Effect of Ethanolic Olive Leaves Extract or Bone Marrow Mesenchymal Stem Cells against Radiation Induced Oxidative Damage in Liver

Hemmat Mansour Abdelhafez, Mona Mohamed El-Tonsy and Eman Mohammed Hassona Dawoud

Department of Zoology, Faculty of Science (for girls), AL–Azhar University, Cairo, Egypt.
dr.hematmansour@yahoo.com

Abstract: This study aimed to investigate the possible therapeutic effects of ethanolic olive leaves extract or bone marrow derived-mesenchymal stem cells (BM-MSCs) on the liver of rats exposed to gamma radiation. **Materials and methods:** 50 adult male albino rats (Sprague dawely strain) were used in this study. They were divided into 5 groups (C group: untreated control rats; R group: rats were exposed to a single dose of gamma-radiation (6 Gy); OLE group: rats were treated with olive leaves extract (15 mg /kg body weight/day for 30 days); R+OLE group: rats were treated with olive leaves extract 15 mg /kg body weight/day for 30 days after irradiation; R+MSCs group: rats were irradiated with 6Gy then injected with bone marrow mesenchymal stem cells (BMSCs) 3×10^6 cells/1ml suspension through the caudal vein. The experimental rats were sacrificed on the 7^{th} and 30^{th} day post irradiation, except **R+MSCs** group which was sacrificed on the 30^{th} day post exposure to radiation. Biochemical parameters, histopathological and quantitative histochemical methods were studied. **Results:** Rats exposed to gamma radiation induced a significant increase in liver MDA level while, liver GSH level showed a significant decrease. Many histopathological lesions were observed in the liver tissue, such as dilated and congested sinusoids with increased Kupffer cells**,** debris of degenerated cytoplasmic organelles with many pyknotic and karyolytic nuclei**,** highly dilated and congested hepatic portal vein which contained hemolysed blood cells, lymphocytic infiltration in the portal area and dilated walls of bile ducts. Highly increased collagen fibers were also observed after gamma irradiation in the liver tissue. In addition, irradiated group induced a significant increase in amyloid β-protein, while a significant decrease in PAS+ve materials, total protein and total DNA content was detected. OLE and BM-MSCs succeeded to minimize the drastic changes which were observed in the liver tissue of the irradiated group. **Conclusion:** According to the results obtained in the current study mesenchymal stem cells proved to be more effective than OLE as it showed more radiotherapeutic effect in improving liver function and structure.

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Key words: Gamma radiation, Albino rats, liver, Ethanolic olive leaves extract (OLE), Bone marrow mesenchymal stem cells (BMSCs).

1. Introduction

Exposure to ionizing radiation causes many health hazardous effects. Such exposure produces biochemical lesions that initiate a series of physiological symptoms. Reactive oxygen species (ROS) such as superoxide (O2-), hydroxyl radical (OH•) and hydrogen peroxide (H2O2) created in the aqueous medium of living cells during irradiation cause lipid peroxidation in cell membrane and damage to cellular activities leading to a number of physiological disorders situation and dysfunction of cells and tissues **(Spitz *et al*., 2004)**. Exposure to gamma radiation caused biochemical, histopathological and histochemical changes in the liver **(kandeal, 2016)**. ROS principal types such as superoxide anions and hydroxyl radicals can react with macromolecules that result in tissue damage and cell dysfunction **(Mansour, 2006)**. ROS mainly targets proteins, lipids and nucleic acids with subsequent DNA strand breakage, DNA–protein cross-linkage and lipid peroxide production **(Sharma, 2013)**. These toxic products unbalance several antioxidant systems like the enzymatic antioxidant defense and glutathione systems **(Qian *et al*., 2010)**, also whole body gamma-irradiation at a dose level of 6Gy produced remarkable decrease in the hepatic antioxidant parameters (GSH and GSH-R) accompanied with elevated lipid peroxidation in liver **(Bashandy *et al*.,2014)**. The same author reported that antioxidant provides considerable radioprotective and radiotherapeutic effects against whole body gamma radiation by preventing oxidative stress through ROS scavenger. OLE increase antioxidant capacity by increase antioxidant enzymes **(Jafaripour *et al*., 2016).** The main constituent of the olive leaves is oleuropein, one of iridoide monoterpenes, which is thought to be responsible for pharmacological effects. Furthermore, the olive leaves contain triterpenes (oleanolic and maslinic acid), flavonoides (e.g., luteolin, apigenine and rutin) and chalcones such as olivine and olivin-

diglucoside (**Pereira *et al*., 2007**). Olive leaves extract have antioxidant activity higher than vitamin C and vitamin E, due to the synergy between flavonoids, oleuropeosides and substituted phenols (**Benavente-Garcia *et al*., 2000**).

Mesenchymal stem cells are a population of adult stem cells and they are promising sources for therapeutic applications. These cells can be isolated from the bone marrow and can be easily separated from the hematopoietic stem cells (HSCs) due to their plastic adherence **(Zhang and chan, 2010)**. Stem cell therapies are a category of regenerative medicine, the promise of which includes innovative therapies for organ failure and degenerative diseases. The first human hematopoietic stem cell transplant (HSCT) or bone marrow transplant (BMT) was performed more than 50 years ago **(Progatzky *et al.*, 2013)**. Due to the capacity of mesenchymal stem cells (MSCs) to differentiate into hepatocytes in *vitro* and in *vivo*, MSCs administration could repair injured liver, lung, or heart through reducing inflammation, collagen deposition and remodeling. These results provide a clue to treatment of liver fibrosis **(Zhao *et al*., 2005).** Moreover**, Seo *et al*. (2014)** reported that mesenchymal stem cells (MSCs) have the ability to migrate into fibrotic livers and differentiate into hepatocytes.

2. Materials and Methods

A total of 50 male Swiss albino rats (Sprague Dawely strain), weighing 120-130 gm, were obtained from Holding Company for Biological products & Vaccines (**Vacsera**), Helwan, Egypt. All animals were kept for about 15 days, before the onset of the experiment, under observation to exclude any intercurrent infection and to acclimatize the laboratory conditions. The animals were kept in metal cage with good aerated covers at normal atmospheric temperature (25+5ºc) and at normal daily 12 hrs dark/light cycles in the experimental animal unit, Zoology Department, Faculty of Science (Boys), Al Azhar University. They were fed commercial food pellets and provided with tap water a*d libitum*. All experiments took place in the laboratories of the Center of Genetic Engineering, Faculty of Science (Boys), Al Azhar University, Cairo.

Gamma-irradiation procedure

Irradiation process was performed using Gamma Cell-40 achieved by Egypt's National Center for Radiation Research and Technology (NCRRT), Cairo. The gamma cell–40 is a caesium-137 irradiation unit manufactured by Atomic Energy of Canada Limited. The unit provides means for uniform Gamma-irradiation of small animals or biological samples while providing complete protection for operating personnel.

The dose rate was 0.54 Gy/min at the time of the experiment.

Olive leaves (*olea europaea*) extraction

Olive leaves were weighed and ground to a fine powder in an electric mixer. The powdered plant material was extracted in 70% ethanol by soxhlet apparatus for 10 hours continuously **(Zahkouk, 2001).** The extract was administrated daily at dose 15mg/kg b. w. for 30 days by ingastric gavages according to the method of **Alirezaei *et al*. (2012)**.

Mesenchymal stem cells (MSCs) transplantation

MSCs cells concentration for transplantation was 3×10^6 cells/ml suspension transplanted into the irradiated rats through caudal vein according to the method of **Abdel-Aziz *et al.* (2007).** A total of ten animals received the 1ml cell suspension.

Experimental design

The experimental animals were divided into 5 groups.

Group 1: untreated control rats (C).

Group 2: irradiated group (R): animals were exposed to single dose of 6Gy of γ-radiation.

Group 3: olive leaves extract (OLE): animals were drenched OLE at a dose of 15 mg /kg body weight/day for 30 days.

Group 4: irradiated rats+ olive leaves extract (R+OLE): animals of this group were irradiated with 6Gy and then treated with OLE (15 mg /kg body weight) daily for 30 days.

Group 5: Irradiated animals+Mesenchymal stem cells (R+MSCs): animals of this group were irradiated with 6Gy of γ- radiation and then injected with MSCs at dose of $3\mathbf{x}10^6$cells/ml cell suspension through the caudal vein.

The experimental rats were scarified after 7 and 30 days post irradiation except R+MSCs group which was scarified after 30 days of irradiation.

Liver homogenate

1gm wet weight of liver was homogenized in 10 ml of distilled water (10% tissue homogenate), then the homogenates were centrifuged at 7000 rpm for 20 minutes and clear supernatants were drawn out and divided into aliquots and stored at -70ºC till the determination of the requested biochemical analysis.

