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# The possible protective effects of olive oil with fig and date-palm fruit extracts as natural antioxidants on some biochemical and hematological parameters of rats treated with doxorubicin and γ-radiation

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## ABSTRACT

*The present study was designed to determine the possible protective effects of the olive oil (7 g/kg) with fig (1 g/kg) and date-palm fruit (1 g/kg) extracts (OFD) against the toxicity hazards of doxorubicin (DOX) which was used as chemotherapy of cancer and/or γ-radiation which was used as radiotherapy of cancer. The DOX-treated groups were injected with doses of 2.5 mg/kg, i.v., weekly for 4 consecutive weeks (10 mg/kg cumulative doses). Rats of irradiated groups were exposed to whole-body γ-radiation with fractioned doses of 2 Gy weekly for 4 consecutive weeks (8 Gy cumulative doses). The OFD-treated groups were received two weeks pretreatment with OFD and during the experimental period, daily via oral gavages. The DOX-treated and/or irradiated groups recorded depletion of the antioxidant parameters (reduced glutathione (GSH) and nitric oxide (NO)) as well as increased thiobarbituric acid reactive substances (TBARS). Also, we observed alterations of lipid profile parameters (triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C)), lipid risk ratios and hematological values (erythrocyte (RBC) count, hemoglobin (Hb) concentration, hematocrit (Hct) percentage, platelet count, total and differential leukocyte (WBC) count) in these groups as compared with control rats. Administration of OFD to DOX-treated and/or irradiated rats significantly ameliorated the oxidative stress markers, lipid profile, risk ratios and the hematological parameters. In conclusion, olive oil with fig and date-palm fruit extracts together could be used synergistically to decrease the bad side effects of chemotherapy and radiotherapy.*

**Keywords:** Olive oil; fig fruit extract; date-palm fruit extract; doxorubicin; γ-radiation; oxidative stress; lipid profile; hematological parameters.

## INTRODUCTION

The oxidative stress is an expression used to describe the various harmful processes resulting from an imbalance between the excessive formation of the reactive oxygen species (ROS) and limited cell's antioxidant defenses **(Turrens, 2003)**. The DOX and/or γ-radiation treatments are known to induce oxidative stress through the generation of ROS, which causes an imbalance of the antioxidant activities and ultimately resulting in cell death **(Srinivasan *et al*., 2006; Elsadek *et al*., 2017)**. ROS can react with biological molecules and destroy the structure of cells **(Baatout *et al*., 2004)**. They are often responsible for protein denaturation, lipid peroxidation and impaired enzyme activities **(Karbowink and Reiter, 2000)**. DOX is a potent antibiotic, also called adriamycin, it is widely used for the treatment of different solid and hematopoietic tumors. However, in addition to its anti-tumoricidal activity, it promotes several well-known side effects that include chronic and irreversible toxicity **(Asmis *et al*., 2005; Patil *et al*., 2008)**.

The chronic administration of plant extracts might augment the major cellular endogenous antioxidants, and so it could be identified as a promising approach to fight the oxidative stress **(Bashandy *et al*., 2014; Bashandy *et al*., 2016)**. The major benefit of the Mediterranean diet is its

high level of antioxidants derived from fruits and vegetables, including olive oil, figs, and date-palm fruits, which contribute antioxidant vitamins, minerals, flavonoids, and polyphenol content **(Solomon *et al*., 2006)**. In addition, mixed plant extracts showed a higher diversity of polyphenols resulted in greater stability and bioaccessibility of antioxidants compared with single extract **(Bashandy *et al*., 2014; Kamiloglu *et al*., 2014; Rubió *et al*., 2014).**

Based on the above hypothesis, the present study aimed to investigate the protective synergistic effects of olive oil with fig and date-palm fruit extracts against the toxicity hazards of doxorubicin and/or γ-radiation-induced oxidative stress in Wistar albino rats.

## MATERIALS AND METHODS

### Ethics statement

All animals in the present study were conducted in accordance with the ethical guidelines for investigations in laboratory animals and comply with the guide for the care and use of laboratory animals **(Institute of Laboratory Animal Resources, 1996)**. The study also approved by an independent ethics committee of the National Research Center, Egypt.

