The Egyptian Journal of Hospital Medicine (Apr. 2017) Vol. 67(1), Page 382- 391

Therapeutic Effects of an Ethanolic Olive Leaves Extract or Bone Marrow Mesenchymal Stem Cells against Liver Injury Induced by Gamma Radiation

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ABSTRACT

Background: Ionizing radiation absorption causes immediate biochemical, sub cellular and cellular damage, while its morphological expression and organ dysfunction are often considerably delayed. This study was aimed to investigate the possible therapeutic effects of ethanolic olive leaves extract or bone marrow derived-mesenchymal stem cells (BM-MSCs) transplanted in the liver of rats exposed to gamma radiation. For this purpose, hematological and biochemical parameters were determined

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 exposed to a single dose of gamma-radiation (6 Gy), OLE group: rats treated with olive leaves extract (15 mg /kg body weight / day for 30 days), R+OLE group: animals of this group were irradiated with 6Gy then treated with OLE(15 mg /kg body weight/ day) after 3 hours post irradiation for 30 days. and R+MSCs group: Mesenchymal stem cells-irradiated animals (MSCs +R): animals of this group were irradiated with 6Gy then injected after 6hours post irradiation with (BM-MSCs) 3×10^6 cells/ml suspension through caudal vein . All these groups were subjected to hematological and biochemical investigations.

Results: Hematological and liver function changes were shown in gamma irradiated rats, these changes included a significant depression in hematological parameters of blood such as (RBCs, Hb, Hct and WBCs)and a significant increase in liver parameters (ALT,AST and ALP) at different intervals of the experiment in comparison with the control group. These changes manifested good amelioration in the exposed groups by using either olive leaves extract (OLE) or bone marrow mesenchymal stem cells (BM-MSCs). **Conclusion:** Ethanolic olive leaves extracts and mesenchymal stem cells have ameliorated hematological and biochemical parameters changes in liver of the irradiated group. their actions may be due to their anti-inflammatory and antioxidant properties.

Keywords: gamma radiation - liver- Ethanolic olive leaves extract (OLE) - Bone marrow mesenchymal stem cells (BM-MSCs).

INTRODUCTION

Ionizing radiation is high-energy radiation that has the ability to break chemical bonds, cause ionization and produce free radicals that can result in biological damage[1]. Low dose of gamma rays could have dangerous on the some hematological parameters (RBCs &WBCs) for blood of female rats exposed to ionizing radiation .It is necessary to review the dose limits recommended by the ICRP-60for radiation workers [2] .Exposure to whole-body gamma radiation causing physiological and histological changes in liver this can progress to fibrosis, cirrhosis and liver failure [3].

Medicinal plants play a key role in the human health care. About 80% of the world population relies on the use of traditional medicine which is predominantly based on plant material[4].Many antioxidant compounds, naturally occurring from plant sources, have been identified as free radical or reactive oxygen scavengers [5]. It has increased considerably in finding naturally occurring antioxidant for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their side effects such as carcinogenicity[6]. Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as retard lipid oxidative rancidity in foods [7] .Olive leaves extract (OLE) or its constituents, especially oleuropein are tissue protective as an antioxidant when administered therapeutically. Although individual phenolic compounds in OLE have strong antioxidant activities, the antioxidant activities of the combination of phenolics are better than the individual phenolics [8] .

Most of the cells in the human body are differentiated and possess a particular function. Stem cells (SCs) are unique cells with the exceptional ability to renew them indefinitely

Received: 24 / 01 /2017 DOI : 10.12816/0036652
Accepted: 31/ 01 /2017

by remaining in an undifferentiated state until receiving signals that lead to a differentiated cell type in maintaining tissue homeostasis. These two properties have to be well regulated and are critical in the ontogeny and the proper maintenance of tissues and organs. SCs are fundamental players in cell biology by allowing tissues to be replenished from freshly created cells throughout their lifetime[9]. 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[10] . This study was aimed to investigate the possible therapeutic effects of ethanolic olive leaves extract or bone marrow derived-mesenchymal stem cells (BM-MSCs) transplanted in the liver of rats exposed to gamma radiation.

MATERIALS AND METHODS

Experimental animals, feeding and maintenance

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 start 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 in70% ethanol by soxhlet apparatus for 10 hours continuously [11]. 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.*** [12].

