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OLIVE OIL: RESEARCH MODEL TO CLARIFY ITS HEALTH BENEFITS AND ITS UNIQUE MECHANISM OF ACTION

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Abstract

The uniqueness of Extra Virgin Olive Oil (EVOO) convincing evidence that its intake has many health aspects by increased longevity and prevention of many age-associated non infectious diseases such as cardiovascular and neurodegenerative diseases. In this study, Twenty-four rats were divided into 4 groups (6 rats each), as a following: Control group, EVOO group: rats were oral administrated with EVOO at a dose of 1ml/100g body weight for two weeks, Irradiated group (IR): rats were exposed to 6Gy of whole body γ-radiation; EVOO and IR group: rats were oral administrated with EVOO for two weeks prior to irradiation (6Gy). EVOO successfully reduces cellular destruction, chromosomal aberrations, pro-inflammatory markers interleukin-1β (IL-1β), and ameliorates heart and coronary arteries tissues damages, accompanied by lowering Vascular Endothelial Growth Factor (VEGF), Total Cholesterol (TC) and Triglyceride levels (TG). Therefore, EVOO provided adequate protection for cells against exposure to harmful ionizing irradiation (6 Gy).

Keywords: Cardiac tissue Chromosomal aberration Cytokines Lipid profile. Olive oil. γ-Radiation.

1. Introduction

The Mediterranean diet (MD) is very healthy and ecological (Shannon et al. 2021). Greater devotion to the MD is related to enhanced long life and avoidance of many age-associated noninfectious diseases such as cardiovascular and neurodegenerative diseases (D'Alessandro and De Pergola 2015). The MD includes many healthy components, but extra virgin olive oil (EVOO) stands out because it is the main source of fat in the MD (Celano et al. 2018). The soluble fraction of EVOO mainly contains phenolic compounds, including phenolic acids, phenolic alcohols, hydroxytyrosol and tyrosol, and their secoiridoid precursors such as oleuropein, its aglycone [oleuropein-aglycone mono-aldehyde] and the dialdehydic form of deacetoxy [oleuropein-aglycone di-aldehyde (Carullo et al. 2020; Venturinia et al. 2022). In addition to, fatty acids and antioxidants (De Santis et al. 2022). Many studies showed that EVOO is the interestingly different oil that offered a different fluorescence spectrum, in contrast to the other vegetable oils (Kyriakidis and Skarkalis 2000).

Ionizing radiation exposure damage starts from cell membrane damage to cell necrosis passing by a number of biological consequences such as inflammation, physiological disorders, immune system

compromise, and carcinogenesis. The purposes of all modern applied radiological research are to minimize radiation exposure risks (Montaser et al. 2019) mediated by ROS production, so the antioxidant system is the key role in treatment-related toxicities (Tan et al. 2022). Therefore, interest remains on the identification and development of nontoxic and effective radio-protectors (Kalekhan et al. 2022).

It has been pointed out that EVOO high consumption is related to a generally lower risk of colon pain, breast or skin cancer as well as favorable effects on aging and coronary diseases (Foscolou et al. 2018).

Several studies indicate that the consumption of EVOO and its phenolic compounds, especially hydroxytyrosol and tyrosol, has healthful effects (Jimenez-Lopez et al. 2020). The mechanisms of action of phenolic compounds are diverse and might be mediated by the gut microbiota (Martin-Pelaez et al. 2017). Examples of proposed mechanisms of action for phenolic compounds include: increased expression and activity of glutathione related enzymes, induction of nuclear factor erythroid 2-related factor 2, inhibition of the pro-inflammatory activity of enzymes such as cyclooxygenase-2, or modulation of different signaling pathways such as nuclear factor k-light chain enhancer of activated B cells (NF-kB) or mitogen activated protein kinase (Pedret et al. 2018).

New research has established epigenetic actions of EVOO and its phenolic compounds. Epigenetic mechanisms are processes that produce reversible heritable variations that are not attributable to changes in the DNA sequence but can regulate gene expression (Casadesús and Noyer-Weidner 2013). Genetics is responsible for approximately 30% of the known variance, with the remaining 70% depending on epigenetics modulated by environmental factors (such as diet). Indeed, numerous cellular processes are influenced by epigenetic modifications. Major mechanisms involved in epigenetic regulation are DNA methylation, histone modification, and regulation by noncoding RNAs [i.e., microRNAs]. These epigenetic changes are relatively stable, tissue-specific, and can be inherited across several generations. Heritability failure of epigenetic marks may result in inappropriate initiation or inhibition of gene expression and lead to pathological conditions. In addition, these epigenetic modifications are modulated by environmental and lifestyle factors such as diet and physical activity (Siddeek and Simeoni 2022).

Several epidemiological studies have confirmed that abundant consumption of foods of plant origin is associated with reduced risk of developing cancers (Lamy et al. 2014).

The present study will manipulate the most useful role of EVOO via its type of extraction (highly refined EVOO from Italy) that contains a high ratio of numerous fluorescent compounds, like pigments that are effective as an epigenetic agent. In addition to other broad beneficial constituents of it that have a valuable health benefits.

2. Material and Methods

Chemicals

- Highly refined olive oil was purchased from LOBA Chemie (Italy).
- Colchicine and other chemicals from Sigma-Aldrich Chem. Co.

