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Therapeutic Role of Folic Acid Loaded Magnetic Nanoparticles Against Gamma-Irradiation Hazards in Rats

E.I. Sobeh [1], H.M. Saleh[1], W.F. Hassanin[1] S.A. Hussein [2] and H.A. Abdel Maksoud [2*]

[1]*Department of Biological Applications, Nuclear Research Center, Atomic Energy Authority, Egypt*

[2]*Department of Biochemistry and clinical Biochemistry, Faculty of Veterinary Medicine, Benha University*

ABSTRACT

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Gamma radiation produces numerous biological perturbations in cells by direct ionization of DNA and cellular targets and by indirect effect through damage by free radical production. The present study has been carried out to investigate the possible therapeutic effect of folic acid loaded magnetic nanoparticles through injection after whole body gamma irradiation. Results showed that whole body gamma irradiation of rats at 6Gy (single dose) induced a significant increase in DNA damage ($P < 0.05$) that was indicated by an increase in tail length, tail DNA% and tail moment as compared to control group. Significant increase in the hepatic cholesterol and triglycerides levels was observed after three weeks post-irradiation. Administration of folic acid loaded magnetic iron oxide (Fe3O4) after irradiation induced significant improvement of the DNA damage and hepatic cholesterol and triglyceride level. These results indicate the possible role of folic acid loaded MNPs as a therapeutic agent against the genetic damage caused by ionizing radiation

Keywords: *Folic acid loaded magnetic nanoparticles/Cholesterol/ Triglyceride/ DNA damage/ Comet* ***assay***

Introduction

Ionizing radiation is known to produce oxidative stress through the generation of reactive oxidative species (ROS) leading to a variety of DNA lesions which consequently leads to lethal effects, Mutagenesis, genomic instability or carcinogenesis [1].This damage can be quarantined or may be cured using different techniques. Efforts has been made during recent years to identify phytochemical or other naturally occurring compounds which can reduce the harmful effect of radiation during accidental exposure or prevent normal tissue injury during radiotherapy [2]. Because of their very small size, Nanoparticles (NPs) can be more chemically reactive, and likely have much greater access into cells, tissues, and organs than larger particles. The important technological advantages of nanoparticles used as drug carriers are the high stability, the high carrier capacity, the feasibility of incorporation of both hydrophilic and hydrophobic substances, and the feasibility of variable routes of administration. Because of their distinct properties compared to the bulk form of the same material, NPs are thus subject to many studies on developments in the fields of biosensors, biomedicine, and bio nanotechnology [3]. Iron oxide nanoparticles (NPs) have been the most extensively investigated, due to their excellent biocompatibility and ease of synthesis for multifunctional biomedical applications such as cellular targeting and drug delivery, tissue repair, magnetic resonance imaging (MRI) and magnetofection[4]. Folic acid (FA) is an essential B vitamin, obtained through dietary sources, such as broccoli, cabbage, fruit and nuts. Folic acid is playing an essential role in nucleic

Corresponding author: walaahassanin@gmail.com
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acid synthesis, methionine regeneration, and in various one-carbon unit reactions required for normal metabolism and regulation [5]. Some reports suggest that folate may act as a radical scavenger; thereby contributing to protection against ?-ray induced chromosomal damage [6,7]. It has been reported that the radical scavenging of folate is equivalent to that of vitamin C.Endoh et al.[7] suggested that the folate could prevent radiation-induced DNA damage by the modification of DNA synthesis and /or repair and as a radical scavenger that traps oxygen radicals produced by radiation. Comet assay (also called, single cell gel electrophoresis, SCGE) is a method used to detect any prospective damage for DNA after various treatments. It detects DNA strand breaks and alkali labile sites by measuring the migration of DNA from immobilized nuclear DNA [8]. Comet assay requires small number of cells to carry out (<10,000) and can be performed on virtually any eukaryotic cell type[8].

The aim of the present study is to investigate the effect of folic acid loaded magnetic NP against DNA damage, triglyceride and cholesterol induced by ionizing radiation.

2. MATERIALS AND METHODS

2.1 Chemicals

Iron (II) chloride tetrahydrate ($FeCl_2$-$4H_2O$), iron (III) chloride hexahydrate ($FeCl_3$-$6H_2O$), polyethylene Glycol 6000, Folic acid, Dimethyl-sulfoxide, anddicyclohexylcarbodiimide were purchased from Sigma-Aldrich (USA). Ammonium hydroxide solution (NH_4OH) was obtained from Merck (Germany).