### Experimental animals and work design

The present study used 120 male Wistar albino rats (150-170 g). The rats were obtained from the Egyptian Holding Company for Biological Products and Vaccines (VACSERA, Giza, Egypt) and allowed to acclimatize in the experimental laboratory for 2 weeks, and then divided into 8 groups (n = 15 rats) according to the treatment and the requirements of the experiment. The rats were maintained under standard laboratory conditions at the animal center, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt. They were kept in a temperature-controlled environment (20-25 °C) and 50%–60% relative humidity with an alternating 12 h light-dark cycle. Five rats were placed into each cage and provided with standard diet pellets and drinking tap water *ad libitum* during the whole experimental period.

**Group I (Control):** rats of this group were neither treated nor irradiated and were provided with standard diet-pellets and drinking tap water *ad libitum* during the experiment (6 weeks).

**Group II (OFD):** rats of this group were administered with extra-virgin olive oil (7 g/kg) and freshly prepared fig (1g/kg) and date-palm fruit (1g/kg) extracts daily *via* oral gavages for 6 weeks.

**Group III (DOX):** rats of this group received DOX in doses of 2.5 mg/kg, iv, weekly for 4 consecutive weeks (cumulative doses of 10 mg/kg body weight).

**Group IV (R):** rats of this group were exposed to whole-body γ-radiation with fractioned doses of 2 Gy every week for 4 consecutive weeks (up to 8 Gy total doses).

**Group V (DOX-R):** rats of this group were irradiated following 20 h of DOX injection as the same schedule mentioned above.

**Group VI (OFD-DOX):** rats of this group were treated with OFD (2 weeks protection and 4 weeks during the experiment) and injected with DOX as the same schedule mentioned above.

**Group VII (OFD-R):** rats of this group were treated with OFD (2 weeks protection and 4 weeks during the experiment) and irradiated as the same schedule mentioned above.

**Group VIII (OFD-DOX-R):** rats of this group were treated with OFD (2 weeks protection and 4 weeks during the experiment) and irradiated following 20 h of DOX injection as the same schedule mentioned above.

### Doxorubicin (DOX)

Adricin® (doxorubicin hydrochloride) vials were obtained from EIMC united pharmaceuticals, Egypt. The cumulative doses of

DOX used in the present study were 10 mg/kg body weight (2.5 mg/kg, i.v., weekly for 4 consecutive weeks).

**Irradiation (R)**

The radiation facility was the Canadian Gamma cell–40 ($^{137}$Cs) housed at the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt. Rats of irradiated groups were exposed to a whole body γ-irradiation with fractioned doses (2 Gy every week for 4 weeks up to 8 Gy cumulative doses). The dose rate at the time of the experiment was 0.45 Gy/min and the time of exposure was 4.44 min exactly.

**Extra-virgin olive oil (*Olea europaea L.; Family Oleaceae*)**

Extra-virgin olive oil was procured from the Grup Pons Company (Spain) with a brand of Monumental extra-virgin olive oils. The purchased extra-virgin olive oil density was 920 g/L and the selected olive oil dose was 7.6 ml/kg (7 g/kg) body weight rat **(Bashandy *et al*., 2014**). The extra-virgin olive oil was used for supplementation to rats by oral gavage.

**Fig fruit extract *(Ficus carica L.; Family Moraceae)***

The dried ripe fruits of fig were procured from Kafoods Ltd Company (Turkey). The fig fruits were cut into small pieces, dried and coarsely grounded by an electrical device. The powdered material was soaked in five folds of 80% ethanol for 72 h with occasional shaking. The soaked material was filtered through a fine filter paper, then subjected to evaporation under reduced pressure on a rotary evaporator until dryness (**Gilani *et al*., 2008)**. The extract was given to rats by oral gavage, and each rat was received fig crude extract in a concentration of 1 g/kg body weight (equivalent to about 3 figs) during the experimental period (6 weeks). The selective dose of the fig fruit extract was based on the human recommended antioxidant dose of dry fig fruits **(Vinson *et al*., 2005)** after conversion to albino rat dose **(Reagan-Shaw *et al*., 2007)**.

