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# Preliminary *in vitro* assessment of cytotoxic and genotoxic effects of avocado (*Persea Americano*) oil in breast cancer cell line (MCF-7)

# Avokado (*Persea Americano*) yağının meme kanseri hücre hattı (MCF-7) üzerindeki sitotoksik ve genotoksik etkilerinin *in vitro* ön değerlendirmesi

Ayça AKTAŞ ŞÜKÜROĞLU[1]

[1]Mersin University, Faculty of Pharmacy, Department of Toxicology, 33169, Mersin-Turkey

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**Abstract**

**Aim:** Toxicological evaluation is required to understand the safety of avocado (*Persea Americana*) oil for use as a food supplement. In this study, cytotoxic and genotoxic effects of avocado oil in MCF-7 cell line were evaluated.

**Materials and Methods:** In this study, the MCF-7 was exposed to avocado oil (1, 10, 25 and 100 ppm) for 24, 48 and 72 hrs to assess the cytotoxic and genotoxic effects.

**Results:** $IC_{50}$ of avocado oil were found to be 68.1, 62.8 and 64.3 ppm for 24, 48 and 72 hrs, respectively. There was a statistically significant decrease in cell polferation between the control and exposed groups ($p$<0.05). Micronucleus frequency was significantly increased compared with negative control ($p$<0.005).

**Conclusion:** Results of the study, avocado oil had cytotoxic and genotoxic effects in a time and concentration dependent manner. Regular use of avocado oil as a dietary supplement has been shown to have a protective effect.

**Keywords:** Avocado oil, Cytotoxicity, Genotoxicity, MCF-7, Cytokinesis-block micronucleus assay.

**Öz**

**Amaç:** Avokado (*Persea Americana)* yağının gıda takviyesi olarak kullanımında güvenliğinin anlaşılması için toksikolojik değerlendirilme yapılması gerekmektedir. Planlanan bu çalışmada avokado yağının MCF-7 hücre hattındaki sitotoksik ve genotoksik etkileri değerlendirilmiştir.

**Gereç ve Yöntem:** Bu çalışmada MCF-7 hücre hattı, avokado yağına (1, 10, 25 ve 100 ppm) ile 24, 48 ve 72 maruz bırakılarak sitotoksik ve genotoksik etkisi değerlendirilmiştir.

**Bulgular:** Avokado yağının $IC_{50}$ değerleri 24, 48 ve 72 saat için sırasıyla; 68.1, 62.8 ve 64.3 ppm olarak bulunmuştur. Avokado yağının bütün maruziyet sürelerinde kontrol grubu ile maruziyet grupları arasında hücre poliferasyonundaki azalma istatiksel olarak anlamlı bulunmuştur ($p$<0,05). Avokado yağına maruziyetine bağlı mikroçekirdek frekansında, tüm dozlarda negatif kontrole göre önemli artış görülmüştür ($p$<0,005).

**Sonuç:** Çalışmanın sonucunda avokado yağının MCF-7 hücre hattında zamana ve konstrasyona bağımlı olarak sitotoksik ve genotoksik etkilerinin olduğu görülmüştür. Avokado yağının gıda takviyesi olarak düzenli kullanılması sonucunda koruyucu etkisinin olabileceği görülmüştür.

**Anahtar Kelimeler:** Avokado yağı, Sitotoksisite, Genotoksisite, MCF-7, Sitokinezin durdurulduğu mikroçekirdek yöntemi.

