**ORIGINAL ARTICLE**

# Comparative Study of Biosynthesizing Selenium Nanoparticles by Gum Arabic and Poly Anionic Cellulose to Prevent Radiation-Induced Death in Chinese Hamster Ovary (CHO) Cells

**Mojgan Hasanzadeh [1], Mohammad-Taghi Bahreyni-Toossi [2], Majid Darroudi [3, 4], Sara Khademi [5], Fereshteh Vaziri-Nezamdoost [1], Hosein Azimian [1, 2*]**

*[1] Department of Medical Physics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

*[2] Medical Physics Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*

*[3] Nuclear Medicine Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*

*[4] Department of Medical Biotechnology and Nanotechnology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*

*[5] Department of Radiology Technology, School of Paramedical Sciences, Mashhad University of Medical Sciences, Mashhad, Iran*

*Corresponding Author: Hosein Azimian
Email: Azimianh@mums.ac.ir

Received: 03 September 2022 / Accepted: 23 January 2023

## Abstract

**Purpose:** In premenopausal women, abdominopelvic radiotherapy may have a direct and profound effect on ovarian function. Stabilized selenium Nanoparticles (NPs) with some natural materials have been demonstrated to have high antioxidant activity and reduce radiation damage as a radioprotector. This study was done to compare the ability for the biosynthesis of selenium NPs by Gum Arabic (Se-GA) and Polyanionic Cellulose (Se-PAC) in the protection of Chinese Hamster Ovary (CHO) cells against radiation damage.

**Materials and Methods:** First, Selenium Nanoparticles (SeNPs) were synthesized in the presence of GA and PAC. Then, CHO cells were cultured in-vitro and were randomly divided into six groups in different concentrations of Se-GA and Se-PAC to measure the biocompatibility of NPs. Finally, cells were treated with NPs and radiation (6MV, 2Gy), and the percentage of cell survival was determined by MTT assay. Both NPs with an average size of 20-30 nm and an absorption absorbance peak at about 300 nm using Ultraviolet-Visible (UV–Vis) spectroscopy.

**Results:** According to the parametric t-test analysis, Se-GA nanoparticles with a concentration higher than 0.4 ppm significantly increased the radioprotective effect on CHO cells compared to the control group ($P<0.05$). However, Se-PAC showed no significant increase in radioprotection in contrast to the control group ($P>0.05$).

**Conclusion:** Se-GA nanoparticles have antioxidant properties, and the radiation protection properties of Se-GA nanoparticles are significantly higher than control. Consequently, Se-GA nanoparticles showed promising results and may be able to play the role of a radioprotector.

**Keywords:** Radiotherapy; Radiation Protection; Gum Arabic; Polyanionic Cellulose; Ovary.

Copyright © 2024 Tehran University of Medical Sciences.
This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International license (https://creativecommons.org/licenses/by-nc/4.0/). Noncommercial uses of the work are permitted, provided the original work is properly cited.
DOI: https://doi.org/10.18502/fbt.v11i2.15340

## 1. Introduction

Radiotherapy (RT), as one of the cancer treatments, is used to treat abdominopelvic cancers. However, in addition to damaging cancer cells, the side effects of RT can cause several complications in healthy tissues [1, 2].

Pelvic radiotherapy, especially for women under 40 years of age, can cause damage to the ovaries and lead to infertility [3]. Also, RT with damage to the ovaries can lead to premature menopause, which in turn reduces the patient's quality of life, and causes osteoporosis and heart problems for the patients [1]. Radioprotectors are substances that can reduce radiation damage. These substances have antioxidant properties and free radical scavengers preventing the activity of free radicals produced by Ionizing Radiation (IR), consequently reducing the side effects [4, 5]. Selenium as an antioxidant has been shown to have a protective effect on healthy cells against IR [6-8]. However, several studies have suggested that selenium may act as a radiosensitizer in cancer cells [9, 10]. This dual property may be effective in improving the therapeutic ratio, which is also seen in its Nanoparticles (NPs). However, SeNPs have attracted more attention due to better bioavailability, excellent biocompatibility, and lower toxicity [11, 12].

The green synthesis method of NPs, in addition to economic benefits, causes the production of NPs that are both non-toxic, and prevents environmental pollution [13]. The antioxidant activity of selenium depends on its size, and smaller NPs have more antioxidant properties [14].

Accordingly, in this paper, two NPs, each synthesized with different materials and the same size, were studied in terms of their ability to increase cell survival against radiation, to find perfect radioprotective; and to find out which of these two substances can have more protection on ovarian cells.

