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In vivo study of nanoparticles as free radical scavengers for radiation protection

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ABSTRACT

Free radicals are formed through ionizing reactions that are then capable of destroying normal tissues during the exposure to radiation. Nanoparticles are gaining interest in the field of radioprotection. Radioprotecting ability of nanoparticle was evaluated in an in vivo model using albino mice. The aim of the present study is the investigation of the ability of silver nanoparticles (AgNPs), gold nanoparticles (AuNPs) and cerium oxide nanoparticles (CeO_2NPs) to protect skin tissues of mice against gamma radiation of whole body exposure to 1.2Sv equivalent dose. The administration of nanoparticles protected the skin cell against radiation-induced damages as revealed by histopathologcal examination of section of skin cell. When administered with nanoparticles at 1 hour prior to whole-body radiation exposure, skin cell were found protected from radiation-induced abnormalities in various cells.The present work will address the effectiveness of nanoparticles in radioprotection in animal models during radiation exposure which will encourage the development of innovative and new approaches to radiation protection, using nanotechnology.

Key words: Radiation protection; nanoparticles; free radicals; skin tissues.

INTRODUCTION

Ionizing radiations are widely used in society, play a key role in the treatment of cancer and are an important diagnostic tool. The radiation affects human body in highly complicated processes. Various degrees of biological effects, from damage to death of living tissues, involve a number of pathological changes in human cells [1]. When exposed to ionizing radiation, large molecules such as nucleic acid and proteins in the cells will be ionized or excited. This may cause changes in the molecular structures which then affect the function and metabolism of the cells [2].Because human tissues contain 80% water, the major radiation damage is due to the aqueous free radicals, such as superoxide, hydrogen peroxide, and hydroxyl radical, generated by the action of radiation on water. These radicals react with cellular macromolecules, such as DNA, RNA, proteins, membrane, etc, and cause cell dysfunction and mortality [3].

Protection against ionizing radiation is of serious importance during accidental and unavoidable exposures to radiation and development the effective approaches to reduce radiation damages using non-toxic radioprotectors are of considerable interest for defense, nuclear industries, radiation accidents, space travels, etc., besides the protection of normal tissues during radiotherapy of tumours and medical diagnostic exposures [4]**.**

Nanotechnology is actually an archivolt of modern technology that covers the whole spectrum of science which includes physics, chemistry, and biology as well as engineering and micro-fabrication techniques. It is best defined as the design, production, and application of structures, devices, and systems through control of size and shape of the materials at 10^{-9} of a meter scale [5]**.**Functionalities can be added to nanomaterials by interfacing them with biological molecules or structures. Because of the size of nanomaterials is similar to that of most biological molecules and structures; therefore, nanomaterials can be useful for both in vivo and in vitro biomedical research

and applications [6].Recently, nanoparticles are gaining interest in the field of radioprotection as cerium oxide nanoparticles, yttrium oxide nanoparticles, carbon nanoparticles, etc. were found to possess antioxidant properties and several works have shown the ability of these nanoparticles to offer protection against radiation damages [7-9].

Silver, gold and ceriumoxide nanoparticles have been known to possess excellent free radical scavenging, antibacterial and anti-inflammatory activities [10-12]**.**

In the present study, silver nanoparticles (AgNPs), gold nanoparticles (AuNPs) and cerium oxide nanoparticles (CeO_2NPs) were used to investigate their ability to protect skin tissues of albino mice against gamma radiation of whole body exposure to 1.2Sv equivalent dose.

MATERIALS AND METHODS

1-Animals

Albino mice of 10-12weeks old, weighing 20-25g was obtained from National Center for Control and Pharmaceutical Research. There were kept under standard condition of temperature and humidity in the Bio-technology Research Center/Al-Nahrin University, Baghdad, Iraq.

2-Chemicals

Silver and gold colloidal synthesized by electrical explosion wire (EEW) method were used, having average particle size 50 nm and 60nm respectively. CeO_2NPs with 40nm particle size were purchased from Sigma Aldrich, Germany by United Tetra Group for Medical and Scientific Supplies / Jordan.

