followed by centrifugation at 6500 rpm, 25°C for 20 minutes to purify the product. The supernatant was separated from the pellets and stored at -18°C. The oil yield was calculated as the percentage of extracted oil weight to wet fish skin weight.

Ethical clearance

The ethical feasibility of this study was given by the Ethical Committee of the Regional General Hospital Dr. Moewardi at Solo, Central Java with approval number 1.805/VII/HREC/2024.

Cell culture preparation

The human colon carcinoma cell line (WiDr) and Vero cells isolated from African green monkey kidney epithelial tissue were obtained from the Laboratory of Parasitology, Faculty of Medicine, Universitas Gadjah Mada, Indonesia. Cells used were from cryopreserved stock and thawed before being placed in tissue culture dishes. The culture media consisted of RPMI 1640 (Gibco, USA), fungizone 0.5% (Gibco, USA), fetal bovine serum 10% (Gibco, USA), penicillin-streptomycin 1% (Gibco, USA), and trypsin-EDTA 0.025% (Gibco, Canada). Cells were cultured in a CO_2 incubator (Heraeus) at a 5% carbon dioxide (CO_2) rate and temperature of 37°C. Neubauer hemasitometers (Sigma Aldrich) were used to count confluent (over 80%) cells. Cells were cultured into 96 well plates (Iwaki, Japan) with a density of 5×10^3 cells per 100 µL.

Cytotoxicity test

This test was carried out according to the method of 3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide MTT Assay.[15] MSSO stock at a concentration of 100,000 µg/mL was made by dissolving 10 mg of oil into 100 µL of 1% dimethyl sulphoxide (DMSO) (v/v in distilled water) (Sigma Aldrich, Germany). The stock solution was diluted with RPMI medium at concentrations of 4000, 2000, 1000, 500, 250, 125, 62.5, and 31.25 µg/mL used for the cytotoxicity test. Doxorubicin was used as a control with a concentration dilution of 25-0.78 µg/mL. Cultured cells (5×10^3 cells per 100 µL) on 96 well plates were incubated for 24 hours in a CO_2 incubator. The medium of WiDr and Vero cells was discarded and then treated according to the respective concentrations of fish oil and doxorubicin with three replications and incubated for 24 hours. All fish oil and doxorubicin in cell cultures were discarded. A hundred µL of MTT solution made from MTT reagent (1:9 PBS solution v/v) was added to each well of 96 well plates and then incubated for 3-4 hours. Next, 100 µL stop solution (10% sodium dodecyl sulfate/SDS in 0.1 N HCl) was added to all cell wells and incubated at room temperature overnight in dark conditions. Absorbance was measured using an ELISA reader (Biorad, USA) at a wavelength of 595 nm.[16]

$$\text{Percentage cell viability} = \frac{(Absorbance\ of\ treated\ cell - Absorbance\ of\ blank)}{(Absorbance\ of\ control - Absorbance\ of\ blank)} \times 100$$

The linear regression equation, y = ax + b, was used to calculate the IC_{50} values for each concentration.[5]

The Vero cell (normal cell line) was used to calculate the selectivity index (SI) of the MSSO using the following equation.[5]

$$SI = \frac{IC50\ (treated\ normal\ cells)}{IC50\ (treated\ cancer\ cells)}$$

Cell cycle termination and apoptosis test

The cell cycle termination and apoptosis test was done using flow cytometry.[16] WiDr cells with a density of 5×10^5 cells on 6 well-plates were grown in 2000 µL of RPMI medium. Test samples (fish oil and doxorubicin) with concentrations of 1/4, 1/2, and 1 IC_{50} cytotoxicity test results were added to the cell well and then incubated for 24 hours. After incubation, the cells were harvested by removing them using 150 µL of trypsin-EDTA followed by 3 minutes of incubation. The suspension was added to 1000 µL of RPMI medium and centrifuged at 2000 rpm for 3 min. Pellets were taken and resuspended with 100 µL of Annexin V-Propidium Iodide reagent for the apoptosis test, while 100 µL of propidium iodide was added for the cell cycle test. The suspension containing cells and reagents was incubated in the darkroom for 10 minutes at room temperature. Following this, the suspension was distributed on a flow cytometry tube. The cell cycle and apoptosis test were done using the FACS-Calibur system, a laser beam of 488 nm, and a flow rate of 500 cells/second. Flow cytometry analysis was performed using MODFIT 3.0 to evaluate the distribution of cells in various phases.

