BENHA VETERINARY MEDICAL JOURNAL, VOL. 34, NO.3: 1-13, AUGUST 2018.

A SPECIAL ISSUE FOR THE 6TH SCIENTIFIC INTERNATIONAL CONFERENCE

Biochemical Role of Curcumin Loaded Magnetic Nanoparticles Versus Gamma Irradiation in Rats

Abdelmaksoud H. A.[1]; Samy Ali Hussein [1]; Saleh H. M.[2]; Hassanin W. F[2]., and Eman I. Sobeh[2]

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

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

A B S T R A C T

Exposure to ionizing radiation is known to have lethal effects in blood cells. It is predicted that an individual may spend days, weeks or even months in radiation field without becoming alarmed. The present study has been carried out to investigate the possible therapeutic effect of curcumin loaded iron oxide nanoparticles through injection after body gamma irradiation of rats. We have shown that the curcumin loaded magnetic nanoparticles administration reduced the lethal effect by gamma radiation. Results showed that whole body gamma irradiation of rats at 6 Gy (single dose) induced significant hematological disorders such as, decrease in the red blood cells count, hemoglobin levels, decline in WBC, lymphocytes, Neutrophils, Monocytes, highly significant increase in malondialdehyde MDA, However decreased in catalase CAT and reduced glutathione GSH was observed after three weeks post-irradiation. Administration of curcumin loaded magnetic iron oxide nanoparticles (Fe_3O_4) after irradiation induced significant improvement of the hematological parameter and MDA, GSH and CAT changes induced by subsequent gamma irradiation.

Key words: Magnetic iron oxide nanoparticles**,** gamma irradiation, curcumin- loaded. In Vivo**.**

(http://www.bvmj.bu.edu.eg) **(BVMJ-34(3):1-13, 2018)**

1. INTRODUCTION

Ionizing radiation (IR) damages biological tissues by exciting or ionizing their atoms and molecules. Depending on the exposure to radiation dose and the biochemical processes, damage may be prompt (expressed minutes to weeks after exposure) or delayed (expressed several months to years later) (El-shanshoury et al., 2016). Ionizing radiation (IR) has a sufficient amount of energy to induce physical symptomatology within minutes of exposure; the prodromal phase includes nausea, vomiting, and fatigue. These prodromal symptoms can be followed by dramatic decrease peripheral blood cell counts, as hematopoietic cells represent a renewal system consisting of cells with fast division rates that are known to be sensitive to IR (Sanzari et al., 2013).

The white blood cells show the most immediate response to IR by exhibiting a dramatic drop 24h (one day) following radiation exposure, in contrast, RBCs and platelets decline more gradually, over a longer time period (Maks et al., 2011, Kennedy et al.,2013).

Exposure to ionizing radiation induces the production of reactive oxygen species (ROS), which include superoxide, hydroxyl radicals, singlet oxygen and hydrogen peroxide (pratheeshkumar and Girija 2010). These free radicals react with critical cellular components, such as DNA, RNA, proteins, and membranes, resulting in cell dysfunction and death (Abdou and El-sayyad, 2012).

Iron oxide nanoparticles (NPs) consider 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 (Gupta & Gupta, 2005, Thorek et al., 2006, Sun et al., 2008, Shubayev et al., 2009, and Bhasskar et al., 2010). Iron oxide (NPs) can be specifically synthesized chemically for a wide variety of applications so-called engineered nanoparticles (Brigitta Szalay, 2012).

Curcumin, (1,7-bis(4-hydroxy-3-methoxyphenyl)- 1,6- heptadiene-3,5-dione, is a linear diarylheptanoid natural pigment isolated from the rhizome of Curcuma Longa (Joe, et al., 2004). Curcumin possesses a wide range of pharamacological activities, such as anticancer, anti-inflammatory, anti-oxidant and antimicrobial activity (Bayomi, et al., 2013, Shen, 2009). In addition, it has been also reported as a potential radio-protective agent (Goel, and aggarwal, 2010, Ozgen, et al., 2012). Curcumin exists in solution as a tautomeric mixture of keto and enol forms. The keto-enol tautomerism is responsible for free radical scavenging property of curcumin (Jha, et al., 2015, Gupta, et al., 2013). The poor aqeous solubility, relatively low bioavailability, intense staining color and rapid metabolism are significant challenges to the clinical use of curcumin (Anand, et al., 2007).

2. MATERIALS AND METHODS:

2.1. Materials

Iron (II) chloride tetrahydrate ($FeCl_2$-$4H_2O$), iron (III) chloride hexahydrate ($FeCl_3$-$6H_2O$), polyethylene Glycol 600, curcumin, were purchased from Sigma-Aldrich (USA). Ammonium hydroxide solution (NH_4OH) was obtained from Merck (Germany).

2.2. Preparation

Iron oxide nanoparticles were prepared using a minor modification of a protocol by Yallapu et al., (2011). Briefly, 480mg of polyethylene glycol (PEG 6000, average molecular weight 6000) dissolved in 45 ml of water containing 810mg of Iron(3+) and iron(2+) ions(molar ratio 2:1) was placed in 100 ml beaker. To this solution, 3ml of ammonium hydroxide (28% ammonia in water) was slowly added at astirring speed of 900 rpm. After 6 hours, after that five washes with water,(Bhattarai, et al., 2008), the nanoparticles were resuspended in 25 ml water and centrifuged at 1000 rpm to remove larger aggregates. This formulation was designed as MNP.

To load CUR in the MNPs, 47 mg CUR in 5ml DMF was added to 10 ml of an aqueous dispersion of MNPs (462 mg). The mixture was stirred overnight at 400 rpm on a magnetic plate to facilitate the penetration of CUR molecules into DMF polymer layer in the formulation. The drug –loaded nanoparticles were washed three times by resuspending them in water and separated with the help magnets, (Bhattarai, et al., 2008) These drug- loaded nanoparticles were

dispersed in 2ml sterile phosphate buffered saline(PBS) solution and stored in a refrigerator until further use.

