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Nanoparticles improved drug radio protective activity

Amir I. Mohamed[a], Mona I. A. El-Assal[b], Mohamed A. Kassem[c], Osama A. A. Ahmed[d*]
[a] Medical services Department, Cairo, Egypt.
[b] Department of Pharmaceutical Technology, Faculty of Pharmaceutical Science & Pharmaceutical industries, Future University, Cairo, Egypt.
[c] Department of Pharmaceutics, Faculty of Pharmacy, Cairo University, Cairo, Egypt.
[d] Department of Pharmaceutics, Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia.

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ABSTRACT

Radioprotective agents are synthetic compounds or natural products that are immediately administrated before irradiation to reduce injuries caused by ionizing radiation. Toxicity, short duration, and the unfavorable routes of administration, have prevented the widespread use of most radioprotective agents in practice. This study aimed to evaluate the use of slowly release- long circulation biodegradable polymer Poly(lactide-co-glycolide) (PLGA) as carrier for certain water-soluble radioprotective agents. Penicillamine and Potassium Iodide (KI) were selected as examples of radioprotectors which can be used to protect against both internal radionuclide (chronic radiation exposure) and external-beam irradiation (acute radiation exposure). Emulsion-solvent evaporation method (ESE) was used to prepare hydrophilic-drug loaded PLGA Nanoparticles (PLGA- NPs) in an efficient and reproducible manner. The radioprotective efficacy was assessed by 30 days-survival percentage, relative body weights, and (liver & spleen) total cell counts. Results revealed that single oral administration of Penicillamine-NPs or KI-NPs was effective as free drug (for 5 successive days) which indicate that PLGA-NPs could be used to modulate radioprotective drug activity in biological system, and to improve drug efficacy in different body organs for longer duration than the equal dose of free drug.

INTRODUCTION

Ionizing radiation, Oxidative stress, and Antioxidants

Nuclear medicine uses radiation to provide diagnostic information about the functioning of a person's specific organs, in addition Radiotherapy used to treat some medical conditions, especially cancer. About 10 millions of nuclear medicine procedures were performed each year in the USA for diagnosis or therapy (http://www.world-nuclear.org/info/inf55.html). Both nuclear medicine and radiation therapy have been associated with increased oxidative stress, generation of reactive oxygen species (ROS), and may further deplete tissue levels of antioxidant such as glutathione and enzymes like glutathione transferase, reductase, peroxidase and superoxide dismutase (Presant *et al.*, 2002). ROS can induce a considerable damage to cellular components such as lipids, proteins and DNA (Hamanna *et al.*, 2009) However, in addition to the intracellular antioxidant enzymes certain chemical substances known as antioxidants can scavenge ROS and reduce the free radical mediated oxidative stress (Bhartiya *et al.*, 2008).The potential of antioxidants to reduce the cellular damage induced by ionizing radiation has been studied in animal models for more than 50 years and the application of antioxidant radioprotectors to various human exposure situations has been generally accepted (JF *et al.*, 1967). The mechanism of antioxidant radioprotectors generally involves two stages of protection: Direct action by oxygen competing/consuming, destruction, and scavenging of lethal intermediates involved in damaging cells (Smoluk *et al.*, 1986; Korystov *et al.*, 1988;Rubin *et al.*, 1996), and In-Direct action which occur slowly after radiation exposure where antioxidant can enhances chemical and enzymatic repair of damaged DNA (Rubin *et al.*, 1996). The repair mechanism involved mainly hydrogen atom donation to radiation-induced "biological" radicals in the damaged macromolecules (Loman *et al.*, 1970). For direct radioprotection effect, antioxidants must be administered prior to radiation exposure to be effective protectors, because the half-life of radiation-induced free radicals is so short that free radical damage is essentially complete by 10^{-3} sec (Coleman *et al.*, 2003). Gamma-ray radiotherapy is one of the most effective treatments for cancer.

* *Corresponding Author*
Dr. Mona I. A. El-Assal, Assoc. Prof.in Pharmaceutical Technology Department, Faculty of Pharmaceutical Science & Pharmaceutical industries, Future University (FUE), Cairo, Egypt.
Tel: + 020 01006424076

Eighty percent of cancer patients need radiotherapy at some time, either for curative or palliative purposes (Pandey *et al.,* 2003). Since gamma rays incite normal tissue toxicity by creating an oxidative stress, protective agents against is essential for treating individuals at risk for environmental exposure or undergoing cancer therapy, but most of radioprotectors developed have limited use due to their inherent toxicity and short active periods (Pandey *et al.,* 2003).With the recent rapid development of nanoscience and nanotechnology, interest in the use of long circulating polymeric PLGA-NPs has been recognized for its ability to sustained drug activity, minimize drug side effects (Kommareddy *et al.,* 2005) , and to be taken up by different body tissues such as brain (Cuia *et al.,* 2005), and bone marrow (Dadochova *et al.,* 2010). In this study, in-vivo radioprotection assays were performed to evaluate the efficacy of PLGA-NPs as a carrier for Penicillamine and KI which can prolong their activity compared to free drugs. The radioprotective efficacy was assessed by 30 days-survival percentage, relative body weights (in-direct radioprotective effect), and by (liver & spleen) total cell counts (direct radioprotective effect).