Biochemical assays

Levels of lipid peroxidation were determined according to the method of Yoshioka ***et al. (*1979).** The assay of glutathione was done using the method of **Beutler *et al.* (1963).**

Histological and histochemical techniques

The animals were sacrificed after 7 and 30 days post irradiation, then the liver was immediately excised and fixed in 10% neutral formalin for 24 hours followed by dehydration in ascending grades of alcohol, clearing in xylene and embedding in paraffin wax. Sections were then cut at 5µ thickness and

stained by hematoxylin and eosin stain for histopathological study **(Bancroft and Gamble, 2002).** Collagen fibers were stained by Mallory's trichrome stain **(Pears, 1977).** Polysaccharides were stained by periodic acid Schiff's (PAS) reagent **(Drury and Wallington, 1980).** Total proteins were stained by mercuric bromophenol blue method **(Mazia *et al.*, 1953).** DNA was stained by Feulgen reaction **(Drury and Wallington, 1980**). Amyloid-β protein was stained by Congo red technique **(Valle, 1986).**

Quantitative histochemical analysis

Optical density of histochemical stained sections of the liver tissue for carbohydrates, total protein, DNA and Amyloid-β protein of the control and treated groups was recorded using IPWIN 32 image analysis software.

Statistical analysis

Results were expressed as mean ± standard error (SE). The significance of differences between means was measured by student's t-test (**Snedecor and Cochran, 1980**). The P values below 0.05 were considered significant while those above 0.05 were considered insignificant. Degree of freedom = (n1+ n2)-2.

3. Results

Biochemical analyses

Figure 1: showed the mean value of MDA level of the studied groups. Highly significant increase was shown in group R on the 7th and 30th day compared to the control. On the other hand, OLE group showed a non significant decrease in the mean value of MDA level on the 7th and 30th day compared to the control group. Treating irradiated group with olive leaves extract showed a significant decrease in MDA level on the 7th day compared to the irradiated group and a significant increase from the control, while on the 30th day showed a non significant decrease from the control group and a significant decrease compared to irradiated group. In addition, group R+ MSCs recorded on the 30th day a non significant increase from the control group and a significant decrease compared to irradiated group.

Figure 2: showed the mean value of GSH level of the studied groups. Highly significant decrease was shown in the irradiated group after 7 and 30 days of treatment compared to the control. On the other hand, OLE group showed a non significant change in the mean value of GSH level on the 7th and 30th day compared to the control group. Group R+OLE recorded a significant increase in GSH level on the 7th day compared to the irradiated group and a significant decrease from the control group, while on the 30th day showed a non significant decrease from the control group and a significant decrease compared to irradiated group. In addition, irradiated group treated with MSCs on the 30th day showed a non significant decrease from the control group and a significant increase compared to irradiated group.

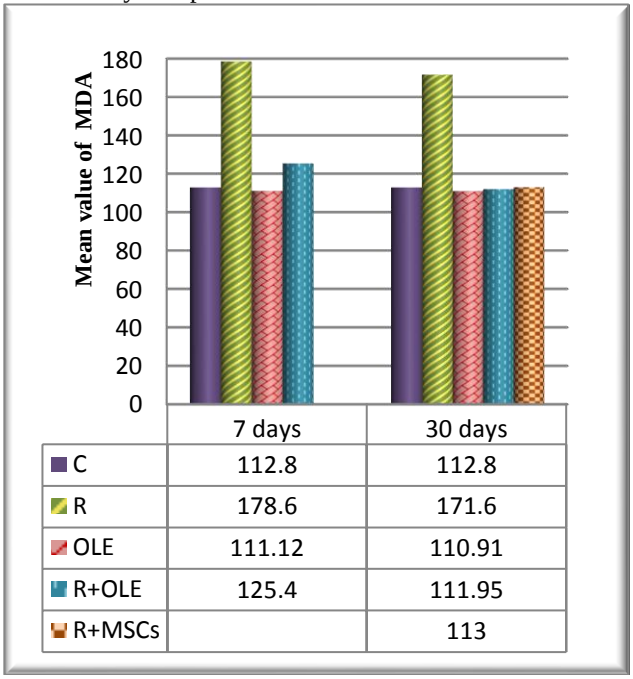


Fig. 1: the mean value of MDA (nm/mg) in the irradiated adult male rats after treatment with olive leaves extract or MSCs.

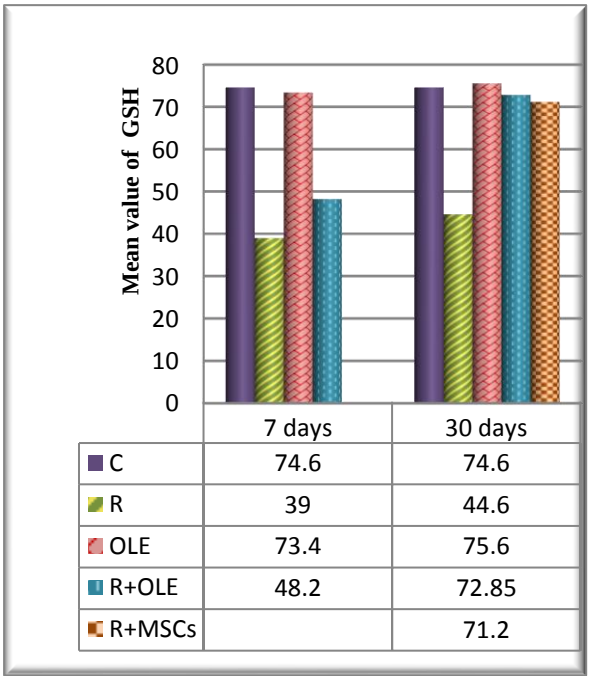


Fig. 2: the mean value of GSH (nm/mg) in the irradiated adult male rats after treatment with olive leaves extract or MSCs.

Histopathological observations:

Control group (C). Figs. 3 showed the normal structure of liver tissue. The normal distribution of collagen fibers were demonstrated in **fig. 4.**

Irradiated group (R). 7 days after irradiation showed many deleterious changes in the liver tissue, such as dilated and congested sinusoids with increased Kupffer cells**,** cellular debris and many pyknotic and karyolytic nuclei**,** highly dilated and congested hepatic portal vein with hemolysed blood cells, lymphocytic infiltration in the portal area and dilated walls of bile ducts **(Figs.5 & 6).** After 30 days of gamma irradiation many drastic changes were observed, delaminated and ruptured endothelial lining of the central vein with hemolysed blood cells, increased proliferation (hyperplasia) in walls of the bile ducts, hemorrhagic areas in between hepatocytes surrounded by many lymphocytes, numerous vacuolated hepatocytes with pyknotic or karyolytic nuclei and increased Kupffer cells **(Figs. 7 & 8)**.

Mallory's trichrome stain showed highly increased collagen fibers after 7 and 30 days of gamma irradiation especially in the congested blood vessels, blood sinusoids and around the portal areas **(Figs. 9 & 10)**.

Olive leaves extract group (OLE). More or less normal structure of the liver tissue **(Figs. 11 & 12)** and normal distribution of collagen fibers **(Figs. 13 & 14)** after 7 and 30 days of treatment were observed.

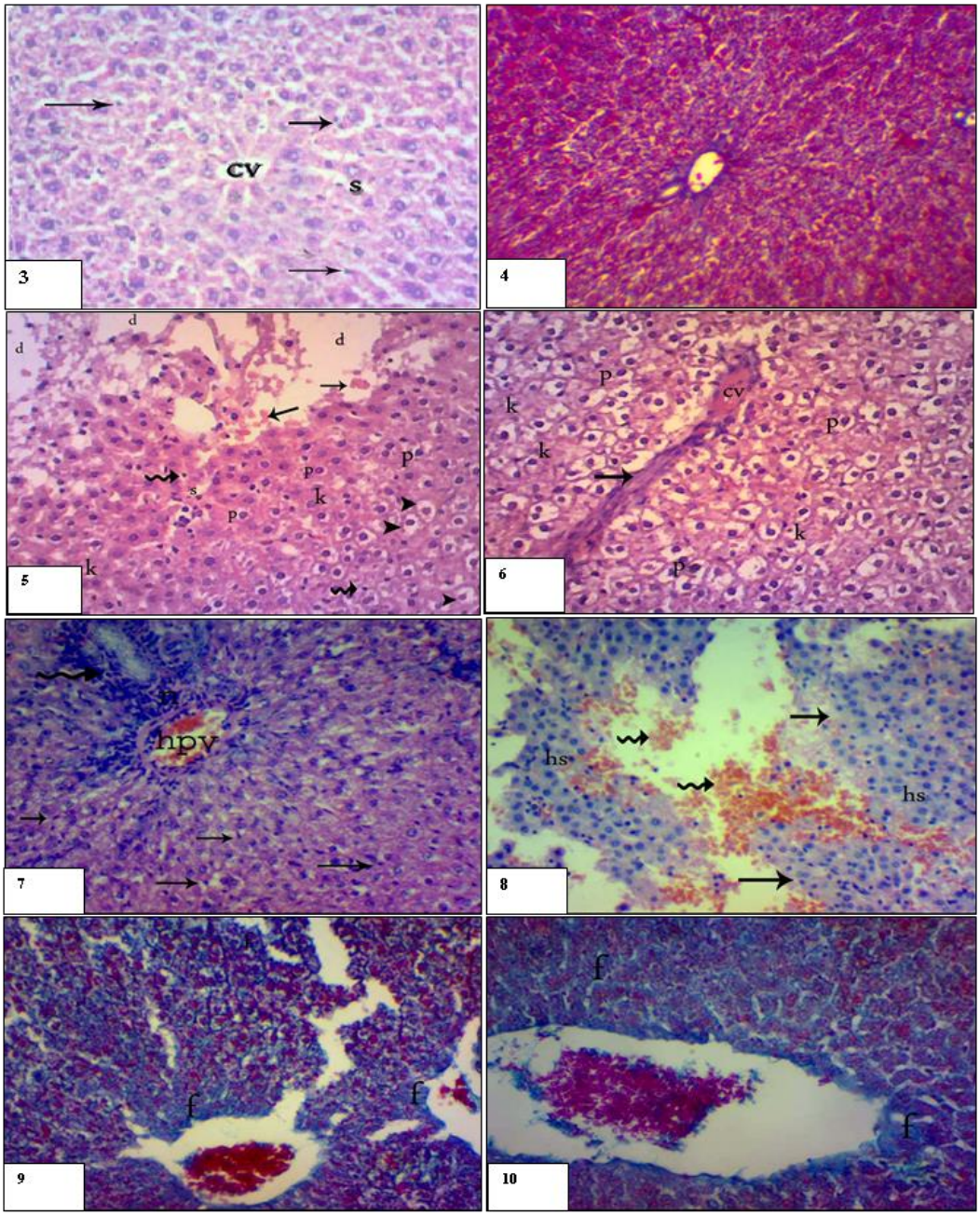
Olive leaves irradiated group (R+OLE). Sections of this group presented improvement in architecture of the liver tissue after 7 days of treatment but some pyknotic nuclei and hemolysed RBCs were still detected (**Fig. 15)**. While, almost normal architecture of liver tissue after 30 days of irradiation were observed (**Fig. 16)**. More or less normal distribution of collagen fibers was observed (**Figs.17 & 18)** after 7 and 30 days of treatment.

