INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print); 2222-5234 (Online)
Website: https://www.innspub.net
Email contact: info@innspub.net
Vol. 27, Issue: 1, p. 291-296, 2025

RESEARCH PAPER OPEN ACCESS

Lipid peroxidation and antioxidant status in 2,4,6-octatrienoic acid treated A549 and HCT-116 cancer cells

Shanmugam M. Sivasankaran[1], Raju Kowsalya[*1], Krishnan Baskaran[2], Chakravarthy Elanchezhiyan[3]

[1]*Department of Biochemistry and Biotechnology, Annamalai University, Annamalainagar, Tamil Nadu, India*

[2]*Department of Biochemistry, Madha Medical College and Research Institute, Chennai, Tamil Nadu, India*

[3]*Department of Zoology, Annamalai University, Annamalainagar, Tamil Nadu, India*

Key words: Lipid peroxidation, Antioxidants, 2,4,6-octatrienoic acid, Colon cancer, Lung cancer

DOI: https://dx.doi.org/10.12692/ijb/27.1.291-296 Published: July 15, 2025

ABSTRACT

The thiobarbituric acid reactive substances (TBARS) and antioxidants status have been utilized as putative biomarkers to assess the *in vitro* antiproliferative effect of phytoconstituents and medicinal plants in cancer cells. This study analysed the status of lipid peroxidation and antioxidant status in untreated and octatrienoic acid treated A549 lung and HCT-116 colorectal cancer cells. The status of lipid peroxidation by-products [TBARS, Conjugated Dienes (CD) and lipid hydroperoxides] and activities of antioxidants [Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx) and Reduced glutathione (GSH)] were analysed using colorimetric assays. While octatrienoic acid increased the TBARS, CD and lipid hydrperoxides levels, it lowered the activities of SOD, CAT, GPx and GSH content in A549 and HCT-116 cells. The results of present study revealed that octatrienoic acid might have suppressed the proliferation of A549 lung and HCT-116 colorectal cancer cells through modulating the oxidative stress biomarkers. The present study concludes that octatrienoic acid facilitates the generation of lipid peroxidation by-products by reducing antioxidant defence mechanism in A549 and HCT-116 cancer cells.

***Corresponding author:** Raju Kowsalya ✉ kowsalyamouli@yahoo.com
[*]https://orcid.org/0009-0008-1328-8409

INTRODUCTION

Cancer arises due to abnormal proliferation of cells induced by physical, chemical and biological agents. Each year, cancer related deaths are increasing globally due to environmental exposures and changes in human lifestyle (Bahrami and Tafrihi, 2023). Cancer imposes a significant health burden and around 20 million new cases and 9.7 million deaths due to cancer are reported in 2022 globally. Recent studies pointed out that around 1,496,972 cancer cases were reported in India by the year 2023. The annual incidence of lung and colorectal carcinomas are sharply increasing in India. Around 66,280 lung cancer deaths were reported by the year 2022 in India. It has also been reported that colorectal cancer caused around 38,300 deaths in India at the year 2022 (Bray *et al.*, 2024). A549 is an epithelial cell line derived from the lung tissue of a 58-year-old male with adenocarcinoma. It resembles type II alveolar cells and serves as a useful in vitro model for studying non-small cell lung cancer, oxidative stress and drug metabolism. HCT 116 is a colon cancer cell line developed from a male patient is widely used in colorectal cancer research, particularly for testing anticancer drugs. Researchers utilized A549 lung cancer cells and HCT-116 colon cancer cells to study the antiproliferative potential of natural products and their bioactive principles under *in vitro* conditions.

Human body generates reactive oxygen species (ROS) during normal cellular metabolism as well as through environmental exposure. The harmful reactive oxygen species are however scavenged by antioxidant defence mechanism (Birben *et al.*, 2023; Sharifi-Rad *et al.*, 2020). During diseased conditions, body generates excessive ROS production thereby leading to insufficient antioxidant potential and causes a disorder termed as oxidative stress. ROS mediated oxidative stress play a major role in oxidative damage to biomolecules and constitutes to various disorders including carcinogenesis (Nakamura and Takada, 2021).