3-Irradiation with Gamma-Radiation

Irradiation was carried out using a ^{137}Cs gamma source at a dose rate of 0.96rad/hr. For in vivo study, the animals were irradiated with whole body exposure at 1.2Sv.

METHODS

1-Nanoparticles toxicity

To monitor the acute toxicity of nanoparticles that used or its well-tolerated in animals, mice were randomized into four groups comprising of 5 animals. The First group is a normal without any dose of nanoparticles, and other three animals groups were injected severally with AgNPs (0.2 ml equivalent to 38.7 µg/ml), AuNPs (0.2ml equivalent to 268 µg/ml) and CeO_2NPs(0.2 ml equivalent to 50 µg/ml)and daily examined for any changes in behavior. The mice were observed over a three-week period. At the end of the treatment, the mice were sacrificed to test the tissues and organs of mice if affected by the dosage of nanoparticles.

All the mice survived throughout the experimental period without exhibiting any abnormalities. The mice did not show any symptoms of toxicity such as fatigue, loss of appetite, change in fur color and weight loss.

2-Protection of mice tissues by nanoparticles

In order to protect the mice tissues due to radiation effect (whole body exposure) by the advantage of nanoparticles, the animals were divided into five groups and treated as follows:

First group is the normal without any irradiation and treatment. Second group: the animals exposed to gamma radiation of ^{137}Cs with equivalent dose 1.2 Sv. Third group: the animals treated with AgNPs (0.2 ml equivalent to38.7 µg/ml).Fourth group: the animals treated with AuNPs (0.2ml equivalent to268 µg/ml).Fifth group: the animals treated with CeO_2NPs (0.2 ml equivalent to 50 µg/ml).

The animals in the last three groups were administered one hour before irradiation with the same radiation dose in the second group. After three days of irradiation, some animals were sacrificed to check the tissues and vital organs that affected by radiation as well as treated by nanoparticles and compared with the normal animals.

Histopathological examination

In order to study the radiation protection by nanoparticles has been proposed this examination. Gamma radiation with equivalent dose 1.2Svand the above treatment dose of nanoparticles were applied to the animals and studied the effect of radiation and the treatment of skin cells, whereas this dose of radiation lead to the influence of inflammatory in the inner tissues of the animals skin that irradiated. The histological analysis over the oxidative stress after the irradiation was performed by examining the morphological changes in skin tissue induced by radiation, further more the analysis of the treatment with nanoparticles.

The present study was carried out at the Bio-technology Research Center/ Al-Nahrin University. After four day of irradiation and treatment, the animals were sacrificed. The tissue of interest, skin, were immediately fixed in 10% buffered neutral formalin solution, embedded in paraffin, and cut into 5-µm-thicksections. The fixed sections were stained for analysis using hematoxylin and eosin (H and E) staining. The sections were examined under light microscope and photomicrographs of the fixed skin tissues were obtained for study the microscopic morphology of the skin.

RESULTS AND DISCUSSION

In vivo nanoparticles toxicity studies are focused mainly on monitoring the behavior of mice during the period of treatment and examining changes in tissues and organs morphology after necropsy. All the mice survived throughout the experimental period without exhibiting any abnormalities. The mice did not show any symptoms of toxicity such as fatigue, loss of appetite, change in fur color and weight loss. At the end of the treatment, the mice were sacrificed. During necropsy no abnormal pathologies were observed in tissues and vital organs (liver, kidney, spleen and lung) about the shape and size of these organs. Comparative observation of various tissues and organs in the silver and gold nanoparticles treated and control animals, clearly showed that there was no significant alteration. Whereas the examined reports obtained from the senior pathologist confirmed that the nanoparticles treated mice did not show any significant morphological changes in comparison to control. Our results corroborate with the previous researches made by Hainfeld et al [13] in using gold nanoparticles which exhibited a non-toxic effect over the blood chemistry and vital organs. Colon et al. were suggested that CeO_2nanoparticles cause limited toxicity and side effects in mice. Therefore, it was suggested that CeO_2nanoparticles causes limited toxicity and side effects in mice [14].Therefore in the present study, after confirmation of the non-toxic nature of the used nanoparticles and its dosage, the effect of the nanoparticles over the oxidative stress induced by 1.2 Sv equivalent dose was investigated.