## 2. Materials and Methods

### 2.1. Cell Culture

This is an original study in which a Chinese Hamster Ovarian (CHO) cell line was purchased from the Pasteur Institute of Iran. The medium used for culturing was Roswell Park Memorial Institute 1640 (RPMI 1640) containing 10% Fetal Bovine Serum (FBS) (CAS no. 9014-81-7), streptomycin (100 µg/ml) (CAS no. 57-92-1), and penicillin (CAS no. 113-98-4) (100 units/ml). These cells were grown in a carbon dioxide incubator (5% of CO2 and at 37 °C). Sub-culturing of the CHO cell line was performed by detaching adherent cells using 0.025 % trypsin-ethylenediaminetetraacetic acid (trypsin-EDTA) (CAS no. 9002-07-7) and cells were washed with Phosphate-Buffered Saline (PBS) (CAS no. 7647-14-5).

### 2.2. Synthesis of Both Types of Nanoparticles

First, 10 mg of Polyanionic Cellulose (PAC) (CAS no. 9004-32-4) was dissolved in 50 ml of distilled water and stirred for 30 min (Solution A). Then, 30 ml of 10 mM solution of Selenium salt (CAS no.10102-18-8 26970-82-1) was added dropwise to solution A and was heated for 1 hr. at 80 °C (Solution B). Then, 30 ml of ascorbic acid (CAS no. 50-81-7) solution (20 mM) was added dropwise to solution B and was left for 24 hrs. at room temperature under stirring conditions. Then, the resulting solution was centrifuged at with 10,000 rpm (Sigma 2-16KL, Germany), and the precipitate was placed in a freezer (Danesh Pajoohesh Fajr, Iran) at -80 °C and was dried by freezer dryer for 48 hrs.

Se-GA NPs were made similarly, with the difference that Gum Arabic (GA) (CAS no. 9000-01-5) was used instead of PAC.

### 2.3. Characterization of Se-GA and Se-PAC NPs

The size and shape of the NPs were obtained by Transmission Electron Microscopy (TEM) (Zeiss EM900 model). Absorption spectra of these NPs were recorded by an Ultraviolet-Visible (UV–Vis) spectrophotometer (UNICO UV-2100, USA).

Of course, more information about the synthesis and characteristics of these nanoparticles can be found in these articles [15, 16].

### 2.4. Toxicity of NPs

First, 6,000 cells were seeded in two 96-well plates and then, were incubated for 24 hrs. After this time, the culture medium of plates was replaced with a new culture medium containing NPs in different concentrations. After 24 hrs, the

plates were emptied, and the MTT assay was performed as follows. For this purpose, cells were washed twice with PBS and were cultured in fresh culture medium without FBS containing 50 µl of MTT solution (5 mg/ml). The plates were covered with aluminum foil and were incubated for 4 hrs. Then, the medium was removed and replaced by 200 µl of dimethylsulfoxide (DMSO). Afterward, it was placed on the rotor for 5 min; finally, absorbance was measured at 570 nm and was compared with 630 nm by an Enzyme-Linke Immunosorbent Assay (ELISA) reader.

### 2.5. Survival Measurement

After ensuring the non-toxicity of selective concentrations, 6,000 cells in plate wells were seeded to determine the effect of NPs against IR. After 24 hrs. of incubation, the culture medium of each well was replaced with a fresh medium containing different concentrations of NPs. Nanoparticles, due to their small size, are easily absorbed by cells. However, the elimination route of absorbed NPs is unclear. The next day, the wells were drained, and then 100µl of fresh culture medium containing 3% FBS was added. Then, the cells were exposed to X-irradiation using a linear accelerator (Siemens, Concord, CA, USA) at a dose rate = 2 Gy/min and energy = 6 MV with a 1.5 cm Plexiglass phantom. Radiation was performed at the following parameters. SSD: 100 cm, Field Of View (FOV): 25 × 25 $cm^2$, and gantry angle: 180°.

After irradiation, a fresh culture medium with 17% FBS was added to the plates and incubated for 24 hrs. Then, the MTT assay was performed again.

### 2.6. Statistical Analysis

The results were analyzed using Graph Pad Prism 8.4.0 software. Information was communicated, and the cruel ± standard mistake of the mean and p < 0:05 was considered to show a statistically noteworthy contrast. Due to the normality of toxicity data, an Analysis Of Variance (ANOVA) test was used. However, due to the abnormality of survival data after irradiation, the Mann-Whitney U test was used to compare differences between every two groups.

## 3. Results

According to TEM, the shape of NPs was similar, and both had rod-shaped morphology with a size of about 20-30 nm (Figure 1a and b). Also, the UV–Vis absorption spectrum of the Se-GA and Se-PAC suspensions with an absorption peak at about 300 nm is illustrated in Figure 1C.

