

Effect of Melanin nanoparticles (MNPs) on radiation-induced cytotoxicity in Chinese hamster ovary cells

Monira M. Rageh¹, Reem H. EL-Gebaly¹, H. Abou-Shady^{2,3}, Doaa G. Amin³

¹Department of Biophysics, Faculty of Science, Cairo University, Egypt

²Department of Physics, Faculty of Science, Cairo University, , Egypt

³Center of Nuclear Studies and Peaceful Application, Egypt

Abstract

Ionizing radiation is known to cause tissue damage in biological systems due to its ability in producing **high** damage free radicals. Melanin nanoparticles (MNPs) are prepared by simple oxidation of dopamine hydrochloride followed by polymerization. In this study protective effects of melanin were assessed using Chinese hamster ovary (CHO) cells, irradiated with 6 **Gy** Gamma radiations. MNPs prevented radiation-induced loss in cell viability. These results suggest that MNPs may be able to attenuate radiation-induced cytotoxicity, possibly by its ability in scavenging free radicals.

Keywords: Radiation protection, Melanin nanoparticles, Chinese hamster ovary cells, free radicals.

Introduction

The search for more effective radioprotectors has intensified recently due to increased use of ionizing radiation. Presently, ionizing radiation is being used in a large number of therapeutic, industrial and other applications like developing new varieties of high-yielding crops and enhancing storage period of food materials [1, 2]. Radiotherapy treatment, is an important therapeutic option for a number of malignancies, **that** relies on the generation and use of reactive oxygen species (ROS) such as hydroxyl radical ($\cdot\text{OH}$), superoxide radicals (O_2^-), singlet oxygen and peroxy radicals ($\text{ROO}\cdot$) to destroy tumors, and in the process, non-target tissues are also damaged [1, 3-5]. Therefore, the application of ionizing radiation to the treatment of malignant tumors has been limited by the need to avoid extensive damage to normal tissues [2]. Hence,

development of novel and effective nontoxic radioprotectors has a great medical importance [7, 6].

Radioprotective agents are synthetic or natural products that are administered to reduce injuries caused by ionizing radiation. Recently, nanoparticles are gaining interest in the field of radioprotection such as carbon (fullerenes), cerium oxide and Silver nanoparticles. They were found to own antioxidant properties and several studies have shown the ability of these nanoparticles in protection against radiation damages [8-16].

Melanin, a high molecular weight pigment that is ubiquitous in nature takes attention as biomaterial because of its interesting properties and diverse biological functions [17, 18]. It has a various number of functions in the biosystem, including, metal ion chelation, photoprotection to absorb a broad range of electromagnetic radiation, thermoregulation and free radical quenching [17-20]. Many fungi synthesize melanin, which is probable to confer a survival advantage in the environment by protecting against environmental predators, heavy metal toxicity, and physical insults such as ultraviolet and solar radiation [21, 22]. Recently there are studies that have suggested that melanin can be a promising radioprotector [23-26]. Schweitzer et al. used silica nanoparticles as carriers of melanin for delivery into the bone marrow to protect hematopoietic cells against radiation fluxes during radiation therapy, but silica is not optimal to radiation therapy due to its persistence in tissues [25].

A novel synthetic method of size-controllable melanin nanoparticles (MNPs) preparation was developed by Ju et al. [27]. They were specified by having a good dispersibility in water and biological media and an efficient radical scavenging activity.

The purpose of the current study is preparing melanin in the nano-range and investigating the possible protective effects of MNPs in radiation challenged Chinese hamster ovary (CHO) K1 cells.

Materials and methods:

Materials

Dopamine hydrochloride (3, 4-Dihydroxyphenethylamine hydrochloride, MW 189.64), 1N sodium hydroxide (NaOH) solution were purchased from Sigma-Aldrich.