2.1.1 MNP Preparation

Iron oxide (Fe_3O_4) nanoparticles were synthesized via a co precipitation process of Fe(III) and Fe(II) salts at 2:1 ratio with ammonium hydroxide reported previously [9].

2.1.2 Modification of MNPs with folic acid

Carboxyl moiety on Folic acid was activated with dicyclohexyl carbodiimide (DCC) according to [10] prior to conjugation to the surface of MNPs. Folic acid and DCC with 1:1 ratio were added in dimethyl-sulfoxide (DMSO) and stirred for 2h. PEG-MNPs sample was added and continuously stirred for 2h. The composites were Then washeddeionized H_2O.

2.2 Irradiation

Whole body irradiation was performed by Gamma-cell 40 (with cesium- 137 source) belonging to theNational Center for Radiation Research andTechnology (NCRRT),Egyptain Atomic Energy Authority. Animals were irradiated with a sublethal dose (6 Gy) as a single dose, by dose rate of 0.957 Rad/sto enforce radiation damage within short time.

2.3 Experimenal design

Adult male albino rats of pure strain ranging from 130-150g body weight were obtained from the animal house of the Animal Nutration Research unit, National Center for Radiation Research and Technology (NCRRT), Egyptain Atomic Energy Authority. Animals were housed in especially designed cages (5 rats/ cage). All rats were kept under good condations, allowed free access to tap water and pellet diet. The animals were divided into six groups(10 rats each):

1- Control group (cont):non-irradiated normal control rats and administered with distilled water.

2- MNPs group:consists of non-irradiated rats that are treated with magnetic iron oxide Fe_3O_4 nanoparticles. This group was used to study the effect of magnetic iron oxide nanoparticles alone on normal (non-irradiated) rats. Each rat received magnetic iron oxide nanoparticles (1.5mg/kg)disolvedin distilled water according its weight for three weeks.

3- Folic acid–MNPs group: consists of non-irradiated rats that are treated with Folic acid loaded magnetic iron oxide Fe_3O_4 nanoparticles. This group was used to study the effect of Folic acid loaded magnetic iron oxide nanoparticles alone on normal (non-irradiated) rats. Each rat received an aqueous solution dose of 1.5mg/kg Folic acid loaded magnetic iron oxide nanoparticles its weight for three weeks.

4- Irradiated group: (Gamma irradiated) Rats (whole body) were exposed to a single dose of 6 Gy gamma irradiation. This group was used to study the effect of gamma irradiation alone on normal rats.

5- Irradiated MNPs group: Rats were first exposed to whole body gamma radiation as a single dose 6Gy and then injected intraperitoneally with iron oxide Fe_3O_4 (1.5mg/kg) twice a week for three successive weeks.
6- Irradiated folic acid- MNPs group: Rats were first exposed to whole body gamma radiation as a single dose 6Gy and then injected intraperitoneally with Folic acid loaded iron oxide Fe_3O_4 (1.5mg/kg) twice a week for three successive weeks.

2.4 Characterizations

The characterization of synthesized nanoparticles was performed using the transmission electron microscopy (TEM),(EFI Netherland, Model Tecani G20), supertwin, double tilt, and Gun type LaB6. The applied voltage was 200 KV andmagnification reached 10000X., and Fourier transform- infrared spectroscopy (FT-IR) using JASCOSPECTROMETERfrom 450cm^{-1} to4000 cm^{-1}, the scanning speed was 0.2 cm^{-1}/s.

2.4.1 Biological sampling

Blood samples were taken from the retro-orbital venous plexus under light ether aneathesia. Each blood sample was collected into tubes without anticoagulant for separation of serum for determination of serum cholestrol and triglyceride.At the end of the experiment,animals in all groups were sacrificed by decapitation and the liver was excised, then perfused with cold saline to exclude the blood cells and then blotted on filter paper, and stored at -20°C for subsequent DNA damage by comet assay.

2.4.2 Determination of serum cholesterol and triglyceride

Cholesterol and triglycerides were determined enzymatically using commercial Kits from Bio Diagnostic, Giza, Egypt. Serum triglycerides and cholesterol were determined enzymatically according to the technique adopted by Fossati and Prencipe[11].