Experimental Animals

24 healthy adult male Swiss Albino rats weighing 240 -250 gm were acquired from the animal house of the NCRRT. They were accommodated in polypropylene cages regarding typical laboratory circumstances and regulated temperature (24±4°C) throughout the experiment (Mansour 2013). All the study's protocols, animal precautions, and treatment were in agreement with the guiding principles allocated by the Research Ethics Committee (REC-NCRRT) with No. (81A/21). Standard pellets and water were delivered ad-labium to the animals.

Radiation Source

Gamma Whole-body irradiation process was performed with a Canadian Gamma Cell. Caesium-137 (^{137}Cs) unit at the National Centre for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority, Cairo. ^{137}Cs source offers a dose rate of 0.604rad/sec at the time of experiment. Whereas regarding the whole shielding for the working staff.

Experimental design

Rats were separated into four groups, six animals each group, and were managed as follows: Group I: control group, Group II: rats were orally treated with EVOO (EVOO group) with dose (1ml/100g) body weight) (Farahat et al. 2019). Group III: rats were irradiated with 6Gy γ-irradiation (El-Deeb et al. 2006), Group. IV: rats irradiated with 6Gy γ-irradiation after EVOO administration as in group II. After the end of treatments, rats were sacrificed under light ether anesthesia.

Collection of samples

Blood, heart, and femoral bone marrow samples were obtained from the experimental animals following normal laboratory procedures and stored at -20^{0}C until used. The mutagenic effect was evaluated by the bone-marrow chromosomal aberration analysis.

Biochemical and Immunological investigations

Biochemical estimations

Total cholesterol content in blood serum was determined according to Allain et al. (1974). Triglycerides were measured according to Fossati and Principe (1982).

Immunological estimations

Determination of IL-1β, IL-10, and VEGF according to their principles of Catalog No (MB5825017, MBS355232,and BMS626-2) respectively, My Bio Source (China) by Elisa Kits Based on sandwich Enzyme-Linked Immunosorbent Assay. Quantitative detection of serum IL -1β, IL -10, and VEGF concentrations in rat serum, a specific antibody for each one well coated plate was used. Standards and samples (plasma) are pipetted into the wells; each one present in a sample bind to the immobilized antibody specific for it.

Then were washed and biotinylated anti-interleukins were added. After washing away, the unbound biotinylated antibody, conjugated streptavidin is pipetted to the wells. Then were washed, finally, a substrate solution was added so color developed in proportion to the amount of interleukin bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

All previous immunological parameters were measured by Eliza reader TECAN Infinite F50 plus and obtained data were analyzed by software Magellan™ Switzerland.

Chromosomal aberration analysis

Animals were injected IP with 0.3 ml/Kg b. wt. of 0.025 % colchicine (Sigma-Aldrich Chem. Co.) two hours before dissection. Both femurs from each animal were removed, bone marrow was flushed out with phosphate buffer by syringe. Samples then centrifuged at 2000rpm for 10 min. then supernatant discarded. Hypotonic solution (0.56% Kcl) added to the pellet for 20 min at 37^{o}C. Samples centrifuged again then fixative (1acetic acid:3methanol v/v) added to the pellet 3 repeated times with centrifuge in between each time. Slides were dried and stained with 10 % Giemsa (Sigma-Aldrich Chem. Co.) at pH 6.8

for 5 min **(**Darwish and Mosallam 2019). Chromosomal aberrations were scored under light microscope (Leitz Wetzlar Ortholux Germany), and fifty cells were examined for each slide.

Histopathology

Whole heart was fixed in 10% formaldehyde fixative for twenty hours washing was done with tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration specimens were cleared in xylene and embedded in paraffin at 56 degrees in hot air oven for 24 h. Paraffin bees wax tissue blocks were prepared for sectioning at 4 micron thickness of rotary LEITZ microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin& eosin stain (Suvarnaet al. 2013) for examination through the light microscope.

Statistical Analysis

The data obtained in the present work are represented in tables as mean ± standard error. Statistical analysis was carried out using one-way analysis of variance (ANOVA) for testing the significance between various treated groups (Lazic 2008).

3. Results

Results in Table 1 showed that EVOO administration caused a significant decrease in the total cholesterol and triglyceride concentration as compared with that of control. On the other hand, radiation induced a significant increase in their values with ≈ 2 folds as compared with control. Meanwhile, treatment with EVOO before irradiation reduced both values to be significantly lower than that of irradiated group, but still significantly higher than that of control group.

The results of VEGF in Table 1 indicated also that EVOO administration induced significant inhibitory effect as compared with control. While gamma irradiation induced a significant elevation of the VEGF concentration when compared with control group. Moreover, current data revealed that intake of EVOO before irradiation can manage the level of VEGF.

Table 1. Effect of EVOO administration on serum total cholesterol, triglycerides levels (mg/dl) and VEGF (pg/ml) in irradiated rats (Mean± S.E).

Groups	Total Cholesterol mg/dl	Triglyceride mg/dl	VEGF pg/ml
Control	130±2.4	98.2±3.8	79.8±1.5
EVOO group	106.7±4.3[a]	83.7±3.1[a]	75.5±0.6[a]
IR	273.5±4.9[ab]	176.0±3.5[ab]	116.5±1.7[ab]
EVOO+IR	208±2.5[abc]	126.8±3.0[abc]	94.3±1.3[abc]

Significant with control (a) Significant with EVOO group (b) Significant with IR group (c)

IL-10 always high in normal control group for immune regulation, while ionizing irradiation group caused a significant decrease when compared with control. EVOO treated group showed non-significant changes with control. EVOO treatment before irradiation verifying significant protection.