**Date-palm fruit extract (*Phoenix dactylifera L.; Family Arecaceae*)**

The plant material was rendered free from soil and the date fruits were manually separated from the pits, the flesh of the fruits was cut into small pieces, dried in an oven at 40 °C and coarsely ground by an electrical device. The ethanol extract of the date fruits was made by adding the coarsely pounded date fruits to ethanol (50%) (1:3 weight to volume) for 48 h in a refrigerator (4 °C) with continuous stirring **(Al-Qarawi *et al*., 2005)**. The whole solution was ground, then centrifuged at 4 °C for 20 min at 1788g. The supernatant was collected and stored at -20 °C until used **(Vayalil, 2002)**. This suspension was given to rats by oral gavage, and each rat was received date-palm crude extract in a concentration of 1 g/kg body weight (equivalent to the flesh of 7 dates) during the experimental period (6 weeks). The selective dose of the date-palm fruits crude extract was based on the human recommended antioxidant dose of date-palm fruits **(Vinson *et al*., 2005)** after conversion to albino rat dose **(Reagan-Shaw *et al*., 2007)**.

**Biochemical study**

At the end of the experiment, blood samples were collected from each animal under anesthesia from the retro-orbital venous plexus puncture using blood capillary tubes. One part of the blood was collected in EDTA tubes for hematological study. The other part of the blood was left to clot at room temperature for 15 min. Sera were separated by centrifugation at 1006g at 20 °C for 15 min where the clear serum was obtained and kept frozen at -80 °C for various biochemical analyses. After blood sampling, animals were sacrificed and livers were isolated, quickly dissected out and washed with isotonic ice-cold saline. A portion of each animal liver tissue was taken from all animal groups. Each tissue was homogenized in ice-cold Tris–HCl lysis buffer,

pH 7.4 containing 1% protease inhibitor cocktail (Cell Signaling Technology, Inc., MA, USA) using Potter-Elvehjem rotor–stator homogenizer, fitted with a Teflon pestle (Omni International, Kennesaw, GA, USA). The homogenates were centrifuged under cooling at 1006g for 20 min. All tissue samples were kept cold on a crushed ice during the preparation, and then supernatants were subsequently aliquoted and stored at -80 °C until used for determination of hepatic thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) and nitric oxide (NO).

The TBARS were measured according to the method of **Yoshioka *et al.* (1979)**. Determination of NO was measured by the method of **Montgomery and Dymock (1961).** GSH was determined according to the method of **Beutler *et al.* (1963)**. The serum lipid profile levels of triglycerides (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were estimated using kits from Elitech diagnostic Co. France. The serum TG was determined according to the method described by **Fossati and Principe (1982)**. The serum TC level was determined according to the method described by **Allain *et al.* (1974)**. Serum HDL-cholesterol level was determined according to the method described by **Burstein *et al.* (1970)**. Low-density lipoprotein cholesterol (LDL-C) was calculated according to the formula of **Wieland and Seidel (1982)**.

The total number of erythrocytes (RBCs), total number of leukocytes (WBCs), differential leukocyte count, platelet count, hematocrit (Hct) percentage, and hemoglobin (Hb) concentration were estimated in the blood by using a CBC analyzer (Sino thinker. sk9000, U.S).

### Statistical analysis

The statistical analysis of the results was performed by using statistical package for social sciences SPSS/PC computer program (version 19, USA). All values were expressed as mean ± SE and the results were analyzed using one-way analysis of variance (ANOVA) test followed by least significant difference test (LSD) for multiple comparisons. Differences were considered statistically significant at $p < 0.05$.

## RESULTS

The DOX injected and/or irradiated (R) rats for four weeks recorded a significant increase ($p < 0.05$) in hepatic TBARS and a significant decrease ($p < 0.05$) in hepatic GSH and NO concentrations as compared with the corresponding values in the control group **(Fig. 1, 2 & 3)**.

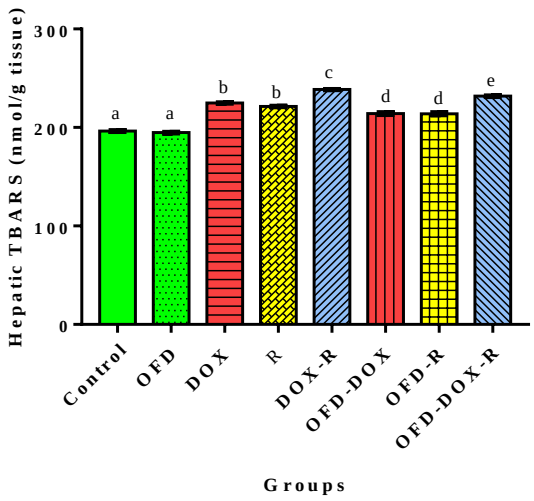


**Fig. 1:** The protective effects of olive oil with fig and date-palm fruit extracts (OFD) on hepatic thiobarbituric acid reactive substances (TBARS) concentration in rats treated with doxorubicin (DOX) and/or γ-radiation (R). Columns not sharing common superscript letters are significant with each other at $p < 0.05$.