MATERIALS AND METHODS

Chemicals and Materials

Poly(lactide-co-glycolide) (50:50) was purchased from Sigma Chemical Company, USA; Polyvinyl alcohol (PVA) from Loba Chemie, Mumbai, India; Penicillamine from Haupt Pharma, Berlin, Germany; Potassium Iodide and Dichloromethne (DCM) from El-Nasr Chemicals Co., Egypt. All other chemicals were of analytical grade.

Nanoparticles preparation and evaluation

Drug-loaded NPs, Penicillamine or KI, and blank NPs were prepared by multiple $w_1/o/w_2$ emulsion solvent evaporation technique and the in-vitro release behaviour of best formulation batches was determined as described before [Mohamed *et al.,* 2010). Briefly, initial drug loading of 10% Penicillamine or 30% KI (drug/polymer ratio) dissolved in 0.1 ml distilled water (w_1-phase) then dispersed in 1ml of o-phase (PLGA in DCM) using ultrasonic homogenization forming primary emulsion (w_1/o). The prepared primary emulsion was immediately injected into a 20 ml glass tube containing 10ml of 3% PVA solution and sonicated for 40 seconds for preparation of the secondary emulsion ($w_1/o/w_2$). DCM was removed by a rotary evaporator (3 hrs, 25^oC) leading to precipitation of polymer and formation of nanoparticles which centrifuged, washed, vacuum dried, and finally stored at 8 ^oC. The entrapment efficiency of drug loaded PLGA-NPs were improved (via buffer adjustment for Penicillamine-NPs and by salt addition for KI-NPs) then the cumulative percent released was observed for 10-12 days.

Animal grouping and drug dosing

10-12 week old male albino mice were used, 20-25 gm average weighed at the start of the experiments and housed in metal cages for 7 days before use under constant environmental and nutritional conditions with free access to food and water (Jeong *et al.,* 2007;singh *et al.,* 2009) In each experiment in this study, animals were divided into 5 groups as shown in and the reported antioxidant doses for Penicillamine (50mg/) (Singh *et al.,* 2007), and KI (10mg/) (Smyth *et al.,* 2003), were used for both free drug and drug loaded-NPs formulation. Blank nanoparticle (drug-free NPs) were used as positive control to exclude the influence of PLGA polymer, if any, on drug estimations. The free drug administrated once daily for 5 consecutive days while the loaded-NPs formulations administrated as a single dose at the first day of the experiment. The amount of loaded-NPs used was calculated from the following formula:

$$\text{Loaded NPs Weight (mg)} = \frac{\text{animal drug dose (mg)} \times 1000}{\textit{NPs drug content } (mg / g)}$$

Irradiation doses

Whole-body gamma-irradiation was performed at National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt, using Canadian Gamma Cell-40 Cesium-137 irradiator at a dose rate of 0.48 Gy/minute. Clinical observations of toxic signs, Mortality percentage , and Body weight of mice exposed to a single total body irradiation (TBI) dose level of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 Gy were carried out to select optimum range (results not shown).

The range of 6-10 Gy was selected to differentiate the biological responses between the free and drug loaded NPs. For irradiation, each mouse was restrained in a removed-end 50ml Falcon tube to ensure that the mice were in the appropriate position during irradiation. Then each group of 10 animals was placed in the central part of irradiation chamber, at the focal distance of 30 cm, and exposed to a single TBI dose level of 6, 7, 8, 9, or 10 Gy.

Effect of NPs formulation on 30 Days-survival percentage and Relative body weight

The 250 mice were randomly divided into 5 groups, 50 animals each, and kept in separate metal cages marked as groups (A, B, C, D & E) for 24hr before the experiment, as shown in Ten mice of each group exposed to a single total body irradiation (TBI) dose level of 6, 7, 8, 9, or 10 Gy at the 3rd day of the experiment. After irradiation, the animals were kept for 30-days in separate metal cages, given free access to pelleted food and water. Cages were examined daily, for animal deaths and expressed as percentage survival and the $LD_{50/30}$ was determined by BioStat software (AnalystSoft Inc.).

Also, the mean body weight of living mice per group was recorded weekly for 30 days after irradiation. Dose-reduction factor (DRF) (Hosseinimehr *et al.,* 2007), and relative body weight (RBW) [Moreno *et al.,* 2010), were used to compare the efficacy of protective formulations in experimental animals.

DRF = $LD_{50/30}$ with drug / $LD_{50/30}$ without drug.