Histological analysis over the skin tissues of albino mice was carried out in order to examine the potency of silver, gold and cerium oxide nanoparticles to prevent the tissues from damage because of free radical formation.

The results of histopathological skin section of normal animal demonstrate no clear lesions and abnormality feature in skin cells as shown in Fig.1. The whole body exposures of animals to gamma radiation with 1.2Sv revealed aggregation of dead and intact neutrophils in the epidermis and in the dermis between hair follicles (Fig.2). Other lesions in the skin characterized by waves of hyperchromatic pleumorphic cells extended from the basal layer of the dermis to dermis with inflammatory cells in the dermis (Fig.3).

For the purpose of avoiding these damages in skin tissues caused by gamma radiation, the animals were treated with nanoparticles (AgNPs, AuNPs, CeO_2NPs) prior of irradiation to study the ability of these materials to prevent the radiation injured the skin cells.

The results of animals treated with AgNPs showed no clear lesion and abnormality of skin cells (Fig. 4). The AuNP streated mice also showed normal skin cells without any significant morphological disruptions in comparison to normal (Fig.5). It was reported moderated inflammatory cells particularly mononuclear cells infiltration in the dermis of animals treated with CeO_2NPs (Fig.6), This disorder is possible to happen even in the absence of exposure to radiation as a result of any dysfunction in the cells or due to CeO_2NPs dosage itself, because this nanoparticles have some toxicity in high range of concentrations [12].

The present study reveal suppurative reaction in the epidermis and dermis, this lesion may be due to gamma radiation induces damages in biological systems either by direct hit or indirectly through generating free radicals especially ROS which damage vital cellular targets. This free radical destroyed the cell wall throw the peroxidation and protein damage or DNA damage. This reaction lead to secreted of pro inflammatory cytosine from epithelial cells and macrophage, these cytosine attract of neutrophillic into the side of injury will lead to suppurative reaction. Also the study show hyper lesion of epidermis due to radiation. In other section hypercromatice, pleomorphic and proliferation cells extend from the epidermis to the dermis; this may be due to damage of sugar DNA and lead to cell division.

Thus the results of AgNPs administration demonstrated the effect of nanoparticles on inhibiting the damages induced by radiation, and the results suggest the radio protecting ability of AgNPs may be attributed to the free radical scavenging property and act as antioxidant materials.

The silver nanoparticle alleviated the extent of acute and chronic inflammation in different skin cells in mice. This may be due to the regulation of different cytokines that are involved in sustaining the inflammatory response along with the combined radical scavenging and anti-inflammatory activities of nanoparticles. This ideas were agreement

with Ramachandran and Nair [3], they suggest the silver nanoparticle - LA complex (SN LA) exhibited anti-inflammatory activity against acute and chronic paw models of edema in mice. SN-LA protected mice from whole body gamma radiation induced body weight losses and mortality revealing its radioprotecting capacity.

Reactive oxygen specious generated by gamma radiation play a vital role in the development of harmful effect complications. It is the resultant of the oxidative stress developed due to the release of free radicals, thereby decreasing the level of antioxidant enzymes. The results of skin section revealed that gold nanoparticles blocked the ROS generation to a maximum extent in the skin cells which is shown in Fig.5. This makes clear the inhibitory effect of gold nanoparticles and act as free radical scavenger during gamma irradiation induced oxidative stress.