MSSO fatty acid profile analysis

The fatty acid profile was analyzed using gas chromatography-mass spectrometry (GC-MS; QP2010S0-Shimadzu) at the UGM Integrated Research and Testing Laboratory (LPPT). MSSO 1µL was dissolved in 1.5 mL sodium methanol 0.5 M and heated at 60°C for 5-10 minutes. Boron trifluoride was added along with heptane and saturated NaCl solution to facilitate the separation of the compound phases. The sample was injected into a column (Rtx 5 MS; length 30 m; diameter 0.25 mm), and the chromatography process was run at 260°C. The fatty acid components were evaporated with helium gas EI 70eV until a chromatogram of the detected compound was formed.

Statistical analysis

The IC_{50} value (compound concentration causing 50% of cell death in the population) of the cytotoxicity test was obtained from the linear regression equation of absorbance data of the three replications. The significance and accuracy of the data were analyzed using the IBM SPSS 26 Tukey HSD method with a confidence level of 95%. Graphs were made using GraphPad Prism 8 (GraphPad Software, Inc.).

Results and Discussion

The extracted oil gained from mackerel scad skin was a bright yellow liquid with a distinctive fish smell. The oil has a slightly acidic pH of 5.01 ± 0.17 and is within the safe oil acidity consumption range of 4.0 to 6.9.[17] The MSSO yield was 7.01 ± 0.28% of 40 grams of wet skin weight, higher than the yield of *Priacanthus tayenus* (1.23%) but lower than *Pangasius hypophthalmus* (14.07%) with the same extraction method.[18, 19] The yield of extracted fish oil can differ due to several factors, such as temperature, time, and adsorbent used during extraction. A higher yield contributes to higher bioactive compound compositions and can possibly affect the bioactivity potential and cytotoxicity effects of compounds in the extracts. The cytotoxicity effects of MSSO were evaluated using WiDr line cells as the primary target and Vero cells as non-target cells. The cytotoxicity parameter was expressed through the IC_{50} value. The results showed that the IC_{50} MSSO value in WiDr cells was 958.04 µg/mL, and Vero cells reached 2865 µg/mL (Figure 1). Extracts of natural compounds as anticancer, especially functional foods such as fish oil, are still considered toxic to cancer cells with an IC_{50} value of less than 1000 µg/ml.[20] A higher IC_{50} value in Vero cells found in this study indicates that MSSO had lower toxicity to healthy cells than cancer cells. As a comparison, the toxicity of doxorubicin, a first-line chemotherapy drug, showed IC_{50} values of 4.88 µg/mL in WiDr and 28.63 µg/mL in Vero cells. In terms of effectiveness, doxorubicin was more potent in killing cancer cells but its toxicity to healthy cells was also much higher than MSSO.

Table 1: Selectivity index of Vero cells against WiDr cells treated with MSSO and doxorubicin.