Mesenchymal stem cells-irradiated group (R+MSCs). Noticeable improvement of liver architecture against deleterious changes induced by irradiation was observed (**Fig.19).**

Using mallory's trichrome stain showed nearly normal distribution of collagen fibers **(Fig.20).**

Quantitative histochemical measurements

Irradiated rats **(R)** exhibited a significant decreased in the mean value of PAS +ve materials, total protein and total DNA content of the liver tissue after 7 and 30 days of irradiation while a significant increase in amyloid β-protein content was recorded during the experimental periods. On the other hand, rats administrated olive leaves extract (OLE) alone or after irradiation and those injected with mesenchymal stem cells (MSCs) post exposed to radiation showed a non-significant change in the mean value of PAS +ve materials, total protein, total DNA and amyloid β-protein content of the liver tissue during the experimental periods when compared to the control rats.



Figs. 3-20: Photomicrographs of sections in liver tissue of the control and treated groups.

Fig. 3: Control rats showing normal structure of liver tissue. Notice: cords of hepatocytes surrounding the central vein **(cv),** sinusoidal space **(s)** in between hepatocytes with many Kupffer cells **(arrow). (H & E X100)**

Fig. 4: Control rats showing normal distribution of collagen fibers in the portal area. **(Mallory's trichrome stain X 100)**

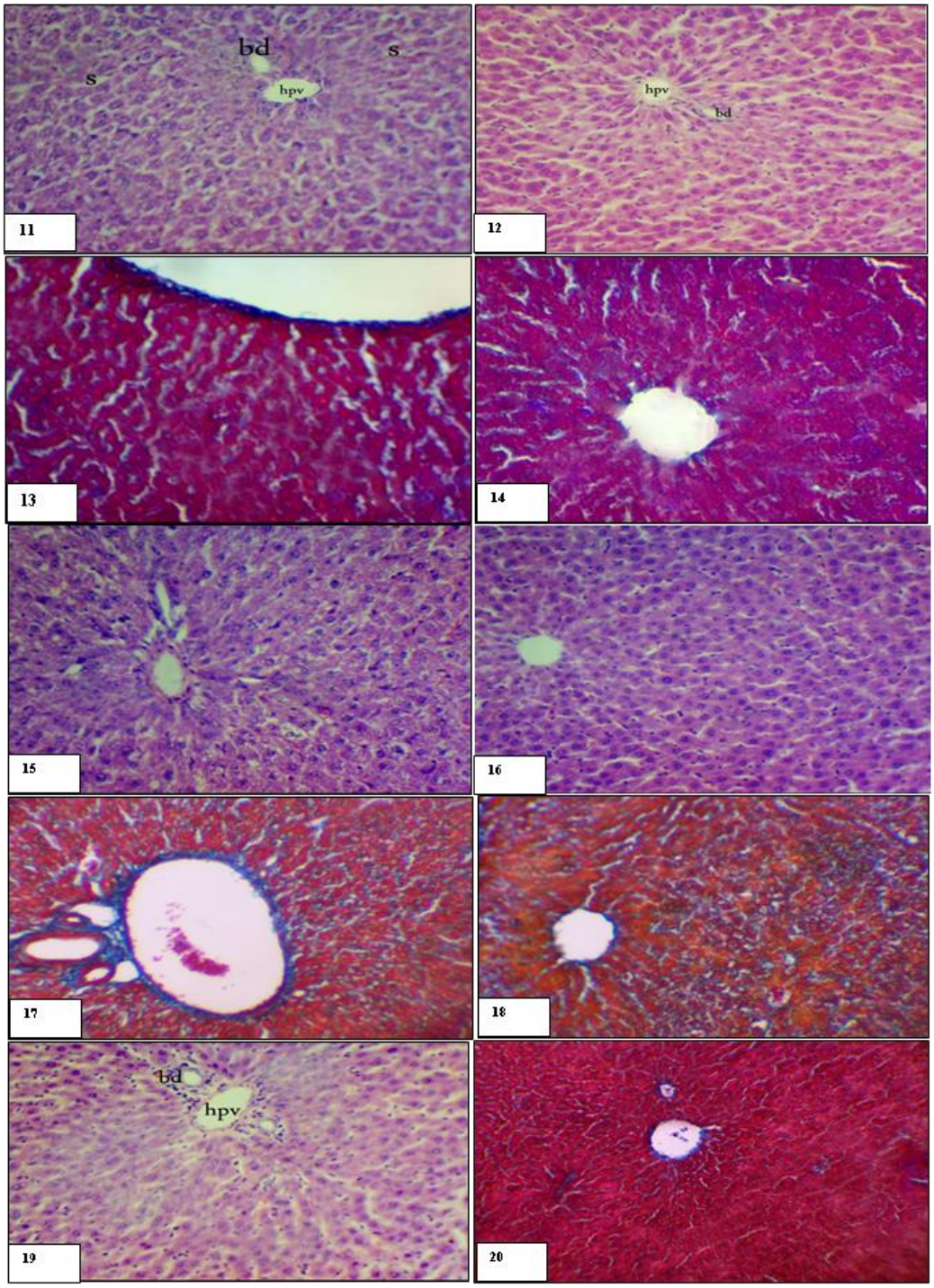
Fig.5: Irradiated rats after 7 days showing considerable number of damaged hepatocytes with cellular debris **(arrow) (d)**, pyknotic **(p)** and karyolitic **(k)** nuclei, number of Kupffer cells **(corrugated arrow)** and vacuolization in the cytoplasm of numerous hepatocytes (**head arrow**). **(H & E X100)**

Fig.6: Irradiated rats after 7 days showing lymphocytic infiltration (**arrow**) around the congested central vein **(cv)**, Pyknotic **(p**) and karyolytic nuclei **(k)** could also be detected. **(H & E X100)**

Fig.7: Irradiated rats after30 days showing congested hepatic portal vein **(hpv)** containing hemolysed RBCs and lymphocytic infiltration around the portal area associated with focal necrosis **(n)**, numerous kupffer cells **(arrow)** and elongated bile duct with degenerated epithelial cells **(corrugated arrow)**. **(H & E X100)**

Fig.8: Irradiated rats after 30 days showing some degenerated hepatocytes (arrow), replaced by numerous blood cells **(corrugated arrow),** most hepatocytes are faintly stained (hs). **(H & E X100)**

Fig. 9 & 10: Irradiated rats after 7 and 30 days showing highly increased collagen fibers **(f)** distribution around the central veins. **(Mallory's trichrome stain X 100)**



Figs. 11 & 12: OLE treated rats after 7 and 30 days of treatment showing: almost normal structure of liver tissue. Notice: normal architecture of hepatic portal vein **(hpv)**, bile duct **(bd)** and sinusoidal spaces **(s). (H & E X100)**