Preparation of Melanin Nanoparticles

MNPs were synthesized according to the method developed by Ju et al., 2011; Lee et al., 2012 [27, 28]. Briefly, 180 mg of dopamine hydrochloride was dissolved in 90 ml deionized water to prepare a dopamine HCl containing aqueous solution (pH 9.8). The solution was mixed by 760 µl of 1 N NaOH solution for neutralization. The resulting product had a faint yellowish color and was stirred with hot plate & magnetic stirrer (MS-300HS, MISUNG SCIENTIFIC CO., LTD, Japan) for 5 hours at 50° C for polymerization. The color of the solution gradually changed from transparent to pale yellow and finally to dark brown. The resulting product was purified by centrifugation three times at 6615 g for 30 min. Finally, pure melanin nanoparticles dispersed in water were obtained after centrifugation at 625 g for 10 min.

Characterization of Melanin Nanoparticles:

The size and morphology of MNPs were studied by scanning electron microscope (SEM) (FEI Quanta FEG 250 SEM, Howland). The mean particle size and the size distribution for MNPs were determined by the light scattering apparatus (Zeta potential/ particle sizer NICOMP-TM 380 ZLS, USA) at 25° C dispersed in deionized water.

The structural characteristic of MNPs was determined by Fourier transform IR spectrophotometer (Basic Vector, 22FT-IR, Germany).

Culture of Chinese hamster ovary (CHO) cells:

Chinese hamster ovary (CHO) K1 cells were obtained from the holding company for biological products and vaccine (VACSERA, Egypt). (CHO) K1 cells were grown in Ham's F-12 culture medium (Lonza) and 10% fetal bovine serum (Biowest). The cells were maintained in a humidified incubator at 37 °c and supplied with 95% O₂ and 5% CO₂.

Irradiation of cells:

The irradiation of the cells was carried out at the **Radiotherapy Department** of the Children's Cancer Hospital, Egypt, using 6 MeV photon beam generated by Siemens linear accelerator model Siemens ONCOR Expression (Siemens Medical Solutions, Malvern, PA), output factors were checked once a week. Flatness of the field was also checked once a week and was maintained within 2%. A 6 Gy radiation dose was used in this study.

Cell viability:

Cell viability was measured by the neutral red assay [29]. CHO cells were cultured into 96-well plate at a density (5,000 cells/ well). After the cells were attached, the original media solution was replaced with a media solution containing four different concentrations (6.25, 12.5, 25 and 50 $\mu\text{g/ml}$). Plain media was used as the control. After 3 hours, cells in the radiation groups were exposed to radiation, while cells in the control group did not receive any radiation. Cells were returned to the incubator and maintained at 37 °C, 95% O₂, and 5% CO₂ for an additional 24 h after irradiation. Then 20 μl of neutral red solution was added to each well and plates were incubated for additional 24 hours, and the absorbance was read at 540 using an ELIZA microplate reader (Meter tech. ξ 960, USA).

Results& Discussion:

MNPs were prepared by simple oxidation of dopamine hydrochloride according to the method of Ju *et al*, 2011; Lee et al, 2012 [24, 25] and were characterized using several techniques. FT-IR spectrum was used to confirm melanin structure, where figure (1) shows the FT-IR spectrum of MNPs. Absorption band with varying intensities were observed at 3456 cm^{-1} , 2357 cm^{-1} , 2194 cm^{-1} , 1640 cm^{-1} , 1531 cm^{-1} , 1424 cm^{-1} , 1321 cm^{-1} , 878 cm^{-1} , 763 cm^{-1} and 665 cm^{-1} . The bands could arise from symmetric and asymmetric stretching and bending of bonds in a variety of functional groups such as amine, carboxylic, carboxylate, phenolic aliphatic CH₃, CH₂, C-H, aromatic C-H, etc. [27, 30, 31]. The shape and size of MNPs were examined by SEM (figure 2).

SEM image shows well dispersed and un-aggregated sphere nanoparticles that have a diameter around 80 ± 7 nm. This result was confirmed by measuring dynamic light scattering (DLS) (figure 3). The figure represents a typical size distribution graph for melanin nanoparticles. As shown in the figure, the size of MNPs is centered at 83.48 nm with a relatively narrow distribution.