Sample	IC_{50} (µg/ml)		Selectivity Index
	Vero cells	WiDr	
MSSO	2865	958.04	2.99
Doxorubicin	28.63	4.88	5.86

MSSO: Mackerel Scad Skin Oil

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Table 2: List of identified compounds in MSSO and their characteristics analyzed through gas chromatography-mass spectrometry

Compound	Chemical Formula	Molecular Weight	Retention Time (min)	Abundance (%)
Saturated Fatty Acid (SFA)				
Dodecanoic acid	$C_{13}H_{26}O_2$	214	22.37	0.18
Pentadecane, 2,6,10,14-tetramethyl	$C_{19}H_{40}$	268	26.43	0.23
Methyl myristoleate	$C_{15}H_{28}O_2$	240	26.54	0.07
Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	26.95	5.72
Pentadecanoic acid	$C_{16}H_{32}O_2$	256	29.03	0.88
Hexadecanoic acid	$C_{17}H_{34}O_2$	270	31.19	20.10
Heptadecanoic acid	$C_{18}H_{36}O_2$	284	32.99	1.20
Methyl stearate	$C_{19}H_{38}O_2$	298	34.91	8.67
Nonadecanoic acid	$C_{20}H_{40}O_2$	312	36.60	0.23
Eicosanoic acid	$C_{21}H_{42}O_2$	326	38.30	0.64
Docosanoic acid	$C_{23}H_{46}O_2$	354	41.66	0.47
Monounsaturated Fatty Acid (MUFA)				
9-Octadecenoic acid	$C_{19}H_{36}O_2$	296	34.43	9.29
15-Tetracosenoic acid	$C_{25}H_{48}O_2$	380	45.77	0.51
Polyunsaturated Fatty Acid (PUFA)				
Linolenic acid	$C_{19}H_{32}O_2$	292	30.40	0.78
Methyl stearidonic	$C_{19}H_{30}O_2$	290	34.07	0.72
5,8,11,14,17-Eicosapentaenoic acid	$C_{21}H_{32}O_2$	316	37.34	9.59
Methyl 8,11,14,17-eicosatetraenoate	$C_{21}H_{34}O_2$	318	37.58	0.36
Ethyl 5,8,11,14,17-icosapentaenoate	$C_{22}H_{34}O_2$	330	38.96	0.34
Methyl 4,7,10,13,16 docosapentaenoic	$C_{23}H_{36}O_2$	344	40.19	0.59
4,7,10,13,16,19-Docosahexaenoic acid	$C_{23}H_{34}O2$	342	40.43	10.70
Methyl 7,10,13,16,19-docosapentaenoic	$C_{23}H_{36}O_2$	344	40.60	3.03
Squalene	$C_{30}H_{50}$	410	49.66	0.25

MSSO: Mackerel Scad Skin Oil

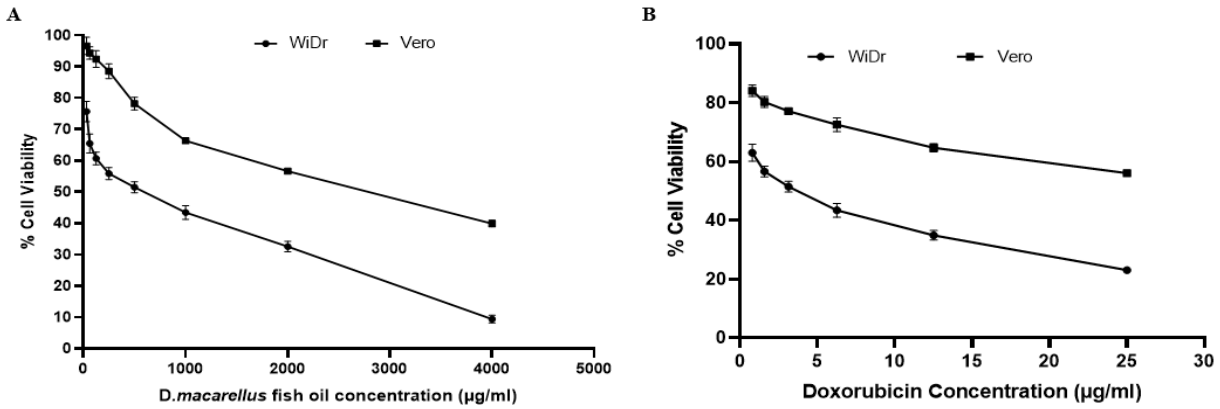

Figure 1: The percent viable WiDr and Vero cells after being treated with (A) MSSO and (B) doxorubicin. Value are mean ± SEM
*P<0.05; MSSO: Mackerel Scad Skin Oil