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 **Abdel-Aziz *et al.*** [13]. A total of ten animals received the 1ml cell suspension.

Experimental design

The experimental animals were divided into 5 groups(each 10 rats): .

Group 1: untreated control rats (C)

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

Group 3: olive leaves extract: animals were treated with dose 15 mg /kg body weight/day for 30 days (OLE) .

Group 4: olive leaves extract irradiated rats(R+OLE): animals of this group were irradiated with 6Gy then treated with OLE(15 mg /kg body weight/ day) after 3 hours post irradiation for 30 days.

Group 5:Mesenchymal stem cells-irradiated animals (R+MSCs): animals of this group were irradiated with 6Gy then treated after 6hours with transplanted (MSCs) 3×10^6 cells/ml suspension through caudal vein .

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

Samples collection

1-Directly, after the animal was anesthetized by ether, blood was collected from the heart by heparinized syringes, and transferred to EDTA vials.

2-Blood sample was used for the determination of CBC.

3-Blood was centrifuged at 3000r.p.m for 15 mints; serum was separated and stored at -20ºC until used for biochemical analysis.

Hematological analyses

The complete blood count was determined according to **Dacie and Lewis [14].**The RBCs, HGB, Hct and WBCs were analyzed using the CELL-DYN 1700 (Abbott Diagnostics, Abbott Park, IL, USA)

Biochemical analyses

The activities of serum alanine aminotransferase (ALAT) and aspartat aminotransferase(ASAT)were assayed by the kinetic method using available commercial kits (Spinreact, Spain) according to the method described by **Young and Friedman [15]** while, the activities of alkaline phosphatase (ALP) in serum were assayed by the kinetic method using available commercial kit (Spinreact, Spain) according to **Schumann *et al* [16].** The study was approved by the Ethics Board of Al-Azhar University.

Statistical analysis

The results were expressed as mean ± standard error (SE). The significance of differences between means was measured by student's t-test [(17)]. The P values below 0.05 were considered significant while those above 0.05 were considered insignificant. Degree of freedom = (n1 + n2)-2.

RESULTS

Hematological parameters

Tables(1.2.3&4) showed that exposure of rats to 6 Gy of γ-radiation induced a significant decrease in the mean values of red blood cells (RBCs), hemoglobin (Hb), hematocrit (Hct) and white blood cells (WBCs) as compared to the control group. The ameliorative effect in the group of rats treated with OLE recorded non-significant change in the mean value of RBCs, Hb, Hct and WBCs. Giving ethanolic extracts of olive leaves to irradiated rats showed a significant decrease in the mean values of the previous hematological parameters of blood after 7 days of irradiation. Rats which treated with OLE or mesenchymal stem cells (MSCs) transplantation restored the values of the previous parameters to the normal value after 30 days of irradiation.

Table (1): The mean ±S.E of RBCs count in irradiated rats after treatment with olive leave extract or MSCs

Parameter	Red Blood Cells ($RBC_S x10^6/mm^3$)					
Time / Groups	7 day			30 day		
	Mean± S.E	% change Vs. control	% change Vs Radiation	Mean± S.E	% change	% change vs Radiation
Control	5.84±0.26	**0.0 %**		5.84±0.26	**0.0 %**	
Radiation (R)	3.40±0.07[a]	-41.78%		4.00±0.07[a]	-31.51	
Olive leave extract (OLE)	5.90±0. 15	1.03%		5.74±0.12	-1.71	
Olive leave extract + Radiation(OLE+R)	4.96±0.07[a]	-15.07%	45.88%	5.58±0.07 ns	- 4.45%	**39.05%**
Mesenchymal stem cells + Radiation(R+MSCs)	–	–		5.61±0.12 ns	-3.94%	**40.25%**

Table (2): the mean ±S.E of Hb (g/dl) level in irradiated rat liver after treatment with olive leaves extract or MSCs.