In order to study the protective effects of MNPs in radiation-induced cytotoxicity, CHO cells were pretreated with 6.25, 12.5, 25 and 50 $\mu\text{g/ml}$ MNPs, followed by a 6 Gy radiation exposure. Figure (4) shows the effects of MNPs on radiation-induced cytotoxicity. The 6 Gy radiation challenge caused a 46% loss in cell viability, as compared to that of the control group. This result was in agreement with the previous investigations which reported a decrease in cell viability caused by ionizing radiation [32]. Pretreatment with MNPs clearly aided in preventing loss of cell viability. At low concentration of MNPs (6.25 $\mu\text{g/ml}$), the viability increased compared to radiation control but this increase is not significant. Increasing the concentration lead to increase the cell viability. At higher concentration of MNPs in irradiated group, there was a significant increase in cell viability. Higher concentrations of MNPs resulted in higher cell viability. At 12.5, 25 and 50 $\mu\text{l/mg}$ cell viability was restored to about 71, 81 and 92 % respectively of the control group. There was a greater protective effect when higher concentrations of MNPs were used. These results clarify the ability of MNPs in protecting normal cells from radiation-induced cytotoxicity. There was no change in the cell viability between the control groups that means MNPs did not have any cytotoxic effects on CHO cells.

Conclusion

The observed improvement of cell viability for different concentrations of MNPs implies that MNPs could investigate in vivo and in the future could be used extensively in radiotherapy.