bioactive compounds as chemotherapeutic adjuvants has already been evaluated in breast, nasopharyngeal, and pancreatic cancers.[21] However, the effectivity of these compounds is still lower than usual chemotherapeutic drugs. The decrease in the percent viability of WiDr cells after 24 hours of treatment in this study indicates that MSSO has dose-dependent cytotoxic activity, consistent with the selectivity index (SI) value calculated.

The calculation of the selectivity index (SI) is necessary to evaluate the toxicity level of tested compounds on cancer cells compared to healthy cells.[22] Potential anticancer and chemopreventive agents are expected to have minimal negative impact on normal cells and, at the same time, can kill targeted cancer cells.[23] The SI value in this study was calculated by comparing the IC_{50} in non-target cells (Vero) with the IC_{50} in the target WiDr cells. Based on the results, the SI score of MSSO (2.99) was lower than doxorubicin (5.86). SI value greater than 1.00 indicates that the tested compound has greater selectivity for cancer cells than normal cells.[24]

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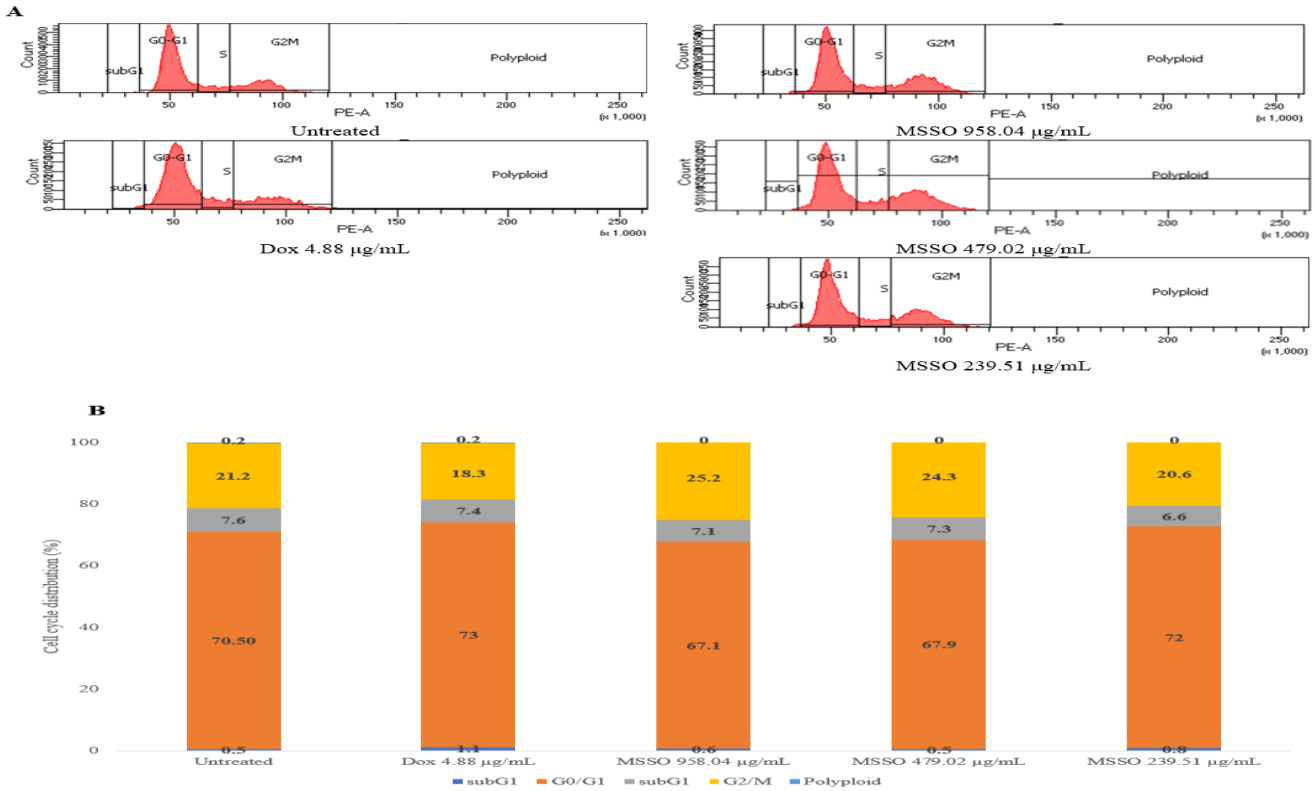