RBW = Body weight / initial weight before starting the treatment.

Effect of NPs formulation on liver and spleen cell counts

125 mice were randomly divided into 5groups of 25 animals each, kept in separate metal cages marked as group (A, B, C, D & E) for 24hr before the experiment, At the 5th day of the experiment 5 animals of each group exposed to a single TBI dose level of 6, 7, 8, 9, or 10 Gy.

After 6 hours of radiation exposure, all mice were sacrificed. Mice livers and spleens were excised, formalin-fixed, and tissue sections (4-5 μm) were stained with hematoxylin and eosin and examined under light microscope. The total number of cells per section was counted using image analysis software (BEL Micro Analyzer) at a magnification of 40X and the mean cell numbers per group were calculated(Jeong *et al.,* 2007).

Data analysis

Data were analyzed by using SigmaPlot 11 software (Systat Software Inc.) and the data were expressed as mean ± SD. To determine the statistical significance of the results, one-way variance analyses (ANOVA) and Holm-Sidak test were performed. The minimum level of statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Penicillamine was first used clinically as a copper chelating agent in the treatment of Wilson's diseases and in lead poisoning. Penicillamine significantly reduces mortality resulting from radiation-induced pulmonary fibrosis in mice irradiated to the whole thorax [(]Ward *et al.,* 1982), and can protect male mice against whole-body irradiation lethality when the drug was injected (ip) [(]Ward *et al.,* 1980).

Different mechanisms can explain the radioprotective effect of Penicillamine which mainly includes: chelation (of radioactive isotopes (Jerrold *et al.,* 2007; NCRP report No.65. http://NCPRpublication.org**)** or serum redox-active metal ions such as iron and copper which act as sources of extracellular oxidants (Rees *et al.,* 2001), antioxidant activity where Penicillamine contain various functional groups (e.g., −SH, −COOH, $-NH_2$) which can serve as a hydrogen and electron donor; receive energy transferred from irradiated molecules; scavenge hydrogen atoms, hydroxyl free radicals, and hydrated electrons; and can form mixed disulfides with macromolecules (ward *et al.,* 1980;ward *et al.,* 1982).

Finally, Penicillamine reduces fibrotic growth of alveoli, improves arterial perfusion, and moderates collagen accumulation and thus significantly reduces mortality resulting from radiation-induced pulmonary fibrosis in mice irradiated to the whole thorax (Ward *et al.,* 1980; Ward *et al.,* 1982; Ward *et al.,* 1988).

Potassium iodide (KI) has been used therapeutically for more than 150 years and continues to be used worldwide because of its effectiveness and low cost. Iodide is an essential component of thyroid hormones (Thyroxine (T4) and Triiodothyronine (T3)) that play an important role in human development, growth and metabolism. [(28)] KI is also used as a protectant of the thyroid gland from exposure to radioactive isotopes of iodine (Franij *et al.,* 1999). Food and Drug Administration (FDA) 1982 recommended the use of KI to reduce the risk of thyroid cancer in radiation emergencies involving the release of radioactive iodine (Jarrett *et al.,* 2005; http://www.chemicalelements.com/elements/i.htm).

The radioprotective mechanisms of KI mainly occur via blocking normal organ (especially thyroid) uptake of different radioactive iodine isotopes (I-125, or I-131, etc.) (Jarrett *et al.,* 2007; Jerrold *et al.,* 2007), or by iodide antioxidant and free radical scavenging properties (Winkler *et al.,* 2000; Ali *et al.,* 2002). The first antioxidant activity of iodide was found in algae which absorbs increased amounts of iodine when placed under oxidative stress for protection against reactive oxygen species (superoxide anion, hydrogen peroxide, and hydroxyl-radical (Miller *et al.,* 2006).

In humans the antioxidant activity of iodide can be explained by a direct effect of iodide as an electron donor to neutralize H_2O_2, and converting it to water during thyroid hormones formation (Smyth *et al.,* 2003). While the in-direct mechanism involved that iodine act as a cofactor for antioxidant enzymes (such as plasma catalase and glutathione peroxidise (Venturi *et al.,* 1999; Winkler *et al.,* 2000). The antioxidant properties of iodide described for the thyroid also apply to other tissues having the ability to concentrate iodide such as salivary glands, gastric mucosa and mammary glands (Smyth *et al.,* 2003). These investigations proved the iodide-antioxidant defence effect in breast cancer, and isolated rabbit eyes (Venturi *et al.,* 1999; Smyth *et al.,* 2003).

Nanoparticles preparation and evaluation

The PLGA NPs were prepared using the emulsion-solvent evaporation method, and the average diameters of blank (w/o/w no drug) and drug-loaded NPs were determined from SEM photos (Philips XL30, Netherlands). Morphological characterization showed that all NPs were spherical in shape, about 200-400 nm in diameter, and almost no polymer remnants. The NPs-drug content was 63 and 192 mg/gm NPs for Penicillamine and KI, respectively and the PLGA-NPs were successfully sustained the in-vitro release of both hydrophilic drugs over 5 days time period (data not shown).