In addition, the DOX injected and/or irradiated groups recorded a significant increase ($p < 0.05$) in the serum levels of TG, TC, LDL-C, TG/HDL-C, TC/HDL-C and LDL-C/HDL-C risk ratios in contrast to a significant decrease ($p < 0.05$) in serum HDL-C as compared with the corresponding values in the control group **(Table 2)**.

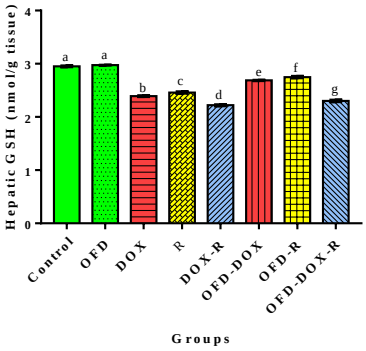


**Fig. (2):** The protective effects of olive oil with fig and date-palm fruit extracts (OFD) on hepatic reduced glutathione (GSH) level in rats treated with doxorubicin (DOX) and/or γ-radiation (R). Columns not sharing common superscript letters are significant with each other at $p < 0.05$.

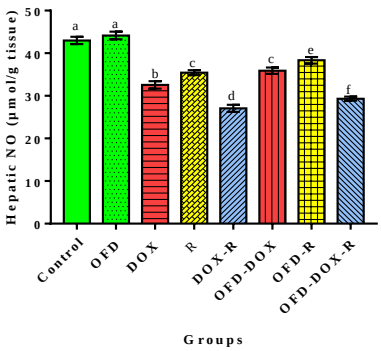


**Fig. (3):** The protective effects of olive oil with fig and date-palm fruit extracts (OFD) on hepatic nitric oxide (NO) level in rats treated with doxorubicin (DOX) and/or γ-radiation (R). Columns not sharing common superscript letters are significant with each other at $p < 0.05$.

**Table 1:** The protective effects of olive oil with fig and date-palm fruit extracts on the serum lipid profile and lipid risk ratios in rats treated with doxorubicin and/or γ-radiation.

| Parameters<br>Groups | TG (mg/dl) | TC (mg/dl) | LDL-C (mg/dl) | HDL-C (mg/dl) | TG/HDL-C risk ratio (mg/dl) | TC/HDL-C risk ratio (mg/dl) | LDL-C/HDL-C risk ratio (mg/dl) |
|---|---|---|---|---|---|---|---|
| **Control** | 80.21±1.15$^{a}$ | 98.62±0.95$^{a}$ | 17.48±1.51$^{a}$ | 65.09±1.62$^{a}$ | 1.23±0.03$^{a}$ | 1.52±0.03$^{a}$ | 0.27±0.02$^{a}$ |
| **OFD** | 81.64±1.62$^{a}$ | 98.09±2.04$^{a}$ | 16.27±1.94$^{a}$ | 65.49±2.05$^{a}$ | 1.25±0.05$^{a}$ | 1.50±0.03$^{a}$ | 0.25±0.03$^{a}$ |
| **DOX** | 129.42±2.09$^{b}$ | 130.57±2.49$^{b}$ | 59.41±2.66$^{b}$ | 45.27±1.32$^{b}$ | 2.87±0.10$^{b}$ | 2.89±0.10$^{b}$ | 1.32±0.08$^{b}$ |
| **R** | 112.78±2.15$^{c}$ | 127.80±1.67$^{bc}$ | 56.34±2.34$^{b}$ | 48.90±1.26$^{c}$ | 2.31±0.07$^{c}$ | 2.62±0.07$^{c}$ | 1.16±0.07$^{c}$ |
| **DOX-R** | 139.88±2.08$^{d}$ | 144.47±1.45$^{d}$ | 80.06±1.66$^{c}$ | 36.42±1.15$^{d}$ | 3.85±0.09$^{d}$ | 3.98±0.12$^{d}$ | 2.21±0.10$^{d}$ |
| **OFD-DOX** | 97.45±1.37$^{e}$ | 115.78±1.19$^{e}$ | 45.68±2.17$^{d}$ | 50.61±2.05$^{c}$ | 1.94±0.08$^{e}$ | 2.30±0.09$^{e}$ | 0.91±0.08$^{e}$ |
| **OFD-R** | 93.02±2.19f | 111.72±2.12$^{f}$ | 38.37±2.31$^{e}$ | 54.74±1.60$^{e}$ | 1.70±0.06$^{f}$ | 2.04±0.06$^{f}$ | 0.70±0.05$^{f}$ |
| **OFD-DOX-R** | 114.74±2.23$^{c}$ | 124.82±1.87$^{c}$ | 59.86±2.31$^{b}$ | 42.00±1.30$^{b}$ | 2.74±0.08$^{b}$ | 2.98±0.10$^{b}$ | 1.43±0.09$^{b}$ |