Figure 2: The cell cycle distribution of WiDr cells over a 24-hour treatment period.
(A) WiDr cell cycle profile with treatments: control, doxorubicin, and IC_{50} MSSO (one, half, and quarter doses) during 24 hours of incubation. The PI solution was added to the cells, and the samples were analyzed using flow cytometry.
(B) The distribution of WiDr cells across different phases of the cell cycle.

Even though MSSO has a lower SI value than doxorubicin, it is still considered toxic to cancer cells. In contrast, doxorubicin has a higher toxicity than MSSO but potentially causes serious side effects on healthy cells. The inhibitory effect of MSSO against WiDr cell viability can be attributed to the modulation of cellular physiological processes such as cell cycle and apoptosis. This study further analyzes the development of the WiDr cell cycle and the induction of apoptosis. In the cell cycle termination test, the total cell distribution of each phase was measured from the IC_{50} dose (full dose of 958.04 µg/ml, half dose of 479.02 µg/ml, and quarter dose of 239.51 µg/ml) over 24 hours (Figure 2A).

MSSO and doxorubicin treatments showed that most cells accumulated in the G0/G1 phase. The decreasing dose of MSSO treatment resulted in more cell accumulation in the G0/G1 phase (one dose: 67.1%, half dose: 67.9%, and quarter dose: 72%). Doxorubicin treatment showed a higher cell accumulation in the G0/G1 phase (73%) than any MSSO treatment. On the contrary, MSSO treatment showed a higher cell accumulation in the G2/M phase (one dose: 25.2%, half dose: 24.3%, and quarter dose: 20.6%) than doxorubicin (18.3%) (Figure 2B). The high cell accumulation in the G0/G1 phase indicates that cells stopped dividing, with twice as many as the number of stops in the G2/M phase. Possibly, the fatty acid compound components in MSSO play an important role in this process. The MSSO treatment showed no polyploidy (failure to separate), and the cells remained diploid. The cytotoxic effects of MSSO were further analyzed to assess the distribution of cell death through apoptosis and necrosis (Figure 3). A single treatment with doxorubicin shows a very high rate of necrosis compared with the apoptosis phase. In contrast, the apoptosis rate of WiDr cells from MSSO treatment showed dominance in the early death phase. This finding aligns with the results of cell cycle termination in the G0/G1 phase. The cytometry approach shows a qualitative difference in cell distribution between the control group and the cells treated with MSSO, as shown in different quadrants (Figure 3A). The cell distribution result showed that the control treatment maintained a high level of viability of 95% (Figure 3B). The results showed that MSSO treatment produces higher levels of apoptosis and lower necrosis as opposed to doxorubicin treatment, aligning with the prior results of induction of G0/G1 phase cell cycle termination and early apoptosis.