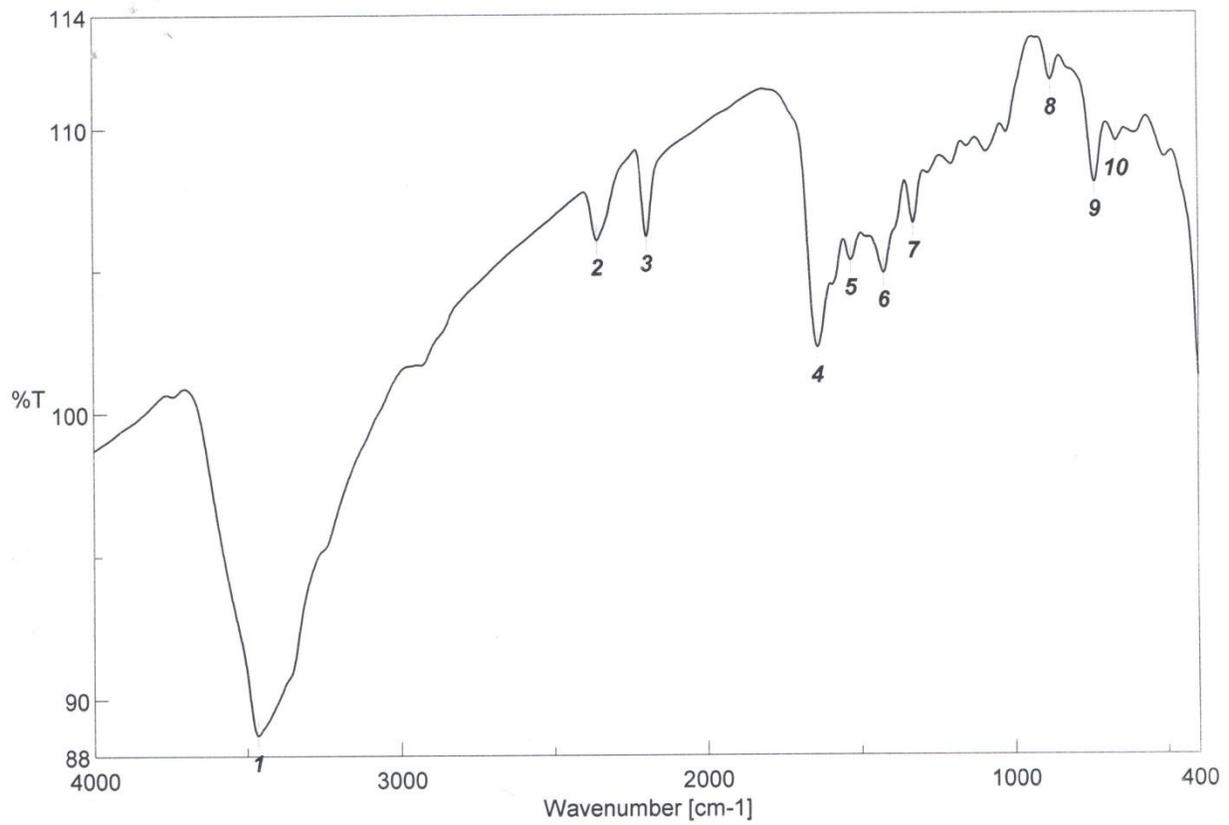


Figure 1: FT-IR spectrum of melanin nanoparticles (MNPs).

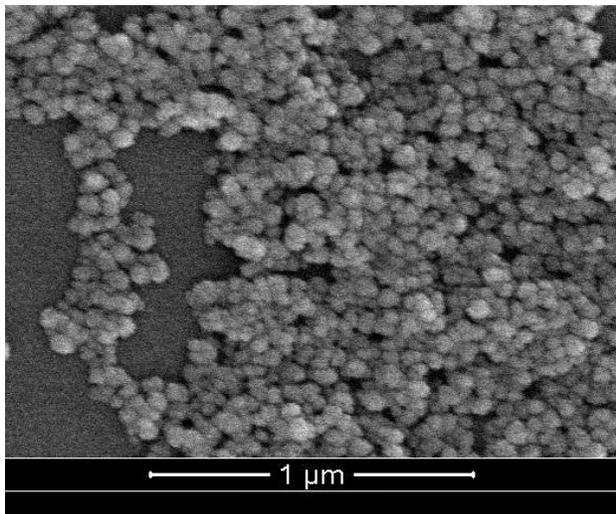


Figure 2: Scanning electron microscope (SEM) image of melanin nanoparticles (MNPs).

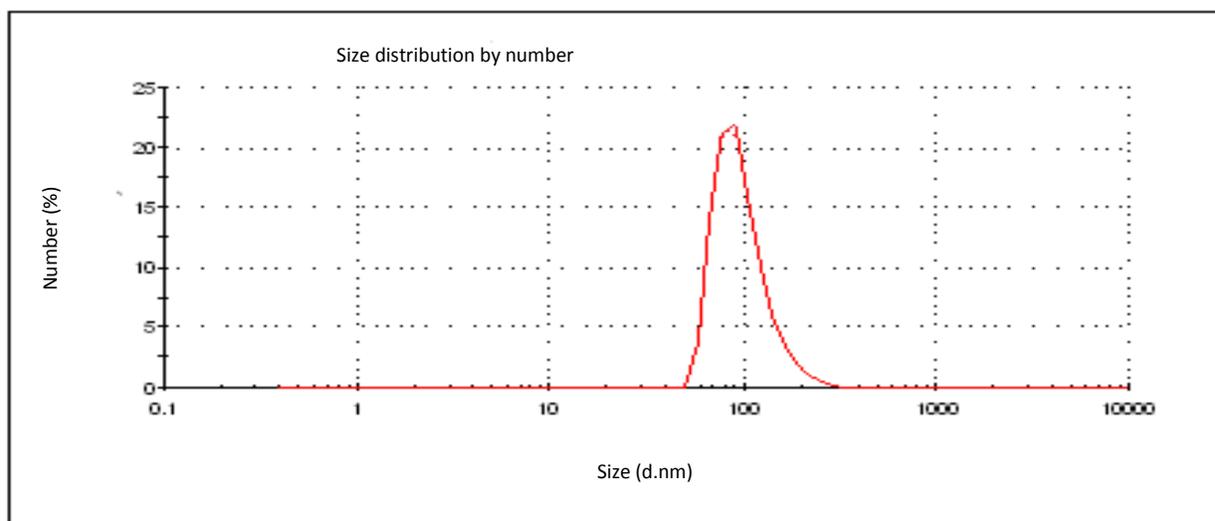


Figure 3: Size distribution of melanin nanoparticles (MNPs) measured by dynamic light scattering. The data points are the means of three independent measurements.