**Note:** Results are expressed as mean ± SE. For each parameter, values not sharing common superscript letters are significant with each other at $p < 0.05$; OFD, olive oil with fig and date-palm extracts; DOX, doxorubicin; R, irradiation; RBC, red blood corpuscle; Hb, hemoglobin; Hct, hematocrit; WBC, white blood cell.

**Table 2:** The protective effects of olive oil with fig and date-palm fruit extracts on hematological parameters in rats treated with doxorubicin and/or γ-radiation.

| Parameters<br>Groups | RBC count X10$^{6}$/mm$^{3}$) | Hb concentration (g/dl) | Hct (%) | Platelet count (10$^{3}$/mm$^{3}$) | WBC count (10$^{3}$/mm$^{3}$) | Differential leukocytic count | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Lymphocytes (%) | Neutrophils (%) | Monocytes (%) |
| **Control** | 8.57±0.06$^{a}$ | 15.62±0.09$^{a}$ | 47.02±0.17$^{a}$ | 936.7±2.79$^{a}$ | 9.71±0.05$^{a}$ | 72.2±0.19$^{a}$ | 19.5±0.16$^{a}$ | 4±0.21$^{a}$ |
| **OFD** | 8.75±0.08$^{a}$ | 15.90±0.14$^{a}$ | 47.75±0.34$^{a}$ | 937.1±3.73$^{a}$ | 9.80±0.06$^{a}$ | 71.6±0.28$^{a}$ | 19.5±0.21$^{a}$ | 4.2±0.37$^{a}$ |
| **DOX** | 6.49±0.06$^{b}$ | 11.85±0.13$^{b}$ | 35.62±0.37$^{b}$ | 702±2.87$^{b}$ | 6.81±0.03$^{b}$ | 65.5±0.21$^{b}$ | 22.4±0.28$^{b}$ | 6.4±0.21$^{b}$ |
| **R** | 6.81±0.04$^{c}$ | 12.44±0.08$^{c}$ | 37.25±0.39$^{c}$ | 714.2±2.38$^{c}$ | 7.37±0.05$^{c}$ | 67±0.27$^{c}$ | 21.6±0.21$^{c}$ | 6.4±0.34$^{b}$ |
| **DOX-R** | 6.05±0.06$^{d}$ | 11.03±0.10$^{d}$ | 33.19±0.31$^{d}$ | 664.9±3.19$^{d}$ | 5.89±0.03$^{d}$ | 62.8±0.25$^{d}$ | 23.2±0.25$^{d}$ | 6.9±0.35$^{b}$ |
| **OFD-DOX** | 7.49±0.12$^{e}$ | 13.66±0.14$^{e}$ | 41.28±0.47$^{e}$ | 728.8±3.48$^{e}$ | 7.48±0.03$^{e}$ | 68.7±0.33$^{e}$ | 20.9±0.30$^{e}$ | 5.4±0.34$^{c}$ |
| **OFD-R** | 7.76±0.08$^{f}$ | 13.95±0.18$^{e}$ | 42.02±0.56$^{e}$ | 745.5±3.66$^{f}$ | 7.78±0.03$^{f}$ | 68.7±0.38$^{e}$ | 20.4±0.21$^{e}$ | 6.2±0.50$^{bc}$ |
| **OFD-DOX-R** | 7.14±0.11$^{g}$ | 12.90±0.16$^{f}$ | 38.62±0.43$^{f}$ | 679.8±3.00$^{g}$ | 6.35±0.05$^{g}$ | 63.8±0.25$^{f}$ | 22.7±0.19$^{bd}$ | 6.9±0.44$^{b}$ |

**Note:** Results are expressed as mean ± SE. For each parameter, values not sharing common superscript letters are significant with each other at $p < 0.05$; OFD, olive oil with fig and date-palm extracts; DOX, doxorubicin; R, irradiation; RBC, red blood corpuscle; Hb, hemoglobin; Hct, hematocrit; WBC, white blood cell.

