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Investigation of the Effects of Polyunsaturated Fatty Acid Ratios in Human SH-SY5Y Cells by *in vitro* Methods

ELÇİ et al. Effects of PUFAs on SH-SY5Y Cells

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ABSTRACT

Objectives: Neuroblastoma, an embryonic tumor of the sympathetic nervous system, is the deadliest type of cancer, accounting for 6-9% of all childhood cancers. The omega (ω)-6 [Linoleic Acid (LA)]: ω-3 [Eicosapentaenoic Acid (EPA)]=13/1 ratio is associated with the presence of chronic disease, and it has been reported that reducing this ratio to 7/1 protects against cancer and cardiovascular diseases.

Materials and Methods: This study aimed to investigate the anticancer effects of different ratios of ω-3 and ω-6 fatty acids in human neuroblastoma cells (SHSY-5Y). For this purpose, SHSY-5Y cells were treated with different ratios of ω-3 and ω-6 fatty acids for 48 and 72 h. The viability of ω-3 and ω-6 fatty acid-treated cells was measured using the Methylthiazolyldiphenyl-Tetrazolium Bromide. The percentage of cell apoptosis was detected using the Fluorescein Isothiocyanate-conjugated Annexin-V/PI assay, and Reactive Oxygen Species (ROS) analysis was performed using flow cytometry. The expression levels of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and *Transforming Growth Factor Beta 1 (TGFβ1)* genes were determined using real-time polymerase chain reaction.

Results: EPA and LA separately significantly inhibited the proliferation of SH-SY5Y cells within 48 h (p<0.001). It was found that apoptosis decreased significantly in all groups due to the application of polyunsaturated fatty acids at different ratios, and the most effective dose was ω-3:ω-6 ratio: 1/1. Although ROS levels were significantly decreased compared with the control group, the lowest ROS level was observed in the ω-3:ω-6 ratio: 1/4 group. Both TNF-α, IL-6, and TGFβ1 mRNA expressions increased significantly after the addition of fatty acid mixtures compared with the control; they were observed to decrease with an increasing ω-6 ratio.

Conclusion: This study is the first to examine the effects of the ω-3:ω-6 ratio on neuroblastoma cancer cells. The application of ω fatty acids decreased apoptosis at all ratios. In contrast, when the ω-3: ω -6 ratio increased, the amount of ROS. Additionally, as the ω-3:ω-6 ratio increases, a decrease in the release of pro-inflammatory cytokines is noted.

Keywords: Apoptosis, IL-6, NF-κB, ROS, SHSY-5Y cell, TGFβ1, ω-3:ω-6 ratio

INTRODUCTION

Neuroblastoma is an embryonic tumor responsible for 6-9% of all pediatric malignancies. Neuroblastoma exhibits a variety of biological and clinical characteristics, ranging from spontaneous remission to exceedingly severe illness with metastatic dissemination.[1] The cure rate for these patients is 50% despite a wide variety of treatment modalities and many relapses due to minimal residual disease.[2] There is a great need for new, effective, and less harmful treatments for patients with neuroblastoma. However, improving current treatment methods may improve overall survival.

Compared with normal nervous tissue, neuroblastoma, glioma, and meningioma have high levels of omega (ω)-6 [Linoleic Acid (LA)] fatty acid arachidonic acid (AA) and low levels of ω-3 [Eicosapentaenoic Acid (EPA)] fat.[3] This proportionate difference may act as an adaptive mechanism in nervous system malignancies.[4] It has been suggested that ω-3 fatty acids contribute to mechanisms that arrest tumor growth progression by accumulating intracellular Reactive Oxygen Species (ROS) and promoting death through antiangiogenic actions.[5]
Clinical trials in adult patients with cancer have shown that ω-3 fatty acids can improve the inflammatory response, nutritional status, and quality of life.[6-8] One study shows that ω-3 supplementation during treatment reduces the side effects of chemotherapy, regardless of the type of drug used.[8-10] ω-3 fatty acids, especially ω-3 (EPA) and Docosahexaenoic Acid (DHA), have been reported to improve tumor response to treatment, protect against toxicity, and reduce secondary complications when administered as an adjunct to chemotherapy in patients with different types of cancer.[11] Lipid mediators such as prostaglandins derived from ω-6 fatty acids act as pro-inflammatory mediators, whereas the products of ω-3 fatty acids are anti-inflammatory. For example, ω-3 reduces the production of proinflammatory cytokines such as interleukin (IL)-1β, tumor necrosis factor-alpha (TNF-α), and IL-6, while the ω-6 series exerts indirect anti-inflammatory effects by inhibiting eicosanoid biosynthesis. Given these reciprocal effects, higher ω-6 to ω-3 ratios increase carcinogenesis risk.[12] Numerous ω-6-derived prostaglandins and leukotrienes have been shown to increase tumor growth by promoting angiogenesis, increasing cellular proliferation, suppressing the immune system, and inhibiting apoptosis.[13,14] Studies have shown that inhibition of the enzymes responsible for producing prostaglandins and leukotrienes suppresses tumor growth.[15,16]
Dietary intake of saturated fatty acids instead of polyunsaturated fatty acids (PUFAs) has recently been encouraged. However, high ω-6/ω-3 PUFA ratios in Western diets increase cancer risk.[17] These results have been confirmed in animal studies. Although some studies have shown that high-fat diets rich in monounsaturated fatty acid and ω-6 PUFAs promote tumor growth and induce liver metastasis, in contrast, administration of ω-3 PUFAs such as EPA or DHA, has been reported to reduce cancer-related symptoms.[18,19] Further research has revealed that ω-3 PUFA induce ferroptosis in cells through lipid peroxidation and inhibit tumor growth when combined with ferroptosis inducers.[19] In one study, dietary supplementation with DHA and EPA increased the antitumor efficacy of chemotherapy.[20] Dietary programs have also been reported to slow the development of cancer. Among these programs, the ketogenic, low-carbohydrate, and high-fat diets have been reported to reduce tumor growth significantly.[21]