2.4.3 Comet assay

Comet assay was carried out according to the technique used by singh et al.[8]. each slide was examined at 400× magnification using an epifluorescence microscope (Zeiss) connected through a camera to an image analysis system (Comet Assay II; Perceptive Instruments Ltd, UK).A total of 100 comets were randomly captured from each slide, computerized acquired images were analysed, computed integrated intensity profiles for each cell and comet cell components were estimated and finally the range of derived parameters were evaluated according to the following formula:

Tail moment (arbitrary unit) = Length of DNA migration (um) × percentage (%) of migrated DNA.

2.5 Statistical analysis

All results were expressed as the mean ± SD. Statistical analysis was performed using statistical package for the social science for windows (SPSS, version 11.0, Chicago, IL, USA). The data were analysed by one-way analysis of variance (ANOVA).

3. RESULTS

The micro-structural features of the MNPs-Folic acid, studied using TEM, showed formation of spherical MNP with size distribution showing two humps at 7.4 nm and 10.4 nm Fig. (1c). The distribution is due to clustering of the nanoparticle during the syntheses process. The TEM images of the MNP-FA showed well defined size distribution around 8.04 nm Fig. (1d). This difference is due to the separation of the attached particles by the layers of FA which enable observation of the separate particles as presented in Fig. (1b)Shapes shape and morphology of MNPs-Folic acid are seen regular and spherical. For accurate comparison between the FTIR spectra, the absorption need to be corrected against the amount of material used in the FTIR measurements. The transmittance of FTIR results is converted to absorption coefficient in the unit of g-1 was obtained from the relation

$$\alpha = \frac{1}{m} \ln \frac{100}{T},$$

Where m is the absolute mass of the material in the KBr pellet and T is the infrared transmittance (%). The comparison is shown quantitatively in Fig.(2b).The spectrum of folic acid (FA) showed a prominent peak (C=O stretching vibrations) at 1694 cm^{-1}, which is attributed to carboxylic group of folic acid. While this peak is robust in the pure

folic acid spectrum, the reduced signal in FA MNP is due to the fractional degree of substitution with folate. The peak for carbonyl stretching of amides of folic acid that appear at 1605 cm^{-1} (related to the bending mode of NH–vibration of amide) is enhanced due to the introduction of additional amide and thereby confirm that MNP is conjugated with folic acid. This is confirmed also from the increase of N–H stretch intensities between 3400-3250 cm^{-1}. We noted that N–H stretch absorptions interfere with the broad band centred at 3400cm^{-1} which is related to the hydroxyl (OH) stretching and with the aromatic C–H stretch from 3100-3000 cm^{-1}. The peak at 1505 cm^{-1}, is attributed to C-C vibration in aromatic ring. The bands at 1489cm^{-1} is attributed to characteristic absorption band of the NH_2–C N– moiety on pterin (PT) ring of FA[12]. This band (at 1489cm^{-1}) totally disappeared on conjunction with MNP which suggests complete conjunction through amines moiety. The aromatic ring stretching vibration appeared at 1413 cm^{-1}. More important, a new mid-strong absorption band appears at 2362cm^{-1}, and it is ascribed to N+–H stretching vibration band of C-N^{+}H– on PT ring. The appearance of the new band demonstrates that the N atom on PT ring is protonated when MNP coexists with folic acid. The observed band at587 cm^{-1} 1and 1085 cm^{-1}correspond to the Fe-O bond vibration of iron oxide nanoparticles [13].

Table (1)and Figure (3) show serum cholesterol concentration in the controls, and animals after MNPs- FA supplementation. Serum cholesterol levels in irradiated animals were increased significantly in the first week, the second week, and the third week when compared with control animals. After the administration of MNPs-FA to irradiated, the enhanced levels of cholesterol were increased in comparison with the irradiated rats and recovered nearly to normal values.

Table (2)and Figure (4)show serum triglyceride levels in controls irradiated animals after MNPs-FA supplementation. TG levels were highly increase significantly in irradiated animals when compared with control animals. Administration of MNPs-folic acid to irradiated animals reduced TG level which is comparable to the control values. However, there were significantly change to best observed after MNPs-FA supplementation of controls compared to irradiated group.