On contrary IL-1β showed low concentration in control group while ionizing irradiation group recorded a significant increase when compared with control. EVOO treated group showed non-significant with control. Also, EVOO treatment before irradiation verifying significant protection as shown in Figure (1).

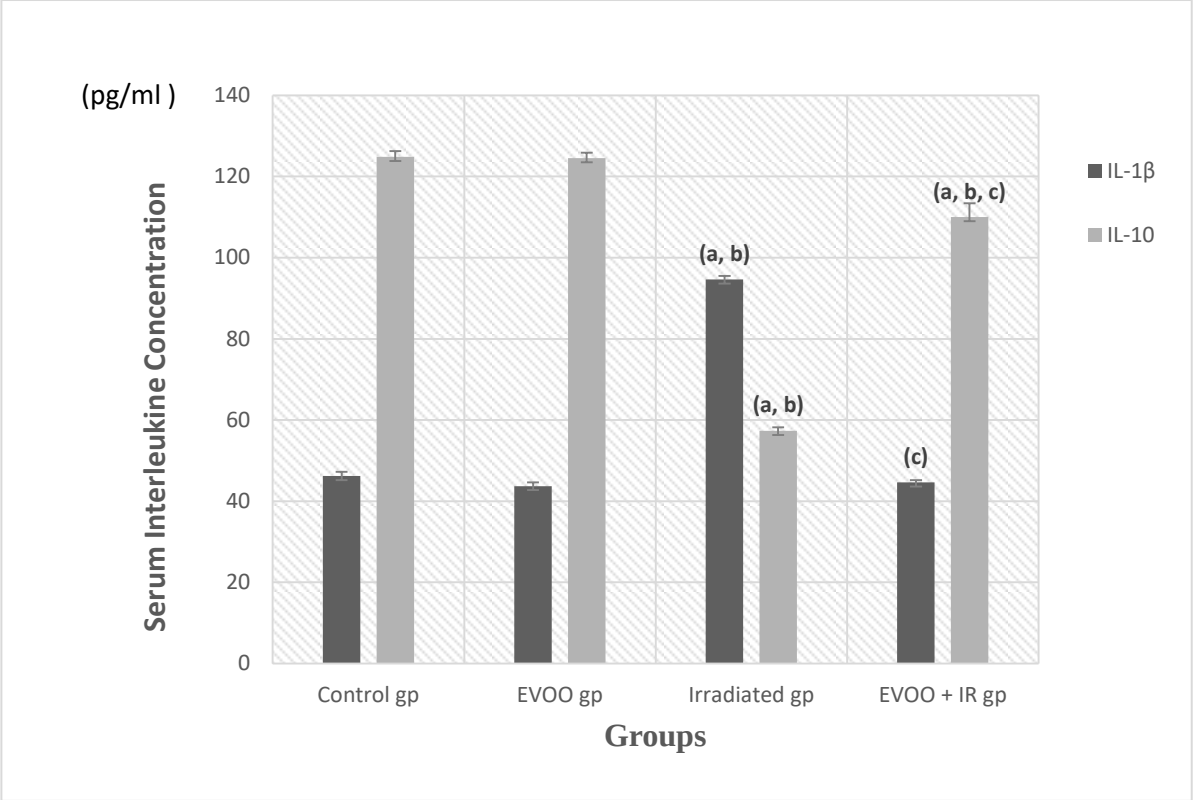

Figure 1. Serum IL-1β and IL-10 Concentration in Irradiated and/or EVOO Treated Rats. a significant when compared with control group. b significant when compared with EVOO group. c significant when compared with irradiated group.

Results in Table 2 revealed that EVOO administration did not cause any significant difference among the scored chromosomal aberrations when compared with the control group. On the other hand, irradiation induced a significant increase in the scored fragments, ring and dicentrics, polyploid and total aberrations by approximately 10, 4, 4, and 11 folds respectively as compared with that of control. While treatment with EVOO before irradiation reduced all of these values by approximately 0.5 fold to be significantly lower than that of irradiated group, but still significantly higher than that of control group.

Table 2. Chromosome aberrations and aberrant cells percentage in rat bone marrow cells in irradiated and/or EVOO treated rats (Mean± S.E).

Aberration types/50 metaphase	Fragment	Ring&Dicentric	Polyploidy	Total Abb.
Control	7.3±0.6	0±0	0±0	7.3±0.6
EVOO	5.5±0.4	0±0	0±0	5.5±0.4
IR	ab 75.2±2.0	ab 3.8±0.3	ab 3.7±0.3	ab 82.7±2.3
EVOO +IR	abc 36.2±2.4	abc 1.3±0.3	abc 1.8±0.3	abc 39.3±2.7