Both direct administration of dietary FAs and FA-related modification of dietary patterns have been shown to improve cancer risk. There are no specific nutritional standards or guidelines for cancer treatment. When prescribing dietary therapy, particular attention should be paid to other nutritional deficiencies and the increased risk of disease due to the specific diet; There is no specific recommendation regarding the type of FA to be taken. This study examined the impact of ω-3:ω-6 fatty acid ratio on neuroblastoma cell release and cytokine release. Fatty acids are known to play a role in tumor cell proliferation, metastasis, and drug resistance; thus, targeting fatty acid metabolism is a promising therapeutic strategy. The
present study aimed to reveal the *in vitro* effects of PUFAs in cancer using various methods. Given the difficulty of anticancer medications entering the blood-brain barrier, the therapeutic effect of ω-3:ω-6 PUFAs against brain cancer might be an excellent possibility to examine. We aim to provide new ideas for drug development and combination therapy.

MATERIALS AND METHODS

Cell culture

The human neuroblastoma cells (SHSY-5Y) (CRL-2266™) cell line was used in this study. Cells were cultured at 37 °C in 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) (Capricorn Scientific, Germany) with 10% Fetal Bovine Serum (FBS) (Capricorn Scientific, FBS-HI11B, Germany), 100 IU/mL penicillin (Capricorn Scientific, PS-B, Germany), 100 µg/mL streptomycin (Capricorn Scientific, PS-B, Germany), and passaged at 70-80% confluence.

Fatty acids and experimental groups

EPA as the ω-3 fatty acid and LA as the ω-6 fatty acid were used in this study. Six experimental groups were created. The first group was the control group, which was not subjected to treatment. The ω-3:ω-6 ratios were 1/1, 1/2, 1/4, 1/8, and 1/16, respectively.

Administration of EPA and LA

200 µL of EPA (item no: 90110, Cayman) were diluted according to the kit protocol. Then, a 4-mM stock solution was obtained. The stock solution was then serially diluted in DMEM to doses of 5 µM, 10 µM, 25 µM, 40 µM, 50 µM and applied to the cells for 48 and 72 h. 3.241 M LA (PC1003367601, Sigma) was diluted according to the kit protocol to obtain a 4-mM stock solution. As with EPA2, the stock solution was serially diluted in DMEM to doses of 5 µM, 10 µM, 25 µM, 40 µM, and 50 µM and applied to cells for 48 and 72 h.

Methylthiazolyldiphenyl-Tetrazolium Bromide (MTT) assay

Cells were seeded in a 96-well plate at 10^4 cells per well and incubated for 24 h at 37 °C in an atmosphere of 5% CO_2. Cells were treated with a combination of ω-6 and ω-3 fatty acids. After 48 h of incubation, each well received 20 µL of MTT solution and incubated for 3-4 h. After incubation, the supernatant was gently removed. Each well was treated with 100 µL of isopropyl alcohol and incubated for 2 h at room temperature in the dark. The plate was then shaken and read at 570 nm.

Annexin-V analysis

Measurement of Annexin-V bound to the cell surface as an indicator of apoptosis should be performed in conjunction with a staining test to determine the integrity of the cell membrane. It distinguishes whole cells Fluorescein Isothiocyanate (FITC-/PI-), apoptotic cells (FITC+/PI-), and necrotic cells (FITC+/PI+). To analyze apoptosis, cells ($1x10^5$) were trypsinized after being treated with varying ratios of ω-6 and ω-3 fatty acids after 48 h. The cell pellet was then dissolved in 100 µL binding solution and added to $5x10^5$ cells in 500 mL. Then, 5 µL of Annexin and 20 µL of PI were added, and the mixture was pipetted. After 30 min of incubation in the dark at room temperature, 400 µL of 1X binding buffer were added and the samples were examined using a BD Accuri C6 (BD Biosciences) flow cytometer.