Moreover, the DOX injected and/or irradiated groups recorded a significant decrease ($p < 0.05$) in RBC count, Hb concentration, Hct percentage, platelet count, WBC count, and lymphocyte percentage in contrast to a significant increase ($p < 0.05$) in neutrophil and monocyte percentage as compared with the corresponding values in the control group **(Table 2)**.

## DISCUSSION

The oxidative stress and generation of reactive oxygen species (ROS) may contribute to doxorubicin (DOX) and/or irradiation (R) cytotoxicity during chemotherapy and radiotherapy **(Bashandy *et al*., 2014; Elsadek *et al*., 2017)**. Among the major forms of cellular damage induced by DOX and/or radiation exposure are DNA damage and lipid peroxidation. The increased levels of hepatic TBARS of DOX and/or R groups and the decreased levels of GSH and NO as compared with the control group indicating high levels of oxidative stress.

Nitric oxide is a small diffusible highly reactive molecule, can generate oxidative stress **(Pryor and Squadrito, 1995; Millar, 2004)**. The significant decrease in NO level recorded in the liver tissue after DOX injection and/or irradiation might be the result of its interaction with superoxide to form the peroxynitrite, a potent oxidant that can react with cellular lipids, proteins and DNA and accelerates cell toxicity **(Pryor and Squadrito, 1995; Kuhn *et al*. 2004)**.

The deficiency of the glutathione (GSH) contributes to oxidative stress. Therefore, may play a key role in aging and many diseases **(Wu *et al*., 2004)**. In addition, depletion in GSH level after DOX and/or radiation exposure may be due to its diffusion through impaired cellular membranes and/or inhibition of GSH synthetase and glutathione reductase enzymes **(Zahran *et al*., 2006)**. Moreover, **Srinivasan *et al*. (2006)** revealed that the decreased levels of GSH at oxidative stress might be due to its utilization by the ROS.

The products of lipid peroxidation (TBARS), is used as an indicator of tissue damage **(Zhou *et al*., 2006).** The increase in TBARS level may be attributed to the increased ROS in the aqueous media of the cells and the interaction of the hydroxyl radical with the polyunsaturated fatty acids of membranes in the phospholipids portion of cellular membranes initiating the lipid peroxidation and consequent damage of cell membranes **(Azab *et al*., 2001)**.

The depletion of NO and GSH, as well as the increase of TBARS at DOX injected and/or irradiated groups are in agreement with those recorded by **Bhatia and Jain (2004), Abd Elbaky *et al*. (2010) and Bashandy *et al*. (2014)** who reported a significant depletion in the antioxidant system accompanied by elevation of lipid peroxides in rats treated with DOX or γ-radiation.

The present results also reported an increase in lipid profile and lipid risk ratios in the serum of rats treated with DOX and/or γ-radiation. The hypercholesterolemia conditions might be due to the stimulation of cholesterol synthesis in the liver due to its release from tissues or destruction of cell membranes and increase the rate of cholesterol biosynthesis in the liver and other tissues **(Fathy, 2014; Al-Saedi *et al*., 2015)** or to the mobilization of fats from the adipose tissues into the bloodstream and mitochondrial dysfunction **(Said and Azab, 2006)**. Moreover, **Bok *et al*. (1999)** contributed the hypercholesterolemia to the increase of activation of 3-Hydroxyl-3-methyl glutaryl coenzyme A (HMG-CoA) reductase enzyme, the key regulatory enzyme in the reduction of the overall process of cholesterol synthesis. **Molchanova and Ahlers (1989)** explained the increase in serum triglyceride level to the inhibition of lipoprotein lipase activity as well as increasing the damage of cells and efflux of triglycerides

from the adipose tissues. The free radicals impair liver functions and cause hormonal imbalance. This imbalance induces hyperlipidemia through its multiple effects on lipid metabolism, including increased synthesis of cholesterol, triglyceride, and LDL-C **(Bowden *et al*., 1989**).