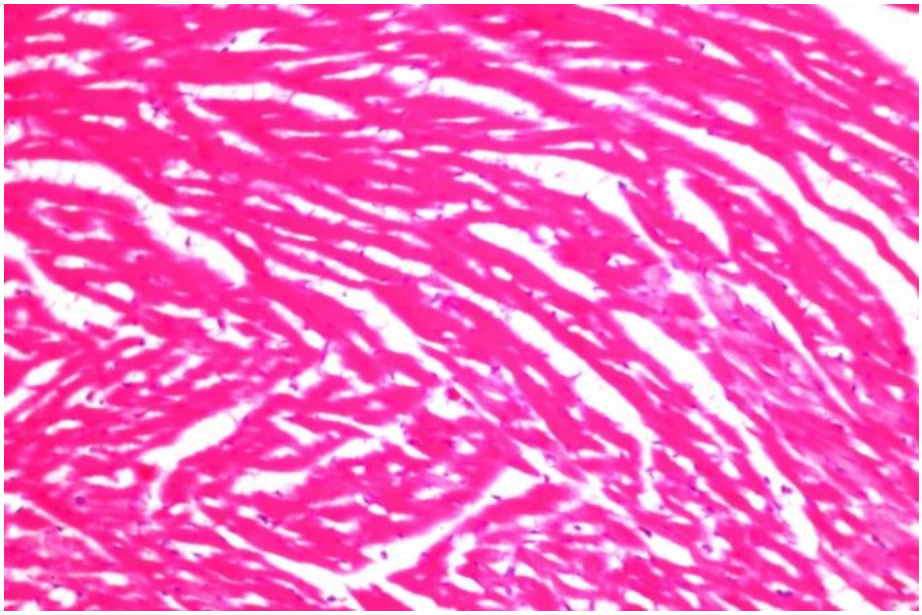

Figure 2. Showed normal histology of myocardial bundles with normal cellular boundary and clear nucleus.

While group of animals exposed to 6 Gy γ-irradiation presented many marked signs as shown in Figure (3A) which reveals degeneration of myocardial muscle bundles, while Figure (3B) showing few inflammatory cells infiltration in between hyalinized myocardial bundles. Also Figure (3C) clearly display deposition of the hemosiderin pigment on the interna of hyalinized vascular wall of myocardial blood vessel.

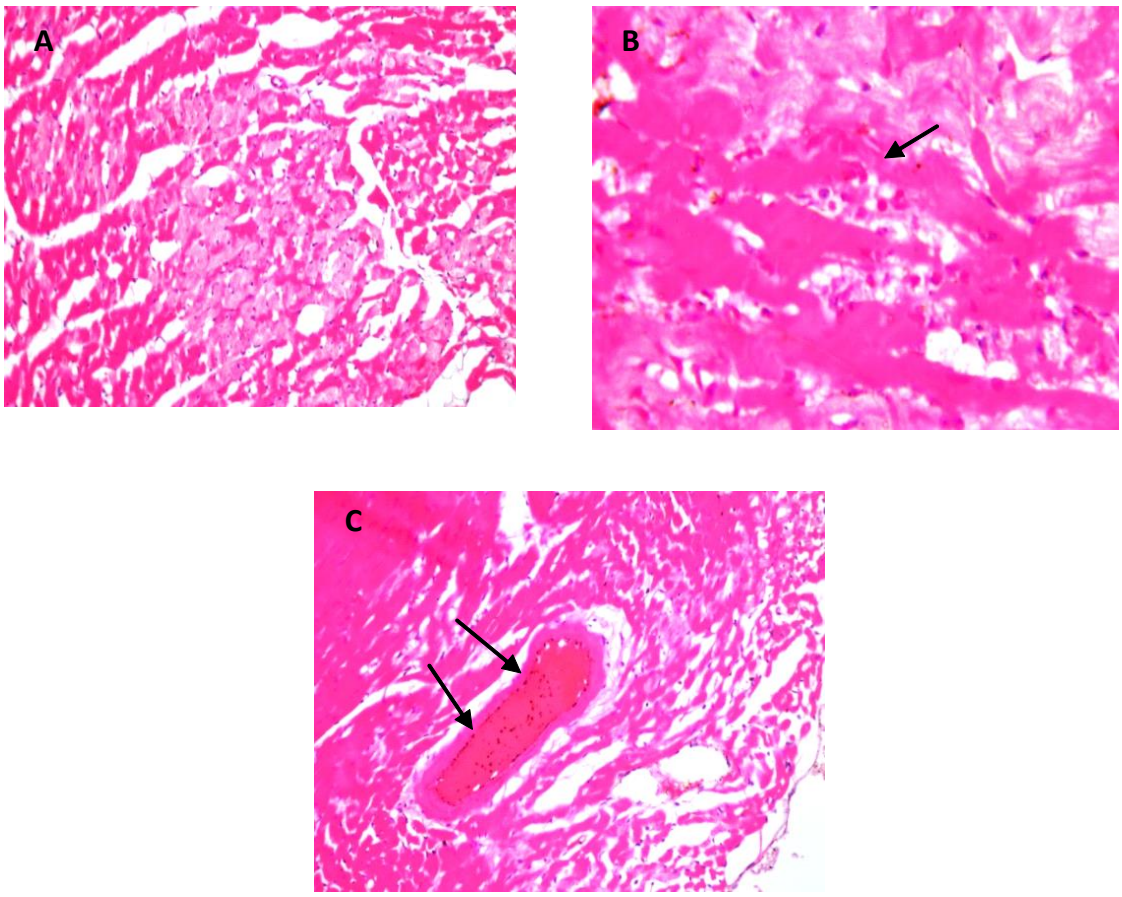

Figure 3. Myocardial bundles of γ-irradiated rat group, showing degeneration (B: arrow), infiltration and hemosiderin deposition (C: arrows), A- (H&E x40); B-(H&E x80); C-(H&E x40).

