http://dx.doi.org/10.14500/aro.11073

# Cytostatic Effects of Avocado Oil Using Single-cell Gel Electrophoresis (Comet Assay): An Evaluation

Subasini Uthirapathy

Department of Pharmacology, Faculty of Pharmacy, Tishk International University,
Erbil, Kurdistan Region, Iraq

***Abstract*—The goal of this paper is to assess the mutagenic and genotoxic potentials of avocado oil made from the fruit pulp of *Persea Americana*, a member of the *Lauraceae* family. Michigan Cancer Foundation-7 (MCF-7) cells are used in the 3-4,5 dimethylthiazol-2yl-2,5-diphenyl tetrazolium bromide (MTT) test to examine the possible antiproliferative and cytostatic qualities of different doses of avocado oil, and MCF-7 cells are used in the comet assay to examine the potential cytostatic effects of avocado oil extracted from the avocado fruit. DNA in human breast cancer cells is partially damaged by avocado oil. However, DNA damage at low, medium, and high levels was discovered in the positive control. Without positive control, the DNA damage level falls in the low, middle, and high ranges. The MTT assay shows that avocado oil exerts a dose-dependent cytostatic impact on human breast cancer MCF-7 cells with an $IC_{50}$ value of 379.2 μg/mL, which is the $IC_{50}$ value that causes genotoxicity in the comet assay.**

***Index Terms*—Avocado oil, *Persea Americana*, Genotoxicity, Mutagenic, Cytostatic**

## I. Introduction

In a multistage process that involves moving from a precancerous lesion to a malignant tumor state, cancer develops when normal cells undergo the change into tumor cells. The interaction of a person's genetic characteristics with environmental elements such as ultraviolet radiation, chemical elements such as cigarettes and asbestos, and biological elements such as viruses and bacteria leads to these alterations (Cragg and Newman, 2009). Deregulation of one or more cellular mechanisms, such as cell division and apoptosis, which are necessary for the healthy cells' normal growth and proliferation, leads to the development of cancer (Elmore, 2007). The main aim of medication development and candidate screening is to identify the differences in regulatory mechanism at play in cancer cells responsible for transformation and particularly target that mechanism (Wiman and Zhivotovsky, 2017) *Persea americana*, often called avocado or gator pear, stands out among the fruits with exceptional dietary and therapeutic properties. According to earlier studies, the total lipid content of fruit was higher than that of seed (Matsuoka and Yashiro, 2018, Imran, et al., 2017).

*P. americana*, avocado or alligator pear, has excellent nutritional and medical applications. Avocado oil is a nutritious oil that is rich in the unsaturated fat oleic acid. It aids in the body's absorption of other fat-soluble vitamins and contains vitamin E. It has been shown to lower low-density lipoprotein cholesterol and raise high-density lipoprotein cholesterol since it is a good source of monounsaturated fat. It contains high amount of fats, proteins, fibers, as well as vitamins and minerals such as phosphorus, sodium, magnesium, potassium, iron, and zinc (Orhevba and Jinadu, 2011; Oluwole, et al., 2013). The bioactive compounds separated from *P. americana* such as linoleic acid, linolenic acid, and oleic acid are all present in abundance in avocado oil that is derived from the fruit's pulp. In addition, it has minerals, vitamins A, C, D, and E, as well as -sitosterol, -carotene, and lecithin (IsabelleSantana, et al., 2019).

Decoctions of avocado seeds have been used in ethnopharmacological investigations of the Aztec and Maya cultures to treat gastrointestinal irregularities, diabetes, inflammation, and mycotic and parasite illnesses. Insecticidal, antibacterial, antidiabetic, and blood pressure-lowering properties of avocado seeds are highlighted in our prior review (Dabas, et al., 2011). Avocado seeds are abundant in polyphenols and have a wide variety of compounds in them. The ethanolic extract of avocado seeds contains a triterpenoid component, and Michigan Cancer Foundation-7 (MCF-7) breast cells were used to study the seed's cytotoxic effects (Abubakar, Achmadi and Suparto, 2017). Dabas, et al., in 2013, discovered that the IC50 values for the triterpenoid fractions were 80.1 g/mL and 99.7 g/mL, whereas the IC50 values for the total extract were 560.2 g/mL and 107.2 g/mL, respectively. Kristanty, et al., in 2014, discovered that the cytotoxicity of aqueous and ethanolic extract of avocado seeds suppressed T47D breast cancer cell line. Using the comet test or single-cell gel electrophoresis (SCGE), one can evaluate DNA damage and repair in individual cells in a flexible, sensitive, easy-to-use, and affordable manner. In the fields of genotoxicity, pharmacology, and molecular investigations, it is necessary to detect DNA damage at the level of eukaryotic cells. The comet assay is useful for measuring DNA breaks at