2,4,6-Octatrienoic acid is a small, reactive molecule ($C_8H_{10}O_2$, 138.16 g/mol) with three double bonds in its carbon chain. The compound known for its ability to counteract harmful ROS. It can take part in various chemical reactions like light-triggered changes, polymerization and oxidation (NCBI, 2025; Flori *et al.*, 2011). This compound is found in *Deschampsia antarctica*, a hardy grass that grows naturally in Antarctica (Zúñiga *et al.*, 1994). One of only two flowering plants in the region, it survives in extreme cold, intense UV light and strong winds by producing protective substances. These include antioxidants, flavonoids and fatty acids including 2,4,6-octatrienoic acid (Ramírez *et al.*, 2024). This compound may help defend against oxidative stress caused by UV exposure, either by directly neutralizing ROS or by supporting the production of other protective chemicals (Flori *et al.*, 2011).

MATERIALS AND METHODS

Cell culture

A549 and HCT-116 cell lines (2.5×10^6 cells) were grown in complete DMEM medium until they reached full confluence. These cells were then treated with 2,4,6-octatrienoic acid at its IC_{50} concentration for 24 hours. Untreated cells maintained for the same durations served as controls. After treatment, the cells were washed twice with PBS (pH 7.2) and detached using a solution of 0.25% trypsin and 2 mM EDTA for 10 minutes. The collected cells were centrifuged at 600 rpm for 10 minutes. The pellets were mixed in 50 mM phosphate buffer (pH 7.0), broken up by sonication on ice for 2 minutes and then centrifuged. The clear liquid (supernatant) was collected to measure enzyme and protein levels.

Lipid peroxidation markers

TBARS assay

Using a modified method (Donnan, 1950), TBARS levels were estimated by measuring the colored complex formed when malondialdehyde (MDA) reacts with thiobarbituric acid. Cells cultured in 6-well plates were scraped, resuspended in PBS and mixed with the TBA reagent.

The mixture was then incubated at 90°C for 20 minutes. After allowing it to cool, TBARS were extracted using n-butanol and measured at 532 nm.

Conjugated dienes

Following the Recknagel and Glende protocol, lipid peroxidation was assessed by detecting conjugated dienes from peroxidized lipids at 233 nm (Recknagel and Glende, 1984). Lipids were extracted from 25 µl of cell lysate using chloroform/methanol (2:1), dried and dissolved in methanol before measuring absorbance.

Lipid hydroperoxides

Using Jiang *et al.* method, lipid hydroperoxides under acidic conditions were detected by their ability to oxidize Fe^{2+} to Fe^{3+} (Jiang *et al.*, 1992). This forms a colored complex with xylenol orange, measured at 560 nm. The reaction mixture (cell lysate + FOX reagent) was incubated for 30 minutes at room temperature before reading.

Antioxidant enzyme activity assays

Reduced glutathione

Using Ellman's method, glutathione was measured based on its ability to form a yellow color with DTNB was read at 412 nm (Ellman, 1959). Cell lysate was treated with 5% TCA and the supernatant was mixed with phosphate buffer, DTNB and water.

Superoxide dismutase

According to Kakkar *et al.* procedure, superoxide dismutase (SOD) activity was assessed by its ability to inhibit the formation of a blue formazan compound (kakkar *et al.*, 1984). The reaction mixture included cell lysate, sodium pyrophosphate buffer, PMS, NBT and NADH. After incubation at 37°C for 90 seconds, the reaction was stopped with glacial acetic acid and extracted with n-butanol and the colored product formed was measured at 560 nm.

Catalase

Catlase activity was determined according to Sinha procedure, by determining the breakdown of hydrogen peroxide at 240 nm (Sinha, 1972). The decrease in absorbance over 4 minutes was used to determine catalase activity.

Glutathione peroxidase

Glutathione peroxidase (GPx) activity was measured based on the breakdown of hydrogen peroxide (Rotruck *et al.*, 1973). The reaction mixture included cell lysate, glutathione, hydrogen peroxide and phosphate buffer and was incubated at 37°C for 10 minutes. The mixture was centrifuged and the remaining glutathione in the supernatant was estimated using DTNB.