In contrast, group of animals treated with EVOO before γ-irradiation showing mild degeneration in myocardial bundle Figure (4).

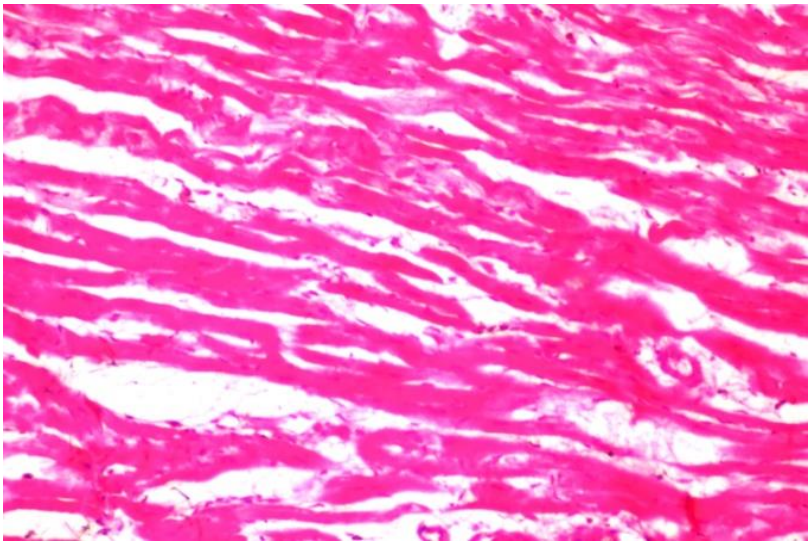

Figure 4. Myocardial bundles of EVOO-treated & γ-irradiated rat is showing mild degeneration.

4. Discussion

EVOO has numerous biological functions owing to its high content of monounsaturated fats and antioxidants, which have various health benefits. EVOO consumption decreases the risk of heart disease and type 2 diabetes, recover brain function, and it also has anti-inflammatory effects, that play a role in reducing the risk of some types of cancer. Besides, it is one of the good sources of vitamin E, K and some minerals (iron, calcium, and zinc) (Schwingshackl et al. 2020).

Exposure to ionizing radiation intensively causes release cytokine and chemokine, increase in the levels of free radicals, tumor suppressor gene inhibition and oncogenes stimulation (Goyes et al. 2018).

In the present study, we examine the biochemical, immunological, cytogenetic and histopathology destruction effects of radiation exposure (6Gy). The results showed that the radiation elevates the cholesterol, triglyceride, IL-1β levels. On the other hand, the levels of IL-10 decreased after irradiation agreed with Nwokocha et al. (2012) investigation.

The presents study results are in covenant with prior studies that proved rises in the inflammatory markers' measurements as IL-1β, IL-6, tumor necrosis- α (TNF-α) and interferon- γ (IFN-γ), after γ-irradiation (Asmaa and Amel 2021). Also, IL-10 recorded in an inhibitory manner associated with radiation oxidative stress (Shmarina et al. 2001). The equilibrium between necrosis and IL-10 level is a vital process to sustain immune homeostasis (Li et al. 2014).

Vascular endothelial growth factor (VEGF) triggers crucial signaling processes that regulate tumor angiogenesis and, therefore, represents an attractive target for the development of novel anticancer therapeutics. VEGF expression is controlled by ROS levels and oxidative stress by related transcription factors (Vanderstraeten et al. 2020). High dose of ionizing radiation was indicated to cause rise in VEGFA transcript levels (Kim et al. 2020) and angiogenesis in several tissues and cell types (Chang et al. 2021).

γ-irradiation also induced genetic alterations and more than one type of chromosomal aberrations, (i.e., dicentrics, rings and deletions) consequently led to cell dysfunction and death (Ballarini and Carante 2016). In the same manner, present study showed increase of the total aberrant cells including fragments, rings, dicentrics and polyploidy.

Histopathology examination of myocardial tissue showed sever damage as myocardial muscle bundles degenerating. Disturbance of permeability is the main reason of all cellular damage or even mitochondrial damage. So, Infiltration of some inflammatory cells in between hyalinized myocardial bundles pointed us to severe cell membrane damage. With hemosiderin pigment spreads in the interna of hyalinized vascular wall of myocardial blood vessel also due to red blood cells damage.

The effect of oxidative stress after irradiation originates mainly from membrane oxidative damages in lipids bilayer of endothelial and smooth muscle cells in blood vessels including heart and allover body cells membrane as mentioned by (Rietjens et al. 2007).

Group treated with EVOO showed no histopathology effects in heart sections that indicate its safety in the treated dose. On the other hand, group of animals given EVOO before irradiation showed mild damage as EVOO played protective role against gamma irradiation.

The gained results of the present study revealed satisfied notable enhancement for all measured biochemical, immunological, cytological, and histological parameters. EVOO administration optimized the levels of lipid profile (total cholesterol and triglycerides) and VEGF not only when compared with irradiated groups but also with the control group.

In the same way, EVOO significantly ameliorate the pro-inflammatory and regulatory mediators as scored for IL-1β and IL-10. EVOO also reduced the frequencies of fragments, rings, and dicentrics as structural aberrations and polyploidy scores as numerical type aberration. In addition, EVOO administration before γ-irradiation show mild degeneration in myocardial bundle compared with myocardium histopathological sections of irradiated group.