Analysis of ROS levels

The intracellular ROS were determined using an ROS detection kit (THORVACS Biotechnology, Cat#; ROS-100T). Cells prepared in the experimental groups created in the SH-SY5Y cell line were collected by trypsinization method. $1x10^5$ cells were suspended in PBS, centrifuged at 1000 rpm for 5 min, and the supernatant was discarded. Cell pellets were resuspended in 200 µL of 100 mM DCF-DA reagent and incubated at 37 °C for 30 min in the dark. The cells were then centrifuged at 1000 rpm for 5 min, after which the supernatant was discarded. The pellet was resuspended in 100 µL PBS and analyzed using the FL1 channel on a BD Accuri C6 (BD Biosciences) flow cytometer.

Gene expression analysis

Real-time polymerase chain reaction (PCR) was used for quantitative investigation of TNFα, IL-6, and TGFβ1 mRNA expression levels. After administering fatty acids, cells were cultured for 24 h before the flask was trypsinized. Total RNA was isolated using an RNA isolation kit (Qiagen, Germany). The multi-mode gave purity and quantity measurements using a microbiological reader. The total RNA amounts obtained from the cells were calculated according to the 260/280 nm ratio using a spectrophotometer, and the results were found as ng/µL. cDNA synthesis was performed from the extracted and thawed samples using a Transcriptor First Strand cDNA Synthesis Kit (Roche) and a T100 PCR System (Bio-Rad, Hercules, CA, USA) thermal cycler. GAPDH was selected as the housekeeping gene. The real-time PCR heat cycle consisted of 35 cycles: 1 min at 95 °C, 15 s at 95 °C, 1 min at 60 °C, and 30 s at 72 °C. Primary designs were created using NCBI and primer3 sites. Table 1 lists the primers used. National Center for Biotechnology Information GenBank Primer-Blast® was built and evaluated using sequence matching analysis (Table 1).

Statistical analysis

The mean and standard deviation (mean ± standard deviation) were used for continuous data as descriptive statistics. The suitability of continuous data for normal distribution was checked using the Kolmogorov-Smirnov test. The distributions of variables in two groups that were suitable for normal distribution were compared with the Student's *t*-test, and those that were not suitable for normal distribution were compared using the Mann-Whitney *U* test. The distributions of variables that did not comply with normal distribution in three or more groups were analyzed using the Kruskal-Wallis test, and the formula developed by Conover (1980) was used as the multiple comparison test. Data were analyzed using the IBM SPSS 23 (IBM SPSS Inc, Chicago, IL) package program. The significance level was set as $p<0.05$.

RESULTS

Determination of cell viability in SHSY-5Y cells after 48-72 h exposure to fatty acid concentrations of 5, 10, 25, 40 and 50 µM via MTT assay

To explore the cell viability of ω-3 and ω-6 fatty acids alone or in combination in the SH-SY5Y cell line, the cells were treated with serial concentrations of EPA and LA (5-50 µM) for 48 and 72 h. Both EPA and LA alone significantly inhibited the proliferation of SH-SY5Y cells within 48 h ($p<0.01$) (Figure 1). On the contrary, cell viability increased significantly as a result of 72 h of treatment with PUFAs ($p<0.001$) Figure 1**.**

Determination of cell viability in SHSY-5Ycells after 48 h of exposure to fatty acid ratios of 1/1, 1/2, 1/4, 1/8, and 1/16 via MTT assay

Treatment of SH-SY5Y cells with fatty acid ratios of 1/1, 1/2, 1/4, 1/8, and 1/16 induced a significant decrease in viable cells within 48 h ($p<0.01$) (Figure 2).

Results of the Annexin-V assay

The Annexin-V assay was used to measure the percentage of apoptotic cells. The percentage of live cells in the control group for SHSY-5Y cells is 99.6%. After 48 h of fatty acid treatmentat ω-3:ω-6 ratio =1/1, ω-3:ω-6 ratio =1/2, ω-3:ω-6 ratio =1/4, ω-3:ω-6 ratio =1/8, ω-3:ω-6 ratio =1/16 the percentages of viability were 27.4%, 45.4%, 49.7%, 54%, 43.6% respectively (Figure 3).

ROS results

For the ROS assay, the ROS value in the control group was 21%, whereas the 48th-h fatty acid treatment (ω-3: ω -6 ratio =1/1, ω-3:ω-6 ratio =1/2, ω-3:ω-6 ratio =1/4, ω-3:ω-6 ratio =1/8, ω-3:ω-6 ratio =1/16) for the SHSY-5Y cell line was 19.8%, 11.5%, 1.5%, 2.7%, and 6.3%, respectively (Figure 4).