Effect of NPs formulation on 30 days-survival percentage

The incorporation of Penicillamine or KI into PLGA-NPs was explored as a method to sustain the effect of these dugs. The results of the survival studies for Penicillamine or KI formulations after gamma-rays irradiations are summarized.

Penicillamine formulations (free drug and loaded NPs) can reduce mortality (induced by radiation) when compared with control or blank NPs treatments. On the other hand, mice treated with blank NPs failed to increase the 30-day survival percentage. Free Penicillamine formulation (gp C) at 50mg/km oral dose shows 90, 70, & 40% survival percentage at 6, 7, & 8 Gy radiation doses respectively, but no survival observed at 9 & 10 Gy

radiation doses, indicating the drug protective action at sub-lethal doses of gamma radiation (6, 7, & 8 Gy). Penicillamine-loaded NPs formulations (gp D and E) showed a higher survival percentage (100, 90, & 70%) and (100, 80, &60%) at lower level of radiations (6, 7, & 8 Gy), respectively. In addition, Penicillamine-NPs formulation (gp D) shows a certain level of protection (10% survival) at 9Gy radiation dose.

Table shows the $LD_{50/30}$ and DRF results for the different groups (A, B, C, D & E). The $LD_{50/30}$ for control (gp A) was 7.4 Gy , for blank NPs (gp B) 7.4 Gy, for free Penicillamine (gp C) 7.8 Gy , for Penicillamine-NPs (gp D) 8.4 Gy and Penicillamine-NPs (gp E) 8.2 Gy. This resulted in a DRF value of 1 for blank NPs, indicating that PLGA polymer has no radioprotective activity and a DRF of 1.05 for 50mg/km free Penicillamine (gp C) indicating the drug radioprotective activity and is consistent with those reported earlier (1.04-1.13) (Afzal *et al.,* 1987).

The DRF value for Penicillamine-NPs (gp E) containing 125mg/ drug was close to that of free Penicillamine (gp C) (total 250mg/ during 5 days) where the DRF was 1.10 for gp E versus 1.05 for gp C. However, in Penicillamine-NPs (gp D) containing 250mg/ drug the DRF value (1.14) was higher than both group C & E.

These results indicated that the single depot administration of Penicillamine-NPs (gp E 125mg/) was effective as free Penicillamine (gp C) and could sustained for 5 days in a lower drug dosing, which proved the suitability of the NPs-PLGA based system to modulate drug activity in-vivo. Finally, the results indicated that the radioprotective activity of Penicillamine increase by dosing increase (gp D containing 250mg/ drug has a higher DRF value than gp E containing 125mg/ drug) which is consistent with previous reports (Ward et el.,1982; Afzal *et al.,* 1987).

The results of KI formulations, free drug and loaded NPs, survival studies are represented . Free KI formulation (gp C) at 10mg/ oral dose shows 90, 80, & 50 % survival percentage at 6, 7, & 8 Gy radiation doses, respectively. These results proved radioprotective activity compared to control group. These result is consistent with earlier reports that in-organic radical scavengers (such as KI, $KMNO_4$, KNO_3, and $K_4Fe(CN)_6$) have an in-vitro radioprotective activity Kesavan *et al.,* 1978). KI-NPs (gp D& E) containing 25-50mg/ drug showed (90, 90, 60%) and (90, 80, 40%) survival percentage at 6, 7, &8 Gy radiation doses, respectively. At 9&10 Gy gamma-ray doses, non of KI-formulations provide any survival indicating minimal iodide activity at higher radiation doses. The $LD_{50/30}$ for free KI (gp C) 7,6 Gy , for KI-NPs (gp D) 8 Gy and KI-NPs (gp E) 7.8 Gy which indicate that NPs formulations have a protective action similar to, or even superior to, that of free drug against mortality. The DRF for KI-formulations were 1.02 for free KI (10mg/ for 5days), 1.08 For KI-NPs (50mg/), and 1.05 for KI-NPs (25mg/). These DRF values indicate that PLGA NPs were able to improve drug activity in contrast to the free drugs at equivalent doses. Several reports referred that biodegradable-NPs can improve the activity of antioxidant drugs such as Ellagic Acid and Melanin (Sharma *et al.,* 2007; Sonaje *et al.,* 2007; Dadachova *et al.,* 2010).