Comet assay was performed to assess DNA damage in the liver of radiation-induced hepatic damage in rats after treatment by magnetic nanoparticles, or MNPs-Folic acidas compared to control. No significant difference in DNA damage (tail length) was observed between normal control and control treated MNPs and Folic acid-MNPs groups. The damaging effect of IR acute dose was significant ($P < 0.05$) in the comet profile of irradiation group, as shown in Table (3) and Figures (5,6).This increased DNA damage was reduced after administration of MNPs, Folic loaded MNPs, in R+MNPs and R+Folic acid MNPs groups for 3 weeks

a **b**

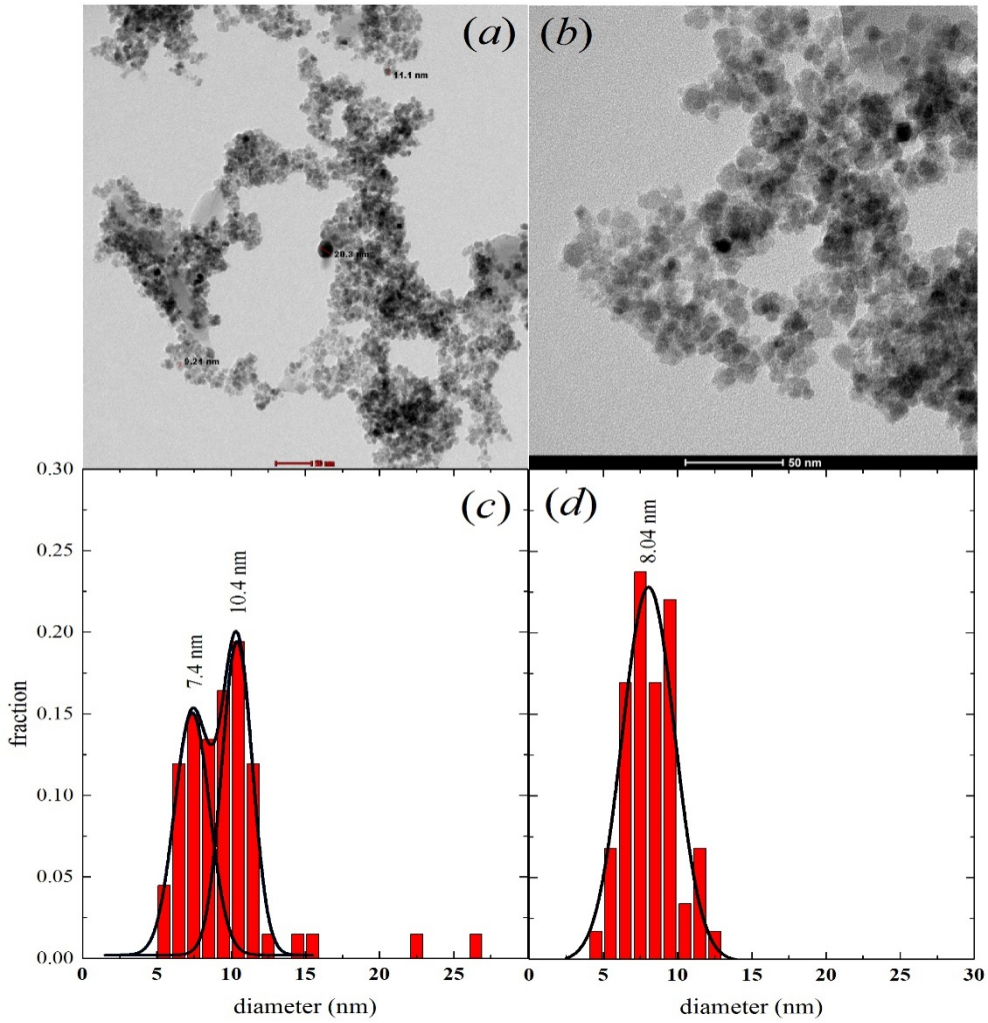

Fig. (1): TEM image of Fe3O4(part a)_Folic acid loaded Fe3O4 magnetic nanostructures (Part b). The corresponding particle size distribution is illustrated in Parts c and d, respectively

Table (1): The effect of MNPs, MNPs-Folic acid, and gamma-irradiation on serum cholesterol in irradiated rats

Parameter	Postirradiation		
	Cholestrol		
Groups	The first week	Thesecond week	The third week
Control	143.6^{b}±17.6	146.2^{c}±15.02	148.9^{c}±4.48
MNPs	139.3^{b} ±8.22	138.8^{c} ±1.71	145.02^{c} ±2.33
MNPS-folic acid	123.6^{b} ±4.52	133.9^{c} ±12.8	141.7^{c} ±2.42
Irradiation	195.1^{a} ±9.04	212.9^{a} ±5.07	225.5^{a} ±10.8
R+ MNPs	182^{a} ± 5.4	188.3^{ab} ±6.82	182.9^{b} ±10.2
R+ MNPS-FA	133.4^{b} ±6.9	163.8^{bc} ± 5.67	167.13^{bc} ±1.76

Means within the same column carrying different superscript letters are significantly different ($P < 0.05$).