Gene expression results

The mRNA expression levels of TNF-α, IL-6, and TGF1β were increased 5.78, 2.14, and 7.3 times at ω-3:ω-6 ratios of 1:4 compared with the control group (p<0.05, p<0.05, p<0.001, respectively) (Figure 5). The mRNA expression levels of IL-6 were increased 2.69, 3.15, and 2.14 times in groups with ω-3/ω-6 ratios of 1:1, 1:2, and 1:4 compared with the control group (p<0.05, p<0.01, and p<0.05, respectively). Compared with the control group, IL1B expression increased 12.9 and 9.29 times in the 1:4 and 1:16 ω-3:ω-6 ratio (p<0.05 and p<0.005, respectively), whereas TGF1β expression increased 7.71 and 6.69 times in the 1:2 and 1:8 groups (p<0.001 and p<0.05, respectively) (Figure 5).

When the ω fatty acid application groups were evaluated within themselves, TNF-α expressions increased 4.77, 4.79, and 5.79 times in the 1:1, 1:2, and 1:4 groups, respectively, but decreased again in the 1:8 and 1:16 groups. While TNF-α expression was observed to be statistically significantly lower in the group with a ω-3:ω-6 ratio of 1:8 compared with the group with 1:4 (p<0.01), it decreased statistically significantly in the group with a ω-3:ω-6 ratio of 1:16 compared with the groups with 1:1, 1:2, and 1:4 (p<0.05, p<0.05 and p<0.005, respectively) (Figure 5).

IL-6 mRNA expression was increased in the group with a ω-3:ω-6 ratio 1:1, but it decreased as the ω-6 ratio increased. Accordingly, IL-6 expression was significantly lower in the group with a ω-3:ω-6 ratio of 1:8 than in the 1:1 and 1:2 groups (p<0.05 and p<0.05, respectively). In addition, IL-6 expression was significantly decreased in the group with a ω-3:ω-6 ratio of 1:16 compared with the 1:1, 1:2, and 1:4 groups (p<0.01, p<0.005 and p<0.05, respectively) (Figure 5).

TGF1β mRNA expressions were observed to be significantly higher in groups with ω-3:ω-6 ratios of 1:2 and 1:4 than in the 1:1 group (p<0.01 and p<0.05, respectively) and than in the 1:16 group (p<0.001 and p<0.001, respectively). Although not statistically significant, TGF1β expressions significantly decreased in the group with ω-3:ω-6 ratio of 1:16 compared with the 1:8 group (p = 0.056) (Figure 5).

DISCUSSION

Western diet contains high amounts of ω-6 PUFAs and small amounts of ω-3 s. The resulting high ω-6:ω-3 ratio is believed to contribute to different diseases such as inflammation and cancer. In particular, evidence that ω-3 PUFA may be inhibitory on neuroblastoma has been shown in both *in vitro* and *in vivo* studies.[21] In contrast, evidence suggests that a high intake of ω-6 PUFAs is associated with an increased risk of developing cancer.[22] The ω-3:ω-6 ratio is important in maintaining an appropriate level of biological membrane fluidity, which is essential for ion channel function, membrane receptor activity, and the release of neurohormones.[23]

EPA is an ω-3 fatty acid with significant anti-cancer effects by regulating the expression of anti-inflammatory mediators, inhibiting cell proliferation, and modulating cell death pathways.[24] Studies have shown that EPA can activate multiple molecular mechanisms, including both classical and alternative apoptotic pathways, and regulate survival and cell growth signaling, ultimately leading to cell death in various *in vitro* and *in vivo* animal models.[25,26] Studies have shown that ω-3 fatty acids reduce neuroblastoma cell proliferation.[27,28] It was revealed that oleic acid treatment significantly reduced SHSY-5Y cells in a time- and dose-dependent manner.[29] In a study of human neuroblastoma LA-N-1 cells, it was reported that ω-3 fatty acids docosahexaenoic acid (DHA) and EPA exert antiproliferative effects depending on time and concentration.[30] In our study, a similar decrease in cell proliferation was observed at 48 h when increasing doses of EPA were administered.

ω-6 PUFAs are also reported to have anticancer activity. In one study, LA as an ω-6, the most abundant PUFA in nature, was reported to suppress cancer cell growth by inducing ROS production and mitochondrial damage.[31] In other studies, unlike this one, they emphasized that LA has a proapoptotic effect on cancer.[32-34] In another study examining the effect of ω-3 and ω-6 fatty acids on human IMR-32 neuroblastoma cells, it was reported that fatty acids significantly reduced the growth rate of cells to different degrees.[35] There are studies suggesting that ω-6 fatty acids have a procarcinogenic effect on increased eicosanoid ratios or, conversely, suppress cancer cell growth by inducing ROS production and mitochondrial damage.[3,36] In our study, we observed a decrease in cell proliferation as a result of increasing doses of LA for 48 h.