ARO-The Scientific Journal of Koya University
Vol. XI, No. 1 (2023), Article ID: ARO.11073. 21 pages
DOI: 10.14500/aro.11073
Received: 19 September 2022; Accepted: 31 December 2022
Regular research paper: Published: 15 January 2023
Corresponding author's e-mail: subasini.uthirapathy@tiu.edu.iq

apurinic and apyrimidinic locations, particularly single-strand DNA breaks. Cells with DNA crosslinks, base-pair damage, and apoptotic nuclei (Nandhakumar, et al., (2011).

Ostling and Johnson, in 1984, provided an explanation of DNA migration from nuclei exposed to an electric field under neutral conditions. Singh, et al., in 1988, significantly improved the specificity and repeatability of this method by modifying and refining it in alkaline conditions. The comet test has since gained popularity and developed into a widely used method for assessing DNA damage. The alkaline comet assay is the method that is most frequently used to evaluate DNA damage (Kaur, et al., 2011).

A quick, easy, visible, and sensitive method for determining and assessing DNA breakage in mammalian cells is the single-cell electrophoresis test, sometimes referred to as the comet assay (Tice, et al., 2000, Kaur, et al., 2011, Collins, 2003). The primary goal of the current study is to assess the anticancer potential of *P. Americana* avocado oil, on human breast MCF-7 cancer cell line using a variety of assay methodologies, including morphological investigations, cell viability tests, and comet assays.

## II. Materials and Methods

### *A. Chemicals and Consumables*

The human breast cancer cell line MCF-7 was bought from NCCS Pune in India. Fetal bovine serum (FBS), Dulbecco's Modified Eagle's Medium (DMEM), the antibiotic solution was from Gibco (USA), dimethyl sulfoxide (DMSO), 3-4,5 dimethylthiazol-2yl-2,5-diphenyl tetrazolium bromide (MTT) was from Sigma, USA, 1X PBS was from Himedia, 96-well tissue culture plate, and wash beaker were purchased from Tarson Products Pvt Ltd, Kolkata, India.

Agarose low melting and normal melting gels, sodium chloride (analytical grade-AR), potassium chloride (AR), disodium hydrogen phosphate (AR), disodium EDTA, trichloroacetic acid, zinc sulfate, sodium carbonate, ammonium nitrate (AR), potassium dihydrogen phosphate (AR), tritron X 100, glycerol, and formaldehyde. Glass measuring cylinders, beakers, conical flasks, staining troughs, straining boxes, micropipettes, and tubes for microcentrifugation.

### *B. Isolation of Avocado Oil Extracted from a Pulp of Avocados by Cold-pressed Method*

Avocado fruits were obtained a local grocery store in Erbil, Iraq. The following is the steps involved in making Avocado oil. After washing the avocados, cut them into two halves, then using a spoon collect the flesh of the avocado such that they will come out having a paste like consistency. Spread the avocado paste on a tray and spread with medium layer in thickness such that the layer is not too thick and not too thin. The tray is placed in a warm shady and airy area in the house. Avoiding placing the tray directly in the sun. After 4 h, the top layer of the avocado paste it has a dark brownish color. At this stage use a spoon and mix the paste such that the upper layer mixes well with the layer using the spoon, the upper layer and lower layer of the paste are mixed well gently. After mixing the avocado paste is spread back on the tray and kept it in an airy, warm, and shady corner in the house. We repeat this procedure whenever we notice that the upper layer of the avocado has a dark brownish color. After the procedure is repeated, whenever noticed that the upper layer of the avocado became a dark brownish color. Once more, the mixture will include almost no avocado paste and only oil. Now take the avocado mixture out of the avocado.

Avoiding too dry paste may lead to difficulty in removing it from the tray and difficult to press out the oil. The oil using cheese cloth is ready and the bowl that use in collecting the oil. Then hold the cloth together and squeeze out the oil, there is so much oil coming out. The oil will be kept in an aseptic area.

### *C. MTT Assay*

The MTT test was used to evaluate the possible cytotoxic and antiproliferative effects of avocado oil on human MCF-7cells (human breast cancer) cell lines. Al-Qubaisi, et al., in 2011, used the MTT experiment with a few minor modifications. According to the methodology, MCF-7 cell lines were grown in liquid DMEM supplemented with 10% FBS, 100 g/mL streptomycin, and 100 g/mL penicillin. These conditions were kept at 37°C in a 5% $CO_2$ atmosphere.