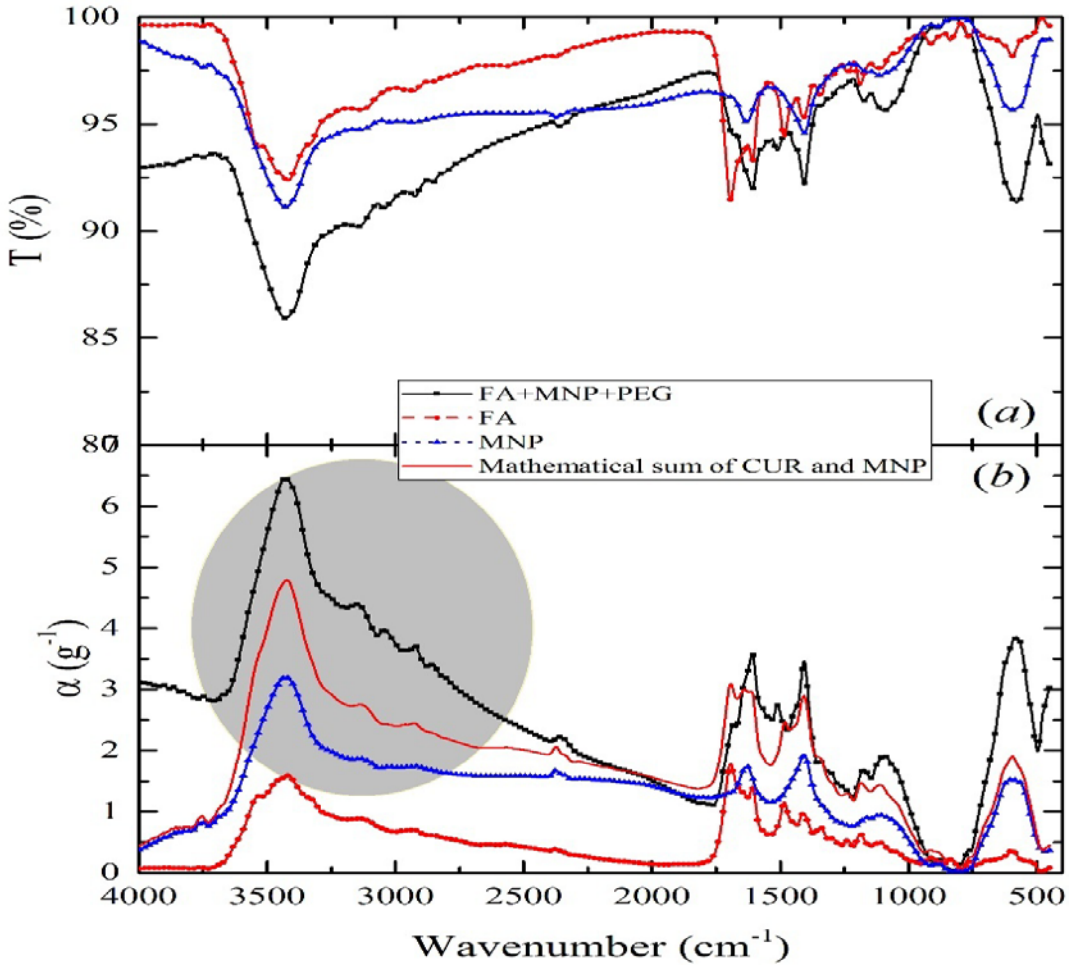

Fig (2): Fourier transform infrared spectra of various agents: folic Acid (FA) Magnetite nanoparticles (MNP); folic acid/MNP mixtures

Table (2): The effect of MNPs, MNPs-Folic acid, and gamma-irradiation on serum triglyceride in irradiated rats