**Yazışma Adresi/Address for Correspondence**: Ayça AKTAŞ ŞÜKÜROĞLU, Mersin University, Faculty of Pharmacy, Department of Toxicology, 33169, Mersin-Turkey, E-mail: aktasayca@mersin.edu.tr

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## Introduction

Avocado (*Persea Americana, Mill*) is a fruit that grows in temperate and subtropical regions worldwide, including Turkey. This fruit's pulp involves approximately 60% oil, 7% peel, and 2% seed. Avocado is a nutritious source of protein, fiber, vitamins (A, E, and C), and other critical compounds (potassium, magnesium minerals and carotenoids, phenolics, phytosterols, terpenoids which are known to exert antioxidant actions)[1,3] Many African countries use *Persea americana* fruit, leaves, and seeds in traditional medicine.[2]

Multiple studies of avocado seeds have been reported such as antioxidant, antihypertensive, larvicidal, fungicidal, bactericidal hypolipidemic, and recently amoebicidal and giardicidal activities.[3-12] Avocado oil have also been shown to effectively treat symptomatic osteoarthritis,[13] periodontal illnesses,[14] and skin problems.[15,16] Avocado is employed in various industries, including food, cosmetics, and medicine. Typically, pulp or avocado oil is utilized for these reasons. Avocado oil is used to marinate salads, sauces, and meat preparations. Compared to olive oil, the usage of cold-pressed avocado oil in cooking is relatively recent.[17]

Breast cancer is the most frequent cancer in women, accounting for 18% of all malignancies [18]. Breast cancer is difficult to cure because there are different classes of tumors that respond differently to treatment.[18-19]

Foods, in addition to medications, play an essential part in cancer treatment. Avocado-based food production and consumption are expanding globally due to rising studies on avocados' nutritional advantages and health benefits.[18-20] Raises questions about using avocado to supplement other foods for cancer treatment.[20]

This study examines the cytotoxic and genotoxic profile of avocado oil, which is widely used today on MCF-7 cells. While the cytotoxic effects of avocado oil in the MCF-7 cell line were evaluated using the xCELLigence system, the genotoxic effect of avocado oil was evaluated using *in vitro* the cytokinesis-block micronucleus (CBMN).

## Materials and Methods

### Materials and chemical reagents

The chemicals used as follows: A brand of avocado oil sold as a food supplement was commercially available obtained from a local shop; dimethyl sulfoxide (DMSO), Dulbecco's modified Eagle's medium, ethanol, fetal bovine serum (FBS), hydrogen peroxide (35%) ($H_2O_2$), Giemsa stain, trypsin-EDTA, RPMI 1640 medium, Dulbecco's phosphate buffered saline (PBS) from Sigma (St. Louis, MO, USA); Millipore filters from Millipore (Billerica, MA, USA).

### Cell culture

Human breast adenocarcinoma cell line MCF-7 (HTB-22) was obtained from ATCC MCF-7 cells were raised in RPMI-1640 medium with 10% heat-inactivated FBS, 1% penicillin-streptomycin solution (10,000 units penicillin and 10 mg streptomycin in 0.9% NaCl), and 2 mM L-glutamine. The medium was replaced every 2-3 days. The xCELLigence system was used to assess the cytotoxicity of avocado oil in the MCF-7 cell line. Cells were incubated at 37°C in a humidified atmosphere containing 5% $CO_2$.

### Avocado oil cytotoxicity on MCF-7 via xCELLigence

The xCELLigence manufacturer's instructions was followed for cytoxicity analyses and MCF-7 cell line was seeded reaching the cell number as $1\times10^4$ cells/well on 16-well plates. Subsequently, cell growth was then observed at a fifteen-minute interval and analysed using RTCA Software 1.2. After 24 hours of transplanted, cells in the 'logarithmic development phase' were treated to varied concentrations of avocado oil (1, 10, 25, and 100 ppm) and examined in real-time for 24, 48, and 72 hours. For positive control 20 mM $H_2O_2$ was used. As a negative control, untreated cells grown in growth medium were used.

All samples were administered in quadruplicate and all processes were carried out in the dark to avoid additional light-induced cellular damage. Using absorbance-

concentration curve, the 50% inhibitory concentration ($IC_{50}$) was determined. After the values of $IC_{50}$ were determined, the genotoxic profiles of Avocado oil on the MCF-7 were evaluated for 24,48 and 72 hrs.