The ω-3:ω-6 ratio is an important nutritional parameter. While this ratio was 1:6.4 at the turn of the last century, it changed to 1:10 in the 2000s.[37] The high ω-6:ω-3 ratio in Western diets increases the risk of cancer.[38] The anticancer effects of different ω-3:ω-6 ratios were investigated using *in vitro* methods. In SH-SY5Y cells treated with different ω-3:ω-6 ratios, although similar results were obtained in separate applications of these fatty acids, we observed a significant and greater reduction in cell proliferation in the ω-3:ω-6 mixture at high levels of ω-3 than in the individual applications. This suggests that they are more effective together, and the effect of ω-3 emerges more strongly.

Apoptosis is referred to as programmed cell death during normal development due to cell aging or homeostasis in a cell population. In a study with liver cancer cell lines, DHA and EPA administration was shown to mediate

apoptosis by causing caspase 3 and caspase 9 activity.[39] Yamamoto et al.[40] reported that EPA increased the expression of pro-apoptotic (BAX and BCL-XS) and decreased the expression of anti-apoptotic (BCL-2 and BCL-XL) proteins in breast cancer cell lines. On the other hand, the role of ω-6 in apoptosis is more complex, with some studies supporting activation of apoptosis and others suggesting inhibition.[41] In the present study, we found that apoptosis was induced by increasing the ω-3 fatty acid ratio. The most effective PUFA ratio was found to be 1/1. In addition, our Annexin-V analysis results also support our MTT results.

It is well known that reactive oxygen and nitrogen species are underlying causes of many diseases, including cancer. Cancer cells produce high levels of ROS because of their rapid metabolism and impaired cellular signaling pathways. In general, the amount of free radicals increases in cancer. A previous study showed that Ppt1-KO neurons treated with a combination of ω-3 and ω-6 had significantly reduced ROS levels compared with the untreated group.[42] However, another study showed that 24 h administration of LA and EPA increased ROS levels in embryonic stem cells.[43] Additionally, a study reported that LA, an n6 PUFA, suppresses cancer cell growth by inducing ROS production and mitochondrial damage, and similar to ω-3 PUFAs, ω-6 PUFAs also have anticancer activity.[44] In our study, it is seen that there is a decrease in ROS values 48 h after the application of ω fatty acids. The ROS level is quite low, especially when the ω-3:ω-6 ratio is 1:4 or 1:8. According to this result, it can be concluded that the use of ω-3:ω-6 in the ratio of 1:4 or 1:8 can make a significant contribution to protecting against free radical damage.

The inflammatory process and cancer development are closely related. Chronic inflammation is a bipolar process that, on the one hand, may stimulate cancer development and progression, and on the other hand, the recruitment of immunocompetent cells and their activation may cause tumor suppression and apoptosis. Tumor-associated cells are a potent source of immunomodulatory molecules, such as the proinflammatory cytokines IL-1, IL-6, and TNF, which are involved in the stimulation of key tumor-promoting factors, such as STAT3 and NF-κB. It has been reported that ω-3 PUFAs modulate the duration and intensity of inflammatory processes, and their increase in the diet reduces proinflammatory cytokines such as IL-1β, TNF-α, and IL-6.[45] In addition, it has been stated that the ω-6 series exerts indirect proinflammatory effects by inhibiting eicosanoid biosynthesis. Considering these mutual effects, higher ω-3:ω-6 PUFA ratios result in increased risk of carcinogenesis.

In a study by Liang et al.,[46] mice inoculated with androgen-sensitive prostate cancer cells were fed a high-fat diet rich in ω-3 fatty acids, and mice in this group were shown to express lower levels of proinflammatory cytokines (IL-6, TNF-α, and IL-10) and chemoattractant protein than animals fed a high-fat diet containing ω-6 fatty acids. While ω-6 fatty acids are known to activate proinflammatory pathways, Lipoxin A4 (LXA4), a common type of lipoxin, is a metabolite derived from endogenous AA in the ω-6 series. LXA4 is reported to be a lipid mediator of endogenous anti-inflammation and resolution.[47] In another study, LipoxinA4 was shown to antagonize TNF-a-stimulated IL-1b and IL-6 synthesis.[48] Another study showed that LXA4 in activated synovial fluid inhibits the synthesis of inflammatory cytokines and MMP and stimulates TIMP production *in vitro*. This result has been interpreted as suggesting that LXA4 is involved in a negative feedback loop that opposes the inflammatory cytokine-induced activation of synovial fluid.[49] In our study, an initial increase in TNF-α and IL-6 expression was observed as the ω-6 fatty acid ratio increased, but a decrease was observed, and similar results were obtained in a repeated study. We believe that this result may be due to an increase in AA-induced lipoxin synthesis due to the increased amount of LA. On the other hand, we observed that as the ω-6 fatty acid ratio increased, the expression of TGFβ1, one of the anti-inflammatory cytokine, decreased.