Trypsinization was used to harvest the cultivated MCF-7 cells, which were, then, gathered in a 15 mL tube. The cells were, then, seeded into a 96-well tissue culture plate at a density of $1 \times 10^5$ cells/mL cells/well (200 μL) in DMEM media with 10% FBS and 1% antibiotic solution for 24–48 h at 37°C. In a serum-free DMEM medium, the wells were cleaned with sterile PBS before being treated with various doses of avocado oil. The cells were incubated for 24 h at 37°C in a humidified 5% CO2 incubator, with each sample being replicated three times.

The cells were, then, treated for a further 2–4 h with MTT (20 μL of 5 mg/mL) until purple precipitates were plainly visible under an inverted microscope. The medium and MTT (220 μL) were then aspirated out of the wells and rinsed with 1X PBS (200 μL). In addition, DMSO (100 μL) was added, and the plate was agitated for 5 min to dissolve the formazan crystals.

With the use of a fluorescence multi-detection reader, the optical density (OD) was measured at 570 nm (Thermo Fisher Scientific, USA). Cells that had not been treated served as a control. Measurements were made, and a graphic representation of the concentration needed to reduce viability by 50% was obtained. The drug concentration was plotted in the X-axis using the standard GraphPad prism 6.0 program, with the relative cell viability being represented on the Y-axis.

$$\text{Cell viability } (\%) = \frac{\text{OD test}}{\text{OD control}} \times 100$$

### *D. Comet Assay (SCGE)*

"Human MCF-7 breast cancer cells were used in the comet test". In a 24-well plate, at a density of 10,000 MCF-7 cells were planted, and they were then cultured for 24 h at 37°C in a humidified 5% CO2 incubator (Nandakumar, et al., 2011). The wells were treated with 379.2 μg/mL of avocado oil sample in a serum-free DMEM medium for 24 h in a CO2

http://dx.doi.org/10.14500/aro.11073

incubator after being treated with sterile PBS. Following incubation, the cells were collected using trypsinization in a 1.5 mL tube, and the comet assay was carried out using, with a few minor modifications of Nandhakumar, et al., (2011) techniques. First, 200 μL of 0.75% normal melting agarose and then 100 μL of 0.5% low melting agarose were progressively applied onto the microscopic slides. The following phase involved adding 20 μL of cell suspension to 60 μL of 0.5% low melting agarose, which served as the third layer on the slides.

After that, the slides were kept at 4°C overnight in cell lysis buffer (2.5 M NaCl, 0.2 M NaOH, 100 mM $Na_2EDTA$, 10 mM Tris-HCl, 1% Triton X-100, and 10% dimethyl sulfoxide, pH = 10.0). The slides were then three times submerged in double-distilled water, followed by a 20-min incubation with an unwinding solution (3M NaOH). Slides were, then, put into a horizontal gel electrophoresis tank with electrophoresis solution (1 mM $Na_2EDTA$ and 300 mM NaOH, pH = 13). For 25 min, the electrophoresis was carried out at 25 V (1 V/cm, 300 mA). The slides were, then, exposed for 10 min to neutralization buffer (0.4 M Tris-HCl, pH = 7.5), immersed 3 times in ultrapure water, and allowed to air dry.

## III. Results and Discussion

The purpose of this study was to look into the cytostatic effects of genuine avocado oil on MCF-7 human breast cancer cells. With the prevalence of malignancies, many herbal plant species demonstrate cytostatic activities (Parasuraman, Raveendran and Kesavan, 2010). Therefore, it is crucial to comprehend the possible toxicity of herbal plants. Some traditional medicinal herbs have secondary metabolite components that have the potential to cause cancer and/or be genotoxic. In developing nations, the majority of medicinal plants are used for homemade medicines. They provide a variety of nutrients and biomolecules important to human health (Nadin, Vargas-Roig and Ciocca, 2001). Five avocado fruits were used in the cold-pressed extraction of avocado oil from the fruit's pulp, yielding more than 20 mL of dark greenish essential oil. Linoleic acid, linolenic acid, and oleic acid are all present in abundance in avocado oil that is derived from the fruit's pulp. In addition, it has minerals, vitamins A, C, D, and E, as well as sitosterol, carotene, and lecithin (IsabelleSantana, et al., 2019). Sterols and hydrocarbons made up the majority of *P. americana* fruits. The smallest components in the fruit other than that seed of *P. americana* were derivatives of other types of sterols, including campesterol, stigmasterol, and sitosterol.