2.3. characterization

2.3.1. A tranamission electron microscope (TEM) of the produced samples was carried out by using EFI Netherland, Modal Tecani G20, supertwin, double tilt with applied valtage 200kv, magnification rang up to 1000,000 X and Gun type LaB_6 Gun was employed to determine the size and morphology of the MNP-CUR nanoparticles.

The particle size, and distribution in aqueous medium of the MNP-CUR formulation (50µL of 500µg/mL dispresd in 3mL of distilled water and ultrasonicated for 30 minutes) were determined for 3 minutes.

2.3.2. Fourier transform infrared (FTIR) spectral study was conducted for dry Curcumin and MNP-CUR. The FTIR of nanoparticles was recorded at a scanning speed of 4 cm^{-1} between 4000-750 cm^{-1} (32 scans) on an Illuminate IR ™ FT-IR microscope (Smiths Detection, Danbury, CT).

2.4. Animals

sixty 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 in the Nuclear center for radiation research, 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.

2.5. Irradiation

Whole body irradiation was performed by Gamma- cell 40 (cesium- 137) source belonging to NCRRT. Animal were exposed to irradiation 6 Gy as a single dose, at a dose rate of 0.957 Rad/s.

2.6. Experimental design

Animal were divided into six groups(10 rats each): non irradiated control group (con); irradiated group exposed to 6 Gy whole body γ- irradiation ; magnetic nanoparticles group injected i.p. by 1.5mg/ kg magentite twice aweek for three weeks. Curcumin MNPs group (n=10) injected i.p. by 1.5mg /kg (Eman, et al., 2014) curcumin loaded magentite twice a week for three weeks. Irradiated magnetic nanoparticles group exposed to sublethal dose (6 Gy) gamma irradiation and then injected i.p.by magentite NPs twice a week for three weeks and irradiated curcumin MNPs Group exposed to sublethal dose (6 Gy) gamma irradiation and then injected i.p.by curcumin loaded magentite NPs twice a week for three weeks.

2.7. Sampling

Blood samples were taken weekly from the retro-orbital venous plexus under light ether aneathesia . each blood sample was collected into tubes containing heparin for complete blood picture .At the end of the experiment after three weeks animals in all groups were sacrificed by decapitation and 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 biochemical analysis (MDA, CAT and GSH).

2.8. Hematolgy

Complet blood counting (CBC) was determined by cell counter fully automated (Sysmex, Japan) according to (Trivelli, Ronney, and Lai,1971).

2.9. Determination of liver (glutathione (GSH), Catalase CAT, and malondialdehyde MDA)

Liver glutathione concentration was determined according to the method described by Paglia and Valentine, (1967). Catalase activity was determined according to the method described by (Aebi, 1984). MDA concentration was determined according to the method described by Ohkawa*et al*., (1979).

2.10. 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:

3.1.Transmission electron microscopy. The micro-structural features of the MNPs-CUR were studied using TEM.

The TEM image in figure (1) revealed an average size of 10-20 nm for MNPs-CUR.

The TEM image of specific areas showed particles with spherical-like shapes, although for a few particles with the largest diameters observed faceted particles. The latter shape is probably related to the high crystallinity of the particles reflecting the cubic crystal habit of the Fe_3O_4 phase, Wladimir et al., 2010.