Effect of NPs formulation on Body weight studies

The body weight gain after 4 weeks was inversely proportional to radiation dose which mainly attributed to radiation damage effect on GIT mucosal membrane and thus reduction of feed nutrients intake (Lappen *et al.,* 1971; Ward *et al.,* 1982; Shoyinka *et al.,* 2008). The results of relative body weight studies for Penicillamine or KI formulations after gamma-rays irradiations are summarized in tables (4&5). In drug untreated mice groups (control and blank NPs), body weight was inversely proportional to radiation dose at all times and the relative body weight of both groups was not significantly different indicating that PLGA polymer has no effect on irradiated animals. Penicillamine formulation treatments clearly show that Penicillamine can improve the relative body weight (RBW) up to 9 Gy dose of radiation. The mechanism of this effect on body weight gain can be related to that Penicillamine protects essential thiol enzymes in the anabolic pathways against inactivation, and thus increase cellular protein levels, by maintaining a suitable intracellular redox state (Chou *et al.,* 1975). At 6 Gy irradiation Penicillamine formulations (free, 250mg NPs, and 125mg NPs) showed a higher relative body weight versus control or blank NPs at all times. The same finding observed at 7,8, & 9 Gy, but at 10 Gy all penicillamine formulations fail to improve RBW. The effect of 250mg NPs (gp D) is relatively higher than free and 125mg NPs (gp C&E) especially at 9 Gy radiation dose. These results proved the positive effect of NPs formulation on penicillamine activity.

In KI formulations table (5), all mice, including control and blank NPs groups, lost weight at the first week as a result of irradiation stress. Thereafter, the body weight of KI formulations treated mice increased during the second week at 6, 7&8 Gy doses. This effect disappears at 9&10 Gy doses indicating KI activity at low level radiation doses only. The protection of intestinal mucosa from radiation damage, likely occurs at the level of the cell membrane as a result of the iodide antioxidant and radical scavenging capabilities. (Mansour *et al.,* 2006). The effect of NP formulation on KI activity is observed in gp D (50mg NPs) and E (25mg NPs), where KI 50mg NPs (gp D) shows a relatively higher retention of body weight compared with free KI or 25mg NPs (gp C & E) indicating the efficacy of NP formulation.

Effect of NPs formulation on liver and spleen cell counts

Radiation therapy is widely used to treat and to prevent the recurrence of many body tumors. Nowadays, it is known that radiation is a kind of oxidative stress for tissues. When water, which constitutes around 80% of the cell, is exposed to ionizing radiation, decomposition occurs through which, a variety of reactive oxygen free radical species (ROS) generated, and interact with cellular molecules, including DNA, lipids, and proteins and resultant apoptosis or cell death (Sezen *et al.,* 2008). Actively dividing cells are at the greatest risk for radiation damage, hence, tissues with generative capabilities and high kinetics are radio-sensitive and first to show damage after exposure (Wyffels *et al.,* 2007). The multiple biological effects of ionizing radiation on sensitive tissues/organs such as liver, bone marrow, and spleen

have been reported (Jordan *et al.,* 1967; jeong *et al.,* 2007; Wyffels *et al.,* 2007). Tissue necrosis and the decrease of total number of cells were induced by the increase of radiation dose in a dose-dependent manner (Jordan *et al.,* 1067;jeong *et al.,* 2007;Wyffels *et al.,* 2007).

Many studies have focused on the prevention of radiation adverse effects on the radio-sensitive organs especially bone marrow, liver, and spleen. Previous experimental studies demonstrated that antioxidant drugs, such as vitamin E, L-Carnitine, and Amifostine were able to reduce injurious effects of irradiation on the radio-sensitive organs and tissues (Mansour *et al.,* 2006; Sezen *et al.,* 2008). In all of the tissues studied, a radioprotective effect was observed, although the degree of protection varied widely among the different tissues according to drug distribution, the antioxidant drugs commonly act through detoxification of oxygen free radicals generated by radiation (Mansour *et al.,* 2006).

This could be protecting cell membrane and prevent lost of membrane permeability induced by oxygen free radicals interaction (Mansour *et al.,* 2006). Currently, with the recent rapid development of nanoscience and nanotechnology, interest in developing of radioprotective NPs has been gradually diverted to protect body organs against radiotoxicity during radiation therapy of cancer. Melanin NPs and Fullerene NPs are recent examples for NPs formulations used to protect bone marrow (Melanin), liver, and spleen (Fullerene) against radiation induced oxidative damage in animal models (Pandey *et al.,* 2003; Dadachovaa *et al.,* 2010). In the present study, the direct radioprotective activity of drug-loaded nanoparticles (Penicillamine-NPs and KI-NPs) was investigated with respect to free drug and blank NPs activity by liver and spleen cell-counting from tissue section microphotographs.

The effect NPs formulations on the total cell count in mice liver and spleen treated with different penicillamine or KI formulations were showed.

In all animal groups (control, blank NPs, free drug (Penicillamine and KI), and drug-loaded NPs) a marked decrease in total cell count (liver/spleen) by elevation of gamma-ray doses from 6 to 10 Gy, indicating the acute/direct damage effect of ionising radiation even with drug treatment. There was no significant difference between control (drug-untreated) and blank-NPs treated groups in both liver and spleen cell count which indicate the absence of any radio-protective response as a result of blank PLGA-NPs administration.