Parameter	Haemoglobin (Hb g/dl)					
Time / Groups	7 day			30 day		
	Mean± S.E	% change vs control	% change vs Radiation	Mean± S.E	% change vs control	% change vs radiation
Control	13.78±0.09	**0.0 %**		13.78±0.09	**0.0 %**	
Radiation (R)	9.00±0.07[a]	-34.69%		10.32±0.16[a]	-25.11%	
Olive leave extract(OLE)	13.84±0. 05	0.44%		13.48±0.05	-2.18%	
Olive leave extract + Radiation(OLE+R)	12.03±0.09[a]	-12.70%	**33.66%**	13.12±0.12ns	- 4.79%	**27.13%**
Mesenchymal stem cells + Radiation(MSCs+R)	–	–		13.34±0.14 **ns**	-3.19%	**29.26%**

Each value is the mean of 10 animal's ± S.E. , Symbol(s) a:The values are considered significant compared to the control group &ns :non significant from control . Percentage of change is in comparison with the control and irradiated group.

Table (3): The mean ±S.E of Hct% in irradiated rats after treatment with olive leave extract or MSCs.

Parameter	Hematocrit (Hct%)					
Time / Groups	7 day			30 day		
	Mean± S.E	% change vs control	% change vs Radiation	Mean± S.E	% change vs control	% change vs radiation
Control	38.38±0.12	**0.0 %**		38.38±0.12	**0.0 %**	
Radiation (R)	28.40±0.07[a]	-26.00%		30.96±0.16	-19.33%	
Olive leave extract(OLE)	38.40±0.09	0.52%		38.22±0. 09	-0.42%	
Olive leave extract + Radiation(R+OLE)	31.35±0.12[a]	-18.32%	**10.38%**	37.24±0.11**n**	- 2.97 %	**20.28%**
Mesenchymal stem cells + Radiation(R+MSCs)	–	–		36.52±0.11**ns**	-4.85%	**17.96%**

Table (4): The mean ±S.E of WBCs (10^3/mm^3) count in irradiated rats after treatment with olive leave extract or MSCs.

Parameter	White Blood Cells (WBCs x 10^3 cell/mm^3)					
Time / Groups	7 day			30 day		
	Mean± S.E	% change vs control	% change vs Radiation	Mean± S.E	% change vs control	% change vs radiation
Control	10.58±0.12	**0.0 %**		10.58±0.12	**0.0 %**	
Radiation (R)	7.92±0.42[a]	-25.14%		8.22±0.16[a]	-22.31%	
Olive leave extract (OLE)	10.49±0. 15	-0.85%		11.02±0.06	4.15%	
Olive leave extract + Radiation(R+OLE)	9.04±0.07[a]	-14.56%	**14.14**	0.12±0.14 ns	- 4.35%	**23.11**
Mesenchymal stem cells + Radiation(R+MSCs)	–	–	-	10.14±0.10 **ns**	-4.16%	**23.36**

Each value is the mean of 10 animal's ± S.E. , Symbol(s) a:The values are considered significant compared to the control group &ns :non-significant from control .Percentage of change is in comparison with the control and irradiated group.

Biochemical parameters

Serum ALAT ,ASAT and ALP levels were measured in all groups. In irradiated group these parameters showed a significant increase in mean value during the experimental periods when compared with the control group. Meanwhile, treatment with OLE alone induced ameliorative effect manifested by non-significant change in the mean values of ALAT ,ASAT and ALP throughout the experimental periods. While, after OLE administration to irradiated animals the level of ALAT,ASAT and ALP showed a significant increase in mean value after 7 days post irradiation and non-significant change after 30 days post irradiation in R+OLE and R+MSCs (tables,5.6&7).

Table (5) The mean ± S.E of ALAT **(U/L)** in irradiated rats after treatment with olive leaves extract or MSCs .

Parameter	Alanine Aminotransferase (ALAT, U/L)					
Time / Groups	7 day			30 day		
	Mean± S.E	% change vs control	% change vs Radiation	Mean± S.E	% change vs control	% change vs Radiation
Control	50.0±0.71	**0.0 %**		50.0±0.71	**0.0 %**	
Radiation (R)	79.00±1.07[a]	**58.0**%		68.80±1.07[a]	37.60%	
Olive leave extract (OLE)	49.52±1. 14	-0.96%		49.21±0.86	-1.58%	
Olive leave extract + Radiation (R+OLE)	58.21±0.78[a]	16.00%	**-26.32**	53.00±1.00ns	6.00%	**-22.97**
Mesenchymal stem cells + Radiation(R+MSCs)	–	–	-	50.20±0.86ns	0.4%	**-27.03**

Each value is the mean of 10 animal's ± S.E. , Symbol(s) a:The values are considered significant compared to the control group &ns :non significant from control.Percentage of change is in comparison with the control and irradiated group.