Statistical analysis

All results are expressed as the mean with standard deviation (mean ± SD) from three independent experiments (n = 3). Statistical differences between groups were evaluated using Student's t-test, with significance set at $p < 0.05$.

RESULTS AND DISCUSSION

The TBARS, conjugated dienes and lipid hydroperoxides in untreated and octatrienoic acid treated A549 and HCT-116 cancer cells are depicted in Fig. 1 to 3. The status of all these biomarkers are decreased in untreated cancer cells. Cancer cells treated with octatrienoic acid increased the levels of TBARS, CD and lipid hydroperoxides.

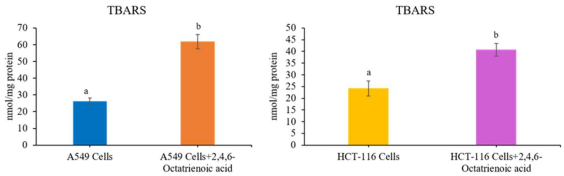

Fig. 1. TBARS levels in untreated and 2,4,6-octatrienoic acid treated cancer cells

Different letters (a, b) on bars show significant changes between groups at P less than 0.05.

The antioxidants status in untreated and octatrienoic acid treated A549 and HCT-116 cancer cells are shown in Fig. 4 to 7. The activities of SOD, CAT, GPx and GSH content were found to be increased in both lung and colorectal cancer cells.

Treatment of these cancer cells with octatrienoic acid decreased the activities of the above said antioxidants.

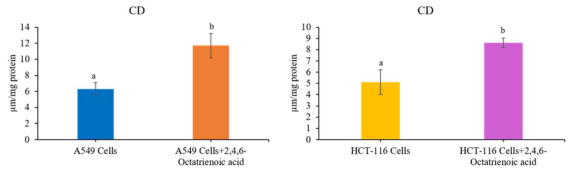

Fig. 2. CD levels in untreated and 2,4,6-octatrienoic acid treated cancer cells

Different letters (a, b) on bars show significant changes between groups at P less than 0.05.

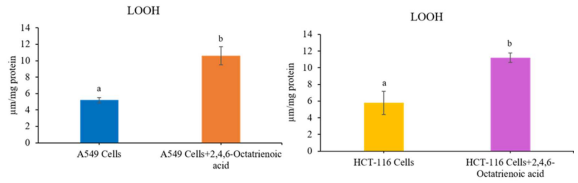

Fig. 3. LOOH levels in untreated and 2,4,6-octatrienoic acid treated cancer cells

Different letters (a, b) on bars show significant changes between groups at P less than 0.05.

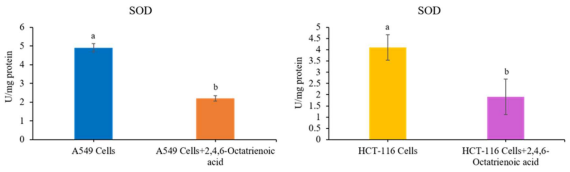

Fig. 4. SOD levels in untreated and 2,4,6-octatrienoic acid treated cancer cells

Different letters (a, b) on bars show significant changes between groups at P less than 0.05.

Multiple evidences documented that the survival of cancer cells are influenced by the status of lipid peroxidation and antioxidants. It has been pointed out that cancer or tumor cells exhibit an inverse relationship between rate of cell proliferation and lipid peroxidation (Gonzalez, 1992; Barrera, 2012). The status of TBARS has been used as an indicator of oxidative damage in cancer cells. Numerous studies investigated the *in vitro* antiproliferative effect of the anticancer bioactive constituents by correlating the status of TBARS and antioxidants in cancer cells (Popovici *et al.*, 2021).

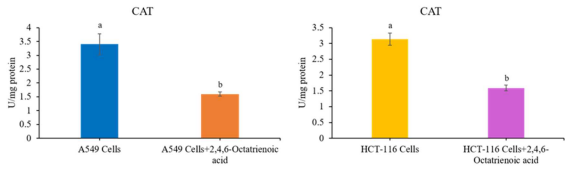

Fig. 5. CAT levels in untreated and 2,4,6-octatrienoic acid treated cancer cells

Different letters (a, b) on bars show significant changes between groups at P less than 0.05.