Tables (6&7) showed the effect of Penicillamine formulations on total cell count at different ray doses. A significant degree of protection is observed at 6 and 7 Gy doses, while no effect at higher doses 8, 9, and 10 Gy. The order of protection degree at 6&7 Gy was gp D (250mg/ NPs) > gp C (free penicillamine 50 mg/ for 5days) > gp E (125mg/ NPs). These sign indicates the need of a higher doses of penicillamine-NPs for protection against the acute effect of radiation on liver and spleen cells, which is in agreement with the commonly accepted view that penicillamine activity is a dose-dependent process (ward *et al.,* 1980;Ward etal.,1982). Tables (8&9) showed the effect of KI formulations on total cell count at different ray doses. The only protective effect is observed at 6Gy, while no radio-protective response at 7, 8, 9&10Gy radiation doses. The KI-NPs gp D (50mg/ NPs) shows a higher degree of protection for the spleen-cells than free KI gp C (10 mg//for 5days) and KI-NPs gp E (25mg/ NPs). In the liver cell count similar results are observed for KI-NPs (gp D) and free KI (gp C).

CONCLUSION

1- Penicillamine and KI formulations (free drug and loaded NPs) significantly reduce mortality induced by radiation when compared with control or blank NPs treatments.
2- Blank PLGA-NPs failed to increase the 30-day survival percentage, indicating that PLGA polymer not show any radio-protective activity.
3- The radioprotective activity of both Penicillamine and KI formulations observed mainly at sub-lethal doses of gamma radiation (6,7,&8 Gy).
4- Single administration of Penicillamine-NPs (125mg/) or KI-NPs (25mg/) were effective as free Penicillamine (total 250mg/ in 5 days) and free KI (total 50mg/ in 5days), respectively, indicate that it possible to have a slow and controlled release of water-soluble radio-protective drugs by NP-encapsulation, a feature not obtained with free drug alone.
5- The radioprotective activity of Penicillamine increase by dosing increase (gp D containing 250mg/ drug has a higher DRF value than gp E containing 125mg/ drug).
6- Penicillamine and KI formulations (free drug and loaded NPs) showed a higher relative body weight versus control or blank NPs at 6,7,&8 Gy and only Penicillamine-NPs (250mg/) can improve the relative body weight up to 9 Gy dose of radiation.
7- In all animal groups (control, blank NPs, free drug (Penicillamine and KI), and drug-loaded NPs) a marked decrease in total cell count (liver/spleen) by elevation of gamma-ray doses from 6 to 10 Gy, indicating the acute/direct damaging effect of ionising radiation even with drug treatment.
8- The radio-protective activity of loaded-NPs (Penicillamine or KI) against the acute radiation effect on liver and spleen cells are equal or slightly lower than free drug of equal doses at 6,7Gy irradiation.
9- Penicillamine-NPs formulation containing 250mg/ drug has a significant degree of (liver and spleen) protection at 6 and 7 Gy doses, while KI-NPs formulation containing 50mg/ drug has only protective effect is observed at 6Gy.
10- The results of drug-loaded PLGA-NPs (Penicillamine or KI) revealed that nanoparticles could be used to modulate radioprotective drug activity in biological system, and could improve the drug efficacy in different body organs for longer duration than the equal dose of free drug.

Fig. 1: Mouse restrained in a removed-end 50ml Falcon tube.