Table (6) The mean ± S.E of ASAT **(U/L)** in irradiated adult male rats after treatment with olive leave extract or MSCs.

Parameter	(ASAT, U/L)					
Time / Groups	7 day			30 day		
	Mean± S.E	% change vs control	% change vs Radiation	Mean± S.E	% change vs control	% change vs radiation
Control	27.40±0.24	0.0 %		27.40±0.24	0.0 %	
Radiation (R)	44.8±0.66[a]	63.50%		36.8±1.39[a]	34.31%	
Olive leave extract(OLE)	26.52±0. 71	-3.21%		27.00±0.86	-1.46%	
Olive leave extract + Radiation(R+OLE)	34.56±0.24[a]	26.13%	**-22.86**	29.20±0.24 ns	6.57%	**-20.65**
Mesenchymal Stem cells + Radiation(R+MSCs)	–	–		27.9±0.86 ns	1.83%	**-24.18**

Each value is the mean of 10 animal's ± S.E., Symbol(s) a: The values are considered significant compared to the control group &ns :non significant from control.Percentage of change is in comparison with the control and irradiated group.

Table (7) The mean ± S.E of ALP **(U/L)** in irradiated liver of adult male rats after treatment with olive leave extract or MSCs .

Parameter	(ALP, U/L)					
Time / Groups	7 day			30 day		
	Mean± S.E	% change vs contro	% change vs Radiation	Mean± S.E	% change vs contro	% change vs radiation
Control	161.20±1.16	**0.0 %**		161.20±1.16	**0.0 %**	
Radiation (R)	221.40±1.03[a]	37.34%		188.20±0.86[a]	16.75%	
Olive leave extract(OLE)	162.00±0.86	0.50%		161.80±0.85	0.37%	
Olive leave extract + Radiation(R+OLE)	178.80±0.79[a]	10.91%	**-19.25**	165.20±1.11	2.48%	**-12.22**
Mesenchymal stem cells + Radiation(R+MSCs)	–	–		161.00±0.71	-0.12%	**-14.45**

Each value is the mean of 10 animal's ± S.E.
Symbol(s) a:The values are considered significant compared to the control group &ns :non significant from control.
Percentage of change is in comparison with the control and irradiated group.

DISCUSSION

Ionizing radiation instantaneously causes formation of water radiolysis products that contain some reactive oxygen species (ROS), ROS are also suggested to be released from the biological sources in their radiated cells. Ionizing radiation increases the mitochondrial reactive oxygen species level [18].Exposure to ionizing radiation induces dose dependent declines in circulating hematopoietic cells not only through reduced bone marrow production, but also by the apoptosis of mature formed elements of the blood [19] .Oxidative damage leads to alteration in both lipid bilayer fluidity and permeability properties [20].

Liver is a very important organ for the health and life of mammals. Electromagnetic field (EMF) induces liberation of free radicals and oxygen species which cause liver disease since it is the main organ of detoxification. Another reason for selection of liver is its sensitivity to waste products [21].Olive leaves extract (OLE) or its constituents, especially oleuropein are tissue protective as an antioxidant when administered therapeutically. Although individual phenolic compounds in OLE have strong antioxidant activities, the antioxidant activities of the combination of phenolics are better than the individual phenolics [8].

Bone marrow transplantation to irradiated rats showed asignificant recovery in blood contents. This may be due to the role played by bone marrow transplantation for replacement or restoration of injured haematopoitic tissue components[22].

Hematological studies

Complete blood count:

The blood is a pathophysiological reflector of the whole body. Therefore, blood parameters are important in diagnosing the structural and functional status of organisms exposed to toxicants [23]. Exposure to ionizing radiation is known to have lethal effects on blood cells [24].

Results of the present study showed a significant decrease in the mean values of RBCs count, Hb, Hct and WBCs upon exposure of rats to single dose of γ-radiation (6 Gy).