Lipid components are the avocado fruit's most significant attributes. Five to six fatty acids are present in substantial concentrations in avocado fruits. These acids include the monounsaturated fatty acids (MUFA) oleic acid and palmitoleic acid, the polyunsaturated fatty acids (PUFA) linoleic acid and linolenic acid, as well as the saturated fatty acids (SFA) palmitic acid and stearic acid. Oleic acid, which was the most prevalent MUFA at both sites, makes up more than 50% of the total lipids. According to McDaniel, et al., (2008), "avocados have been found to have a fruit oil that is high in MUFAs (MUFA), PUFA, and SFAs, with 71% MUFA, 13% PUFA, and 16% SFAs."

### *A. MTT Assay*

MCF-7 cells were tested using the MTT assay to determine their cytotoxicity against human breast cancer cells. Human breast MCF-7 cells' ability to proliferate was decreased by avocado oil. Fig. 1 shows the MTT assay findings. Within 24 h, the proliferation of cells toward MCF-7 cells was inhibited at different concentrations (31.25–1000 μg/mL). The $IC_{50}$ value of avocado oil for MCF-7 cell lines at 24 h was 379.2 μg/ml. MTT was a water-soluble substance that the live cell can take up. For calorimetric measurement, a water-insoluble blue formazan that was the reduction product of MTT must be dissolved. Effects of avocado oil on human breast cancer cells that suppress cancer cell growth (MCF-7), it exhibited the most cytotoxic effects. Due to the oil's bioactive components, particularly MUFA such as oleic acid and linoleic acid, the antiproliferative properties of avocado oil are enhanced. Avocado oil has an $IC_{50}$ of 379.2 μg/mL, with MCF-7 cells being the most sensitive. MCF-7 cells were used in this experiment due to their sensitivity to growth inhibition caused by avocado oil. To find out how normal, non-tumorigenic cells respond to avocado oil in terms of growth.

As shown in Fig. 2a, the untreated MCF-7cells maintained their original morphology and close closeness to one another even when the incubation time was extended to 24 h. In contrast, after being exposed to avocado oil for 24 h, MCF-7 cells started to lose their normal shape. The MCF-7 cells' characteristic elongated spindle-shaped morphology was no longer visible. When the treatment was extended to 48 h, dead cells were discovered; more were discovered at 24 h (Fig. 2b-g). The results of the present study show that avocado essential oil possesses cytostatic properties. The same observational results agreed with those published by Sahranavard, Naghibi and Ghafari (2012) and Jayaprakash, et al. (2010). Numerous natural compounds have been shown to be able to cause apoptosis in different tumor cells with human origin (Aigner, 2002; Shiezadeh, et al., 2013). Apoptotic inducers originating from plants must be thoroughly screened, whether they were

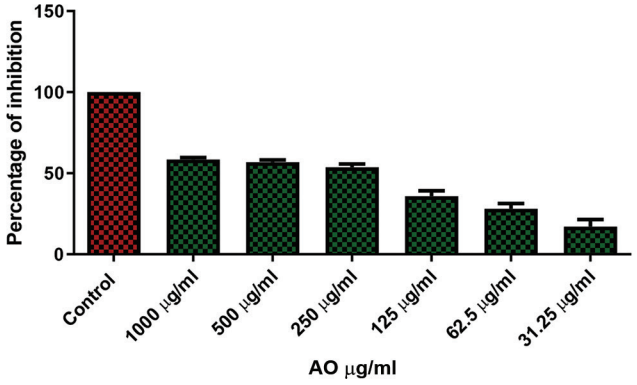


Fig. 1. Cytostatic effect assessed by 3-4,5 dimethylthiazol-2yl-2,5-diphenyl tetrazolium bromide assay of Avocados oil (data were presented as the mean of triplicate determinations ± standard deviation.