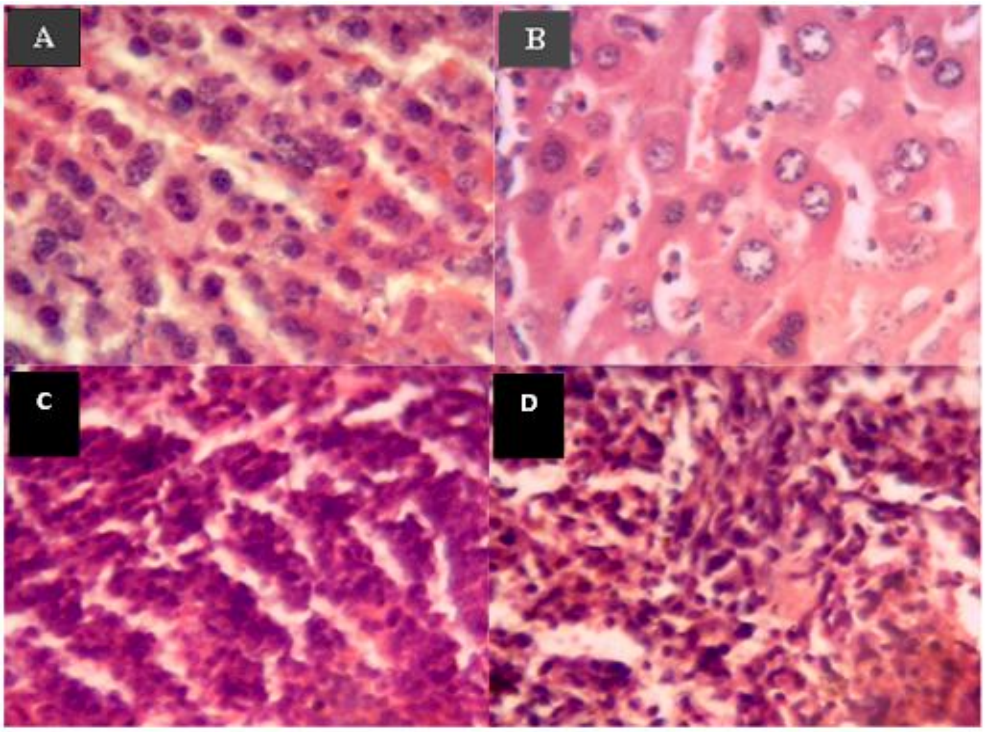

Fig. 2: Microphotographs of mice liver and spleen sections at magnification of 40 X; (A) Normal hepatocytes, (B) Hepatocytes after gamma-ray irradiation, (C) Normal spleen cells and (D) Spleen after gamma-ray irradiation.

Table. 1: Penicillamine and KI formulations tested in this study.

Group	Formulation*	Penicillamine Dose (mg/)	KI Dose (mg/)	Day of administration
A	Control (D. water)	---	---	1,2,3,4,5
B	Blank NPs (50mg)	---	---	1,2,3,4,5
C	Free drug	50	10	1,2,3,4,5
D	Loaded- NPs	250	50	1st only
E	Loaded- NPs	125	25	1st only

Table. 2: Penicillamine formulations 30-days Mortality.

Group	6Gy	7Gy	8Gy	9Gy	10Gy	$LD_{50/30}$	DRF
A (control)	20%	40%	70%	100%	100%	7.4	1
B (blank NPs)	20%	40%	70%	100%	100%	7.4	1
C (free drug 50mg/)	10%	30%	60%	100%	100%	7.8	1.05
D (drug-NPs 250mg/)	0%	10%	30%	90%	100%	8.4	1.14
E (drug-NPs 125mg/)	0%	20%	40%	100%	100%	8.2	1.10

Table. 3: KI formulations 30-days Mortality.

Group	6Gy	7Gy	8Gy	9Gy	10Gy	$LD_{50/30}$	DRF
A (control)	20%	40%	70%	100%	100%	7.4	1
B (blank NPs)	20%	40%	70%	100%	100%	7.4	1
C (free Drug 10mg/)	10%	20%	50%	100%	100%	7.6	1.02
D (drug-NPs 50mg/)	10%	10%	40%	100%	100%	8	1.08
E (drug-NPs 25mg/)	10%	20%	60%	100%	100%	7.8	1.05

Table. 4: Relative body weight (RBW) for Penicillamine formulations.

Dose (Gy)	Weeks after irradiation	Group(A) Control	Group(B) Blank NPs	Group(C) Free drug	Group(D) Loaded- NPs	Group(E) Loaded- NPs
6	0	1	1	1	1	1
	1st	0.94	0.943	0.967	0.995	0.967
	2nd	1.123	1.126	1.153	1.162	1.139
	3rd	1.059	1.06	1.172	1.194	1.176
	4th	0.889	0.892	1.204	1.208	1.2
7	0	1	1	1	1	1
	1st	0.843	0.848	0.897	0.885	0.894
	2nd	0.834	0.834	0.925	0.912	0.903
	3rd	0.82	0.811	0.939	0.935	0.917
	4th	0.792	0.797	0.911	0.908	0.903
8	0	1	1	1	1	1
	1st	0.807	0.8	0.86	0.892	0.869
	2nd	0.784	0.781	0.893	0.915	0.901
	3rd	0.775	0.777	0.855	0.878	0.864
	4th	0.766	0.767	0.828	0.836	0.822
9	0	1	1	1	1	1
	1st	0.776	0.768	0.841	0.868	0.858
	2nd	0.72	0.717	0.813	0.924	0.82
	3rd	---	---	---	0.83	---
	4th	---	---	---	0.792	---
10	0	1	1	1	1	1
	1st	0.705	0.705	0.778	0.788	0.777
	2nd	---	---	0.735	0.755	0.75
	3rd	---	---	---	---	---
	4th	---	---	---	---	---

Table. 5: Relative body weight (RBW) for KI formulations.