The number of cells undergoing apoptosis or necrosis in this study depends on the dose and intensity of the treatment time.[25] According to Patino-Ruiz et al.[26] cell death can be categorized into programmed cell death (PCD) and non-PCD. An example of PCD is apoptosis, which occurs from the normal physiological process of cells to maintain homeostasis. In contrast, necrosis is part of non-PCD, where pathological conditions arise due to exposure to reactive oxygen species, ischemia, hypoglycemia, and hypoxia. The cellular pathophysiological forms of necrosis include extensive cell swelling, organelle distension, DNA degradation, and accumulation of plasma membrane damage. Uniquely, necrosis can be programmed with specific mechanisms that can be its main potential therapeutic application in cancer treatment.

The distribution of apoptosis at early and late stages in this study was higher than the occurrence of necrosis, indicating that the fatty acid composition in MSSO can intervene in the homeostasis condition in WiDr cells. MSSO showed more selective results in induced programmed cancer cell death, thus potentially suppressing tumor growth without triggering systemic inflammation. In contrast, the high necrosis phase in doxorubicin treatment indicates that in addition to attacking cancer, this drug can also trigger unwanted inflammatory effects on non-target cells. This effect occurs by intercalating DNA and inhibiting topoisomerase II, which contributes to oxidative stress and cell damage.[26] Thus, the cell cycle development is correlated with the cessable division activity with MSSO.

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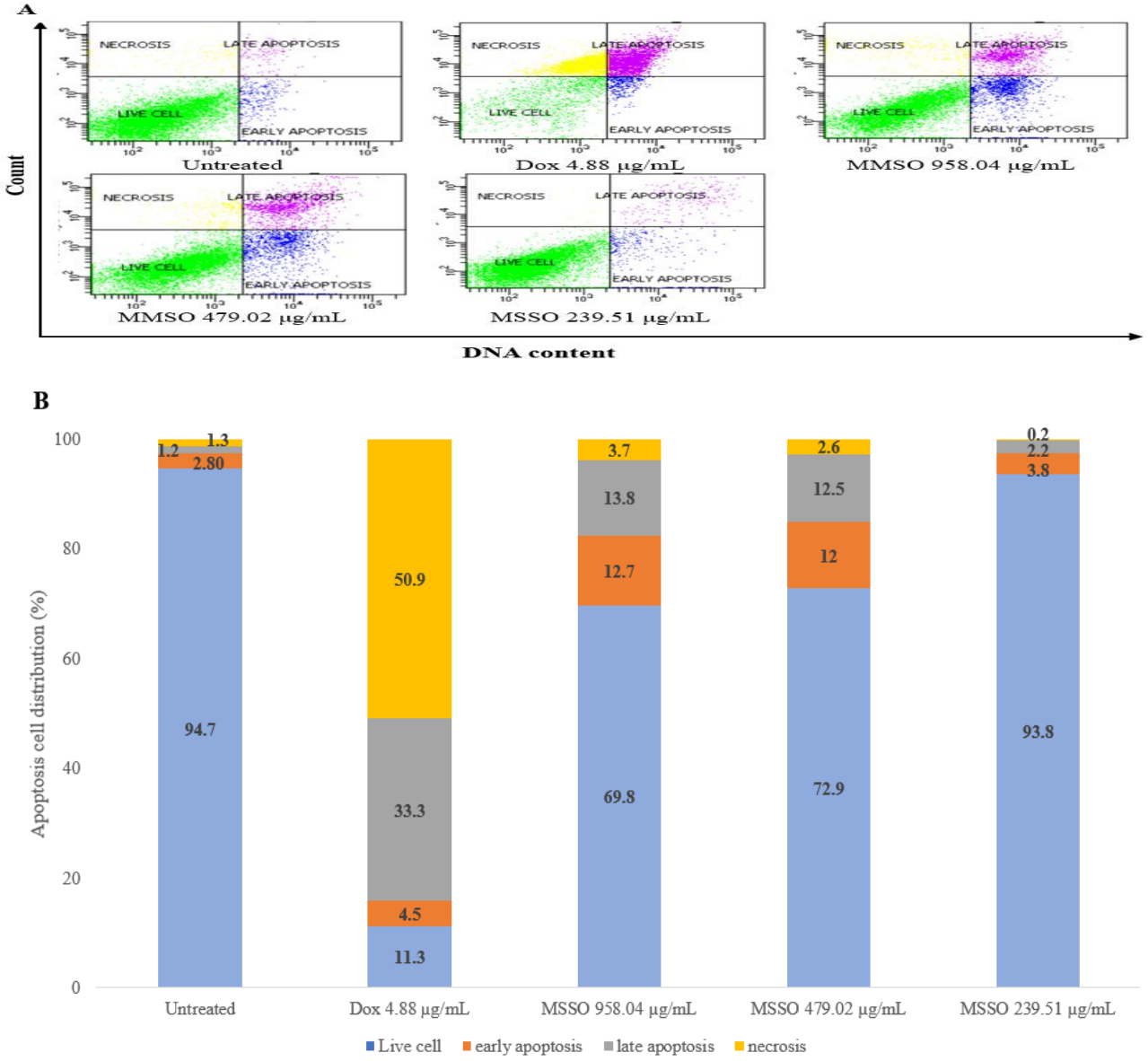