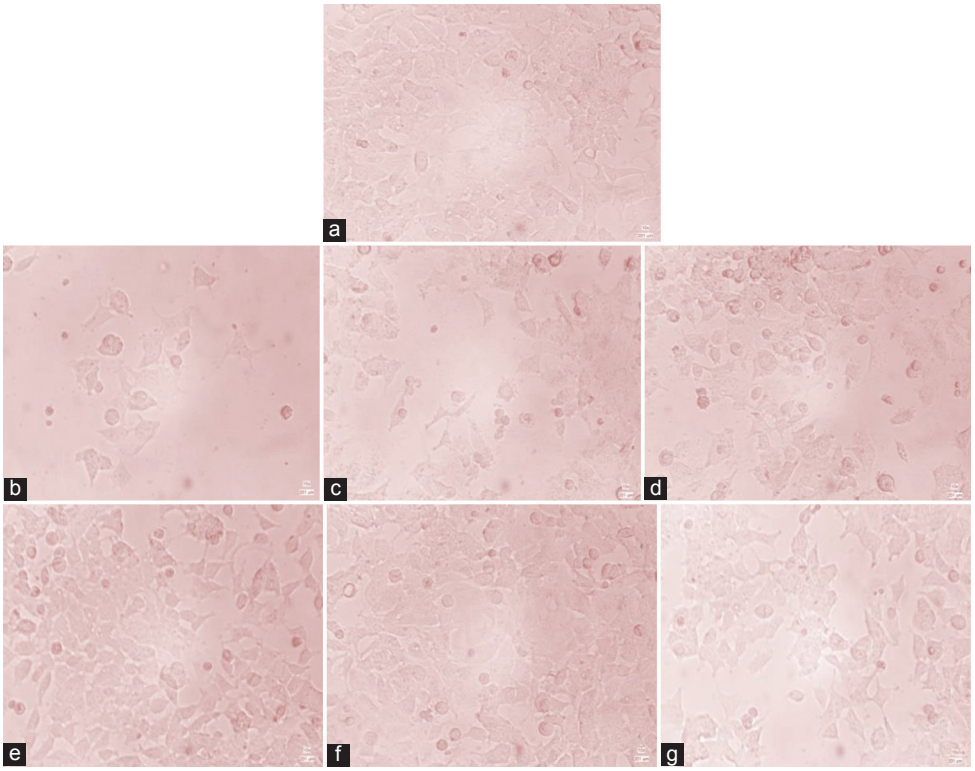


Fig. 2. Cytostatic produced by essential oil of Avocados against human breast cancer MCF-7 cells. Where, figure (a) control cells; and figure (b-g) essential oils of Avocados fruits (conc. 31.25–1000 µg/mL, respectively.

TABLE I
Genotoxicity of Avocado Oil in Human Breast Cancer Cell Line in the Comet Assay

| S. No | Test sample | Number of comet events | | | | | | | | | | DNA damage events (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Class 0 | | Class 1 | | Class 2 | | Class 3 | | Class 4 | | |
| 1. | Control | 100 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2. | Treated 79.2 µg/mL of AO | 61 | 58 | 20 | 26 | 7 | 5 | 10 | 8 | 2 | 3 | 40.5% |
| 3. | Average | 59.5 | | 23 | | 6 | | 9 | | 2.5 | | |

raw extracts or part of components that have been extracted from their original natural plant sources (Taraphdar, Roy and Bhattacharya, 2001).

*B. Comet Assay (SCGE)*

At a concentration of 379.2 µg/mL of avocado oil, 59.5% of cells (Class 0) showed no damage, 23% of cells (Class 1) showed light damage, and 9% of cells (Class 1) showed medium damage (Class 3). Cells in 6 and 2.5 were severely damaged by avocado oil (Class 2 and Class 4). The results are shown in Table I. The cells were stained with 50 µL of ethidium bromide (5 mg/L), then viewed under a fluorescence microscope. Fluorescent slides are used to see ethidium bromide dyed slides. The utilization of a fluorescent microscope with a ×200 magnification and an excitation filter of 590 nm allows for the observation of ethidium bromide-stained slides. All procedures were done in low light to prevent extra DNA damage (Nadin, Vargas-Roig and Ciocca, 2001).

*C. Evaluation of DNA Damage*

Using an ocular scale affixed to the microscope's eyepiece, the length of the comet tail can be measured. Alternatively, the degree of damage can be visually graded from Class 0–4 based on how the comet appears Fig. 3. Additional option is to use image analysis tools to quantify other DNA damage characteristics as the percentage of DNA damage in the head and tail, the length of the tail movement, the area of the tail, etc. Per sample, a total of 50–70 randomly chosen cells are examined. Comets must be chosen at random to encompass the entire gel. Comets observed in overlaps, air bubbles, and edges are discarded. Fig. 4a-c and Fig. 5 shows the comet's length, tail length, head diameter, and% of DNA content in the head, tail, and tail movement (Collins, 2003). Twenty fatty acids were found in avocado oil, of which six compounds could be isolated from them, whereas 14 fatty acids remained unrecognized. According to their percentages of 8.87%, 44.61% and 6.40%, respectively, the discovered fatty acids were categorized as saturated, monounsaturated, and polyun SFAs. The discovered fatty acids were categorized as saturated, monounsaturated, and polyun SFAs. The main MUFA from the saponifiable materials that could be isolated was oleic acid methyl ester (Louw, 2012). Other secondary metabolic phytoconstituents such as flavonoids, alkaloids, triterpenes, glycosides, tannins, and steroids are among the phytochemicals found in avocado oil (McDaniel, et al., 2008, Cardoso, et al., 2004).