Oxidative stress is recognized to plays a foremost role in a wide range of diseases, including cancer. The ability of gold nanoparticles in inhibiting lipid peroxidation and preventing the ROS generation has restored the imbalances in the antioxidants. Our results are suggesting gold nanoparticles potential as antioxidant is coinciding with previous reports delivering the control effects of gold nanoparticles as an antioxidant. The potential ability of AuNPs to inhibit the oxidative stress mediated ROS generation is highly supported by existing evidences of various other nanoparticles such as platinum nanoparticles that had an immense ability to inhibit the pulmonary inflammation led by oxidative stress due to their antioxidant properties [15].

The *in vivo* experiment of mice treated by CeO_2 nanoparticles reinforces the conclusion that CeO2 nanoparticles confer tolerable protection from ionizing radiation. Thus far, studies have shown that CeO_2decrease the accumulation of ROS, and prevent the activation of the ROS-induced inflammatory, aggregation and proliferation of the skin cells, the antioxidant capability of CeO_2nanoparticles has been suggested as the key mechanism by which CeO_2nanoparticles as radioprotection. This ability of CeO_2nanoparticles offer many active sites for free radical scavenging due to their large surface to volume ratio and, more importantly, due to their mixed valence states for unique redox chemistry. Results supporting the antioxidant properties of CeO_2nanoparticles is mounting, and many studies suggest that nanoparticles act as free radical scavengers, whereas the hestopathological study of Cheryl H. Baker [12] in the lung of mice receiving CeO_2nanoparticlesshowed no visible lesion and appeared normal in compare with control (radiation alone). Thus, it has been proposed that CeO_2nanoparticles may enhance radioprotection by scavenging the free radical produced during radiotherapy. In vitro studies have suggested that CeO_2 NP may also have toxic effects at higher concentration. Lin et al. have reported that nanoceria at a concentration between 3.5 and 23.3 µg was able to induce reactive oxygen species production in lung cancer cell causing cell death [16]. Babu et al. have reported the radical scavenging capacity of ceria NPs at a lower, optimum concentration [17]. Therefore, both higher and lower doses can have different applications on different cell types.

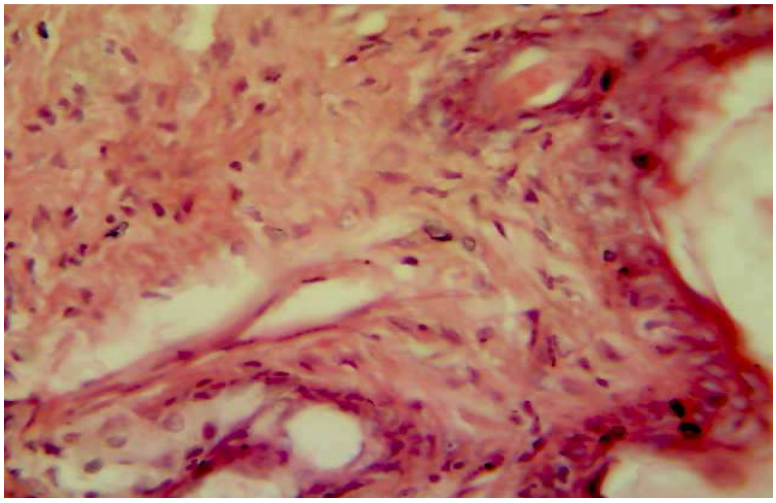

Fig .1: Histopathological section in the skin of normal animal shows no clear lesions (H &E stain 400X)