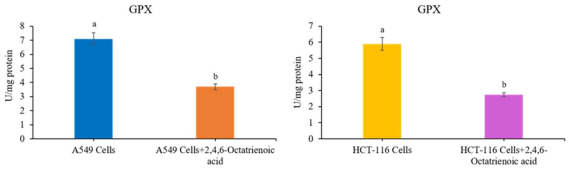

Fig. 6. GPX levels in untreated and 2,4,6-octatrienoic acid treated cancer cells

Different letters (a, b) on bars show significant changes between groups at P less than 0.05.

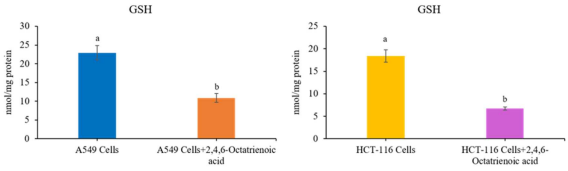

Fig. 7. GSH levels in untreated and 2,4,6-octatrienoic acid treated cancer cells

Different letters (a, b) on bars show significant changes between groups at P less than 0.05.

Su *et al.* reported that treating HCT-116 colon cancer cells with zingerone significantly increased the levels of thiobarbituric acid reactive substances (TBARS), indicating higher lipid peroxidation (Su *et al.*, 2019). This was also linked with a notable reduction in important antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH), showing that zingerone induced oxidative damage by impairing the cellular antioxidant defense system. Similarly, Yue *et al.* observed comparable effects in A549 lung cancer cells treated with pelargonidin, a naturally occurring anthocyanidin. Their study demonstrated a notable increase in TBARS levels along with a suppression of antioxidant enzyme activities, further confirming the pro-oxidant

potential of pelargonidin in cancer cells. The authors proposed that pelargonidin exacerbates oxidative stress, thereby compromising the redox balance within the cells (Yue *et al.*, 2024). In another study, Crespo *et al.*, found that geraniol treatment in A549 lung cancer cells resulted in a significant elevation in TBARS content, indicating increased lipid peroxidation. No significant changes were however noticed in enzymatic antioxidants status. These findings reinforce the concept that certain phytochemicals can induce oxidative damage in cancer cells by enhancing lipid peroxidation without necessarily impairing antioxidant enzymes (Crespo *et al.*, 2020).

Additionally, Kachadourian *et al.* demonstrated that treatment with chalcones in A549 cells caused a pronounced depletion of intracellular glutathione (GSH). As GSH plays a vital role in preserving the redox balance within cells, its depletion indicates that chalcones may promote cytotoxicity by disrupting antioxidant defenses and increasing oxidative damage (Kachadourian *et al.*, 2006).

This study highlights the regulatory effect of octatrienoic acid on oxidative stress markers and the antioxidant defense system in A549 lung and HCT-116 colorectal cancer cells.

Treatment with octatrienoic acid significantly increased lipid peroxidation byproducts, along with a decrease in the activities of key antioxidant enzymes and the level of GSH, which resulted in disruption of cellular redox equilibrium. These findings suggest that octatrienoic acid may suppress cancer cell proliferation by inducing oxidative damage and weakening the antioxidant defense system, ultimately affecting cell growth and survival.

CONCLUSION

This study demonstrated that octatrienoic acid modulated the markers of oxidative stress in a way that might have helped to suppress the proliferation of both A549 and HCT-116 cancer cells under in vitro conditions. Further studies are needed to evaluate the modulatory role of octatrienoic acid on oxidative markers in in vivo experimental models.

ACKNOWLEDGEMENTS

The authors wholeheartedly acknowledge Dr. K. Suresh, a distinguished member of the research advisory committee, for his insightful guidance and constructive suggestions.

REFERENCES

Bahrami H, Tafrihi M. 2023. Global trends of cancer: The role of diet, lifestyle and environmental factors. Cancer Innov. **2**, 290–301.

Barrera G. 2012. Oxidative stress and lipid peroxidation products in cancer progression and therapy. ISRN Oncology **2012**, 137289.

Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. 2012. Oxidative stress and antioxidant defense. World Allergy Organ J. **5**, 9–19.

Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A. 2024. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians **74**, 229–263.