Parameter	Postirradiation		
	Triglyceride		
Groups	The first week	Thesecond week	The third week
Control	83^{b} ±3.4	76.4^{bc} ±2.3	80.1^{bc} ±2.2
MNPs	67.3^{c} ±7.02	71.9^{cbd} ±8.0	79.2^{bc} ±7.8
MNPS-folic acid	41.7^{d} ± 4.5	42.4^{cd} ±5.3	47.2^{cd} ±5.29
Irradiation	116.5^{a} ±6.22	120.4^{a} ±5.6	123.0^{a} ±5.8
R+MNPs	107.5^{a} ±5.91	85.3^{b} ±31.4	89.6^{b} ±30.9
R+MNPs-FA	73.6^{bc} ± 2.8	49^{cd} ±19.3	54.6^{cd} ±18.8

Means within the same column carrying different superscript letters are significantly different (P < 0.05).

Table (3): Comet assay parameters obtained by image analysis in cells of all groups after treatment experiment

Group	Tailed %	Untailed %	Tails length µm	Tail DNA%	Tail moment
control	2	98	1.45±0.12 ^{d}	1.54	2.23
MNPs	5	95	2.03±0.35 ^{d}	1.85	3.75
Folic acid - MNPs	4	96	1.85±0.22 ^{d}	1.71	3.16
Irradiation	29	71	9.42±0.68 ^{a}	8.11	76.40
R+MNPs	16	84	5.72±0.29 ^{b}	4.73	27.05
R+Folic acid-MNPs	12	88	4.51±0.21^{b,c}	3.56	16.06

Different superscript letters in the same column of tail length showed significance difference at P< 0.05

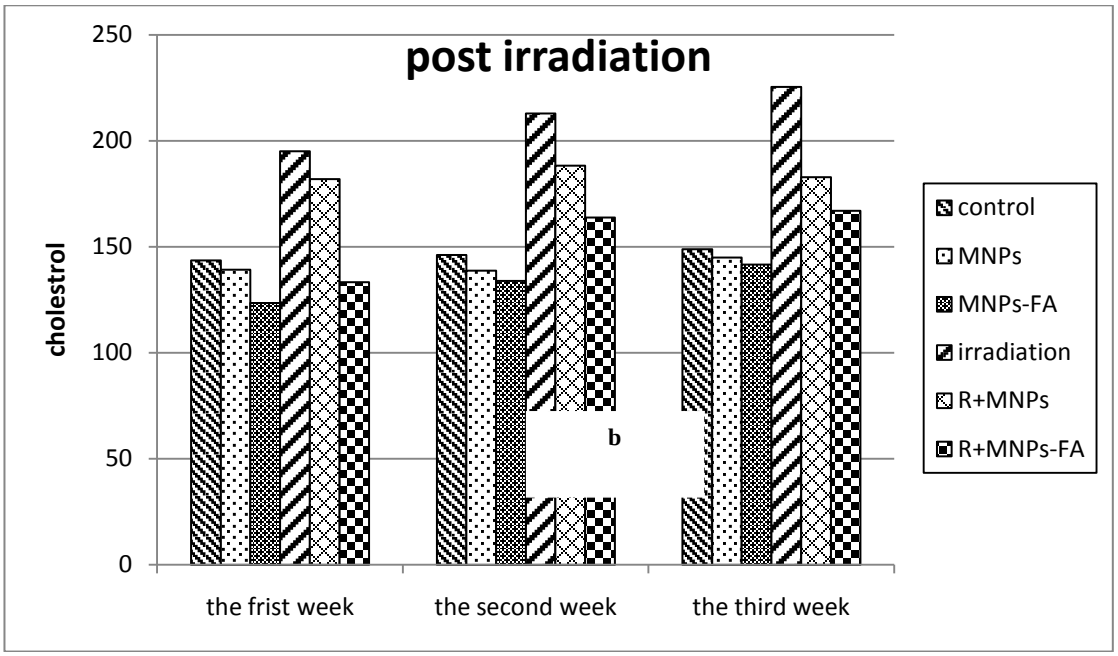

Fig (3): The effect of MNPs, MNPs-folic acid, and γ- irradiation on seurm cholestrol in irradiated rats