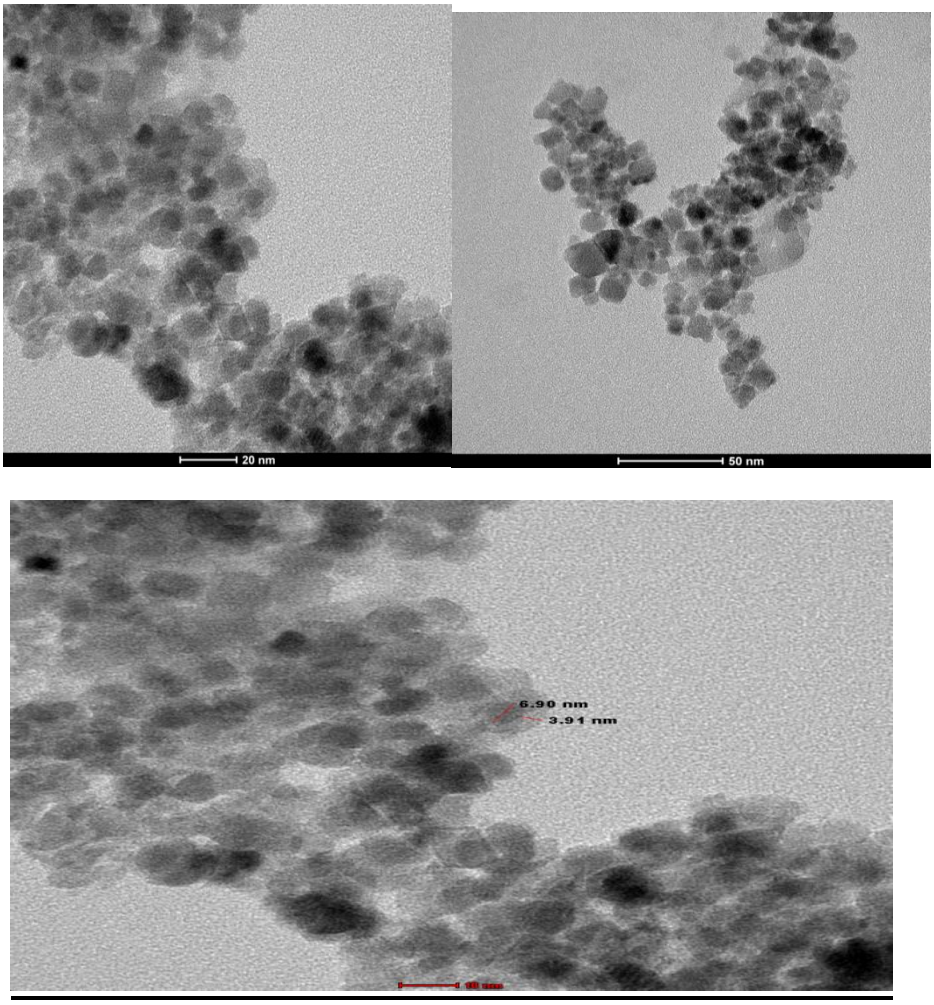


Fig (1) TEM image of curcumin loaded Fe_3O_4 magnetic nanostructures

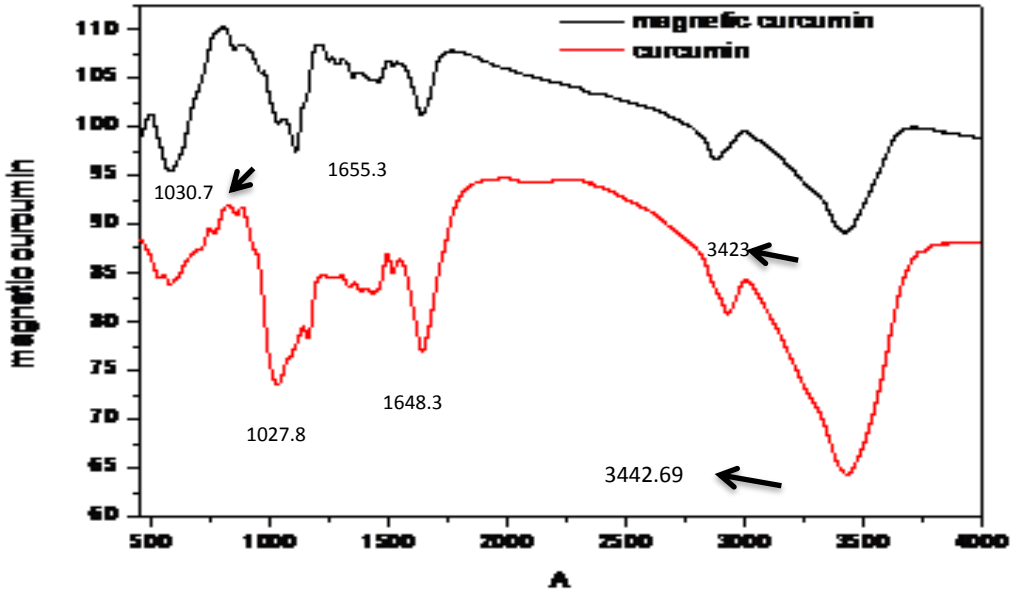
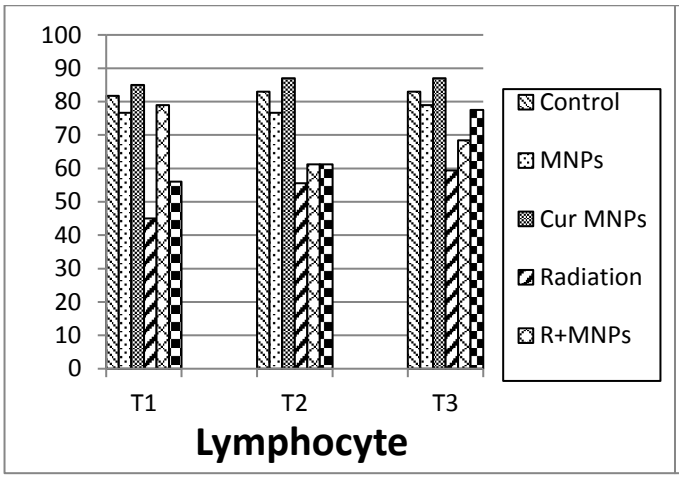


Fig (2) FTIR of curcumin and curcumin MNPs

3.2. (Fig2) FTIR spectra of curcumin showed characteristic band at around 3443cm^{-1} which is attributed to the O-H stertching vibration of curcumin while in the loaded system in presence of PEG assisted nanoparticles , the O-H stertching vibration shifted to 3423.4cm^{-1}. This result indicates there is an interaction among the components namely.PEG, curcumin and iron oxide nanoparticles. The higher intensity of this band in loaded system compared to the curcumin in absence of PEG assisted NPs confirmed the presence of more number of such bonds. Further shifting of the strong band at 1655 cm^{-1}of curcumin to 1649 cm^{-1} incojugated system indicated the participation of the dione moiety in conjugation. Moreover , the shifting of the stertcing vibrational band from 1027.8cm^{-1} to 1030.7 cm^{-1}in the loaded system was indication of interaction of oxygen of DMF with iron oxide nanoparticles (Salmaso, et al., 2007). Its relevant to mention that the binding of magnetic particles to bioactive substances also involves various chelating interactions between the biological moiety and metal's centers. Such binding pave the way for the coupling of biomolecular entities with enhanced stability (Konwarh, et al., 2009).

The loading efficiency of therapeutic drugs in nanoparticles directly influences the therapeutic outcome of the system. CUR is a known antioxidant, and anti-inflammatory molecule (Anand, et al., 2008). However, its use is limited due to its low bioavailability and poor pharmacokinetics. Therefore, a MNP-CUR nanosystem was developed to increase the bioavailability, pharmacokinetics and subsequently improve the therapeutic efficacy of CUR.