In healthy humans, the mother's intake of olive oil during pregnancy can affect placental histone acetylation in immune regulatory genes (Fabiani et al. 2008).

Its oleuropein content stabilizes the proteasome, during initiation of replicative senescence, and prolonged life span (Acevedo et al. 2019). EVOO is furthermore important in diminishing the accumulation of genetic alterations in radiotherapy-treated patients or subjects exposed to high but non-lethal levels of radiation (5-12 Gy) (Koukourakis 2012).

In addition, EVOO polyphenols are capable to minimize oxidative stress and related inflammatory effects related to chronic degenerative diseases (Calabriso et al. 2016). Zrelli et al. (2011) studied precisely the hydroxyl tyrosol role in regulation of the intracellular reactive oxygen species levels in vascular endothelial cells and prevention of cardiovascular diseases. EVOO reduced inflammation by decreasing inflammatory markers in dendritic cells (De Santis et al. 2021).

Other important unsaturated fatty acid residues in EVOO are the essential linoleic and linolenic acids which are present in plasma membranes, prevent cellular proliferation and trigger the synthesis of IL-6, IL-8 and VEGF (inflammatory and angiogenic factors), along with production of nitrite (Smith et al. 2012).

Novel study has confirmed epigenetic activities of EVOO and its phenolic compounds. Epigenetic mechanisms are manners that induce reversible heritable alterations that are not associated with modifications in the DNA sequence but can control gene expression. Genetics is responsible for ∼30% of the identified variations, and∼70% are subjected to epigenetics modulation by environmental aspects. Main mechanisms of epigenetic are DNA methylation, histone modification, and regulation by non-coding RNAs(microRNAs). These epigenetic alterations are moderately stable, tissue specific, and can be hereditary through numerous generations (Del Saz-Lara et al. 2022).

According to all aforementioned traditional interpretations, which are, satisfy the unique role of EVOO on many health aspects. Our conclusion and opinion coincide with the recent epigenetic theory that explains its role via changes in gene expression (Ahmed et al. 2022). The present research model of our study was designed according to the useful role of EVOO via its type of extraction (highly refined EVOO LOBA Chemie from Italy) that contains a high ratio of numerous fluorescent compounds, like pigments such as chlorophyll and beta-carotene that be effective as an epigenetic agent. Adding up to the other broad beneficial constituents of it (lipophilic compounds such as tocopherols, carotenoids, lutein, and carotene) that it has a valuable health benefit especially for acting as an antioxidant (Ahmed et al. 2018).

Therefore, modern nutritional strategies display the use of EVOO as epigenetic agent. This point of discussion is clearly in view of its ability to prevent the development of certain chronic pathologies, such as cancer or cardiovascular disease, through epigenetic mechanisms. Of course, more research is required using appropriate *in vitro* and *in vivo* models to clarify the epigenetic effects of EVOO and its potential future role as adjunct "therapeutic" agents.

5. Conclusions

Elucidating the epigenetic effects of EVOO and its OOPCs may contribute to identifying and developing different nutritional strategies focused on the use of these compounds as epigenetic agents. As we suppose the existence of another explanation factor that related to the physical energy nature of

EVOO, which produced from its stereochemistry composition in the configuration and frequency of its molecules, so EVOO considered one of the epigenetic agents.

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Conflicts of Interest: The authors declare that there are no any conflicts or potential conflicts of interest are applicable

Ethics Approval: All the study's protocols, animal precautions, and treatment agreed with the guiding principles allocated by the Research Ethics Committee of Egyptian Atomic Energy Authority (REC-NCRRT) with approval No. (81A/21).

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References

ACEVEDO, N., et al. Histone acetylation of immune regulatory genes in human placenta in association with maternal intake of olive oil and fish consumption. *International Journal Molecular Science*. 2019, **20**(5), 1060. https://doi.org/10.3390/ijms20051060

AHMED, M.M., et al. Another Concept of Cancer Interpretation in View of the Interaction between Plasma Radiation and DNA. Clinical Cancer Investigation Journal. 2022,**11**(4), 33-43. https://doi.org/10.51847/Pms16qq3BK

AHMED, M.M., et al. Radioprotective role of α-tocotrienol against oxidative damage in γ-irradiated human blood cultures. *Bioscience Journal.* 2018, **34**(2), 400–409.

ALLAIN, C.C., et al. Enzymatic determination of total serum cholesterol.*ClinIcal Chemistry.* 1974, **20**(4), 470-475. PMID: 4818200.

ASMAA, A.S. and AMEL, F.M.I. Protective impact of Spirulinaplatensis against γ-irradiation and thioacetamide-induced nephrotoxicity in rats mediated by regulation of micro-RNA 1 and micro-RNA 146a, *Toxicology Research*. 2021, **10**(3), 453–466. https://doi.org/10.1093/toxres/tfab037

BALLARINI, F. and CARANTE, M. Chromosome aberrations and cell death by ionizing radiation: Evolution of a biophysical model. *Radiation Physics and Chemistry.* 2016, **128**, 18-25. https://doi.org/10.1016/j.radphyschem.2016.06.009

CALABRISON, MASSARO, M., et al. Extra virgin olive oil rich in polyphenols modulates VEGF-induced angiogenic responses by preventing NADPH oxidase activity and expression. *The Journal of nutritional biochemistry*. 2016, **28**: 19-29. https://doi.org/10.1016/j.jnutbio.2015.09.026

CARULLO, G., et al. Mantonico and pecorello grape seed extracts: Chemical characterization and evaluation of in vitro wound-healing and anti-inflammatory activities. *Pharmaceuticals*. 2020, ***13***, 97. https://doi.org/10.3390/ph13050097

CASADESÚS, J. and NOYER-WEIDNER, M., Epigenetics. Brenner's Encyclopedia of Genetics. 2nd Ed. San Diego: Academic Press; 2013.