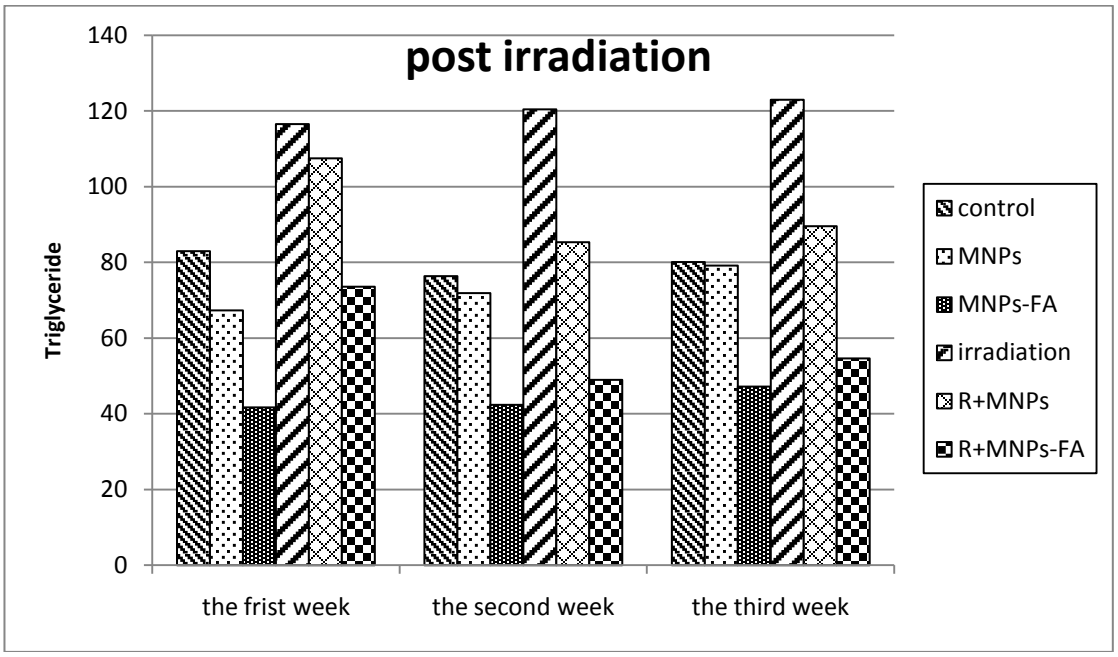

Fig (4) The effect of MNPs, MNPs-folic acid, and γ- irradiation on seurm trigylcerides in irradiated rats