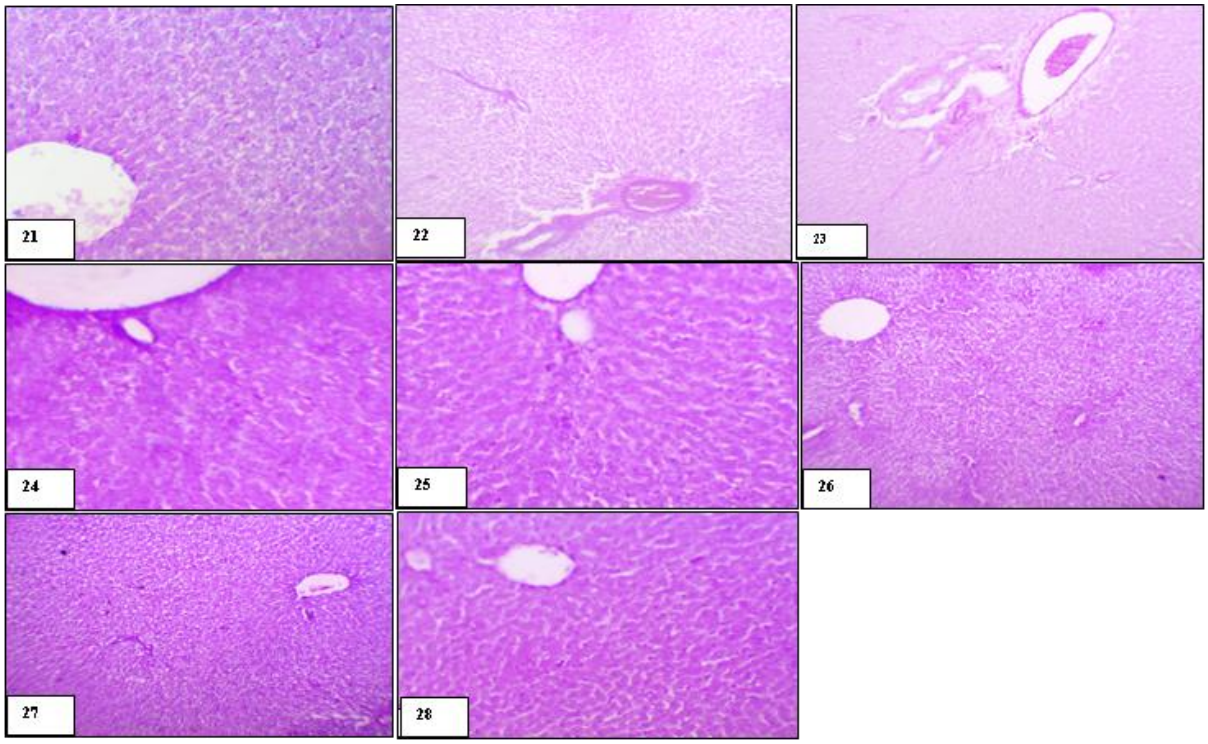
Figs. 13 & 14: OLE rats after 7 and 30 days of treatment showing normal distribution of collagen fibers around the central vein**. (Mallory's trichrome stain X 100)**

Fig. 15 & 16: **R+OLE treated rats after 7 and 30 days of irradiation showing** almost normal architecture of hepatocytes around the portal and central veins. **(H & E X100)**

Fig. 17 & 18: **R+OLE treated rats after 7 and 30 days of irradiation showing** slightly increased collagen fibers around the central and portal veins and bile duct, especially in the wall of the central, portal veins and sinusoidal spaces. **(Mallory's trichrome stain X 100)**

Fig. 19: R+MSCs treated rats showing almost normal architecture of hepatocytes but some pyknotic nuclei were still present. **(H & E X100)**

Fig. 20: R+MSCs treated rats showing slightly increased collagen fibers distribution around the central vein especially around its wall and sinusoidal spaces. **(Mallory's trichrome stain X 100)**



Figs. 21-28: photomicrographs showing distribution of PAS +ve materials in the liver tissue of the control and treated groups after 7 and 30 days of irradiation. **(PAS X 100).**
Fig. 21: Control rats showing moderately stained PAS +ve materials in the central area.
Figs. 22 & 23: irradiated rats showing faintly stained PAS +ve materials in the portal area after7 and 30 days respectively.
Figs. 24 & 25: OLE treated rats showing almost moderately stained PAS +ve materials after 7 and 30 days respectively.
Figs.26 & 27: R+OLE treated rats showing almost moderately stained PAS +ve materials in the central area after 7 and 30 days respectively.
Fig. 28: R+MSCs treated rats showing almost moderately stained PAS +ve materials after 30 days.

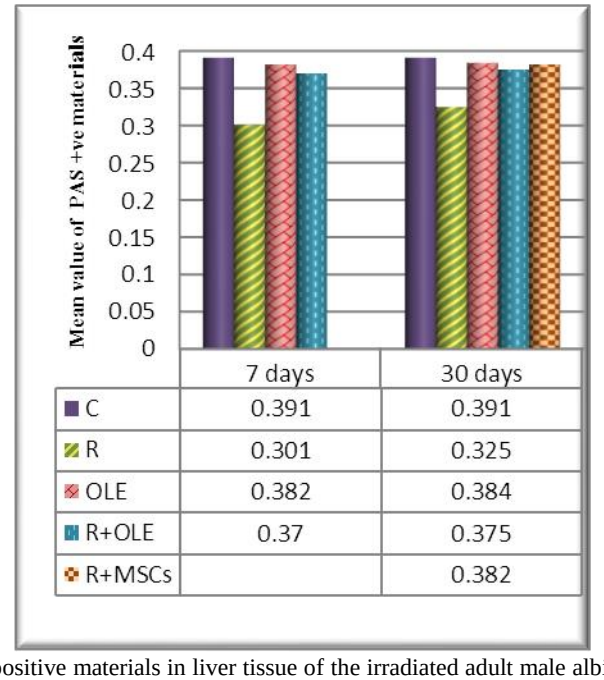
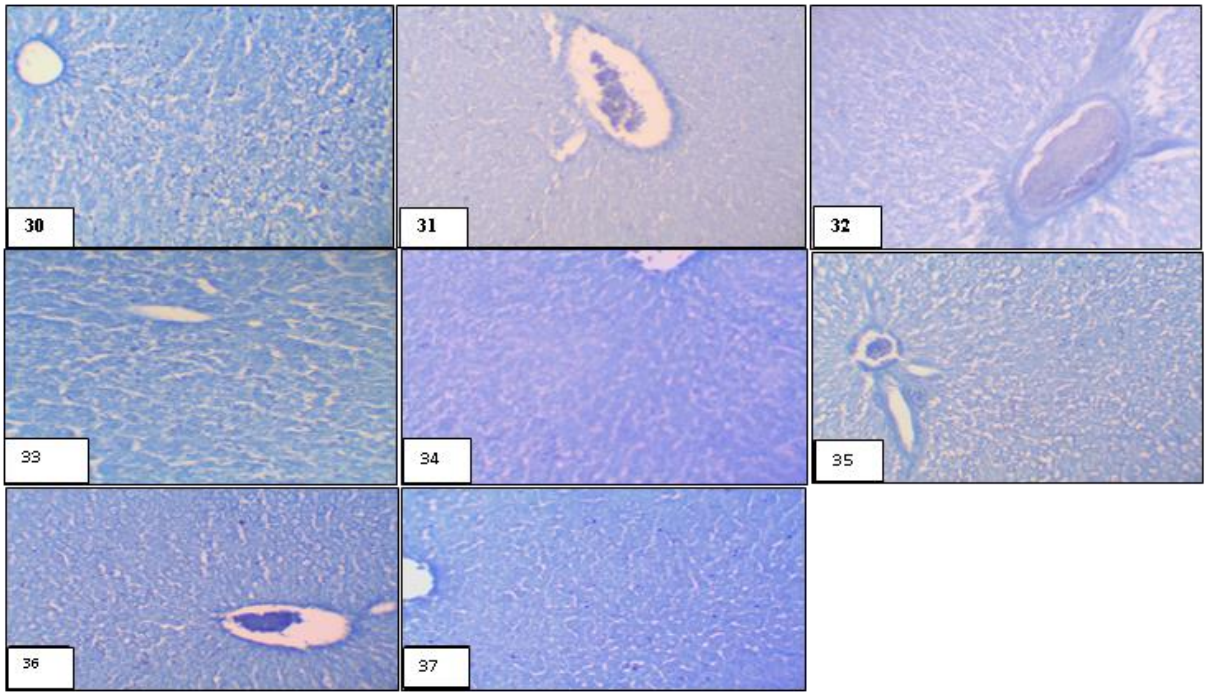


Fig. 29: the mean value of PAS positive materials in liver tissue of the irradiated adult male albino rats after treatment with olive leaves extract or mesenchymal stem cells.



Figs. 30-37: photomicrographs showing distribution of total protein in liver tissue of the control and treated groups after 7 and 30 days of irradiation **(Bromophenol blue X 100).**

Fig 30: control rats showing normal distribution of total protein in portal area.

Figs. 31 & 32: irradiated rats showing faintly stained total protein in most hepatocytes after 7 and 30 days of γ- irradiation respectively.

Figs. 33 & 34: OLE treated rats showing more or less normal distribution of total protein in the hepatocytes after 7 and 30 days of treatment respectively.

Figs. 35 & 36: R+OL treated rats showing almost normal total protein content in hepatocytes after 7 and 30 days of γ- irradiation respectively.

Fig. 37: R+MSCs treated rats showing almost normal total protein content after 30 days of γ- irradiation.