CONCLUSION

In conclusion, in our study, although significant decreases in apoptosis were generally observed with the application of ω fatty acids, we observed a decrease in the amount of ROS with an increase in the ω-6 ratio. As expected, there was a certain decrease in anti-inflammatory TGF1β levels as the ω-6 ratio increases. However, consistent with some studies, the observed decrease in the release of proinflammatory cytokines starting from a 1/4 or 1/8 ω-3:ω-6 ratio was confusing. A limitation of our study was that the protein expression of cytokines could not be measured because mRNA expression may not always fully express protein expression. Considering our findings, further studies are needed to suggest a clear ω-3:ω-6 ratio.

Ethics

Ethics Committee Approval: Not required.
Informed Consent: Not required.

Footnotes

Authorship Contributions

Surgical and Medical Practices:
Concept:
Design:
Data Collection or Processing:
Analysis or Interpretation:

Literature Search:
Writing:
Conflict of Interest:
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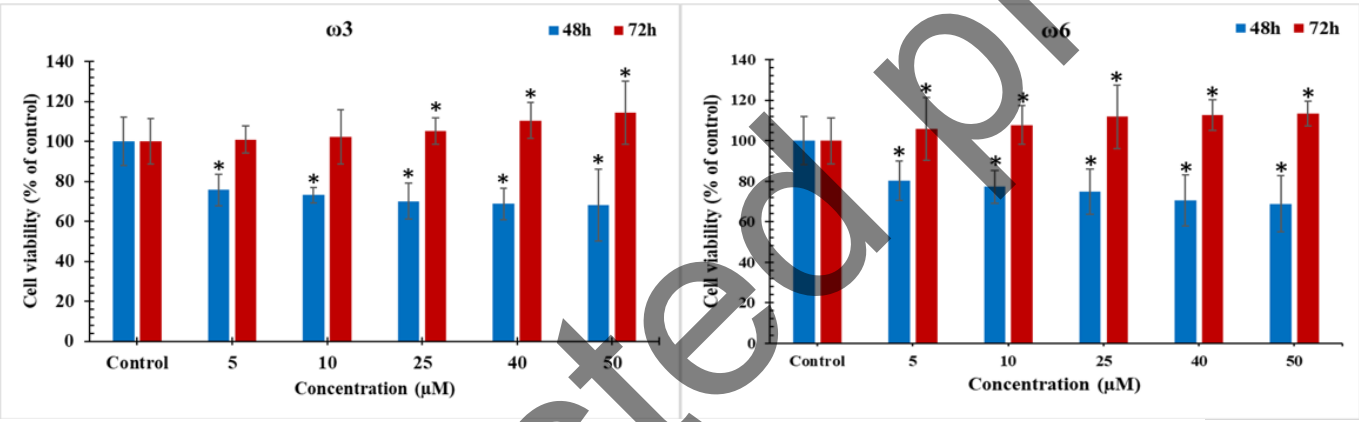

Figure 1. SHSY-5Y cells MTT assay. Fatty acids (5, 10, 25, 40 and 50 µM concentrations were added to SHSY-5Y cell culture. After 48-72 h treatment with fatty acids, MTT tests were performed for each concentration. **p*<0.01 significance level obtained by comparing the control group
MTT: Methylthiazolyldiphenyl-Tetrazolium Bromide

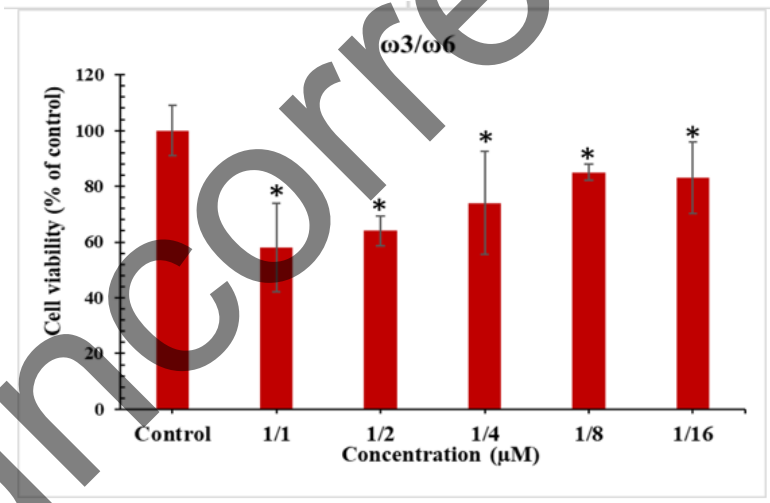

Figure 2. SHSY-5Y cells MTT assay. Fatty acid ratios were added to SHSY-5Y cells. After 48 h of treatment with fatty acids, MTT tests were performed for each concentration. **p*< 0.01 significance level obtained by comparing the control group
MTT: Methylthiazolyldiphenyl-Tetrazolium Bromide