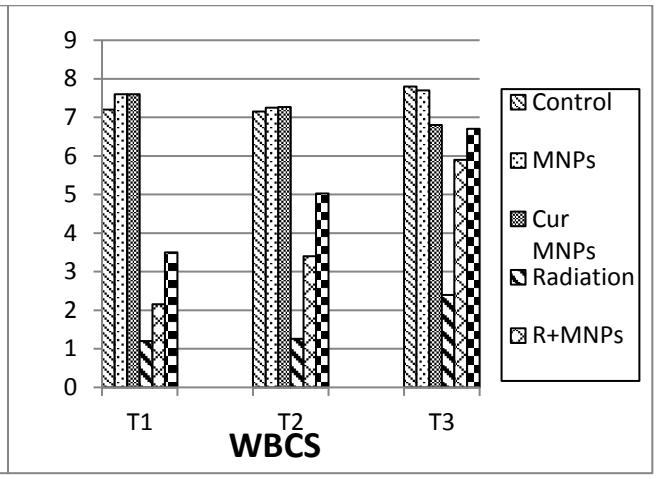
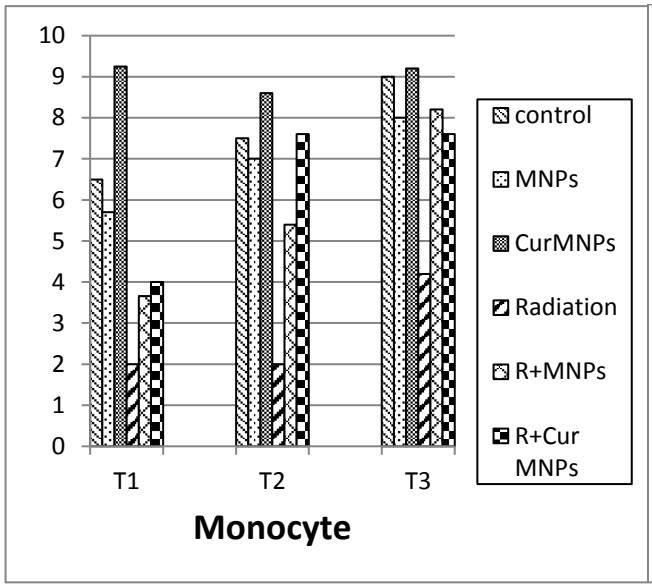


Fig. (3) the effect of γ-irradiation and MNPs-CUR On WBC count Fig. (4) the effect of γ-irradiation and MNPs-CUR On lymphocyte



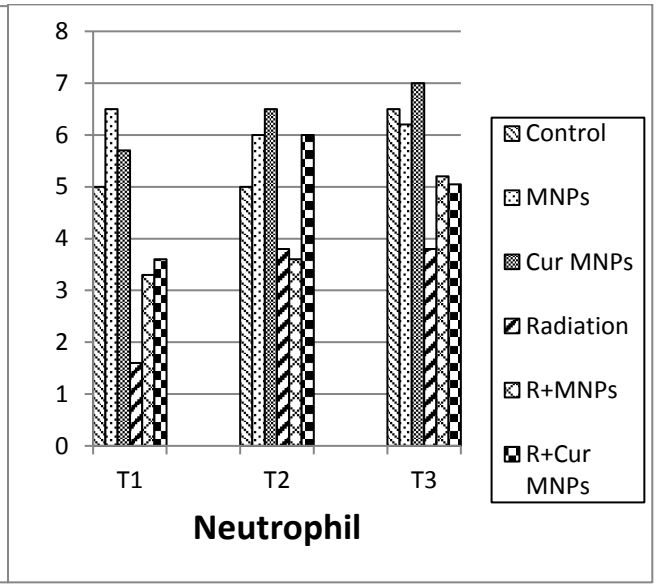
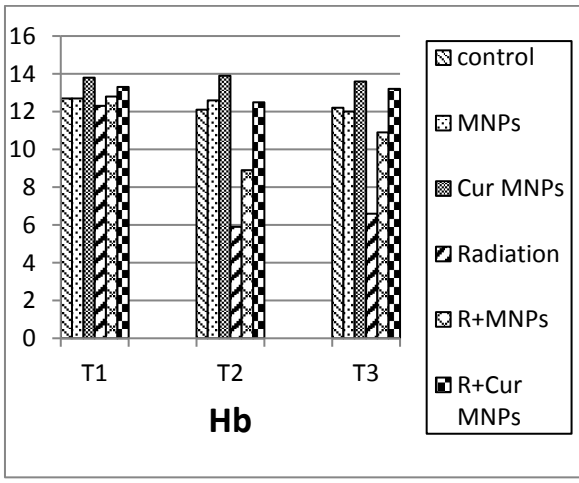


Fig. (5) The effect of γ-irradiation and MNPs-CUR On neutrophil count Fig. (6) The effect of γ-irradiation and MNPs-CUR on Monocyte



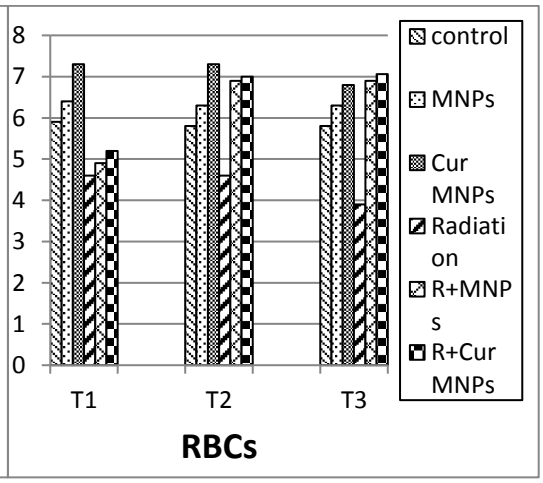


Fig. (7) The effect of γ-irradiation and MNPs-CUR On RBC Fig. (8) The effect of γ-irradiation and MNPs-CUR On Hb level