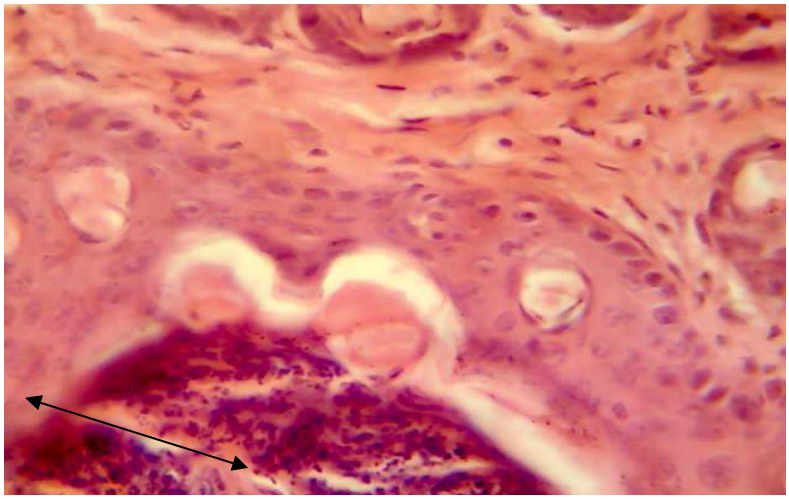

Fig.2: Histopathological section in the skin of animal with whole body radiation (1.2Sv) shows aggregation of dead and intact neutrophils ↔ in the epidermis (H &E stain 400X).

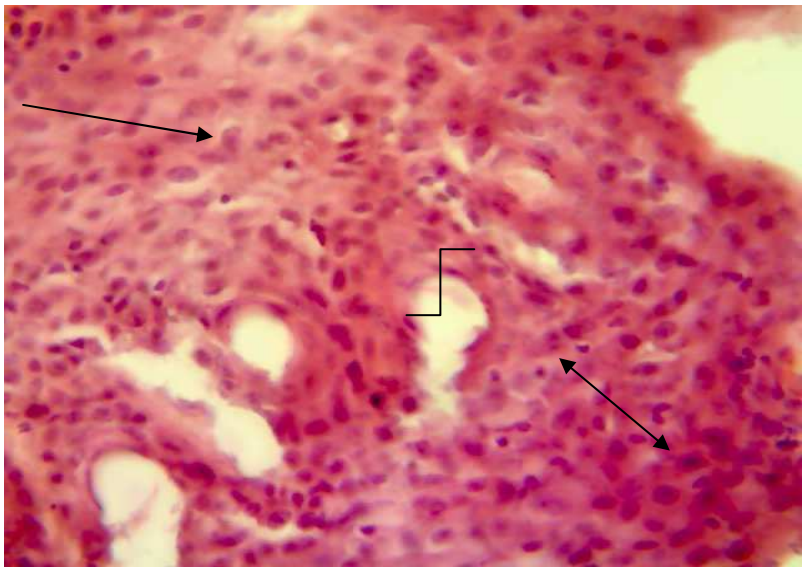

Fig. 3: Histopathological section in the skin of animal with whole body radiation (1.2Sv)shows masses, ↗ sheat ⌐ and ↔ cords of hyperchromaticpleumorphic abnormalcells extended from epidermis to dermis (H &E stain 400X).

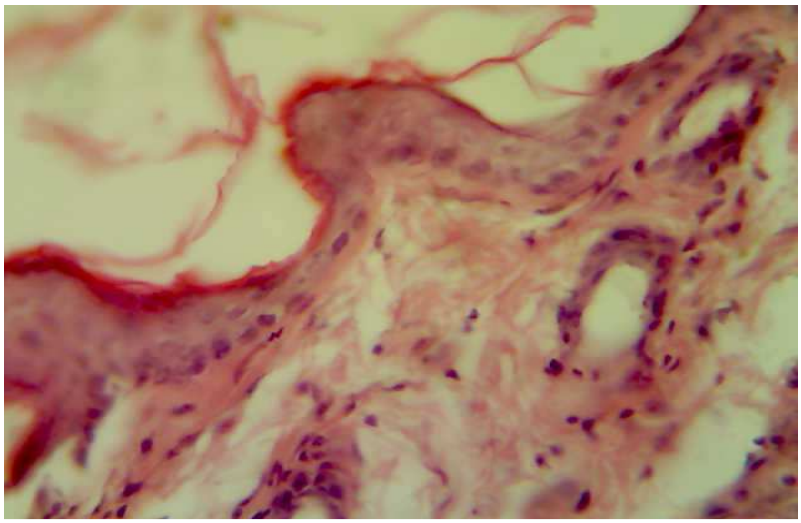

Fig.4: Histopathological section in the skin of animal with whole body irradiation (1.2Sv) and treated with AgNPs shows no clear lesions in dermis and epidermis (H &E stain 400X).