CASADO-DÍAZ, A., DORADO, G., and QUESADA-GÓMEZ, J.M. Influence of olive oil and its components on mesenchymal stem cell biology. *World Journal of Stem Cells* 2019, **11**(12), 1045-1064. https://doi.org/10.4252/wjsc. v11.i12.1045

CELANO, R., et al. Insights into the Analysis of Phenolic Secoiridoids in Extra Virgin Olive Oil. *Journal of Agricultur and Food Chemmisrty*. 2018, **66**, 6053–6063.https://doi.org/10.1021/acs.jafc.8b01751

CHANG, G.L., et al. Mast cell degranulation and vascular endothelial growth factor expression in mouse skin following ionizing irradiation. *Journal of Radiation Research* 2021, **62**(5), 856–860. https://doi.org/10.1093/jrr/rrab067

D'ALESSANDRO, A. and DE PERGOLA, G. Mediterranean Diet and Cardiovascular Disease: A Critical Evaluation of a Priori Dietary Indexes. *Nutrients*. 2015, **7**, 7863–7888. https://doi.org/10.3390/nu7095367

DARWISH, I.A.E. and MOSALLAM, S.A.E. Chromosome aberrations in bone marrow cells of rats treated with MTBE. *Pakistan journal of pharmaceutical sciences*. 2019, ***32***(1), 89–93. PMID: 30772795.

DE SANTIS, S., CLODOVEOM, L., and CORBO, F. Correlation between chemical characterization and biological activity: An urgent need for human studies using Extra Virgin Olive Oil. *Antioxidants*. 2022, **11**(2), p.258. https://doi.org/10.3390/antiox11020258

DE SANTIS, S., et al. Extra virgin olive oil extracts modulate the inflammatory ability of murine dendritic cells based on their polyphenols pattern: Correlation between chemical composition and biological function. *Antioxidants* 2021, **10**, (7),1016. https://doi.org/10.3390/antiox10071016

DEL SAZ-LARA, A., et al. Nutri-Epigenetic Effects of Phenolic Compounds from Extra Virgin Olive Oil: A Systematic Review. *Advances in nutrition*. 2022, **13**(5), 2039–2060. https://doi.org/10.1093/advances/nmac067

EL-DEEB, A.E., ABD EL-ALEEM, I.M., Abd EL-RAHAMAN,A.A. The curative effect of some antioxidants on γ-irradiated rats. *Journal of the Egyptian Society of Toxicology*. 2006, **35**: 79-89.

FABIANI, R., et al. Oxidative DNA damage is prevented by extracts of olive oil, hydroxytyrosol, and other olive phenolic compounds in human blood mononuclear cells and HL60 cells. *Journal of Nurtition*. 2008, **138**, 1411-1416. https://doi.org/10.1093/jn/138.8.1411

FARAHAT, A. A., SAWIRESS, F. A., and AGHWIDER, A. A. Effect of Virgin Olive Oil Supplementation on Some Hematologic and Thyroid Hormones, Levels in Rats. *Journal of Veterinary Medical Research*. 2019, **26** (1), 41-47. https://doi.org/10.21608/jvmr.43332

FOSCOLOU, A., CRITSELIS, E., and PANAGIOTAKOS, D. Olive oil consumption and human health: A narrative review. *Maturitas*. 2018, **118**, 60–66. https://doi.org/10.1016/j.maturitas.2018.10.013

FOSSATI, P., and PRENCIPE, L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical Chemistry*. 1982, **28**(10),2077-2080. https://doi.org/10.1093/clinchem/28.10.2077

GOYES, J.C.R., et al. Biologic effect of ionizing radiation. *InterventionalCardiology*. 2018,**10**(6), 133–138.

JIMENEZ-LOPEZ, C., et al. Bioactive compounds and quality of extra virgin olive oil. *Foods*. 2020, **9**(8),1014. https://doi.org/10.3390/foods9081014

KALEKHAN, F., et al. Traditionally Used Natural Products in Preventing Ionizing Radiation-Induced. *Medicinal Chemistry-Anti-Cancer Agents*. 2022, **22**(1), 64-82. https://doi.org/10.2174/1871520621666210405093236

KIM, E. J., et al. Ionizing Radiation regulates vascular endothelial growth factor-A transcription in cultured human vascular endothelial cells via the PERK/eIF2α/ATF4 pathway. International Journal of Radiation Oncology and *Biology* Physics. 2020, **107**(3), 563-570. https://doi.org/016/j.ijrobp.2020.03.003

KOUKOURAKIS, M.I. Radiation damage and radioprotectants: new concepts in the era of molecular medicine. *The British Journal of Radiology*. 2012, **85**(1012), 313–330. https://doi.org/10.1259/bjr/16386034