### Avocado oil genotoxicity on MCF-7 via cytokinesis-block micronucleus assay (CBMN)

*In vitro* Mammalian Cell Micronucleus Test (OECD Test 487) was performed with minor modifications.[21] MCF-7 cells were seeded at a density of $5\times10^4$ cells per well in a T-25 flask and exposed to (1, 10, 25, and 100 ppm) avocado oil for 24, 48, and 72 hrs. 3 µg/mL Cytochalasin B was added to inhibit cytoplasmic division in 38th hours. As previously described, 2000 binucleated cells for each sample were examined microscopically and evaluated toxicity by classifying cells according to the number of micronucleus (MN) compared to the negative control.[22,23]

### Statistics

The Kolmogorov-Smirnov test was used to determine the normality of the data distribution. The means of the data were compared using the One-way variance analysis test, and the least significant difference test was used for post hoc analysis of group differences. The results were displayed as the mean and standard deviation from three experiments in triplicate. GraphPad Prism Software version 5.0.1 (San Diego, CA, USA) for Windows was used for statistical analyses. A *p*-value of under 0.05 was determined to be statistically significant.

### Results

The avocado oil used in the study was purchased from a national producer and the company's analytical characterisation values were accepted. The characterisation values obtained are shown in Table 1.

The xCELLigence technique analyzes the net adhesion of cells on a specifically designed gold electrode as the impedance of electricity fluctuates to quantify cellular growth in real-time. As a result, it provides better pre-sized data regarding the viability over the long term for cell screening that minimizes erroneous responses caused by material-dye interactions.[24] For the cytotoxicity study, the xCELLigence method was preferred. Unlike MTT, this approach provides more information regarding the long-term viability of cell assessment and avoids incorrect responses based on material-dye interaction.[24]

**Table 1.** Avocado oil content components.

| Components | % |
|---|---|
| Myristic Acid | 0.01 |
| Palmitic Acid | 18.33 |
| Palmitoleic Acid | 9.27 |
| Steraic Acid | 0.61 |
| Oleic Acid | 56.89 |
| Linoleic Acid | 13.16 |
| Linolenic Acid | 0.86 |
| Eicosanoic Acid | 0.08 |
| 11- Eicosanoic Acid | 0.16 |

This study evaluates the cytotoxic response of the MCF-7 cell line to avocado oil application. The time-dependent graph of proliferation curves acquired from the real-time cell analyser was displayed. The device's software digitized the collected data according to the definition of the cell value. According to these graphic drawings, by looking at the $r^2$ values, a cell index ($IC_{50}$) was found for avocado oil. $IC_{50}$ value correlates with the viability of cells. The results were obtained by taking logarithms of all administered dose groups at 72 hrs and plotting a graph against cell index values. $IC_{50}$ values; 68.1 for 24 hrs; 62.8 ppm for 48 hrs and 64.3 ppm for 72 hrs.

The doses in all 24, 48, and 72 hours of incubation were statistically significant compared to the negative control($p$>0.05). After 48 and 72 hours of incubation, the doses were statistically significant within themselves, while no significant difference was observed between 25 ppm and 50 ppm in 24 hours of exposure. Furthermore, the viability of cells decreases with duration in all exposure durations. Figure 1 shows the standardized cell index of MCF-7 cells treated with varied doses of avocado oil (1, 25, 50, 100 ppm) in contrast to medium as the negative control.