Crespo R, Rodenak-Kladniew BE, Castro MA, Soberón MV, Lavarías SML. 2020. Induction of oxidative stress as a possible mechanism by which geraniol affects the proliferation of human A549 and HepG2 tumor cells. Chemico-Biological Interactions **320**, 109029.

Donnan SK. 1950. The thiobarbituric acid test applied to tissues from rats treated in various ways. Journal of Biological Chemistry **182**, 415–420.

Ellman GL. 1959. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics **82**, 70–77.

Flori E, Mastrofrancesco A, Kovacs D, Ramot Y, Briganti S, Bellei B, Paus R, Picardo M. 2011. 2,4,6-Octatrienoic acid is a novel promoter of melanogenesis and antioxidant defence in normal human melanocytes via PPAR-γ activation. Pigment Cell & Melanoma Research **24**, 618–630.

Gonzalez MJ. 1992. Lipid peroxidation and tumor growth: An inverse relationship. Medical Hypotheses **38**, 106–110.

Jiang ZY, Hunt JV, Wolff SP. 1992. Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. Analytical Biochemistry **202**, 384–389.

Kachadourian R, Day BJ. 2006. Flavonoid-induced glutathione depletion: Potential implications for cancer treatment. Free Radical Biology & Medicine **41**, 65–76.

Kakkar P, Das B, Viswanathan PN. 1984. A modified spectrophotometric assay of superoxide dismutase. Indian Journal of Biochemistry and Biophysics **21**, 130–132.

Nakamura H, Takada K. 2021. Reactive oxygen species in cancer: Current findings and future directions. Cancer Science **112**, 3945–3952.

National Center for Biotechnology Information. 2025. PubChem compound summary for CID 5368831, Octa-2,4,6-trienoic acid. Retrieved June 17, 2025 from https://pubchem.ncbi.nlm.nih.gov/compound/Octa-2_4_6-trienoic-acid.

Popovici V, Bucur L, Vochita G, Gherghel D, Mihai CT, Rambu D, Calcan SI, Costache T, Cucolea IE, Matei E, Badea FC, Caraiane A, Badea V. 2021. *In vitro* anticancer activity and oxidative stress biomarkers status determined by *Usnea barbata* (L.) F.H. Wigg. dry extracts. Antioxidants (Basel) **10**, 1141.

Ramírez CF, Cavieres LA, Sanhueza C, Vallejos V, Gómez-Espinoza O, Bravo LA, Sáez PL. 2024. Ecophysiology of Antarctic vascular plants: An update on the extreme environment resistance mechanisms and their importance in facing climate change. Plants (Basel) **13**, 449.

Recknagel RO, Glende EA Jr. 1984. Spectrophotometric detection of lipid conjugated dienes. Methods in Enzymology **105**, 331–337.

Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. 1973. Selenium: Biochemical role as a component of glutathione peroxidase. Science **179**, 588–590.

Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, Rajkovic J, Tsouh Fokou PV, Azzini E, Peluso I, Prakash Mishra A, Nigam M, El Rayess Y, Beyrouthy ME, Polito L, Iriti M, Martins N, Martorell M, Docea AO, Setzer WN, Sharifi-Rad J. 2020. Lifestyle, oxidative stress and antioxidants: Back and forth in the pathophysiology of chronic diseases. Frontiers in Physiology **11**, 694.

Sinha AK. 1972. Colorimetric assay of catalase. Analytical Biochemistry **47**, 389–394.

Su P, Veeraraghavan VP, Krishna Mohan S, Lu W. 2019. A ginger derivative, zingerone—a phenolic compound—induces ROS-mediated apoptosis in colon cancer cells (HCT-116). Journal of Biochemical and Molecular Toxicology **33**, e22403.

Yue L, Li Y, Luo Y, Alarfaj AA, Shi Y. 2024. Pelargonidin inhibits cell growth and promotes oxidative stress-mediated apoptosis in lung cancer A549 cells. Biotechnology and Applied Biochemistry **71**, 1195–1203.

Zúñiga GE, Alberdi M, Fernández J, Móntiel P, Corcuera LJ. 1994. Lipid content in leaves of Deschampsia antarctica from the maritime Antarctic. Phytochemistry **37**, 669–672.