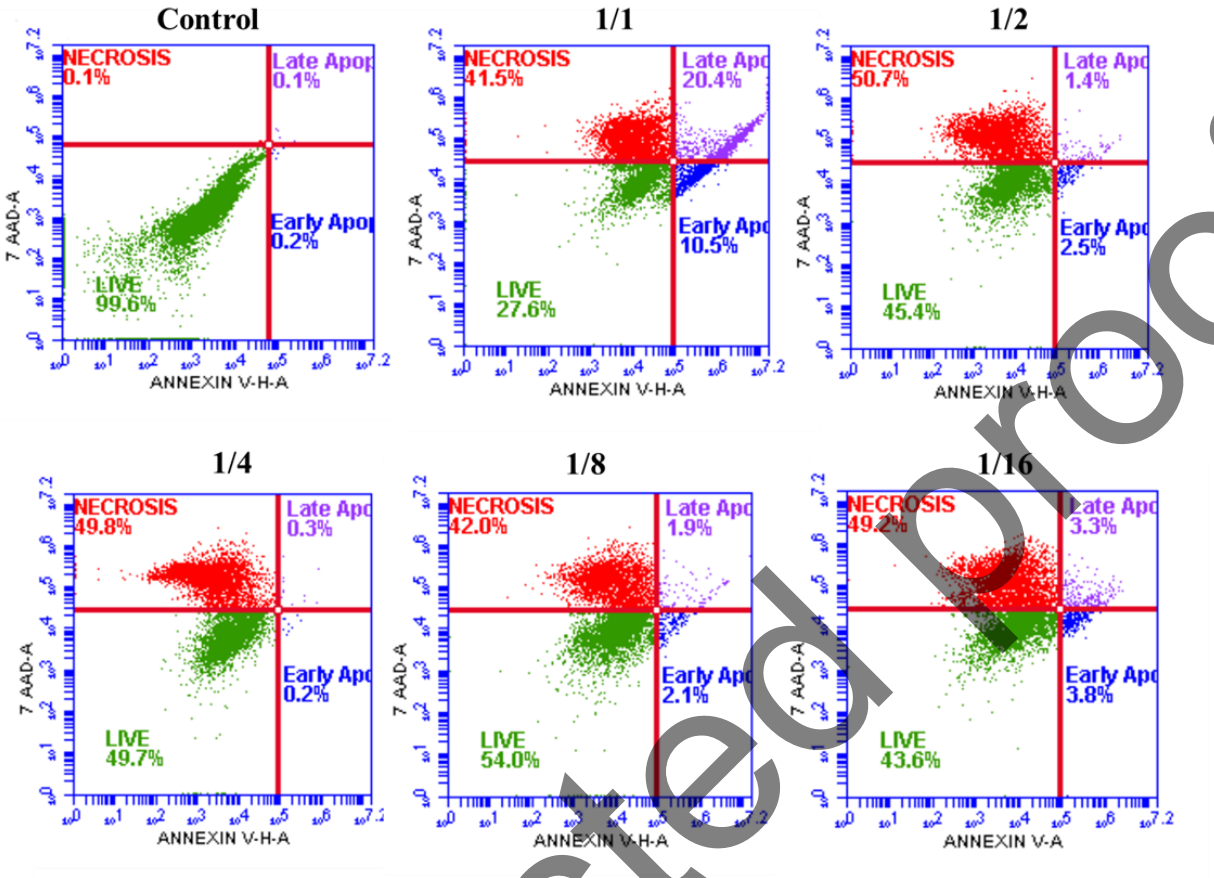

Figure 3. B1 (Annexin -, PI +): Necrotic cells; B2 (Annexin +, PI +): Late apoptosis; B3: Live cells (Annexin -, PI -); B4 (Annexin +, PI -): Early apoptosis