Figure 3: The apoptosis cell distribution of WiDr cells over a 24-hour treatment period.
(A) The apoptosis profile with control, doxorubicin, and MSSO (one, half, and quarter of IC50) in WiDr cells for 24 hours incubation. Annexin-V and PI solution were added to the cells, and the samples were analyzed using flow cytometry.
(B) The apoptosis cell distribution (%) in each treatment of WiDr cells.

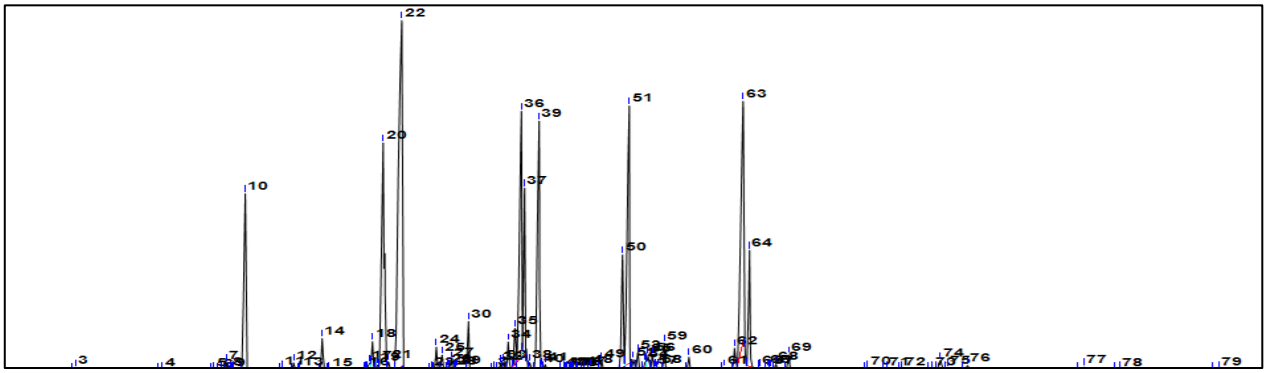

Figure 4. Chromatogram of fatty acids identified from mackerel scad's skin oil samples

In line with this, apoptosis induction can reduce the viability of WiDr cells without causing inflammation of non-target cells and also reduce side effects. These two physiological processes can occur sequentially but can also be analyzed separately, depending on the exploration approach. The profile and composition of fatty acids analyzed by the GC-MS method are necessary to understand the underlying mechanism of MSSO's anticancer potential. According to Alinafiah et al.[27], GC-MS is a widely used instrument to measure and identify fatty acids profile because of its accuracy, sensitivity, and ability to handle complex mixtures with specific and fast results. The results showed that 79 fatty acids were detected in this study (Figure 4). The MSSO fatty acid composition analysis focused on the percent abundance listed in Table 2. The most abundant fatty acids detected include polyunsaturated fatty acids, e.g., eicosapentaenoic acid (9.59%), docosahexaenoic acid (10.70%); monounsaturated fatty acids, e.g., 9-octadecenoic acid (9.29%); and saturated fatty acids with different carbon chains including hexadecanoic acid (20.10%) and, methyl stearate (8.67%). These identified fatty acids are suspected to have a role in cancer treatment.