The present findings are supported by research work of **Eshak and Osman** [25] who realized a significant decreases in RBCs, Hb, Hct% and WBCs in groups exposed to 4 and 6 Gy gamma-radiations when compared to the control. The depletion in the values of hematological parameters following radiation exposure may be attributed to direct damage caused by radiation and due to overproduction of ROS by gamma radiation interaction. Also, **Samarth *et al*.** [26] stated that these variations may be due to direct destruction of mature circulating cells, loss production of cells and also loss of cells by hemorrhage or leakage through capillary walls. Reduction in erythrocyte count value was explained by **Sleim *et al*.** [27] who proved that gamma irradiation of red blood cells induced alterations at three different functional units of the membrane: lipid bilayer, protein components and cytoskeleton at the membrane surface. In addition, radiation induced shortening in the fatty acid chains by lipid peroxidation. The present results agreed with **El-Desouky *et al*.** [28] who reported decreased white blood cells (WBCs), red blood cells (RBCs), platelets count and hemoglobin concentrations in blood of albino rats exposed to gamma radiation (4&6 Gy) for 2 weeks. Moreover, **it was noticed** a decrease in the hematological parameters of blood of female rats exposed to 0.055, 0.11 and 0.165 Gy gamma rays[2].

The current study reported that treatment of normal rats with olive leaves extract (OLE) showed in significant change in mean value of RBCs, Hb, Hct and WBCs after 7 and 30 days post treatment.While,treating irradiated rats with OLE showed significantly decrease of RBCs, Hb, Hct and WBCs after 7 days and non-significantly decrease after 30 days as compared to the control group these results could attributed to the protective effect of phenolic antioxidant present in OLE.This antioxidant may be able to reduce leukocyte lipoxygenase enzymes and the damaging consequences of their ability to release ROS whilst leaving unimpaired the generation of prostaglandins ,which promote microvascular blood flow and act as immune-modulators [29].Also ,these findings are in agreement with the research work of **Geyikoglu *et al.[*** [30]who reported that treatment with OLE showed non significantly increase in hematological parameter of normal rats, similar results obtained by **Zari and AL-Attar** [31] they revealed that there is insignificant changes in hematological parameter in normal rat treated with olive leaves extract compared to control.

agreement with **Ashour** [32] who noticed that OLE increased RBCs count ,Hb,Hct and WBCs to normal value after exposed to gamma radiation 4 and 6Gy, this investigated the radio protective role of orally administrated ethanolic olive leave extract after one and two weeks of gamma radiation at dose 4 and 6 Gy. According to **Geyikoglu *et al*.**[30] the efficacy of supplementation of OLE on the hematological parameter against cisplatin induced hematological damages by increase the hematological parameter to near normal value. OLE is an antioxidant and strong free radical scavenger[33].

The present results illustrated that RBCs, Hb , Hct and WBCs were in significantly decrease in irradiated rats treated with MSCs 3×10^6 cells/ml suspension for 30 days as compared to the control group .

It was also noticed that bone marrow transplantation to irradiated rats at a dose level of 6Gy showed a significant recovery in red blood cells towards their normal levels. she added that this may be due to replacement or restoration of injured hematopoietic tissue components**.** [34]

These finding are in agreement with research work of **El-Ganzuri *et al.*** [22] who reported that BM transplantation after 3 hours to whole body gamma-radiation restored the value of the hematocrit, partially ameliorated the other blood component

(WBCs, RBCs, HGB, HCT, PLT) and demonstrated a significant preservation of the bone marrow components and scanty adipose cells replacement. Irradiation of animals at 5 Gy induced a significant depression in RBCs, WBCs and lymphocytes. Bone marrow transplantation (BMT) and/or white germ oil (WGO) together with irradiation showed a significant elevation in RBCs, WBCs and lymphocytes as compared to irradiated group [35]

Biochemical studies

The results obtained in the present study, revealed that exposure of rats to gamma radiation (6 Gy) increased ALAT, ASAT and ALP activities significantly in serum when compared to the control group. The rise in the serum transaminases activities and alkaline phosphatase in irradiated animals may be due to the drastic physiological effect caused by irradiation, either directly by interaction of cellular membranes with gamma rays or through the action of free radicals produced by radiation. Liver damage may increase the cell membrane permeability accompanied with rise in the transaminases activities. Accordingly, the observed increase in serum transaminase activities and ALP is expected as a consequence for the increase in activities of the liver enzymes [36]. **Ramadan *et al.*** [37] [38] explained that changes in the enzymatic activities after irradiation may be due either to the release of enzymes from radiosensitive tissues or to changes in its synthesis and may be related to the extensive breakdown of liver parenchyma and renal tubules. Results of the present study are agreement with those described previously by **Ramadan *et al.*** [39] who stated that whole body gamma-irradiation (3 or 6 Gy) induced hepatotoxicity 1, 3 and 7 days. A gradual increase in serum ALAT, ASAT as well as GGT activities were observed. Moreover, the damage of cellular membranes of hepatocytes, which in turn leads to an increase in the permeability of cell membranes and facilitates the passage of cytoplasmic enzymes outside the cells lead to the increase in the hepatic enzyme activities in liver and blood serum. Ionizing radiations induced significant elevations in the physiological and metabolic processes, as well as, disorders in blood biochemical parameters [40] and caused chain reaction of oxidation .[41] Moreover, it was found that exposure of rats to a single dose of 6 Gy gamma-radiation induced a hepatic damage and a significant elevation in activities of ALAT and ASAT [42].