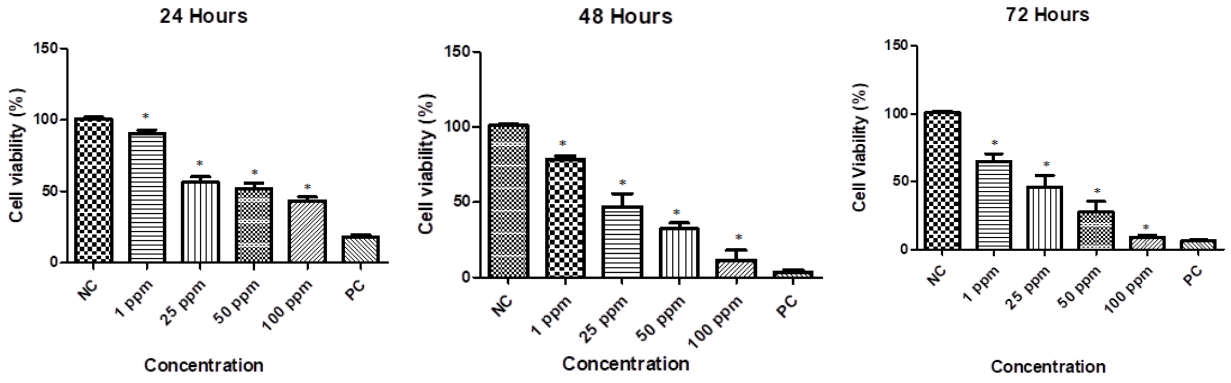


**Figure 1.** Effects of avocado oil on the cell viability of MCF-7 cells for 24 h, 48 h and 72 h. *Significant difference as compared to the negative control ($p$<0.05).

Avocado oil significantly increased the frequency of MN regardless of doses to the negative control after 24, 48, and 72 hours of exposure ($p$< 0.005).

When the MN frequencies were found at the most in the 48 hrs evaluated depending on time. In addition, the MN frequency with the avocado oil increased compared to the negative control, statistical difference was seen only at 100 ppm ($p$<0.005). According to doses, the MN frequency is evaluated, the MN increases with each exposure time depending on the concentration (Table 2).

**Table 2.** Changes in micronucleus frequencies in MCF-7 cell line treated with different concentrations of avocado oil depending on the exposure time 24, 48 and 72 h.

| Groups | | 24 hours | 48 hours | 72 hours |
|---|---|---|---|---|
| | Concentration(ppm) | MN (Mean ± SD) | MN (Mean ± SD) | MN (Mean ± SD) |
| **NC** | | 3.83 ± 1.04 | 4.83 ± 1.04 | 4.67 ± 0.76 |
| **PC** | | 18.50 ± 0.50* | 17.50 ± 0.50* | 18.00 ± 0.01* |
| **Doses** | **1** | 7.83 ± 0.76 | 7.67 ± 0.29 | 7.67 ± 0.76 |
| | **25** | 10.33 ± 0.58 | 11.67 ± 0.29 | 11.00 ± 0.50 |
| | **50** | 13.00 ± 0.50 | 13.50 ± 0.87 | 11.17 ± 0.29 |
| | **100** | 15.33 ± 0.58 | 18 ± 0.50* | 14.33 ± 1.04 |

MN: Micronucleus. SD: Standart deviation.NC: Negative Control. PC: Positive Control. *Significant difference as compared to the negative control ($p$<0.05). Negative control (1% PBS). positive control (50 µM $H_2O_2$).

## Discussion

This study investigates the cytotoxicity and genotoxicity profile of avocado oil in the MCF-7 cell line as a function of exposure and time.

Several biological activities of the avocado seed have been reported such as antioxidant, antihypertensive, larvicidal, fungicidal, hypolipidemic, and recently amoebicidal and giardicidal activities.[25-28] Treatment of MDA-MB-231 human breast cancer cells with a methanolic extract of avocado seed led to induction of apoptosis as measured by increased caspase-3, caspase-7, and poly(ADPribose) polymerase (PARP) cleavage and increased DNA laddering.[27] Abubakar, Achmadi, & Suparto (2017) isolated a triterpenoid fraction from an ethanolic extract of avocado seeds and studied its cytotoxic effects in MCF-7 breast cells.[28] They found that the triterpenoid fraction and the whole extract had $IC_{50}$ values of 80.1 $\mu$g/mL and 99.7 $\mu$g/mL, respectively. Kristanty, Suriawati, & Sulistiyo (2014) found that the cytotoxicity of aqueous and ethanolic extract of avocado seeds inhibited T47D breast cancer cell line with $IC_{50}$ values of 560.2 $\mu$g/mL and 107.2 $\mu$g/mL, respectively.[29]