Dose (Gy)	Weeks after irradiation	Group(A) Control	Group(B) Blank NPs	Group(C) Free drug	Group(D) Loaded- NPs	Group(E) Loaded- NPs
6	0	1	1	1	1	1
	1st	0.94	0.943	0.977	0.981	0.995
	2nd	1.123	1.126	1.133	1.144	1.134
	3rd	1.059	1.06	1.142	1.158	1.143
	4th	0.889	0.892	1.156	1.176	1.152
7	0	1	1	1	1	1
	1st	0.843	0.848	0.893	0.897	0.885
	2nd	0.834	0.834	0.902	0.925	0.899
	3rd	0.82	0.811	0.916	0.939	0.908
	4th	0.792	0.797	0.856	0.906	0.853
8	0	1	1	1	1	1
	1st	0.807	0.8	0.853	0.865	0.861
	2nd	0.784	0.781	0.88	0.907	0.898
	3rd	0.775	0.777	0.848	0.884	0.861
	4th	0.766	0.767	0.798	0.861	0.8
9	0	1	1	1	1	1
	1st	0.776	0.768	0.819	0.814	0.811
	2nd	0.72	0.717	0.75	0.759	0.751
	3rd	---	---	0.699	0.708	0.7
	4th	---	---	---	---	---
10	0	1	1	1	1	1
	1st	0.705	0.705	0.758	0.76	0.762
	2nd	---	---	0.707	0.714	0.707
	3rd	---	---	---	---	---
	4th	---	---	---	---	---

Table. 6: Total number of hepatocytes after Penicillamine formulations treatment.

Dose (Gy)	No. of total cells per field (Mean ± SD)				
	Group(A) Control	Group(B) Blank NPs	Group (C) Free drug	Group(D) Loaded-NPs	Group(E) Loaded-NPs
6	67.8±7.7	70.4±8.3	91.5±6.3*	92.7±6.4*	89.6±5.6*
7	62.9±7.2	68.1±7.7	84.9±7.8*	86.2±6.6*	83.4±6.5*
8	59.8±6.7	64.2±7.3	69.8±8.2	69.5±8.6	68.7±8.6
9	56.8±6.8	62.1±7.5	67.2±7.3	67.6±7.5	66.9±8.2
10	53.8±6.4	58.3±6.8	64.6±8.2	64.5±6.4	63.5±8.3

*Significantly different from control (gp A) at P<0.05.

Table. 7: Total number of splenic-cells after Penicillamine formulations treatment.

Dose(Gy)	No. of total cells per field (Mean ± SD)				
	Group(A)Control	Group(B)Blank NPs	Group(C)Free drug	Group(D)Loaded- NPs	Group(E) Loaded- NPs
6	358.7±20.2 330.6±19.7	385.3±18.6	429.7±18.3*	438.8±19.2*	421.6±19.8*
7	312.7±17.3 295.4±18.9	350.3±20.1	409.4±19.7*	419.6±19.8*	392.7±20.1*
8	277.6±18.6	340.4±19.3	344.7±18.3	347.5±18.3	345.4±17.8
9		322.2±18.4	322.8±19.2	327.4±19.4	323.7±18.4
10		299.6±19.7	306.4±18.6	309.2±19.2	309.6±18.9

*Significantly different from control (gp A) at P<0.05.

Table. 8: Total number of hepatocytes after KI formulations treatment.

Dose(Gy)	No. of total cells per field (Mean ± SD)				
	Group(A)Control	Group(B)Blank NPs	Group(C)Free drug	Group(D)Loaded-NPs	Group(E)Loaded-NPs
6	67.8±7.7	70.4±8.3	90.7±6.4*	91.3±6.3*	88.7±6.8*
7	62.9±7.2	68.1±7.7	73.2±6.5	73.1±6.6	72.8±6.5
8	59.8±6.7	64.2±7.3	70.1±6.6	69.9±6.2	69.5±7.1
9	56.8±6.8	62.1±7.5	66.5±6.4	67.1±7.3	66.3±6.7
10	53.8±6.4	58.3±6.8	63.6±6.5	64.5±6.1	63.2±6.4

*Significantly different from control (gp A) at P<0.05.

Table. 9: Total number of splenic-cells after KI formulations treatment.

Dose(Gy)	No. of total cells per field (Mean ± SD)				
	Group(A)Control	Group(B)Blank NPs	Group(C)Free drug	Group(D)Loaded- NPs	Group(E)Loaded- NPs
6	358.7±20.2 330.6±19.7	385.3±18.6	420.3±16.3*	428.8±17.2*	415.6±15.8*
7	312.7±17.3 295.4±18.9	350.3±20.1	362.7±15.3	362.1±15.4	360.5±14.3
8	277.6±18.6	340.4±19.3	343.3±14.7	342.5±16.3	342.1±15.1
9		322.2±18.4	324.6±15.2	324.8±17.4	325.7±16.3
10		299.6±19.7	305.9±14.4	305.4±19.2	304.7±15.7

*Significantly different from control (gp A) at P<0.05.

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