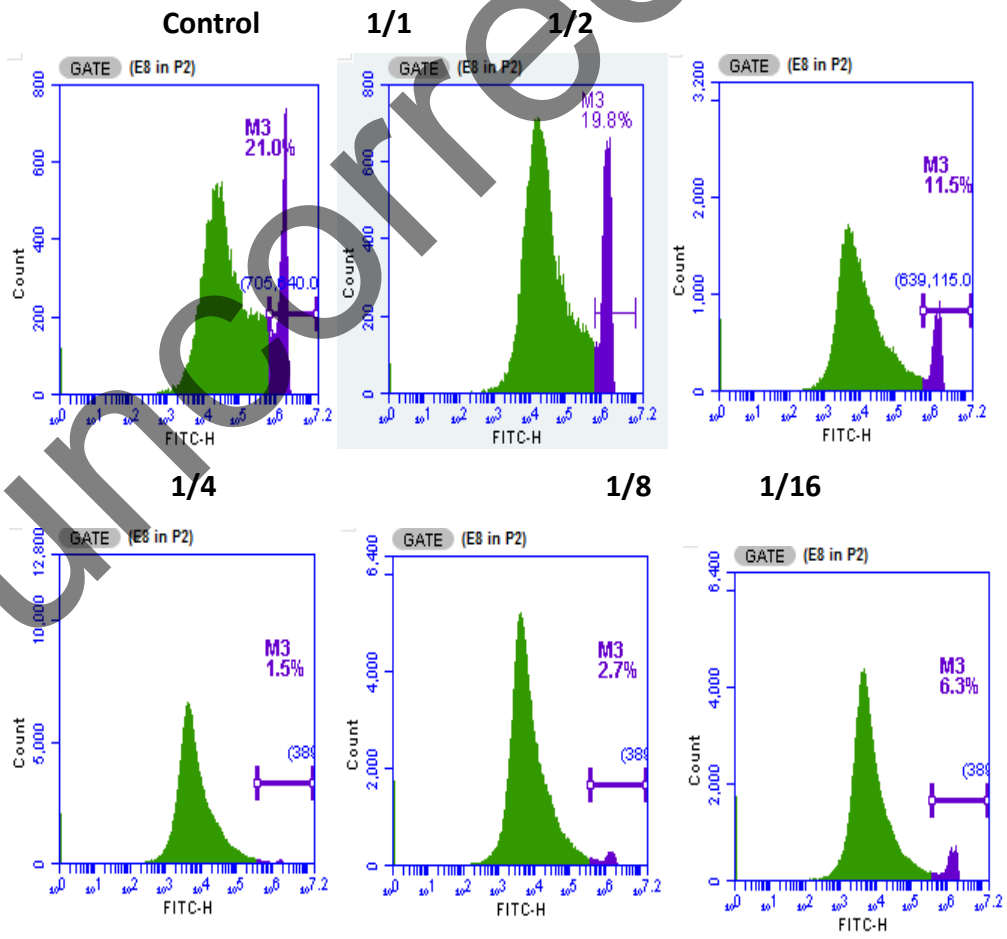

Figure 4. SHSY-5Y cell line ROS results
ROS: Reactive Oxygen Species

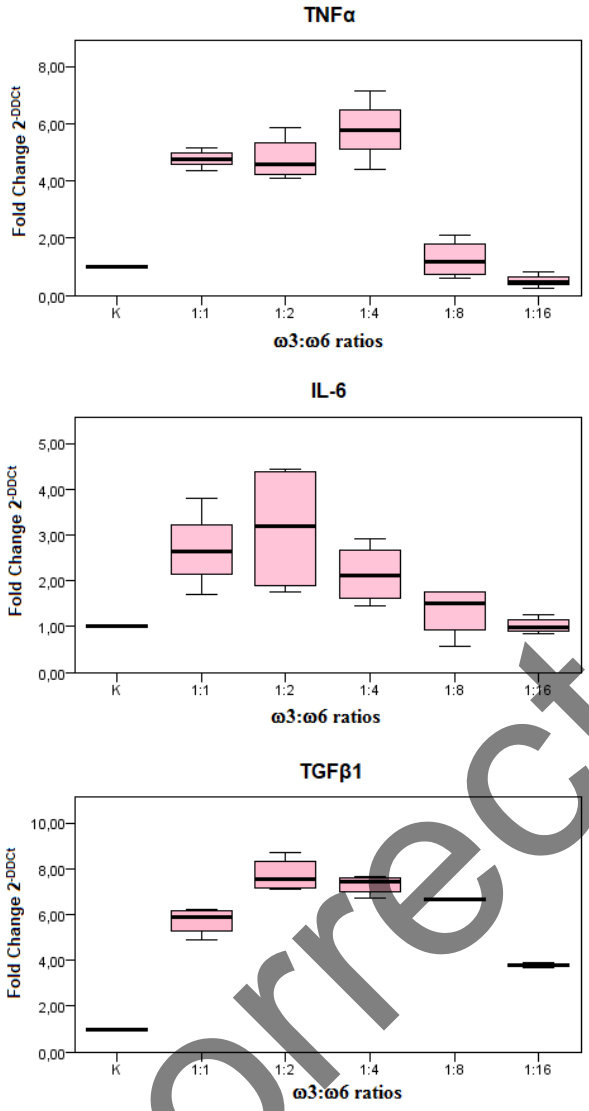

Figure 5. mRNA expression levels of TNF-α, IL-6 and TGFβ1 in the SHSY-5Y cell line
TNF-α: Tumor necrosis factor-alpha, TGFβ1: Transforming Growth Factor Beta, IL-6: Interleukin-6

Table 1. Forward and reverse primers of genes		
GAPDH	F: TTTTGCGTCGCCAGCC	R: ATGGAATTTGCCATGGGTGGA
TNF-α	F: AAAACAACCCTCAGACGCCA	R: TCCTTTCCAGGGGAGAGAGG
IL-6	F: CTTCGGTCCAGTTGCCTTCTC	R: GGCATTTGTGGTTGGGTCAG
TGFβ1	F: ACCTGCCACAGATCCCCTAT	R: GAGCAACACGGGTTCAGGTA
TNF-α: Tumor necrosis factor-alpha, TGFβ1: Transforming Growth Factor Beta, IL-6: Interleukin-6		