The hematopoietic system is highly sensitive to DOX and ionizing radiation. The reduction in the hematological parameters due to DOX and/or ionizing radiation treatments might be due to the damage in the hematopoietic system **(Fathy**, **2014**) or may be due to the increased permeability of cell membrane, which in turn caused osmotic swelling and erythrocyte hemolysis **(Asmis *et al*., 2005)**. In addition, it might be due to the increased destruction of mature cells or increased plasma volume **(Patil *et al*., 2008**) or decreased hemoglobin affinity for oxygen that induced hypoxia *via* diminished $O_2$ transport from the lungs to the blood and decreased $O_2$ release from oxyhemoglobin to the tissues **(Jagetia *et al*., 2006)**.

The olive oil with fig and date-palm extracts (OFD) are rich in polyphenolics substances; polyphenolics have received widespread attention because of their potential for preventing some highly prevalent chronic diseases. In fact, it has been reported that polyphenols are endowed with interesting biological activities such as anti-inflammatory, antioxidant, antidiabetic, and hepatoprotective activities **(Cicerale *et al*., 2012; El-Arem *et al*., 2014; Bashandy *et al*., 2014; Bashandy *et al*., 2016)**.

The two weeks pretreatments of DOX and/or irradiated rats with OFD for six weeks of successive supplementation protect against oxidative stress. This evidenced by significantly ameliorated oxidative stress markers (TBARS, GSH and NO) in the liver tissue as compared with the DOX and/or irradiated rats. This might be due to the protective action of OFD active ingredients as factors modifying membrane organization and their ability to scavenge the oxidation-initiating agents **(Bashandy *et al*., 2014; Bashandy *et al*., 2016)**. In addition, the antioxidant effect of OFD is mainly due to their phenolic compounds, which are able to donate a hydrogen atom to the free radicals, thus stopping the propagation chain reaction during the lipid peroxidation process **(Sanchez-Mareno *et al*., 1998)**. The two weeks pretreatment of DOX and/or irradiated rats with OFD was also found to increase GSH and NO production. The antioxidant role of GSH and NO comes from its reaction with oxygen, carbon, and nitrogen-centered radicals and can be seen to have a scavenger role against the free radicals attack **(Grisham *et al*., 1999)**. In addition, NO is important in the modulation of the inflammatory response by inhibiting the formation of proinflammatory lipids **(Rubbo *et al*., 1994)**.

Olive oil has been shown to protect LDL-C from lipid peroxidation *in vitro* experiments **(Owen *et al*., 2000)**. The evidence is accumulating to demonstrate that OFD is remarkably rich in effective phenolic antioxidants that could provide protection by radical scavengers and inhibiting the oxidative damage **(Bashandy *et al*., 2014; Rubió *et al*., 2014; Bashandy *et al*., 2016)**.

The present results are in agreement with the findings of **Gorinstein *et al*. (2002)** who reported that the polyphenols decreased plasma LDL-C levels and prevent their oxidation *in vivo*. The mechanism of this hypocholesterolemic action may be due to the inhibition of dietary cholesterol absorption in the intestine or inhibition of cholesterol production by the liver **(Krzeminski *et al*., 2003)** or due to stimulation of the biliary secretion and cholesterol excretion in the feces **(Prasad and Kalra, 1993; Katan *et al*., 1995)**. Regarding existing evidence on the effect of dietary fat type on blood lipids, the intake of unsaturated fat decreases plasma-cholesterol in opposition to what saturated fat does **(Grundy and Denke, 1990; Visioli *et al*.,**

**2005)**. Moreover, **Fathy (2014)** found that the administration of fig extract and/or olive oil to irradiated rats significantly ameliorates the lipid profile parameters and lipid risk ratios.

The administration of OFD to DOX-treated and/or irradiated rats improves the hematological parameters (RBC, Hb, Hct, platelets, total, and differential WBCs) which might be attributed to the amelioration in the antioxidant enzymes leading to diminished oxidative stress in bone marrow and spleen. The present results are also in agreement with **Viola and Viola (2009**) who attributed the improved hematological parameters to the oleuropeine, a component of the olive oil, which also exerts a favorable action on the platelets.

In conclusion, according to the results obtained in the present study the administration of the extra virgin olive oil with fig and date-palm extracts to the rats treated with DOX and/or γ-radiation ameliorated the oxidative stress markers in the liver tissue and improved the hematological parameters as well as lipid profile and lipid risk ratios. These novel findings revealed the synergistic effect of the combination between them to produce a broad spectrum of antioxidative activities that create an effective defense system against the free radical attack and fighting the oxidative stress.

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