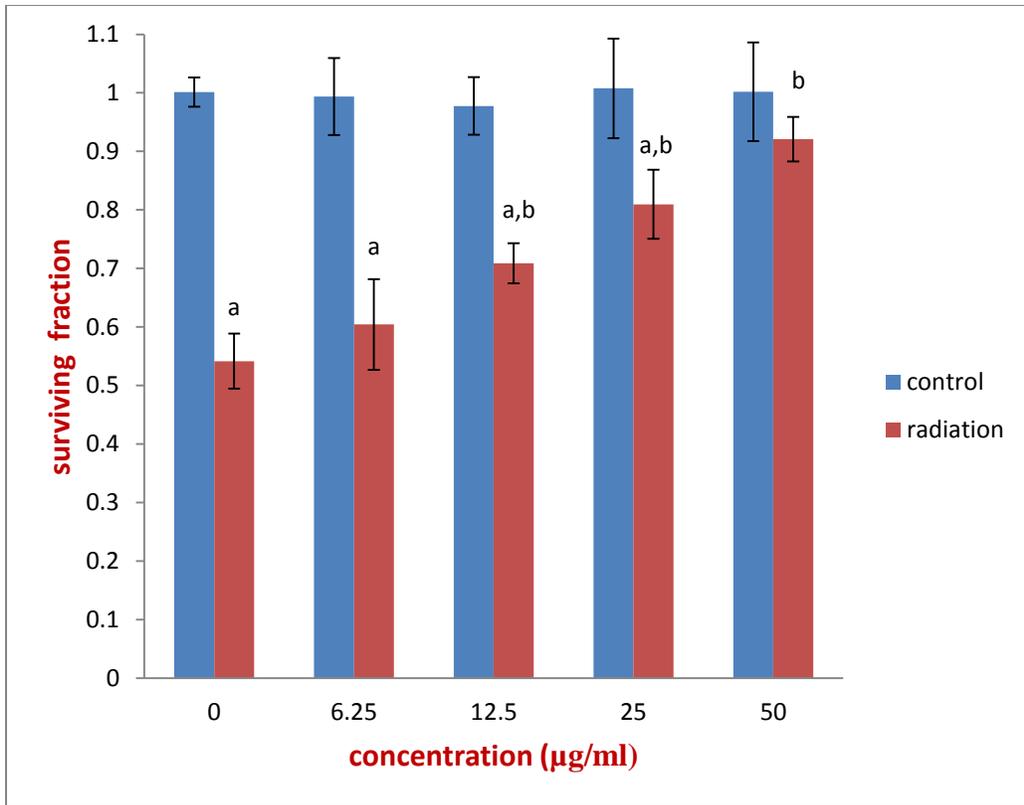


Figure 4: Protective effects of MNPs in radiation-induced cytotoxicity: survival fraction of CHO cells after 6 Gy of radiation in the presence of different concentrations of MNPs compared to controls (with different conc. of MNPs or with and without MNPs). The data points are represented as mean \pm SD (n=3). (a-P < 0.05) when compared to the control (zero conc. of MNPs) group; (b-P < 0.05) when compared to the irradiated (zero conc. of MNPs) group.