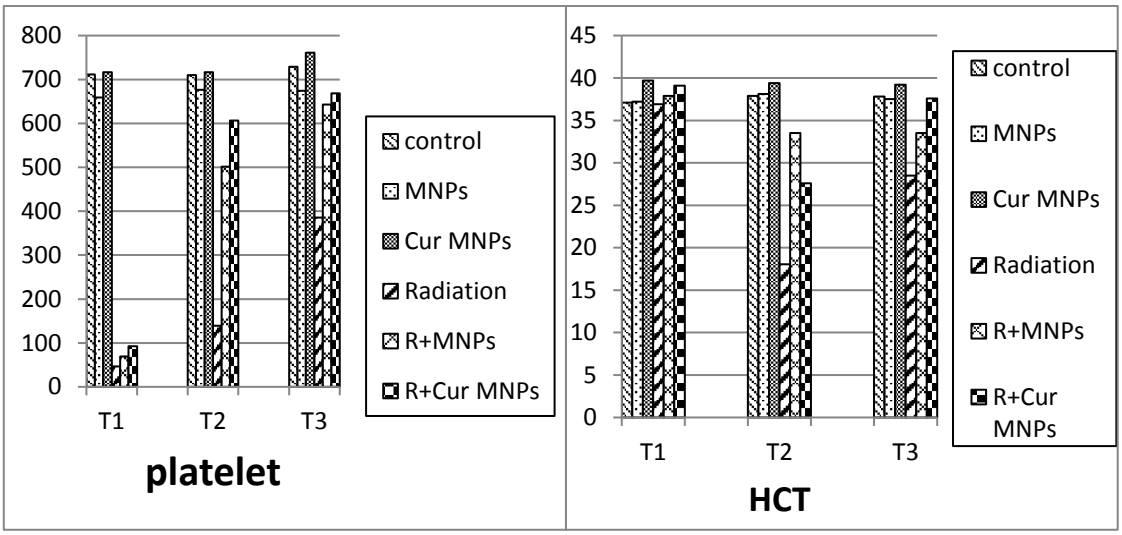


Fig. (9) the effect of γ-irradiation and MNPs-CUR On HCT Fig. (10) the effect of γ-irradiation and MNPs-CUR On platelet

3.1.Hematologicalparameters

3.1.1. Radiation and MNPs-curcumin effects on white blood cell counts (WBCs)

The recorded data in figures (3) showed that: the effect of irradiation dose and MNPs-CUR on WBCs count at different post-irradiation periods is revealed that,

- Normal rats administrated with iron oxide NPs, and MNPs-CUR in G (2, and 3) for three weeks exhibited a non-significant increase of WBCs count, lymphocyte, Neutrophil and Monocyte after three weeks (T_1, T_2, and T_3) in comparison with control. While The mean value was the highest significant decline in WBCs count in irradiated rats at (6Gy) in irradiated group in the first week of irradiation (T_1), the second weeks of irradiation (T_2) and the third weeks of irradiation (T_3) as compared to normal control group(G1).

- Administration of MNPs (G5), or MNPs-Curcumin (G6) after irradiation revealed significant improvement in WBCs count in the second week of irradiation (T1), with best improvement in G6 (MNPs-CUR) as compared to irradiated group (4).

- The obtained data in the second week and the third week of irradiation showed that the irradiated group was still significantly declined in WBCs count. But the data showed significantly increase in G (5, and 6) (MNPs group and MNPs-CUR) as compared to G (4) (irradiated group). With the best significant improvement WBCs count in G (6) and (7) (MNPs) and (MNPs-CUR) which reached to the count in normal control group.

- The mean value of WBCs count in G6 in (T_2 and T_3) was highly significant increased on comparison with irradiated group.

- The presented data in the second week and the third week of irradiation showed that the irradiated group was still significantly declined in WBCs lymphocyte, neutrophil and monocyte count. But the data showed significantly increase in G (5, and 6) (MNPs group and MNPs-CUR) as compared

to G (4) (irradiated group). With the best significantly improvement WBCs count in G (6) and (7) (MNPs) and (MNPs-CUR) which reached to the count in normal control group.

- The presented data in the first week of irradiation showed that RBC, Hb level HCT% and platelet significantly decline. While at the second week and the third week the irradiated group was still highly significantly declined in RBCs count Hb level,HCT%,and platlets. But the data showed significantly increase in G (5, and 6) (MNPs group and MNPs-CUR) as compared to G (4) (irradiated group). With the best significantly improvement RBCs, Hb level, HCT% and platelet count in G (6) and (7) (MNPs) and (MNPs-CUR) which reached to the count in normal control group.

Table (1) the effect of γ- irradiation and MNPs-CUR on L-Malnodialdehyde, Glutathione and catalase.

	Post irradiation					
parameter	MAD		GSH		CAT	
group	T_1	T_2	T_1	T_2	T_1	T_2
Control	33.3^{e} ±0.73	33.8^{e} ±0.55	33.73^{a} ±0.91	33.03^{ab} ±0.37	4.75^{b} ±0.15	4.75^{b} ±0.38
MNPs	32.0^{ef} ±0.58	31.1^{f} ±0.38	29.07^{b} ±0.52	29.56^{c} ±0.54	4.98^{ab} ±0.08	5.12^{b} ±0.18
CUR MNPs	30.8^{f} ±0.73	30.13^{f} ±0.44	33.67^{a} ±0.88	34.24^{a} ±0.33	5.33^{a} ±0.37	6.15^{a} ±0.08
Radiation	68.4^{a} ±0.72	65.47^{a} ±0.88	10.41^{e} ±0.30	10.41^{e} ±0.30	1.07^{e} ±0.06	0.98^{e} ±0.02
R+MNPs	68.4^{a} ±0.72	49.30^{b} ±0.35	24.10^{d} ±0.55	24.10^{d} ±0.55	2.23^{d} ±0.12	2.83^{d} ±0.12
R+Cur MNPs	41.6^{d} ±0.68	41.6^{d} ±0.68	26.40^{c} ±0.83	27.30^{d} ±0.51	2.97^{c} ±0.15	4.06^{c} ±0.10