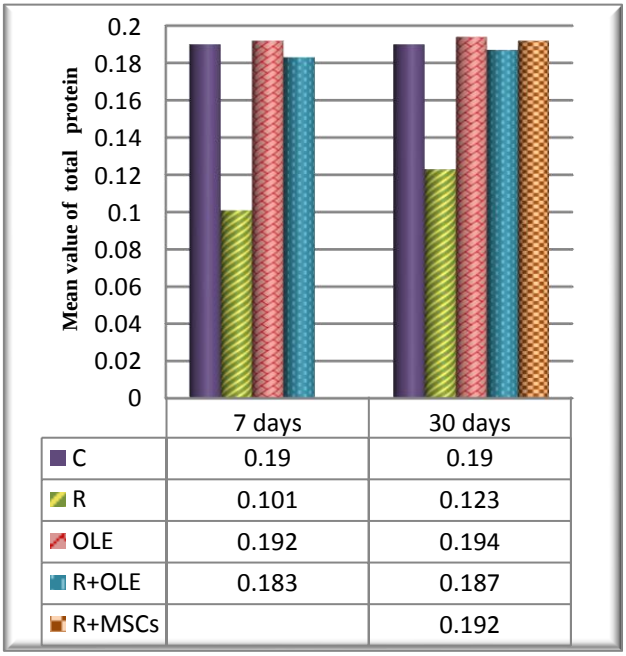
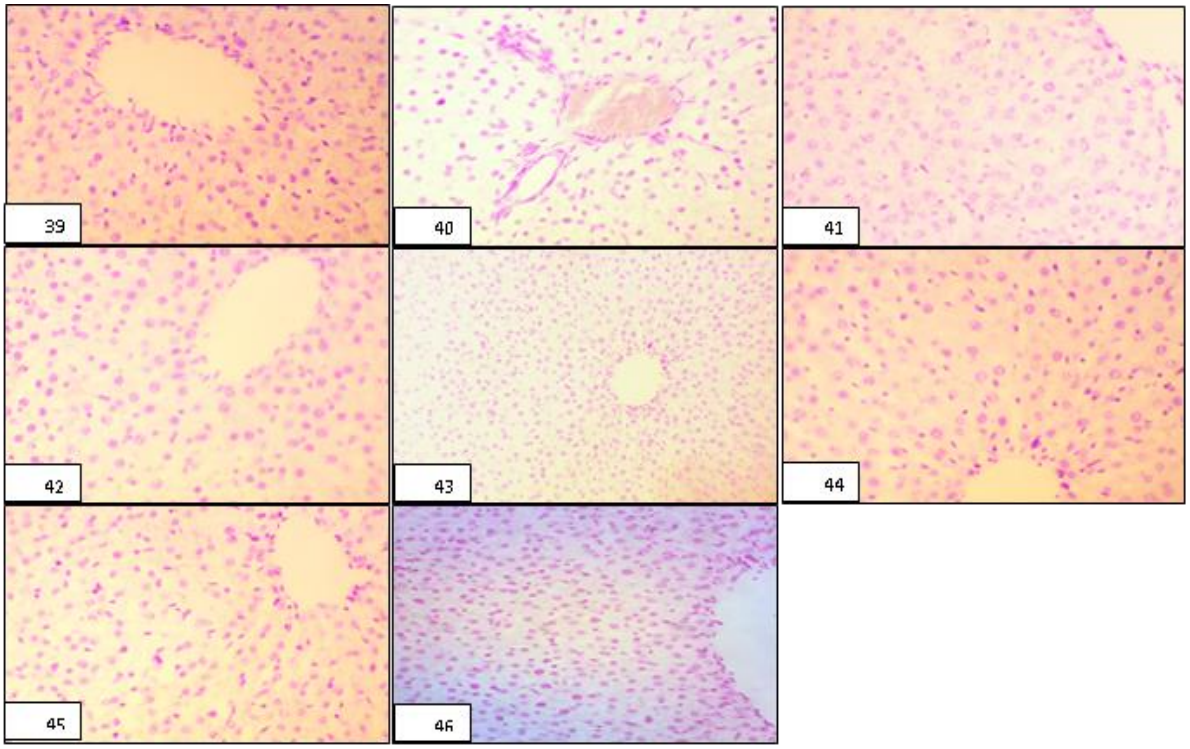


Fig.38: the mean value of total protein content in liver tissue of the irradiated adult male albino rats after treatment with olive leaves extract or mesenchymal stem cells.



Figs. 39-44: photomicrographs showing distribution of total DNA content in the liver tissue of the control and treated groups. **(Feulgen stain X 200)**

Fig. 39: control rats showing moderately stained DNA content in nuclei of hepatocytes, Kupffer cells, nuclei of endothelial lining of the blood vessels.

Figs. 40 & 41: irradiated rats showing faintly stained total DNA content in the hepatocytes of the central and portal areas, but deeply stained DNA content is observed in walls of the blood vessel and bile ducts after 7and30 days of irradiation respectively.

Figs. 42 & 43: OLE treated rats showing moderately stained total DNA content after 7 and 30 days of treatment respectively.

Figs. 44 & 45: R+OLE treated rats showing almost moderately stained DNA content after 7 and 30 days of γ- irradiation respectively.

Fig. 46: R+MSCs treated rats showing moderately stained total DNA content after 30 days of γ- irradiation.

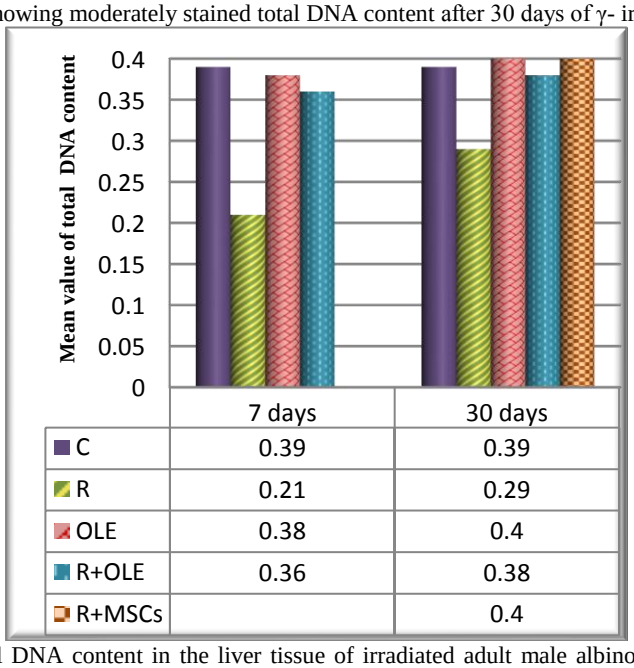
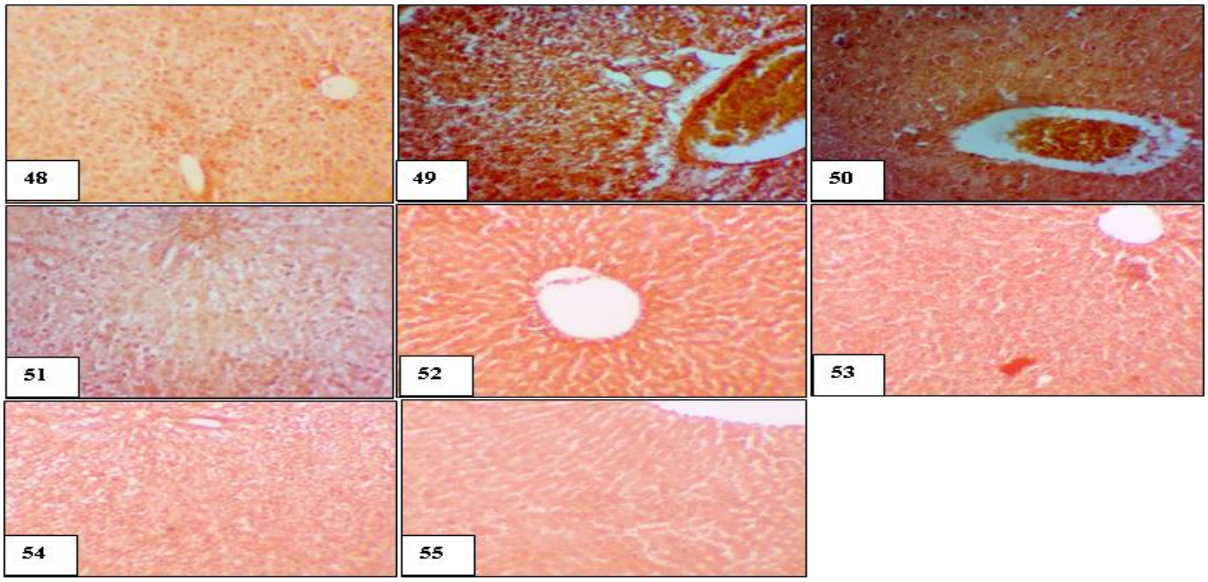
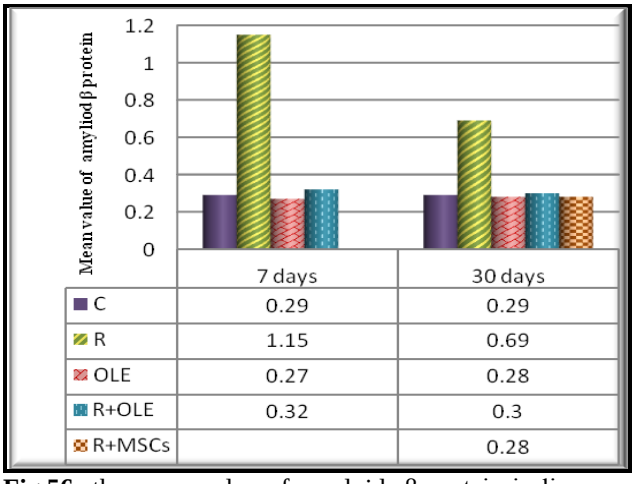


Fig.47: the mean value of total DNA content in the liver tissue of irradiated adult male albino rats after treatment with olive leaves extract or mesenchymal stem cells.