The $IC_{50}$ value of avocado oil data showed that there was low, medium, and high damage at concentrations of 379.2 g/mL in comet assay; this may be related to variations in cell physiology, such as cell cycle (Jayaprakash, et al., 2010,

http://dx.doi.org/10.14500/aro.11073

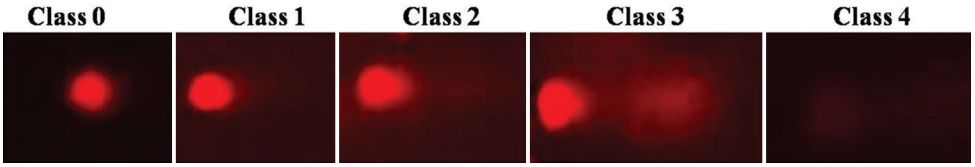


Fig. 3. Visual scoring of DNA damage from Class 0–4 according to comet appearance. Magnification ×200.

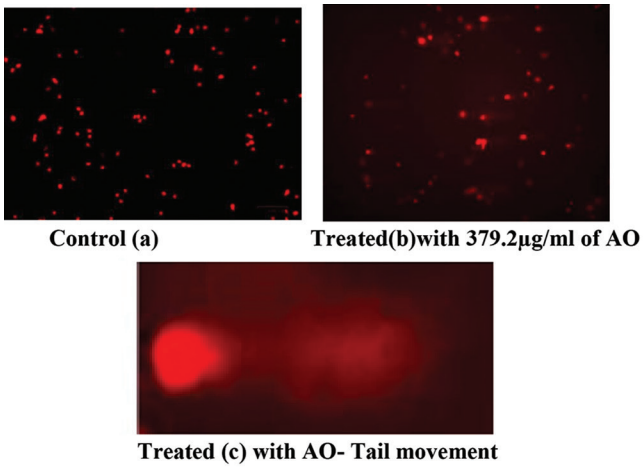


Fig. 4. Photomicrographs of stained DNA human breast cancer cell (MCF-7) for alkaline COMET assay. (a) Untreated showing no DNA damage, (b) treated with Avocados oil (379.2 µg/mL) showing DNA damage, (c) tail movement.

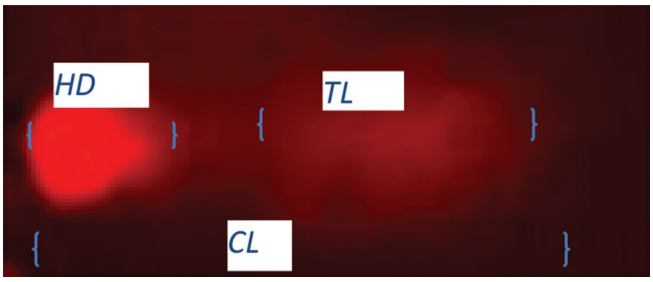


**Fig. 5.** Various comet parameters - CL: Comet length, TL: Tail length, HD: Head diameter - Magnification ×200.

Kaskoos, et al., 2021). Results showed that there was a decrease in cell damage compared with control group, there was less cell damage. However, a different study using the MTT test showed that avocado oil exhibits a strong cytostatic impact in a dose-dependent manner (JavedAhamad, et al., 2021). Avocado essential oil has medicinal properties and has been used for many years to treat many ailments. With the COMET assay (Kaur, et al., 2011, Jakobsen, et al., 2009, Barakat, et al., 2013), it did not demonstrate total DNA damage. The results of this study show that genotoxicity is caused in Comet assay and MTT assay models by the original essential oil from avocado fruits. Despite the fact that it has antimutagenic properties, more research on molecular signaling pathways is needed. Barakat, et al., in 2013, proved that the water extract of avocado exhibited small $IC_{50}$ values of 13.3 µg/mL in HepG-2 and 22 µg/mL in HT-29 cell lines. MTT assay and COMET assay, which are very sensitive tests for estimating DNA damage and which directly identify single or double strand breaks in individual cells, were used to evaluate the cytotoxicity and genotoxicity of avocado oil in human breast cancer cell lines.