EPA and DHA from MSSO and other fish oils are known to inhibit the proliferation of cancer cells by intervening in the molecular pathways of the cell cycle. This process occurs by decreasing the expression of cyclin D1 and CDK4, which are required in the G1 to S cleavage phase, thereby inhibiting DNA replication.[28] These two fatty acids are also known to induce apoptosis through increased mitochondrial membrane permeability, thereby triggering the release of cytochrome c and activating the caspase pathway, decreased expression of anti-apoptosis proteins such as Bcl-2 and increased pro-apoptotic proteins such as BAX, as well as inhibiting pro-inflammatory pathways such as NF-kB and COX-2.[29,30] Most cancer studies on unsaturated fatty acids in fish oil did not differentiate EPA and DHA separately, and most studies believe that the two have similar biochemical effects. In addition, 9-octadecenoic acid (oleic acid), a monounsaturated fatty acid that can modulate the PI3K/Akt and MAPK pathways, which play a role in cell proliferation, was also detected.[30]

GC-MS also detected saturated fatty acids that act as cytotoxic agents of colorectal cancer, e.g., hexadecanoic acid, 9 octadecenoic acid, and methyl stearate. Hexadecanoic acid or palmitic acid is known to induce apoptosis in cancer cells by increasing BAX (pro-apoptotic) expression and decreasing Bcl-2 (anti-apoptotic) expression.[31, 32] Such regulation of expression leads to the formation of mitochondrial membrane pores and the release of cytochrome-C to cytosols, triggering the activation of caspase-9 and 3, which are key to the apoptosis process. Methyl stearate was also detected and thought to cause changes in cell morphology typical of apoptosis, such as chromatin compaction and DNA fragmentation.[33] Thus, it can be concluded that both polyunsaturated and monounsaturated fatty acids as well as some saturated fatty acids, are responsible for the beneficial effects of fish oil on tumor growth.

Various mechanisms have been proposed to explain how fatty acids can suppress the growth of tumor cells. New mechanisms will continue to be reported as we understand the regulation of gene expression by fatty acids.[34] Inhibition of cancer cell growth due to fatty acid function is likely due to a combination of mechanisms of each compound component. The formation of cytotoxic compounds in the form of the role of peroxidation of long-chain fatty acids has been proposed as the main mechanism of fatty acids against cancer.[35,36] In addition, unsaturated fatty acid chemopreventive agents can induce oxidative stress in colorectal cancer cells.[37]

The fatty acid composition of MSSO, especially EPA and DHA, can decrease the WiDr cell line viability. This effect is probably due to the induction of high levels of oxidative stress. The ability of unsaturated fatty acids to induce oxidative stress that leads to the cessation of the cell cycle and apoptosis has great potential in developing anticancer agents, including chemopreventative. These findings provide a basis for deeper exploration focusing on the synthesis of MSSO fatty acid components and investigating the molecular pathways that drive cell cycle termination and apoptosis.

Conclusion

Though often considered waste, the skin of mackerel scad fish is a rich source of beneficial fatty acids known to provide cytotoxic and colorectal anticancer effects. This study showed that fish oil extracted from mackerel scad skin can decrease the viability of WiDr cells, stop the cell cycle in the G0/G1 phase, and cause higher apoptosis than necrosis. Therefore, it indicates a low risk of inflammation in non-target cells, resulting in minimal side effects. This natural product has high potential as a promising source for nutraceutical products inhibiting cancer growth.

Conflict of Interest

Authors declare no conflict of interest.

Authors' Declaration

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

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