Figs. 48-55: photomicrographs showing distribution of the amyloid β-protein in the liver tissue of the control and treated groups. **(Congo red stain X 100)**
Fig. 48: control rats showing moderately stained amyloid- β protein in portal and central areas.
Figs. 49 & 50: irradiated rats showing deeply stained amyloid- β protein in hepatocytes of the portal and central areas and in the hemolysed RBCs inside veins after 7 and 30 days respectively.
Figs.51 & 52: OLE treated rats showing moderately stained amyloid -β protein after 7 and 30 days respectively.
Figs. 53 & 54R+OLE treated rats showing almost moderately stained of amyloid -β protein after 7and 30 days respectively.
Fig.55: R+MSCs treated rats showing almost moderately stained amyloid -β protein in hepatocytes after 30 days.



	7 days	30 days
C	0.29	0.29
R	1.15	0.69
OLE	0.27	0.28
R+OLE	0.32	0.3
R+MSCs		0.28

Fig.56: the mean value of amyloid- β protein in liver tissue of the irradiated adult male albino rats after treatment with olive leaves extract or mesenchymal stem cells.

4. Discussion

Free radicals formed during irradiation can cause a variety of membrane changes including lipid peroxidation, hydrolysis of phospholipids head groups, lipid-lipid cross links, disulfide bridge formation and amino acid residue damage in membrane proteins and lipid protein cross links **(Cakmak *et al.*, 2012)**.

MDA and GSH in liver tissue

The present study showed a significant increase in MDA level in the liver tissue of rats of the irradiated group during the experimental periods when compared to the control. These results are in agreement with the results of **Karslioglu *et al.* (2004)** who revealed that rats exposed to whole body gamma radiation (5 Gy) showed a significant increase of MDA level after 10 days post-irradiation. **Parivar *et al.* (2006)** attributed this increase in tissue MDA to the susceptibility of lipids to free radical attack leading to MDA formation, an important marker of radio toxicity. Elevated lipid peroxides in the irradiated rats is quite correlated with the disturbance in the concentration of Na^+ and K^+ as recorded by **Abu-Safi *et al.* (2006)**. The present findings are also supported by the research work of **Abdel- Rahman (2013)** who found that whole body gamma irradiation of rats at 7 Gy produced a significant increase in the level of MDA as compared to the control group. In addition, **Kandeal (2016)** reported that MDA level was increased in liver homogenate after whole body gamma radiation exposure at dose 4Gy as compared to the control. In the present study, supplementation with OLE induced a non significant decrease in the MDA level as compared to the control group after 7 and 30 days of exposure. These results are supported by the results obtained from histological and histochemical studies. Moreover, **Abaza *et al.* (2015)** indicated that the oral administration of OLE showed decrease in the

level of MDA marker for lipid peroxidation after treatment with OLE as compared to that of the control. In the present study the irradiated group treated with OLE showed a significant increase compared to the control group and a significant decrease compared to irradiated group on the 7th day of treatment. On the other hand after 30 days of treatment this group induced a non significant decrease in level of MDA as compared to the control group and a significant decrease as compared to the irradiated group.

These results agreed with the research work of **Kumral *et al.* (2015)** who found that oral administration of OLE reduce the level of MDA in liver of doxorubicin-treated rats near to normal value. **Alirezaei *et al.* (2012)** also, indicated that oleuropein consumption suppresses oxidative stress as monitored by the elevation activity of the main antiperoxidative enzyme, catalase and decreases lipid peroxidation products in the rat liver. However, the protective effect of this extract may be attributed to the presence of flavonoid compounds and their antioxidant effects and free radical scavenging properties **(El- Lakkany *et al*., 2011; El-Depsi, 2016)**.

The present results revealed a significant depletion in glutathione after radiation exposure, which might be a result from diffusion through impaired cellular membranes and/or inhibition of GSH synthesis **(Pulpanova *et al*., 1982). Said *et al*. (2005)** explained that the depletion in glutathione (GSH) content by irradiation isdue tothe diminished activity of glutathione reductase and the deficiency of NADPH which is necessary to change oxidized glutathione to its reduced form.

Dahm *et al*. (1991) attributed the decrease in liver GSH content to the inhibition of GSH efflux across hepatocytes membranes. Moreover, reduced glutathione has been reported to form either nucleophil-forming conjugates with the active metabolites or act as a reductant for peroxides and free radicals which might explain its depletion **(Moldeus and Quanguan, 1987)**. The reduction in GSH level may thus increase susceptibility of the tissue to oxidative damage including lipid peroxidation. Interaction of radiation with biological molecules produced toxic free radicals lead to structural and functional damage to cellular membranes. Consequently, a dramatic fall in glutathione and antioxidant enzymes leads to membrane lipid peroxidation and loss of protein thiols **(Devi and Ganasoundari, 1999)**. Inaddition, irradiation has been reported to cause renal GSH depletion and lipid peroxides accumulation in different organs **(Yanardag *et al*., 2001).**

In the present study improvement in GSH was noticed in OLE group during the experimental periods while, the irradiated group treated with OLE showed a significant decrease after 7 days and a non significant decrease after 30 days as compared to the control group. Also, a significantly increase in GSH level of this group was observed when compared to the irradiated group after 7 and 30 days of treatment. The high availability of oleuropein in its active form *in vivo* might explain the positive impact on the enzymatic and non-enzymatic GSH content antioxidants observed in this study. Oleuropein is able to chelate metal ions, such as Cu^{2+} and Fe^{2+}, which catalyse free radical generation reactions **(Andrikopoulos *et al*., 2002).** Oleuropein can be considered as effective quenchers of singlet oxygen and related reactive oxygen species. In addition to quenching ROS directly, oleuropein was reported to effectively prevent protein, lipid or DNA from oxidative damage by regulating other cellular antioxidant systems **(Fatani *et al*., 2015)**. Also, oleuropein has been detected in plasma only in its glycoside form, suggesting that it is absorbed intact from the intestine. Oleuropein and its metabolite, hydroxytyrosol, both possess the structural requirement (a catechol group) needed for optimum antioxidant and/or scavenging activity. Indeed, both oleuropein and hydroxytyrosol have been shown to be scavengers of superoxide anions and inhibitors of the respiratory burst of neutrophils and hypochlorous acid derived radicals **(Al-Azzawie and Alhamdani, 2006).** These results are in agreement with the research work of **Kumral *et al.* (2015)** who found that oral administration of OLE increased the level of GSH, SOD & GSH-Px in liver of DOX-treated rats near to normal value. **Geyikoglu *et al.* (2016)** reported that oleuropein is a major phenolic compound and used as a possible natural antioxidant which decreased total oxidative stress and increased total antioxidant capacity.

In the present study, transplantation of mesenchymal stem cells to the irradiated rats induced a non significant increase in lipid peroxidation (MDA) compared to the control group and a significant decrease when compared to the irradiated group. On the other hand, a non significant decrease was observed in the level of glutathione (GSH) of the irradiated rats injected with MSCs when compared to the control group and a significant increase as compared to the irradiated group. It was noticed that MSCs reduced oxidative stress through expressing high basal level of active forms of catalase (CAT), glutathione peroxidase (GPx) and SOD, which confers the resistance against acute ROS-mediated cellular damage **(Valle-Prieto and Conget,2010).**

In these respect **Shi *et al*. (2012)** showed that MSCs injection reduced ROS production in the injured liver decreasing oxidative stress by altering microenvironment of liver and activated transcription

factor Nrf2 which protect against oxidative stress and induced SOD production which decreased ROS in liver. These findings are in agreement with **Francios *et al*. (2014)** who reported that hMSC reduced the level of lipid peroxidation (MDA) against radiation-induced liver injury by increasing SOD gene expression which might reduce ROS production in the injured liver. Administration of MSCs induced an increase in the activities of antioxidant enzymes including superoxide dismutase (SOD) and decreases malondialdehyde (MDA) levels in lung tissues **(Ni *et al*., 2015)**. It was also noticed that MSCs have shown more resistance to ROS than differentiated cells and their redox status depends on complex and abundant anti-oxidant mechanisms by increasing levels of mRNAs for antioxidant proteins such as SOD-1, catalase, thioredoxin and peroxiredoxin **(Román *et al*., 2017**).

The histopathological changes

In the present study liver tissue of the irradiated rats after seven and thirty days of gamma irradiation showed many drastic changes such as corrugated and ruptured endothelial lining of the central vein which contained hemolysed blood cells, numerous vacuolated hepatocytes with increased signs of karyolysis and pyknosis in nuclei of hepatocytes, highly dilated and congested hepatic portal vein, thickened arterial walls, numerous hemorrhagic areas and malformed bile ducts.