## IV. Conclusion

Avocado oil is an herb with medicinal characteristics, compared to lipid extract from the seeds, the oil from *P. american's* fruit displayed surprisingly prominent antioxidant, anti-inflammatory, and anticancer properties. This could be explained by the substantial amounts of sterols, hydrocarbons, and un SFAs. This study's MTT and COMET assay were used to assess the cytostatic and genotoxic effects of avocado oil at various concentrations. These findings imply that the real avocado oil exhibited low, medium, and high levels of DNA damage. The DNA was not completely damaged, though. These show that the actual avocado oils are mutagenic.

## Acknowledgment

I gratefully acknowledge the Tishk International University, Erbil, Iraq, for providing research facilities and also acknowledge Trichy Research Institute of Biotechnology Pvt. Ltd., Trichy, Tamil Nadu, India, for conducting anticancer cell line study.

## References

Abubakar, A.N.F., Achmadi, S.S. and Suparto, I.H., 2017. Triterpenoid of avocado (*Persea Americana*) seed and its cytotoxic activity toward breast MCF-7 and liver HepG2 cancer cells. *Asian Pacific Journal of Tropical Biomedicine*, 7(5), pp.397-400.

Aigner, T., 2002. Apoptosis, necrosis, or whatever: How to find out what really happens? *Journal of Pathology*, 198(1), pp.1-4.

Al-Qubaisi, M., Rozita, R., Yeap, S.K., Omar, A.R., Ali, A.M. and Alitheen, N.B., 2011. Selective cytotoxicity of goniothalamin against hepatoblastoma HepG2 cells. *Molecules*, 16(4), pp.2944-2959.

Barakat, H.S., El-Hallouty, S., Khalifa, N.S. and Salem, D., 2013. Effect of the water extracts of avocado fruit and cherimoya leaf on four human cancer cell lines and *Vicia faba* root tip cells. *Journal of Agricultural Science*, 5(7), p.245.

Cardoso, C.R.B., Souza, M.A., Ferro, E.A.V., Favoreto, S. Jr. and Pena, J.D.O., 2004. Influence of topical administration of n-3 and n-6 essential and n-9 nonessential fatty acids on the healing of cutaneous wounds. *Wound Repair and Regeneration*, 12(2), pp.235-243.

Collins, A.R., 2003. The comet assay. Principles, applications, and limitations. *Methods in Molecular Biology*, 203, pp.163-167.

Cragg, G. and Newman, D.J., 2009. Nature: A vital source of leads for anticancer drug development. *Phytochemistry Reviews*, 8, pp.313-331.

Dabas, D., Elias, R.J., Lambert, J.D. and Ziegler, G.R., 2011. A colored avocado seed extract as a potential natural colorant. *Journal of Food Science*, 76(9), pp.C1335-C1341.

Dabas, D., Shegog, R.M., Ziegler, G.R. and Lambert J.D., 2013. Avocado (*Persea Americana*) seed as a source of bioactive phytochemicals. *Current Pharmaceutical Design*, 19(4), pp.6133-6140.

Elmore, S., 2007. Apoptosis: A review of programmed cell death. *Toxicologic Pathology*, 35, pp.495-516.

Imran, A., Qamar, H.Y., Ali, Q., Naeem, H., Riaz, M., Amin, S., Kanwal, N., Ali, F., Saba, M.F. and Nasir I.A., 2017. Role of molecular biology in cancer treatment. A review article. *Iranian Journal of Public Health*, 46(11), pp.1475–1485.

IsabelleSantana, I., Castelo-Branco, V.N., Guimarães, B.M., de Oliveira Silva, L., Di Sarli Peixoto, V.O., Cabral, L.M.C., Freitas, S.P. and Torres, A.G., 2019. Hass avocado *(Persea Americana* Mill.) oil enriched in phenolic compounds and tocopherols by expeller-pressing the unpeeled microwave dried fruit. *Food Chemistry*, 286, pp.354-361.

Jakobsen, M.U., O'Reilly, E.J., Heitmann, B.L., Pereira, M.A., Bälter, K., Fraser, G.E., Goldbourt, U., Hallmans, G., Knekt, P., Liu, S., Pietinen, P., Spiegelman, D., Stevens, J., Virtamo, J., Willett, W.C., Ascherio, A., 2009. Major types of dietary fat and risk of coronary heart disease: A pooled analysis of 11 cohort studies. *American Journal of Clinical Nutrition*, 89(5), pp.1425-1432.

JavedAhamad, J., Uthirapathy, S., Ameen, M.S.M. and Anwer, E.T., 2020. Essential oil composition and antidiabetic, anticancer activity of *Rosmarinus officinalis* L. leaves from Erbil (Iraq). *Journal of Essential Oil Bearing Plants*, 22(6), pp.1544-1553.