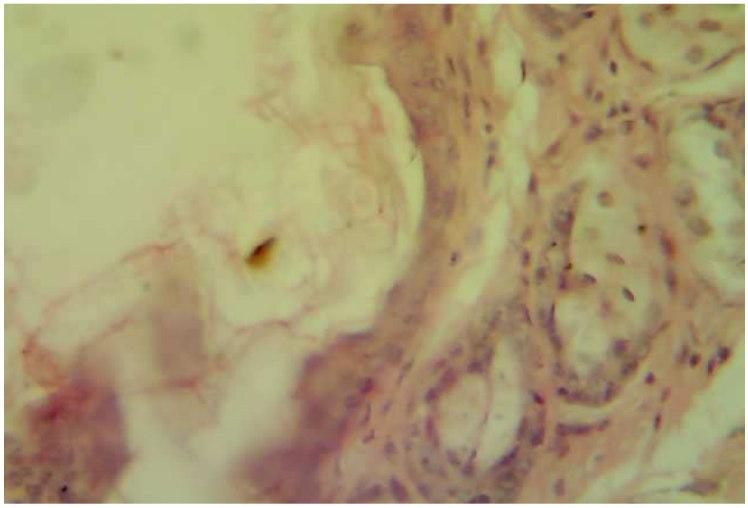

Fig.5: Histopathological section in the skin of animal with whole body irradiation (1.2Sv) and treated withAuNPs shows no clear lesions in dermis and epidermis (H &E stain 400X).

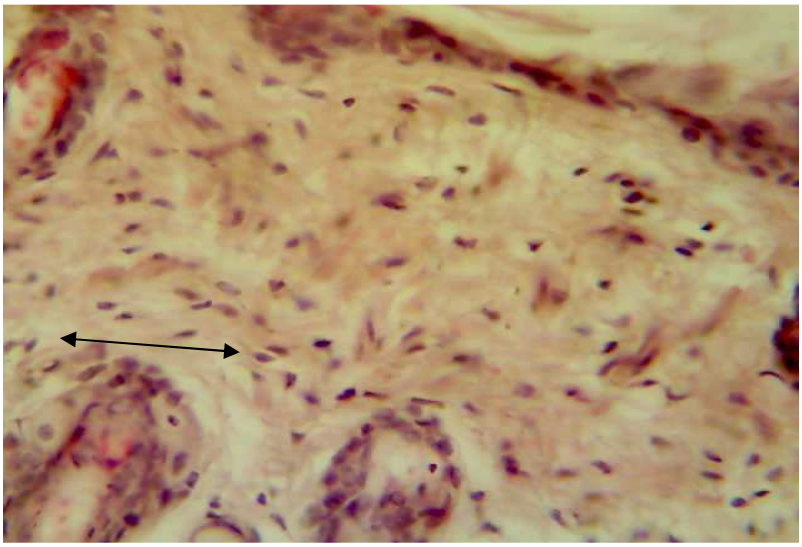

Fig.6: Histopathological section in the skin of animal with whole body irradiation and treated with CeO_2NPs shows inflmammatory cells particularly mononuclear cells infiltration in the dermis ↔ (H &E stain 400X).

CONCLUSION

The present work will address the effectiveness of nanoparticles in radioprotection in animal models during radiation exposure which will encourage the development of innovative and new approaches to radiation protection, using nanotechnology. Thus, nanoparticles are believed at the forefront of the effort to utilize emerging nanotechnology to improve quality of life and healthcare, and that they hold great potential for future clinical trials.

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