Dilatation and congestion of blood vessels with increased proliferation of bile ducts in the portal areas were observed post-irradiation with lymphocytic infiltration between the degenerated hepatocytes (**Abdel-Mottaal and Abdel Maguid, 2007; Nakajima *et al*., 2016)**. According to **Levier *et al.* (1993)** the pyknotic and darkly stained nuclei may be as a result of coiling and shortening of chromosomes which became inactive especially in protein synthesis. Hepatocellular damage obtained in the present results also are in agreement with those described previously by **Waer and Shalaby (2012)** who observed changes in hepatic cells after exposed rats to accumulated dose of γ-radiation. This change was represented by congestion, dilatation of the central and portal veins with ruptured endothelial lining, lymphocytic infiltration, internal hemorrhage, fragmentation of nuclei with vacuolated cytoplasm, many necrotic and pyknotic areas**.** Hemorrhage and extravasated blood elements post-radiation exposure were also observed by **Ozguner *et al*. (2006).**

It was reported that following exposure of rats liver to radiation, distortion in the architecture of hepatic lobules, degeneration of hepatocytes and lymphocyte infiltration was recorded. Also hepatic cells showed necrosis, the nuclei showed pyknosis and karyolysis, in addition to dilation of portal spaces and blood vessels **(Abdel–Rahman, 2013; Kandeal, 2016)**.

Supplementation with OLE in the present study showed normal structure of the central and portal areas of the liver tissue. OLE showed a trend toward lowering incidence of hepatic pathological changes induced by γ-radiation, a remarkable restoration of normal cell structure. The cells restored their regularity and sizes with normal homogenous cytoplasm and rounded nucleus.

Meanwhile, Flavonoids mitigate the radiation damage to DNA *in vitro* which is registered by the reduction of chromosomal aberrations (CA) in the bone marrow, in all probability, due to their anti-radical activity **(Janjua *et al*., 2009).** Furthermore, oleuropein consumption suppressed oxidative stress as monitored by the elevation activity of the main antiperoxidative enzyme, catalase and decreased lipid peroxidation products caused by ethanol in the liver rat **(Alirezaei *et al*, 2012).** Administration of OLE decrease lymphocyte infiltration and activation of kupffer cells in a dose dependent manner. Thus, their suppression with OLE might contributed to deactivation of fibrogenic potential. These results are attributed to immunmodulatory properties of OLE in the liver by oxidative stress regulation **(Kobatake *et al*., 2015)**.

In the present study mesenchymal stem cells (MSC) showed improvement of the liver tissue of rats exposed to γ-radiation with a remarkable restoration of normal cell structure**.** These results are supported by the research work of **Abdel Aziz *et al*. (2007)** who found that administration of MSC decreased fibrosis of liver and improved liver tissue of rats exposed to CCL_4 due to infusion of allogeneic MSC which is of great value for liver fibrosis. MSCs are an attractive candidate to regenerate and repair liver injury. Evidence from *in vitro* and *in vivo* studies has indicated that MSCs have the ability to enhance fibrous matrix degradation by producing secreted factors that stimulate the regeneration of endogenous parenchymal cells. (**Kuo *et al*.,2008).** The presence of liver injury recruits homing of bone marrow derived stem cells through up regulation of cytokines such as stem cells factor-1, hepatocytes growth factor and matrix metalloproteinase **(Porada *et al*., 2004)**.

Kinnaird *et al.* (2004) thought that MSCs secrets a wide array of arteriogenic cytokines, e.g., vascular endothelial growth factor and contribute to reducing fibrosis through paracrine mechanisms rather than by cell incorporation. MSC showed anti apoptotic activity in hepatocytes and played an essential part in the regulation of the liver **(Ueki *et al.*, 1999; Baligar *et al.* 2016)**.

Kubo *et al*. (2015) found that human amnion-derived mesenchymal stem cells (hAMSC)

transplantation ameliorated liver fibrosis, suppressed the activation of hepatic stellate cells (HSCs), suppressed the infiltration of kupffer cells and regulated the activation of kupffer cells.

The present study showed increased collagen fibers inside the highly dilated hepatic portal vein, in the detached endothelial lining, in walls of the bile ducts and arterial walls with numerous scattered collagen fibers in between the hepatocytes of the irradiated group.

Horn *et al*. (1985) reported that the presence of collagen in the presinusoidal spaces might affect the blood supply to liver cells and would reduce the exchange of metabolites, perhaps causing hepatocellular dysfunction and necrosis. **Enzan *et al*. (1995)** also attributed a similar finding to the activation of myofibroblast- like cells present normally within the hepatic and renal parenchyma.

Hepatic stellate cells play a major role in fibrogenesis by synthesising increased amounts of collagen when activated by profibrogenic factors such as oxidant stress **(Ramadori *et al*., 2008)**.

In the present study, almost normal distribution of collagen fibers was demonstrated in the central and portal areas of liver tissue of OLE & OLE+R groups. These results are in agreement with those obtained by **Abd El-Hady and Al Jalaud (2015**) who observed moderate amount of collagen fibers in irradiated lung tissue after treatment with OLE. Also **Alirezaei *et al*. (2012)** observed somewhat normal appearance of collagen fiber in liver tissue after administration of OLE due to their free radical scavenging activity and inhibition of lipid per oxidation. Also**, Cerig *et al*. (2016)** found that administration of OLE decreased fibrosis of liver tissue similar to the normal in cisplatin treated rats.

In the present study, nearly normal distribution of collagen fibers was demonstrated in the central and portal areas of liver tissue of MSCs+R group. These findings are in agreement with **Abdel Aziz *et al*. (2007)** who reported that MSC have potential therapeutic effect against the fibrotic process, their effect in minimizing collagen deposition in addition to their capacity to differentiate into hepatocytes. MSCs have the ability to enhance fibrous matrix degradation and produce secreted factor that stimulate the regeneration of paranchymal cells (**Parekkadan *et al*., 2007).**

Seo *et al*. (2014) also reported that MSCs reduce the density of collagen fibers in fibrotic liver due to the promotion of MSC migration and incorporation into liver tissue as well as the acceleration of hepatocytes growth by hepatic growth factor expressed by MSCs.

The present investigations are supported by **Kubo *et al*. (2015)** who found that transplantation of hAMSCs significantly reduced the fibrotic area, deposition of typ-1collagen, the number of α-smooth muscle actin-positive hepatic stellate cells and kupffer cells in fibrotic liver through the inhibition of kupffer cells and hepatic stellate cell activation.

The immunmodulatory function of MSCs suppresses inflammation of cirrhotic liver leading to lower fibrolytic effect of CCL_4. MSCs could also take part indirect (paracrine effect on HSCs) and direct (differentiation into hepatocytes) in liver regeneration process (**Baligar *et al*., 2016)**.

The histochemical changes

The present study revealed significant decreased polysaccharides in the hepatocytes of the central and portal areas of the liver tissue of the irradiated group, but they were increased in walls of the hepatic portal veins, bile ducts, arterial walls of the portal areas and in the hemolysed RBCs inside the hepatic portal vein. Decreased glycogen content in the fetal liver tissue post-irradiation was also noted by **Eid and Aldossary (2007)**. They postulated this decrease to vacuolation and degeneration in hepatocytes. In contrast, **Gorczynska and Wegrynowicz (1991)** noticed increased glycogen content in the liver cells post-irradiation. They stated that this increase may be due to increased cortisol which usually leads to an accumulation of glycogen in hepatocytes.

Saeid *et al.* (2010) observed the reduction of PAS +ve materials around the central vein of liver of the white rabbits and concluded that electromagnetic fields (EMFs) can decrease liver glycogen stores.

Similarly**, Abdel Azeem (2011)** showed decreased glycogen content in liver tissue after 6Gy of gamma irradiation. The decrease in glycogen content in liver of irradiated rats induced hyperglycemia in these animals. This may be explained in two ways: it may be due to direct effect of irradiation on liver and stimulation in hepatic rate of glucose production because of the abnormalities in liver function which induced as a result of hepatitis. Secondly, the release of corticosteroid hormone (glucocorticoid) deregulate carbohydrate metabolism and decrease the cellular synthesis of glycogen in the liver tissue.

Reduced glycogen in cells post-irradiation may be due to decreased T3 and T4 hormones of the thyroid glands, which reduce the entrance of glucose to the cells **(Abuo El Naga and Abd Rabou, 2012)**.

In additions, reduction of PAS +ve materials was noticed by **Eid *et al.* (2015)** who observed a significant decrease of PAS +ve materials in the central and portal areas in liver of adult male albino rats exposed to RF-EMF from mobile phone radiation 900 MHz. The present findings are in agreement with **Kandeal (2016)** who reported highly significant decreased polysaccharides in the hepatocytes of the

central and portal areas of the liver tissue after exposed to 4Gy of gamma.

Administration of OLE in the present study showed normal distribution of PAS +ve materials in the liver tissue. Olive leaves extract given to mature male rats in 100-, 250and 500mcg doses increased T3 levels in a dose-dependent manner and significantly reduced circulating thyroid stimulating hormone levels at all doses after 14 days of treatment. This study suggested a possible use of OLE for hypothyroidism **(Al-Qarawi *et al*., 2002).**

Administration of OLE to the irradiated group showed almost normal appearance of PAS +ve materials in the liver tissue. This finding are in agreement with **Mansour (2014)** who reported that oral administration of OLE improved carbohydrate content in liver and kidney tissues in diabetic rats treated with OLE when compared to diabetic group. These effects might be due to the antioxidant nature of this plant. OLE improved liver functions which intermediate the conversion of energy from the food and supply extra-hepatic tissues **(Nico *et al*., 2013)**. In addition, **Geyikoglu *et al*. (2016)** showed that treatment with 50mg/kg OLE for 3days increased glycogen content in renal cells near normal control in rats exposed to cisplatin.