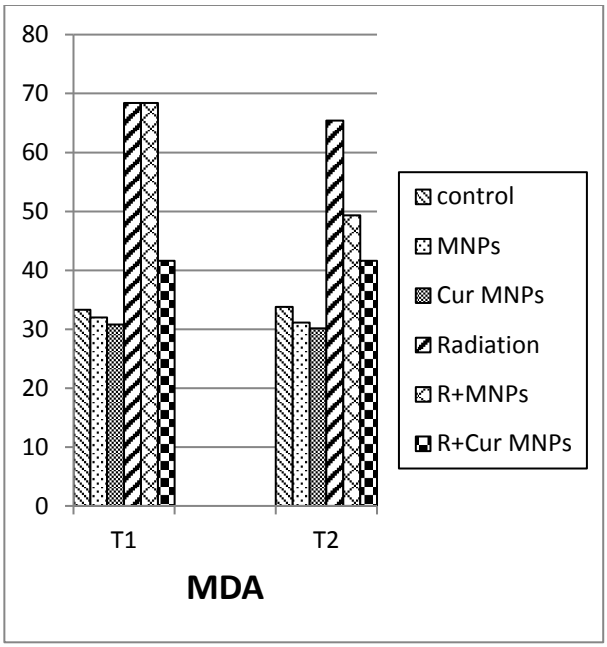


Fig. (11) the effect of γ-irradiation and MNPs-CUR On MDA

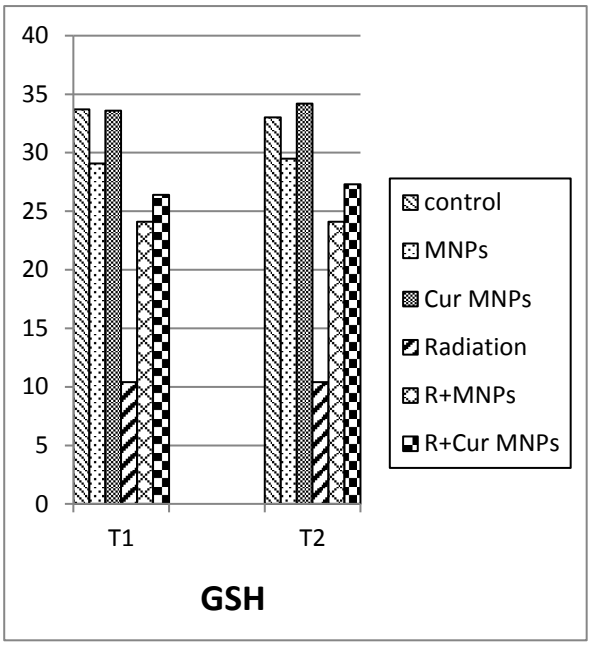


Fig. (12) the effect of γ-irradiation and MNPs-CUR On GSH

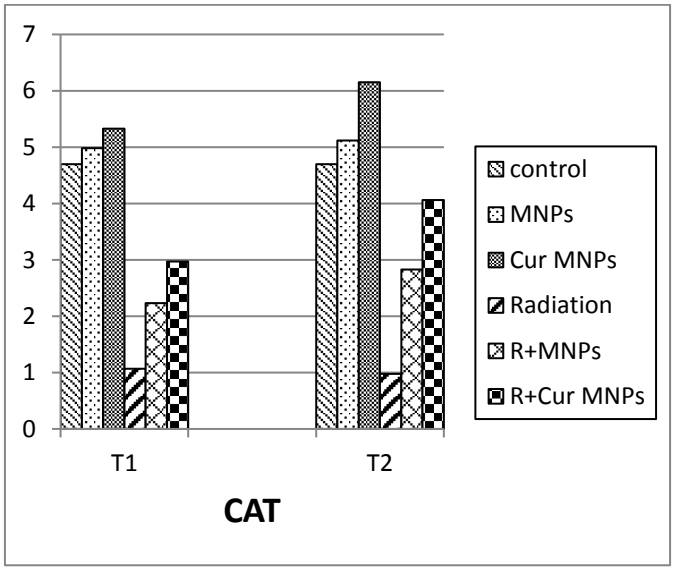


Fig. (13) the effect of γ-irradiation and MNPs-CUR On CAT

3.2.2 Antioxidant status

The recorded data in table (1) and figures (11 and 12) showed that: At the first week (T_1) and the second week (T_2) post exposure of rats to gamma- irradiation, the activity of the antioxidant enzymes; CAT, GSH in the liver tissue in irradiation group were significantly decreased ($P < 0.05$), as compared to the control group, However, the liver tissue MDA in irradiated group was significantly increased ($P < 0.05$) when compared to control group. Similar results have been reported by previously (Gorbunov, et al., 2000, Voevodskaya, and Vanin, 1992). Conversely, the irradiated rats treated with MNPs and MNPs- curcumin showed significant improvement in the antioxidant status via enhancement of the antioxidant enzymes activities (GSH, CAT).

4. DISCUSSION:

Ionizing radiation has well documented effects on blood cells and it's generally assumed that these effects contribute to the hematopoietic syndrome, observed in animals and humans, following exposure to total body irradiation (Billings et al., 2014). WBCs appeared to be the most sensitive to γ- ray irradiation among the type of cells evaluated (Sanzari et al., 2013). This result was comparable with that of thrall et al., (2013) who detect a statistically significant reduction in WBC counts at 24h (one day) post-irradiation for animals at 6Gy gamma irradiation. This can be explained by continuous radiation induced excess cell losses in the stem (and precursor) cell pools (Graessele, 2002). However, previousely, Rozgaj et al., (1999) reported that long- term exposure to low doses of ionizing radiation may affect the cells and tissues and result in the blood count drops soon after irradiation and recovers within several weeks. Seed et al, (2002) reported that IR is one of the cytotoxic agents that particularly cause damage to cell renewal systems. They also demonstrated that the lymphocytes, Neutrophiles, Monocytes showed decrease within the first days after irradiation (seed, et al., 2002). The platelet counts typically decline 5-10 days following exposure to gamma rays.