References

- [1] C. K. K. Nair, D. K. Parida, and T. Nomura, "Radioprotectors in radiotherapy," *Journal of radiation research*, vol. 42, pp. 21–37 2001.
- [2] DK Maurya and ThP Devasagayam (2011). Role of Radioprotectors in the Inhibition of DNA Damage and Modulation of DNA Repair After Exposure to Gamma-Radiation. In Selected Topics in DNA Repair Edited by Clark C. Chen, University of California, San Diego, USA , ISBN, p.p 483:496 publisher: InTech
- [3] Borek, C., 2004. Antioxidants and radiation therapy. *Journal of Nutrition* 134 (11), 3207S–3209S.
- [4] Mishra, K.P., 2002. Fluorescence studies on radiation oxidative damage to membranes with implications to cellular radiosensitivity. *Journal of Chemical Sciences* 114 (6), 705–711.
- [5] Delanian, S., Lefaix, J.L., 2002. Reversibility of radiation-induced fibroatrophy. *Revue de médecine interne* 23 (2), 164–174.
- [6] SJ Hosseinimehr. Trends in the development of radioprotective agents. *Drug Discov.* 12 (2007) 794–805. / .(2007) *Today* 12, 794–805. [Drug Discov Today](#). 2007 Oct;12(19-20):794-805. Volume 12, Issues 19–20, October 2007, Pages 794–805
- [7] S. N. Upadhyay, B. S. Dwarakanath, and T. Ravindranath, "Chemical Radioprotectors," *Defence Science Journal*, vol. 55, pp. 403-425, 2005.
- [8] B. A. Rzigalinski, "Nanoparticles and cell longevity," *Technol Cancer Res Treat*, vol. 4, pp. 651-9, Dec 2005.
- [9] S. S. Ali, J. I. Hardt, K. L. Quick, J. S. Kim-Han, B. F. Erlanger, T. T. Huang, C. J. Epstein, and L. L. Dugan, "A biologically effective fullerene (C60) derivative with superoxide dismutase mimetic properties," *Free Radic Biol Med*, vol. 37, pp. 1191-202, Oct 15 2004.
- [10] R. W. Tarnuzzer, J. Colon, S. Patil, and S. Seal, "Vacancy engineered ceria nanostructures for protection from radiation-induced cellular damage," *Nano Letters*, vol. 5 pp. 2573–2577, 2005.
- [10] S. Trajkovic, S. Dobric, V. Jacevic, V. Dragojevic-Simic, Z. Milovanovic, and A. Dordevic, "Tissue-protective effects of fullerene C60(OH)24 and amifostine in irradiated rats," *Colloids Surf B Biointerfaces*, vol. 58, pp. 39-43, Jul 1 2007.

- [11] B. Daroczi, K. Gabor, M. F. Mary, W. C. Jeffrey, R. Ulrich, P. Adam, and Dicker, "In vivo radioprotection by the fullerene nanoparticle DF-1 as assessed in a zebrafish model," *Clinical Cancer Research*, vol. 12, 2006.
- [12] D. Schubert, R. Dargusch, J. Raitano, and S. W. Chan, "Cerium and yttrium oxide nanoparticles are neuroprotective," *Biochem Biophys Res Commun*, vol. 342, pp. 86-91, Mar 31 2006.
- [13] S. Deshpande, S. Patil, S. V. N. T. Kuchibhatla, and S. Seal, "Size dependency variation in lattice parameter and valency states in nanocrystalline cerium oxide," *Applied Physics Letters*, vol. 87, pp. 133113-3, 2005.
- [14] Ramachandran L and Nair CKK. Therapeutic Potentials of Silver Nanoparticle Complex of α -Lipoic Acid. *Nanomaterials and Nanotechnology*. 2011;1:17-24.
- [15] Chandrasekharan DK, Khanna PK, Nair CKK. Cellular radioprotecting potential of silver nanoparticle - glyzyrrhizic acid complex. *Genetic Toxicology and Environmental Mutagenesis*. 2011;723:51-57.
- [16] Kannan Badri Narayanan, Hyun Ho Park. Pleiotropic functions of antioxidant nanoparticles for longevity and medicine. *Advances in Colloid and Interface Science* 201–202 (2013) 30–42.
- [17] Hill HZ (1992) The function of melanin or six blind people examine an elephant. *Bioessays* 14: 49–56.
- [18] Simon, J. D.; Peles, D. N. The Red and the Black. *Acc. Chem. Res.* 2010, 43, 1452–1460.
- [19] Simon JD. Spectroscopic and dynamic studies of the epidermal chromophores trans-urocanic acid and eumelanin. *Acc Chem Res* (2000) **33**: 307–313.
- [20] P. Meredith and T. Sarna, "The Physical and Chemical Properties of Eumelanin," *Pigment Cell Research*, vol. 19, pp. 572-594, 2006.
- [21] Dadachova, E., Casadevall, A., 2008. Ionizing radiation: how fungi cope, adapt, and exploit with the help of melanin. *Curr. Opin. Microbiol.* 11, 525–531.