The present findings are in agreement with **Cerig *et al*. (2016)** who indicated that OLE resulted in the overall improvement of glycogensis in liver tissue as evident by increasing glycogen deposits as compared to CIS treated rats.

In the present study hepatocytes of liver tissue treated with 6Gy of γ-rays followed by bone marrow mesenchymal stem cells injection restored their normal content of glycogen, while, congested blood vessels contained deeply stained RBCs and this might be due to the high content of glycogen in those cells.

Moreover, **Shams *et al.* (2014)** reported that transplantation of MSCs restored high glycogen levels in liver tissue of animals treated with CCL_4 to normal value. **Abd El-Hady and Al Jalaud (2015)** suggested that somewhat normal appearance of PAS +ve materials was demonstrated in lung tissues of irradiated rats treated with MSCs. Furthermore, **Baligar *et al.* (2016)** suggested that MSCs secrete transforming growth factor β and insulin-like growth factor-1. Additionally, the transplantation of MSCs led to functional improvement of the liver through repair and regeneration.

The present results revealed reduced total protein in most hepatocytes of the liver tissue of the irradiated group, but they increased in the thickened walls of the blood vessels and bile ducts with mild staining affinity in the hemolysed blood cells.

In this respect, **Kilberg and Nachaus (1978)** found that whole body irradiation of rats led to degeneration of tissue protein. It has been found that ionizing radiation usually inhibits the protein synthesis and the decline recorded could be attributed to degeneration of cellular tissues **(Eid *et al*., 2015)**. This decrease in total protein may be due to highly affected RER, mitochondria and Golgi apparatus with increased lysosomes in fetal hepatocytes exposed maternally to EMF radiation **(Eid and Al-Dossary, 2007)**.

Decreased protein content post-irradiation was realized by **Chen *et al.* (2006)** they reported that hypo-staining affinity may be due to damaged DNA. Gamma rays caused lesions on template DNA strand which result in impaired gene transcription, therefore the synthesis of functional mRNA is impaired and this may alter the pattern of protein synthesis either by stimulation or by inhibition **(Ali *et al*., 2007)**. Furthermore, **Abdel Azeem (2011)** showed that 6Gy of gamma radiation induced a clear inhibition in protein contents in liver due to the possible decrease in DNA biosynthesis as a result of coiling and shortening in the chromosomes. Irradiation of animals at 900-1800 MHz resulted in a marked reduction in the total protein content giving weak to moderate reaction in some areas **(Mohamed, 2014).** The present study is in agreement with **Kandeal (2016)** who revealed reduction in total protein in most hepatocytes of the liver tissue after 4Gy whole body gamma irradiation.

Administration of OLE alone or after irradiation (OLE+R) in the present work showed normal total protein content in the central and portal areas of liver tissue. The antioxidants components of OLE protected biologically important molecules such as DNA, proteins and lipids from oxidative damage and consequently reduced the risk of several chronic diseases **(Janani *et al*., 2010; Myung *et al*., 2013)**.

Mansour (2014) also showed that administration of OLE improved protein content in liver tissue in diabetic rats. This improvement may be due to the antioxidant activity of olive leaves extract. In this respect, **Carluccio *et al*. (2003)** reported that oleuropein stimulated endothelium formation as well as synthesis of mRNA and protein. Meanwhile, It may also cause increase in the amount of ribososmes in the rough endoplasmic reticulum in cells, reflecting their ability to stimulate protein synthesis **(Tuenz *et al*., 2003)**. However, **Abd El-Hady and Al Jalaud (2015)** suggested that improvement of total protein content was noted in irradiated rats following olive leaves extract application and such improvement may be due to the action of olive leaves extract on the lung tissue by enhancing protein synthesis.

In the present study hepatocytes of liver tissue treated with 6Gy of γ-rays followed by bone marrow mesenchymal stem cells injection restored their normal total protein content. Restoration following

gamma radiation exposure could be due to the therapeutic effect of BMSCs. As the stem cells can be transplanted to replace nonfunctional or lost stem cells in tissues to enhance tissue healing and restore their original function **(Burt *et al.*, 2008)**, **Abd El-Hady and Al Jalaud (2015)** also revealed that bone marrow mesenchymal stem cells treatment restored total protein content near normal in the lung tissue in irradiated group treated with MSCs.

Results of the present study showed decreased nuclear DNA content in hepatocytes of liver of the irradiated group, but they were increased in the walls of the hepatic portal veins, bile ducts and arterial walls of the portal area.

These findings are in agreement with **Fouda *et al.* (2009)** who found that lower doses of irradiation did not lead to any obvious injury, but a number of the cells that survived had incorrectly repaired DNA so that they carry mutations while, at high doses, damage of DNA induced cell death. The harmful effects resulting from ionizing radiation are related in most cases to increased production of free radicals that cause damage to the cellular macromolecules, especially DNA. Thus, a comprehensive approach has been made in the scientific community to identify antioxidant agents for their potential protective effect at the cellular level **(Hosseinimehr, 2010)**. Similar to the present study, **Kandeal (2016)** also observed reduction in DNA content in most hepatocytes of the liver tissue after 4Gy whole body gamma irradiation.

Drenching OLE to normal or exposed rats showed almost normal content of DNA materials in the liver tissue.

Efficiency of treatment with the OLE against DNA damage might be explained by the mechanisms reported by **Ghanema and Sadek (2012)** who showed that OLE increased cells antioxidant capacity by stimulating the synthesis of antioxidant enzymes and helped maintain their activity during oxidative stress. Additionally, OLE influenced the structure and stability of the DNA helix **(Mohamdi *et al.*, 2015**)

The present investigations are in agreement with **Abd El-Hady and Al Jalaud (2015)** who found an improvement of DNA content which was noted in lung tissue following olive extract application and such improvement may be due to the action of olive leaves extract on the lung tissue by DNA repairing system. Also oleuropein acts as a free radicals scavenger, since DNA materials are their main target.

Cerig *et al.* (2016) also revealed that the highest concentration of OLE exhibited the highest decrease in the number of cells with DNA damage in the liver tissue. **Geyikoglu *et al.* (2016**) reported that OLE decrease DNA damage by exerting its antioxidant effect which reduced ROS levels, indicating the prevention of oxidative DNA damage.

In the present study, treatment of irradiated group by bone marrow mesenchymal stem cells restored their normal DNA content. MSCs are able to quickly and efficiently recognize radiation-induced DNA damage via different recognition pathways. **(Nicolay *et al.*, 2015**).

To maintain genomic stability and avoid transmission of mutations into progenitors cells, stem cells have DNA damage response signaling, a contrast to somatic cells. Stem cell transplantation may protect against radiation-induced late effects (**Mujoo *et al.*, 2016**).

The current study recorded a significant increase in the amyloid–β protein content in hepatocytes of the central and portal areas of the irradiated animals and in the hemolysed RBCs inside the hepatic portal veins.

Eid *et al.* (2013) recorded slightly increased amyloid β deposits in hepatocytes of the central and portal areas and in the blood cells inside the blood vessels of the liver tissue of the exposed rats to RF-EMF from mobile phone (45min/day) for one month.

In addition, **Kandeal (2016**) indicated that a significant increase in the amyloid–β protein content in hepatocytes of the central and portal areas of the liver tissue after exposed to gamma radiation.

The present findings showed normal appearance of amyloid β-protein in the liver tissue of groups OLE & OLE+R during the experimental periods.

Oleuropein may exert a protective action counteracting the amyloid plaque generation and deposition **(Bazoti *et al.*, 2008; Kostomoiri *et al.*, 2013)**. A number of dietary factors including antioxidants, vitamins and polyphenols have been characterized for their ability to protect cells stressed by several factors including the presence of amyloid deposits as well as to inhibit amyloid self-assembly and cytotoxicity **(Casamenti *et al.*, 2015)**.

Oleuropein aglycone compound has radical-scavenging activity and antioxidative effects and it is considered a promising target to prevent amyloid toxicity as an inhibitor of the oligomer nucleation and growth. Oleuropein aglycone counteracts amyloid aggregation and toxicity affecting different pathways: amyloid precursor protein processing, amyloid-β peptide and tau aggregation, autophagy impairment andneuro inflammation **(Martorell*et al.*, 2016)**. Further, **Geyikoglu *et al.* (2016)** reported that OLE decreased amyloid aggregation in kidney tissue due its antioxidant properties. OLE has an ability to modify the path of amyloid aggregation.

The present findings showed normal appearance of amyloid β-protein in the liver tissue of groups MSCs+R during the experimental periods. In these respect**, Harach *et al.* (2016)** found that human mesenchymal stem cells (hMSC) treatment significantly reduced cerebral Aβ plaques and neuro-

inflammation in Alzheimer mice model, without increasing cerebral amyloid angiopathy or micro hemorrhages, due to anti -inflammatory impact of MSCs.

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