The present findings are supported by the work done **by Eshak and Osman** [25] who realized a significant increase in serum ALAT, ASAT and ALP activities in groups exposed to 4 and 6 Gy gamma radiations when compared to the control.

In this respect, **El-Desouky *et al*.** [28] noticed increases in ALAT, ASAT and ALP in serum of albino rats exposed to gamma radiation (4& 6 Gy).This was in parallel with hepatocyte degeneration and lymphocytic infiltration and necrosis of the liver.

Kandeal[3] reported that a significant increase in serum ALAT, ASAT and ALP activities in groups exposed to 4 Gy of gamma radiations when compared to the control.

In the present study non significant changes in the activities of ALAT, ASAT and ALP were recorded in olive leaves extract group while , the irradiated groups supplemented with olive leaves extract showed a significant increase after 7 days and in significant change after 30 days as compared to control group. this indicate that supplementation of olive leaves extract manifested good ameliorative effects in liver enzymes activities. These results study go in parallelism with previous studies that revealed a potential hepatoprotective effect of oleuropein in rats with induced hepatitis [12].

Administration of olive leaves extract reduced the elevation of level plasma AST ,ALT and liver glycogen caused by carbendazim due to preventing the decline of antioxidant defense system and direct free radical scavenging activity [31]. Olive leaves extract improved biochemical alterations induced by liver tocixity due to the main constituent of olive leaves extract (oleuropein) which is thought to be responsible for pharmacological effect [43] .Oleuropein has high antioxidant activity *in vitro* ,comparable to a hydrosoluble analog of tocopherol [44]

In addition, olive leave extract exhibited a hepatoprotective effect against thioacetamide which caused liver cirrhosis

[45]. It also decreased cardiac and hepatic injury together with the amelioration in metabolic and oxidative stress parameters in rat model of diet-induced obesity and diabetes. [46]

Administration of OLE 200 mg/kg body weight, significantly ($p<0.05$) lowered the elevation of serum enzymes induced by cisplatin in relation to the control group. [47]

In the present study transplantation of bone marrow derived MSCs reduced the elevation of liver enzymes ALT,AST and ALP caused by exposure to 6Gy gamma radiation . In these respect, **Matsuda-Hashii *et al*.** [48] suggested that MSC may protect against CCL_4-induced injury by altering the microenvironment of liver at sites of engraftment.MSC could secrete many cytokines and growth factors such as hepatic growth factor. This shows antiapptotic activity in hepatocytes and plays an essential part in the regeneration of liver [49].

This result is in agreement with those of **Abdel Aziz *et al*.** [13] who demonstrated the effective role of bone marrow-derived mesenchymal stem cells (MSC) in improving liver fibrosis induced by CCl_4 where MSC transplantation reduced ALT,AST and ALP to normal value through up regulation of cytokines such as stem cell factor-1,hepatocytes growth factor and matrix metalloproteinase. Moreover, MSCs protect against injury by altering the oxidative microenvironment of liver.NrF2 is a transcription factor that positively regulates the basal and inducible level of cyto-protictive genes.NrF2 activation is protective against oxidative stress and induce SOD production which decreased ROS in liver [50].Further studies by **Francois *et al*.** [51] showed that irradiation induced a significant increases in plasma indices of liver (ALAT, ASAT and ALP activities),an increase of the apoptosis process in liver vascular system and increase oxidative stress. Also , transplantation of MSCs lowered the elevation of serum liver enzymes induced by CCL_4 in relation to the control group. [52] [53]

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