- [22] Dadachova, E., Bryan, R.A., Howell, R.C., Schweitzer, A.D., Aisen, P., Nosanchuk, J.D., Casadevall, A., 2007. The radioprotective properties of fungal melanin are a function of its chemical composition, stable radical presence and spatial arrangement. *Pigment Cell Melanoma Res.* 21, 192–199.
- [23] Dadachova, E., Bryan, R.A., Huang, X., Moadel, T., Schweitzer, A.D., Aisen, P., Nosanchuk, J.D., Casadevall, A., 2008. Ionizing radiation changes the electronic properties of melanin and enhances the growth of melanized fungi. *PLoS One* 2, e457.
- [24] Schweitzer, A.D., Howell, R.C., Jiang, Z., Bryan, R.A., Gerfen, G., Chen, C.C., Mah, D., Cahill, S., Casadevall, A., Dadachova, E., 2009. Physico-chemical evaluation of rationally designed melanins as novel nature-inspired radioprotectors. *PLoS One* 4, e7229.
- [25] Schweitzer, A.D., Revskaya, E., Chu, P., Pazo, V., Friedman, M., Nosanchuk, J.D., Cahill, S., Frases, S., Casadevall, A., Dadachova, E., 2010. Melanin-covered nanoparticles for protection of bone marrow during radiation therapy of cancer. *Int. J. Radiat. Oncol. Biol. Phys.* 78, 1494–1502.
- [26] A. Kunwar, B. Adhikary, S. Jayakumar, A. Barik, S. Chattopadhyay, S. Raghukumar, K.I. Priyadarsini. Melanin, a promising radioprotector: Mechanisms of actions in a mice model. *Toxicology and Applied Pharmacology* 264 (2012) 202–211.
- [27] Ju KY, Lee Y, Lee S, Park SB, Lee JK. Bioinspired Polymerization of Dopamine to Generate Melanin-Like Nanoparticles Having an Excellent Free-Radical-Scavenging Property. *Biomacromolecules* 12 (2011) 625–632.
- [28] Lee et al, Nano-sized melanin particles and method of producing same patent NO. US 2012/ 0205590 A1 (2)
- [29] Borenfreund, E., Puerner, J.A., 1984. A simple quantitative procedure using monolayer culture for toxicity assays. *J. Tissue Cult. Meth.* 9, 7–9.
- [30] Sarna, T., and Swartz, H.M. (2006). The physical properties of melanins. In *The Pigmentary Systems: Physiology and Pathophysiology*, J.J. Nordlund, R.E. Boissy, V.J. Hearing, R.A. King, W.S. Oetting, J.-P. Ortonne, eds (Oxford: Blackwell Publishing Ltd), pp. 311–341.

[31] Centeno, S.A., Shamir, J., 2008. Surface enhanced Raman scattering (SERS) and FTIR characterization of the sepia melanin pigment used in works of art. *Journal of Molecular Structure* . 87,149-159.

[32] Wright, J.A., Keegan, K.S., Herendeen, D.R., Bentley, N.J., Carr, A.M., Hoekstra, M.F., Concannon, P., 1998. Protein kinase mutants of human ATR increase sensitivity to UV and ionizing radiation and abrogate cell cycle checkpoint control. *Proceedings of the National Academy of Sciences* 95 (13), 7445–7450.