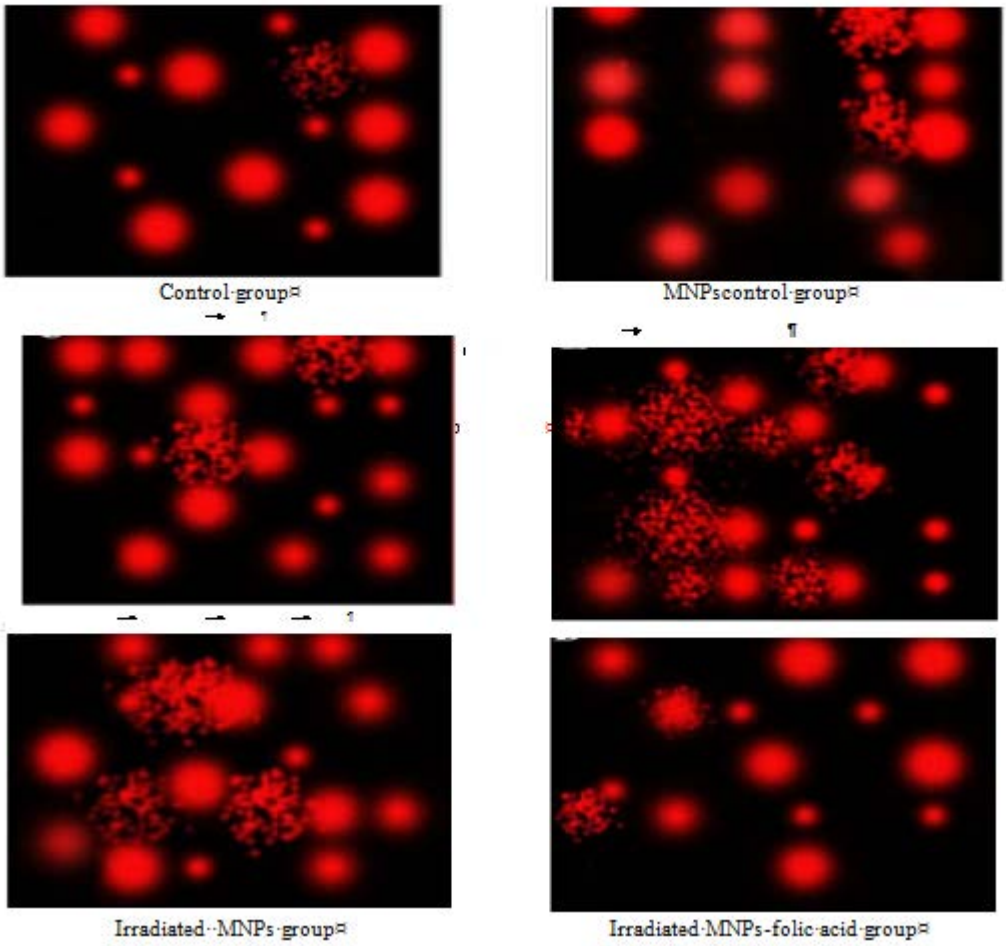

Fig. (6): The effect of MNPs, MNPs-Folic acid, and γ - irradiation on hepatic DNA damage in irradiated rats

4. DISSCUSION

The damaging effect of Ionizing radiation in the living cell occurs, collectively, through two mechanisms. The first mechanism is the direct effect on DNA, proteins and lipids. IR induces DNA breaks, particularly double strand break, and the incidence of this direct action can also affect proteins resulting in modification and deactivation, and lipids resulting in the production of radicals. The second is an indirect mechanism through the radiolysis of water resulting in the production of free hydroxyl radicals which subsequently undergo reactions with cellular components resulting in depletion of folate, other electron scavenging molecules and systems, peroxidation of cellular membrane lipids and proteins, and the induction of several damages to DNA, like, the generation of basic sites and single strand breaks[14]. Because of its function in the methylation of homocysteineinto methionine, the folate depletion under stress of free radicals results in accumulation of homocysteine which is believed to be one of the factors that cause elevation in levels of serum triglycerides and cholesterol [15]. In the present study we observed that irradiated animals supplemented with MNPs-folic showed great reduction in the TG and cholesterol levels. The reduction of cholesterol and TG levels nearly to normal levels may result from the recovery of the of the physiologically required folate levels which in turn regained the function of in the methylation of homocysteine into methionine resulting in the reduction of homocysteine to normal levels and subsequently regain of associated normal cellular metabolic pathways-Folate is required in the anabolic pathways of purine and pyrimidine nitrogenous bases in the cell which are crucially important in the synthesis and repair of genomic DNA [16], as observed in the comet assay results of the MNPs-folic acid group showed noticeable reduction in the DNA damage caused by IR when compared to the MNPs and irradiated groups. Iron oxide has impenetrable contribution in metabolic

pathways which leads to the development of the damaging effect of IR inside the cell, sense, it has a role in the development of oxidative stress through the Fenton effect.On the other hand, iron oxide contributes as cofactor for many enzymes and cellular electron transfer systems which are responsible for reduction of ROS, DNA synthesis and repair. In the current study, the sole administration of iron oxide in the form of MNPs, or, as conjugate with Folic acid showed improvement in the lipid and the comet profiles of the treated groups. These results suggest that administration of iron oxide in the form of MNPs may have a role in the treatment of the damaging effect of the acute whole-body gamma irradiation incidents. The highest improvement in the lipid and comet profiles were noticed in MNPs-Folic acid treated group. These results suggest that possible effective contribution of MNPs-folate conjugate in the treatment γ radio toxicity.

5. CONCLUSION

The present study suggests that folic acid loaded MNPs controlled the excess production of free radicals produced by gamma irradiation and have a protective effect against oxidative stress by decreasing triglyceride and cholesterol concentrations and increasing the antioxidant system. The comet assay results gave preliminary evidence of the protective effect folic acid loaded MNPs against damaging effect of IR on genomic DNA which can be used for future investigations on mechanisms of their radio protective action on molecular bases. The results of the present study also suggest that folic acid loaded MNPs may be used to protect the liver cells from oxidative damage and preserve the integrity of tissue functions. It is recommended that further studies should be carried out to investigate and confirm the action of Folic acid loaded in the treatment γ radio toxicity.

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