KYRIAKIDIS, N.B., and SKARKALIS, P. Fluorescence spectra measurement of olive oil and other vegetable oils. *Journal of AOAC International*. 2000, **83**(6), 1435-1439. https://doi.org/10.1093/jaoac/83.6.1435

LAMY, S., et al. Olive oil compounds inhibit vascular endothelial growth factor receptor-2 phosphorylation. Experimental cell research. 2014, ***322***(1), 89–98. https://doi.org/10.1016/j.yexcr.2013.11.022

LAZIC, S.E. Why we should use simpler models if the data allow this: relevance for ANOVA designs in experimental biology. *BMC Physiology*. 2008, **8**, 16. https://doi.org/10.1186/1472-6793-8-16

LI, W., et al. Blocking the function of inflammatory cytokine s an d mediator s b y usin g IL -10 and TGF-β: A potential biological immunotherapy for interverte-bral disc degeneration in a beagle model. *International Journal of Molecular Science*. 2014,**15**,17270-17283. https://doi.org/10.3390/ijms151017270

MANSOUR, H.H. Protective effect of ginseng against gamma-irradiation –induced oxidative stress and endothelial dysfunction in rats. *EXCLI journal*. 2013, **12**, 766-777.

MARTIN-PELAEZ, S., et al. Effect of virgin olive oil and thyme phenolic compounds on blood lipid profile: Implications of human gut microbiota. *European journal of nutrition*. 2017,**56**,119–31. https://doi.org/10.1007/s00394-015-1063-2

MONTASER, S.A., AHMED, M.M., and IBRAHIM, S.I. Antioxidant Capacity of Achilleamillefolium L. against Cytogenetic and Immunological Disorders in Irradiated Human Blood Cultures. *Egyptian Journal of Radiation Science and Applications* .2019, **32**, (2): 137-144. https://doi.org/10.21608/ejrsa.2019.14648.1077

NWOKOCHA, C.R., et al. Serum lipids, proteins and electrolyte profiles in rats following total body irradiation. *The West Indian medical journal*. 2012, **61**(2), 117–121. PMID: 23155954

PEDRET, A., et al. Cardiovascular benefits of phenol-enriched virgin olive oils: New insights from the Virgin Olive Oil and HDL functionality (VOHF) study. *Molecular nutrition & food research*. 2018, **62**(16), 1800456. https://doi.org/10.1002/mnfr.201800456

RIETJENS, S.J., et al. The olive oil antioxidant hydroxytyrosol efficiently protects against the oxidative stress-induced impairment of the NO • response of isolated rat aorta. *American Journal of Physiology-Heart and Circulatory Physiology*. 2007, **292**(4), H1931-6. https://doi.org/10.1152/ajpheart.00755.2006

SCHWINGSHACK, L.L., MORZE, J., and HOffMANN, G. Mediterranean diet and health status: Active ingredients and pharmacological mechanisms. *British Journal of Pharmacol*ogy. 2020, **177**, 1241–1257. https://doi.org/10.1111/bph.14778

SHANNON, O.M., et al. Mediterranean diet and the hallmarks of ageing. *European Journal Clinical Nutrition.* 2021, ***75***, 1176–1192. https://doi.org/10.1038/s41430-020-00841-x

SHMARINA, G.V., et al. Tumor necrosis factor-a/interleukin-10 balance in normal and cystic fibrosis children. *Mediators of Inflammation*. 2001, **10**, 191–197. https://doi.org/10.1080/09629350123387

SIDDEEK, B., and SIMEONI, U. Epigenetics provides a bridge between early nutrition and long-term health and a target for disease prevention. *Acta Paediatrica*. 2022, **111**, 927–34. https://doi.org/10.1111/apa.16258

SMITH, A.N., et al. Unsaturated fatty acids induce mesenchymal stem cells to increase secretion of angiogenic mediators. *Journal of Cell Physiology*. 2012, **227**, 3225-3233. https://doi.org/10.1002/jcp.24013

SUVARNA, S.K., CHRISTOPHER, L., and JOHN, D. B. "Theory and practice of histological techniques." Pbl London, Churchill Livingstone, Elsiver 2013, 173-187**.**

TAN, F.C., et al. Protective effect of pycnogenol against gamma radiation-induced lung injury in rat: DNA damage, lipid peroxidation, antioxidant levels, and histopathological changes. *Journal of cancer research and therapeutics*. 2022, **18**(6), 1658-1665. https://doi.org/10.4103/jcrt.JCRT_1062_20

VANDERSTRAETEN, J., et al. Modulation of VEGF Expression and Oxidative Stress Response by Iodine Deficiency in Irradiated Cancerous and Non-Cancerous Breast Cells. *International journal of molecular sciences.* 2020, **21**(11), 3963. https://doi.org/10.3390/ijms21113963

VENTURINIA, F., et al. Physico-chemical properties extraction from the fluorescence spectrum with 1D-convolutional neural networks: application to olive oil. *arXiv preprint arXiv*. 2022**,** 2203.07229. https://doi.org/10.48550/arXiv.2203.07229

ZRELLI, H., et al. Hydroxytyrosol reduces intracellular reactive oxygen species levels in vascular endothelial cells by upregulating catalase expression through the AMPK-FOXO3a pathway. *European Journal Pharmacology*. 2011, **660**, 275–282. https://doi.org/10.1016/j.ejphar.2011.03.045

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