Jayaprakash, T., RamachandraRao, K., Bhat, B.V., Chand, P. and Kumar, S.N., 2010. DNA damage studies in cases of trisomy 21 using comet assay. *Current Pediatric Research*, 14, pp.1-4.

Kaskoos, R.A., Ahamad, J. and Uthirapathy, S., 2021. Chemical composition and cytotoxic activity of *Pistacia atlantica* var. kurdica fruits. *ARO The Scientific Journal of Koya University*, 9(2), pp.91-95.

Kaur, R., Kaur, S. and Lata, M., 2011. Evaluation of DNA damage in agricultural workers exposed to pesticides using single cell gel electrophoresis (comet) assay. *Indian Journal of Human Genetics*, 17(3), pp.179-187.

Kristanty, R.E., Suriawati, J. and Sulistiyo, J., 2014. Cytotoxic activity of avocado seeds extracts (*Persea Americana* mill.) on t47d cellines. *International Research Journal of Pharmacy*, 5(7), pp.557-559.

Louw, L., 2012. Effects of conjugated linoleic acid and high oleic acid safflower oil in the treatment of children with HPV-induced laryngeal papillomatosis: A randomized, double-blinded and crossover preliminary study. *Lipids Health and Disease*, 11, p.136.

Matsuoka, T. and Yashiro, M., 2018. Biomarkers of gastric cancer: Current topics and future perspective. *World Journal of Gastroenterology*, 24(26), pp.2818-2832.

McDaniel, J.C., Belury, M., Ahijevych, K. and Blakely, W., 2008. Omega-3 fatty acids effect on wound healing. *Wound Repair and Regeneration*, 16(3), pp.337-345.

Nadin, S.B., Vargas-Roig, L.M. and Ciocca, D.R., 2001. A silver staining method for single-cell gel assay. *Journal of Histochemistry Cytochemistry*, 9, pp.1183-1186.

Nandhakumar, S., Parasuraman, S., Shanmugam, M.M., Rao, K.R., Chand, P. and Bhat B.V., 2011. Evaluation of DNA damage using single-cell gel electrophoresis (comet assay). *Journal of Pharmacology Pharmacotherapeutics*, 2, pp.107-111.

Oluwole, S., Yusuf, K., Fajana, O. and Olaniyan, D., 2013. Qualitative studies on proximate analysis and characterization of oil from *Persea Americana* (Avocado pear). *Journal of Natural Science Research*, 3(2), pp.68-73.

Orhevba, B.A. and Jinadu, A.O., 2011. Determination of physico-chemical properties and nutritional contents of Avocado pear (*Persea Americana* M.). *Academic Research International*, 1(3), p.372.

Ostling, O. and Johanson, K.J., 1984. Microelectrophoretic study of radiation-induced DNA damage in individual mammalian cells. *Biochemical and Biophysical Research Communications*, 123(1), pp.291-298.

Parasuraman, S., Raveendran, R. and Kesavan, R., 2010. Blood sample collection in small laboratory animals. *Journal of Pharmacology Pharmacotherapeutics*, 1(2), pp.87-93.

Sahranavard, S., Naghibi, F. and Ghafari, S., 2012. Cytotoxic activity of extracts and pure compounds of *Bryonaia aspera*. *International Journal of Pharmacy and Pharmaceutical Sciences*, 4(3), pp.541-543.

Shiezadeh, F., Mousavi, S.H., Amiri, M.S., Iranshahi, M., Tayarani-Najaran, Z. and Karimi G., 2013. Cytotoxic and apoptotic potential of rheum turkestanicum Janisch root extract on human cancer and normal cells. *Iranian Journal of Pharmaceutical Research*, 12(4), pp.811-819.

Singh, N.P., McCoy, M.T., Tice, R.R. and Schneider, E.L., 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. *Experimental Cell Research*, 175(1), pp.184-191.

Taraphdar, A.K., Roy, M. and Bhattacharya, R.K. 2001. Natural products as inducers of apoptosis: Implication for cancer therapy and prevention. *Current Science*, 80(11), pp.1388-1896.

Tice, R.R., Agurell, E., Anderson, D., Burlinson, B., Hartmann, A., Kobayashi, A., Miyamae, Y., Rojas, E., Ryu, J.C. and Sasaki, Y.F., 2000. Single cell gel/comet assay: Guidelines for *in vitro* and *in vivo* genetic toxicology testing. *Environmental and Molecular Mutagenesis*, 35(3), pp.206-221.

Wiman, K.G. and Zhivotovsky, B., 2017. Understanding cell cycle and cell death regulation provides novel weapons against human diseases. *Journal of Internal Medicine*, 281(5), pp.483-495.