Redox state of the cell is primarily a consequence of the precise balance between the levels of ROS and endogenous thiol buffers, present in the cell, such as glutathione, which protect cells from oxidative damage. Dramatic elevation of ROS, exceeding compensatory changes in the level of the endogenous thiol buffers, may result in the sustained activation of signaling pathways and expression of genes that induce apoptosis in affected cells (Davis, et al., 2001).

Glutathione acts synergistically with other endogenous antioxidants and acts as a co-factor with the enzyme glutathione peroxidase to scavenge free radicals, and detoxify xenobiotics (Aquilano, et al., 2014).Thus, the depletion of GSH contents after exposure to γ-radiation may be due to reaction of GSH with free radicals resulting in the formation of thiyl radicals that react to produce GSSG as reported earlier (Navarro, et al., 1997).

The significant reduction in liver GSH following exposure. This could be due to an enhanced utilization of the antioxidant system as an attempt to detoxify the free radicals generated by radiation, the intra-peritoneal administration of MNPs- curcumin significantly enhanced the endogenous GSH level in liver of irradiated rats (Pratheeshkumar and Kuttan,2010).

The mitigation of cytotoxic effect of ROS by MNPs- curcumin may be due to the direct scavenging of free radicals and subsequent inhibition of oxygenation reaction, as curcumin has been reported to be a good antioxidant, free radical scavenger and a radioprotective agent (Inano, and Onoda, 2002).

Curcumin may have lowered lipid peroxidation by maintaining the activities of antioxidant enzymes and glutathione at higher levels as these enzymes play an important role in the regulation of lipid peroxidation (jagetia, and Rajanikant, 2015). Moreover, the radio-protective activity of MNPs-curcumin is attributed to the scavenging of free radicals, enhancement of the activity of the antioxidant enzymes; CAT, up-regulation of GSH level, and inhibition MDA production, resulting in inhibition of lipid peroxidation. (Goel A., et al., 2010, Ozgen S.C., et al., 2012, Mathews V.V., 2012).

2. CONCLUSION:

Turmeric as naturally occurring antioxidants was studied to reduce the cellular damage induced by ionizing radiation. MNPs-Curcumin ameliorates the oxidative damage of γ-radiation. MNPs- Curcumin provides radio-resistance effect to bone marrow cells in animals received γ-radiation. Use of commercial turmeric may be limit due its poor oral bioavailability and solubility in plasma. Our study with these naturally antioxidant loaded nanoparticles provide an extended window of protection against sublethal-dose irradiation. It could be concluded that MNPs- curcumin can be used as a radio protector to the professional radiation and remediation workers who may be exposed to low level of ionizing radiation especially if it is administered after irradiation for therapeutic purpose.

3. REFERENCES

Abdou M. I., and El-Syyad H., (2012). The protective effect of oyster Mushroom *(Pleurtus pulmonarus*) aginst sublethal gamma radiation dose in rats. Arab j. of nuclear sci. and Applic, (45): 234-255.

Aebi H., (1984). Catalase in vitro. J. Methods in Enzymology. (105), 121-126.

Anand P, Sundaram C, Jhurani S, Kunnumakkara AB, and Aggarwal BB, (2008), Curcumin and cancer: an “old-age” didease with an “ age- old” solution. Cancer Lett. 267(1):133-164.

Anand P., Kunnumakkara AB, Aggarwal BB, and Newman R.A., (2007). Bioavailability of curcumin: Problems and promises, Molecular Pharmaceutics. (4). 807-818.

Aquilano, K., Baldelli, S.,and Ciriolo, M.R.(2014) Glutathione: New roles in redox signaling for an old antioxidant. Front. Pharmacol. 5,196.

Bayomi S. M., El-kashef H. A., El-Ashmawy M. B., Nasr M. N. A., El-Sherbeny M. A., Badria F. A., Abou-Zeid L. A., Ghaly M. A., and Abdel- Aziz N. I., (2013). Synthesis and biological evaluation of new curcumin derivatives as antioxidant as antioxidant and antitumor agents, Medicinal Chemistry Resesrch, (22).1147-1162.

Bhasskar S., Tian F., Stoeger T., Kreyling W., Fuenteet J. M. de la, Grazu V., Brom P., Estrada G., Ntziachritos V.,and Razansky D., (2010). Multifunctional nanocarriers for diagnostics, durg delivery and targeted treatment across blood-brain barrier: Perspectives on tracking and neuroimaging. Part fibre Toxicol, (7): 1-3.

Bhattarai SR, Kc RB, and Kim SY, (2008) N-hexanoyl chitosan stabilized magnetic nanoparticles: implication for cellular labeling and magnetic resonance imaging. J Nanobiotechnology.6:1.

Billings P. C., Romero-Weaver A. L., and Kennedy A. R., (2014). Effect of gender on the radiation sensitivity of murine blood cells. Gravitational and Space Research, 2(1), 25-31.

Brigitta S., PhD Thesis, (2012). Iron oxide nanoparticles and their toxicological effects; in vivo and in vitro studies. Department of Public Health Faculty of Medicine University of Szeged.

Davis, W., Jr., Ronai, Z., and Tew, K., D., (2001) cellular thiols and reactive oxygen species in drug-induced apoptosis.j. pharmacol. Exp.Therapeut.296,1-6.

El-shanshoury H. , El-shanshoury G., and Abaza A., (2016) Evaluation of low dose ionizing radiation effect on some

blood components in animal model. J. of radiation research and applied Sciences. 282-293.

Eman Sobeh, Souad El Feky, Rehab Amin, Eldemeryaty A. S., (2014). Studying the biostimulatory effect of magnetic nanoparticles on irradiated rats. M.S.C. Thesis, cairo univ.

Goel A., and Aggarwal B.B., (2010), Curcumin, the golden spices from Indian saffron, is a chemosensitizer and radiosensitizer for tumors and chemoprotector and radioprotector for normal organs, Nutrition and cancer, 62, 919-930.