Our study investigated the cytotoxicity of avocado oil and according to our results, our $IC_{50}$ values were found 68.1 for 24 hrs, 62.8 ppm for 48 hrs, and 64.3 ppm for 72 hrs in the MCF-7 cell line, respectively. Additionally, several studies have focused on the evaluation of acute toxicity of the fruit and leaves.[29] Avocado leaves showed cardiotoxic effects in mammals and birds.[30-33] Queiroz Junior et. al., (2021) found that avocado extract and oil in the presence of rotenone increased cellular viability at all tested concentrations compared to cells exposed only to rotenone. In addition,

extract and avocado oil exhibited antioxidant action as evidenced by decreased levels of reactive oxygen species (ROS), superoxide ion, and lipid peroxidation, generated by rotenone.[34]

Kulkarni et al.[35] showed that the extracts of both avocado fruit and leaves can potentially cause genomic instability and some genetic damage *in vitro* human lymphocytes. Padilla-Camberos et al.[36] showed that the genotoxic potential of an ethanolic seed extract of *Persea americana* in rats using a MN test. There were no differences in the incidence of micronuclei in rodent groups given an avocado seed extract against the negative control.[36] This is the first study of avocado oil genotoxicity in the MCF-7 cell line using CBMN. According to our results, the MN frequency increases with each exposure time depending on the concentration against the negative control.

The cytokinesis-block micronucleus (CBMN) assay according to the OECD 487 guideline was the preferred method for measuring MN in MCF-7 cell line.[21] In addition to its reliability, the CBMN assay has evolved into one of the industry-standard cytogenetic techniques for genetic toxicity testing *in vivo* and *in vitro* research. MN is an effective genotoxic biomarker; [37,38] hence, the staining procedure with Giemsa dye assists in differentiating micronucleated cells. Examining micronucleus frequencies *in vitro* is one of the significant genotoxicity assays regulatory organizations suggest for product safety evaluation.[37]

According to toxicity data, many plants used as food or in traditional medicine contain cytotoxic, mutagenic, and genotoxic characteristics.[39,40] The results highlight the need to comprehend thetoxicological effects of substances that come into contact, either directly or indirectly, with humans.[40] Other cell lines and toxicity tests must be investigated to complete the toxicological evaluation of avocado oil.

## Conclusion

The study results shows that avocado oil has both cytotoxic and genotoxic effects in MCF-7 cell line. Genotoxicity results was found statistically significant, especially for 100 ppm dose of avocado oil at 48th hour.

Recently, new alternative agents for treating and preventing breast cancer have been investigated. The focus has been on therapeutically effective foods based on the induction of apoptosis in cancer cells, especially those containing natural products or herbs. This study will provide a new perspective on the mechanisms between the use of avocado oil as a food supplement and breast cancer and new approaches to forming potential cancer drugs for managing breast cancer.

However, the study was only conducted in the MCF-7 cancer cell line. Avocado oil concentrations should also be conducted in healthy cell lines to better evaluate the results obtained and in order to verify our results, we believe it is important to conduct a safety study with *in vivo* experimental animals in further studies.

## Ethics Committee Approval

In the current work, there was no animal or human experiments conducted.

## Informed Consent

Informed consent forms were obtained from all participants.

## Author Contributions

All of the authors contributed at every stage of the study.

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## Conflict of Interest

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

## Financial Disclosure

The authors declared that this study has received no financial support.

## Statements

These results have not been presented anywhere previously.

## Peer-review

Externally peer-reviewed

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