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

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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 (Fe₃O₄) 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.

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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 pharmacological 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 aqueous 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$_2$O), iron (III) chloride hexahydrate (FeCl$_3$-6H$_2$O), polyethylene Glycol 600, curcumin, were purchased from Sigma-Aldrich (USA). Ammonium hydroxide solution (NH$_4$OH) 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 stirring speed of 900 rpm. After 6 hours, after that five washes with water, 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
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dispersed in 2ml sterile phosphate buffered saline(PBS) solution and stored in a refrigerator until further use.

2.3. characterization

2.3.1. A transmission electron microscope (TEM) of the produced samples was carried out by using EFI Netherland, Modal Tecani G20, supertwin, double tilt with applied voltage 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, Egyptian 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 magnetite twice aweek for three weeks. Curcumin MNPs group (n=10) injected i.p. by 1.5mg /kg (Eman, et al., 2014) curcumin loaded magnetite twice a week for three weeks. Irradiated magnetic nanoparticles group exposed to sublethal dose (6 Gy) gamma irradiation and then injected i.p.by magnetite 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 magnetite 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 Fe₃O₄ phase, Wladimir et al., 2010.

Fig (1) TEM image of curcumin loaded Fe₃O₄ magnetic nanostructures
3.2. (Fig2) FTIR spectra of curcumin showed characteristic band at around 3443 cm\(^{-1}\) 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 3423.4 cm\(^{-1}\). 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 particpation of the dione moiety in conjugation. Moreover , the shifting of the stertcing vibrational band from 1027.8 cm\(^{-1}\) 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 count  

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 count  

Fig. (7) The effect of γ-irradiation and MNPs-CUR on RBC count  

Fig. (8) The effect of γ-irradiation and MNPs-CUR on Hb level
Fig. (9) the effect of $\gamma$-irradiation and MNPs-CUR On HCT  Fig. (10) the effect of $\gamma$-irradiation and MNPs-CUR On platelet

3.1 Hematological parameters

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 (T1, T2, and T3) 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 (T1), the second weeks of irradiation (T2) and the third weeks of irradiation (T3) 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 (T2 and T3) 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 platelets. 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-Malondialdehyde, Glutathione and catalase.

<table>
<thead>
<tr>
<th>parameter</th>
<th>MAD</th>
<th>Post irradiation</th>
<th>CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>group</td>
<td>T1</td>
<td>T2</td>
<td>T1</td>
</tr>
<tr>
<td>Control</td>
<td>33.3±0.73</td>
<td>33.8±0.55</td>
<td>33.73±0.91</td>
</tr>
<tr>
<td>MNPs</td>
<td>32.0±0.58</td>
<td>31.1±0.38</td>
<td>29.07±0.52</td>
</tr>
<tr>
<td>CUR</td>
<td>30.8±0.58</td>
<td>30.13±0.38</td>
<td>33.67±0.28</td>
</tr>
<tr>
<td>MNPs</td>
<td>30.8±0.44</td>
<td>30.13±0.68</td>
<td>33.67±0.28</td>
</tr>
<tr>
<td>Radiation</td>
<td>68.4±0.72</td>
<td>49.30±0.35</td>
<td>24.10±0.55</td>
</tr>
<tr>
<td>R+MNPs</td>
<td>68.4±0.72</td>
<td>49.30±0.35</td>
<td>24.10±0.55</td>
</tr>
<tr>
<td>R+Cur</td>
<td>41.6±0.68</td>
<td>41.6±0.68</td>
<td>26.40±0.55</td>
</tr>
<tr>
<td>MNPs</td>
<td>41.6±0.68</td>
<td>41.6±0.68</td>
<td>26.40±0.55</td>
</tr>
</tbody>
</table>
3.2.2 Antioxidant status

The recorded data in table (1) and figures (11 and 12) showed that: At the first week (T1) and the second week (T2) 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, previously, 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 thyl 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-curtumin significantly enhanced the endogenous GSH level in liver of irradiated rats (Pratheeshkumar and Kuttan, 2010).

The mitigation of cytotoxic effect of ROS by MNPs-curtumin 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 radioprotective activity of MNPs-curtumin 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 limited 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


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