Gorobunov N.V., Pogue-Geile, Epperly M.W., BigbeeW.L., Draviam R., Day B.W., Wald S.C.,Watkins J. S., and Greenberger S.C., (2000), Activation of nitric oxide synthase 2 pathway in the response of bone marrow stromal cells to high doses of ionizing radiation, Radiation research, 154,73-86.

Graessle D. H., (2002). Mathematical modeling of the blood platelet renewal system as an approach to analyzing the effects of chronic irradiation on haematopoiesis. The British Journal of Radiology Supplement, 26, 202-207.

Gupta A. K., and Gupta M., (2005). Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. Biomaterials J., (18): 3995-4021.

Gupta S. C., Patchva S., and Aggarwal B.B., (2013). Therapeutic roles of curcumin: Lessons learned from clinical trials, The AAPS journal, (15).195-218.

Inano, H., and Onoda, M., (2002) Radioprotective action of curcumin extracted from curcuma longa linn: inhibitory effect on formation of urinary 8-hydroxy- 2-deoxyguanosine, tumorigenesis, but not mortality, induced by γ-ray irradiation. Int.J. Radiat. Oncol.Biol. phys. 53,735-743.

Jagetia G. C., and Rajanikant G. K., (2015) curcumin stimulates the antioxidant mechanisms in mouse skin exposed to fractionated γ-irradiation. antioxidants, 4, 25-41.

Jha N.S., Mishra S., Jha S.K., and Surolia A., (2015). Antioxidant activity and electrochemical elucidation of the enigmatic redox behavior of curcumin and its structurally modified analogues, Electrochimica Acta, (151). 574-583.

Joe B., Vijaykumar M., and Lokesh B.R., (2004). Biological properties of curcumin-cellular and molecular mechanisms of action, Critical reviews in food Science and Nutrition, (44),97-111.

Kennedy A. R., Romero-Weaver A.L., Wan X.S., Diffenderfer E.S., and Lin L., (2013). Kinetic of neutrophils in mice exposed to radiation and / granulocyte colony stimulating factor treatment. Radiation Research, 180(2), 177-188.

Konwarh R., Karak N., Rai S.K., and Mukherjee A. K., (2009). Nanotechnology 20. 3621-3623.

Maks C. J., Wan X. S., Ware J. H., Romero-Weaver A. L., Sanzri J. K., and Wilson J. M., (2011). Anlysis of white blood cell counts in mice after gamma- or proton- radiation exposure. Radiation Research, 176(2), 170-176.

Mathews V.V., Binu P., Sauganth M. V., Abbilash M., Manju A., and Nair R.H.,(2012). Modulator effect of turmeric on oxidative damage in whole body gamma irradiated rats, Arab journal of nuclear science an application 2, 706-711.

Navarro, J., Obrador, E., Pellicer,J.A.,Aseni, M., Vina,J., and Estrela,M. (1997).

Blood glutathione as an index of radiation –induce oxidative stress in mice and humans. Free Radic.Biol.Med. 22, 1203-1207.

Ozgen S.C., Dokmeci D., Akpolat M., Karadag C. H., Karadag O., Gunduz H., Erbas H., Benian O., Uzal C., and Turan F.N.,(2012), The protective Effect of curcumin on ionizing Radiation- induced cataractogenesis in rats, Ballkan Medical Journal, 29, 358-363.

Pagila D. E., and Valentine W. N., (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. (1): 158-69.

Pratheeshkumar P., and Girija K (2010). Protective role of Vernonia Cinerea L. against gamma radiation- induced immunosuppression and oxidative stress in mice. J. Human and experimental toxicology 30(8) 1022-1038.

Rozgaj R., Kasuba V., Sentija K., and Prlic I., (1999). Radiation- induced chromosomal aberrations and hematological alterations in hosoital workers. Occupational Medicine, 49(6), 353-360.

Salmaso, S., Bersani A., Semenzato P., and Caliceti J., (2007). Drug Target 15. 379-390.

Sanzari J. K., Wan X. S., Krigsfeld G. S., Wroe A. J., Gridley D. S., and Kennedy A. R. (2013a). The effects of gamma and proton radiation exposure on hematopoietic cell counts in the ferret model. Gravitational and space Research, 1(1),79-94.

Seed T. M., Fritz T. E., Tolle D. V., and Jackson W. E., (2002). Hematopoieteic responses under protracted exposures to low daily dose gamma irradiation. Advances in Space Research, 30 (4), 945-955.

Shen L.,Ji H-F, (2009). Insights into the inhibition of xanthine oxidase by curcumin, Bioorganic and Medicinal Chemistry Letters, (19). 5990-5993.

Shubayev V. I., Pisanic T. R., and Jin S., (2009). Magnetic nanoparticles for theragnostic. Adv. Drug Deliv. Rev., (61): 467-477.

Sun C., Lee J., and Zhang M., (2008). Magentic nanoparticles in NMR imaging and drug delivery. Adv Drug Deliv Rev60, (11): 1252-1265.

Thorek D. L., Chen A. K., Czypryna J., and Tsourkas A., (2006). Superparamagnetic iron oxide nanoparticles probes for molecular imaging Ann Biomed Eng., (34): 23-38.

Thrall K. D., Lovaglio J., Murphy M. K., Cataneo R. N., Chaturvedi A., and Mundada M., (2013). A dose-dependent hematological evaluation of whole-body gamma-irradiation in the Gottingen minipig. Health Physics, 105(3), 245-252.

Voevodskaya N.V., and Vanin A.F., (1992) Gamma-irradiation potentials L-arginine-dependent nitric oxide formation in mice, Biochemical and biophysical research communications, 186, 1423-1428.

Yallapu M M, Othman SF, Curtis ET, Gupta BK,Jaggi M, and Chauhan SC, (2011), Multi-functional magnetic nanoparticles for magnetic resonance imaging and cancer therapy. Biomaterials;32(7):1890-1905.