A comparison of toxicity results of different concentrations showed that all concentrations had a non-toxic effect and no significant difference between the groups (Figure 2).

As shown in Figure 3, in the groups irradiated for 24 hrs. after receiving the NPs with 2 Gy of X-ray, concentrations of 0.4, 0.8, and 1.7 ppm of Se-GA NPs caused a significant increase in survival compared to the control group (Mann-Whitney U test $P<0.05$) (Figure 3A). However, different concentrations of Se-PAC did not have any significant change in the viability of irradiated CHO cells (Figure 3B). Also, a Simple linear regression analysis was performed (Figure 3C) and the change of cell viability as a linear function of Se-GA NPs concentrations was obtained with the following formula (Equation 1):

$$Y = 0.3093 * X + 0.8026 \quad (1)$$

## 4. Discussion

Selenium plays a crucial role in human's and animal's health, but receiving more than 3-5 µg of selenium can be toxic to the body. This element acquires new properties in the Nano-state, such as more antioxidant capacity and less toxicity [11]. To help the readers in making better comparisons, in Table 1, the results of the current study were compared with the recent in-vitro and in-vivo studies carried out by SeNPs in response to IR.

It should be noted that smaller SeNPs have been proven to have free radical scavenging, low toxicity, and more antioxidant activity [17-19]. In this study, Se-GA and Se-PAC NPs with the sizes of 20-30 nm did not show any toxicity up to a concentration of 1.7 ppm, and Se-GA NPs also had an antioxidant effect.

Our findings are consistent with those of the study by Torres *et al.*, who demonstrated that smaller SeNPs with sizes less than 100 nm could potentially reduce oxidative stress [20]. In addition, based on the assumption that GA has potent antioxidant and free radical scavenging properties, the synthesis of small-sized NPs in the presence of GA can be promising to create synergistic effects in antioxidant capacity [21].

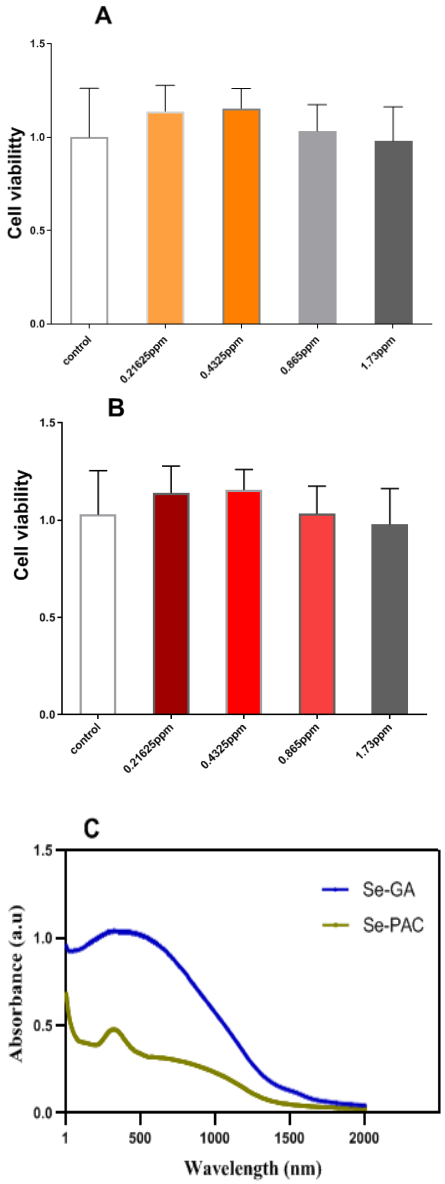


**Figure 1.** A) TEM of biosynthesis Se nanoparticle by gum arabic (Se-GA). B) TEM of biosynthesis Se nanoparticle by PAC (Se-PAC). C)UV–visible absorption spectrum of the Se-GA and Se-PAC suspension

TEM: Transmission Electron Microscopy, PAC: Polyanionic cellulose, UV: Ultra Violet.

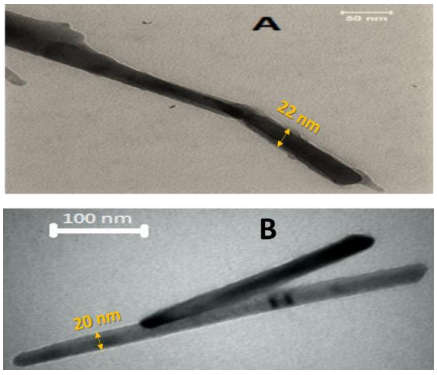


**Figure 2.** Cell viability of the CHO cells incubated with different concentrations of NPs (A) Se-GA (B) Se-PAC

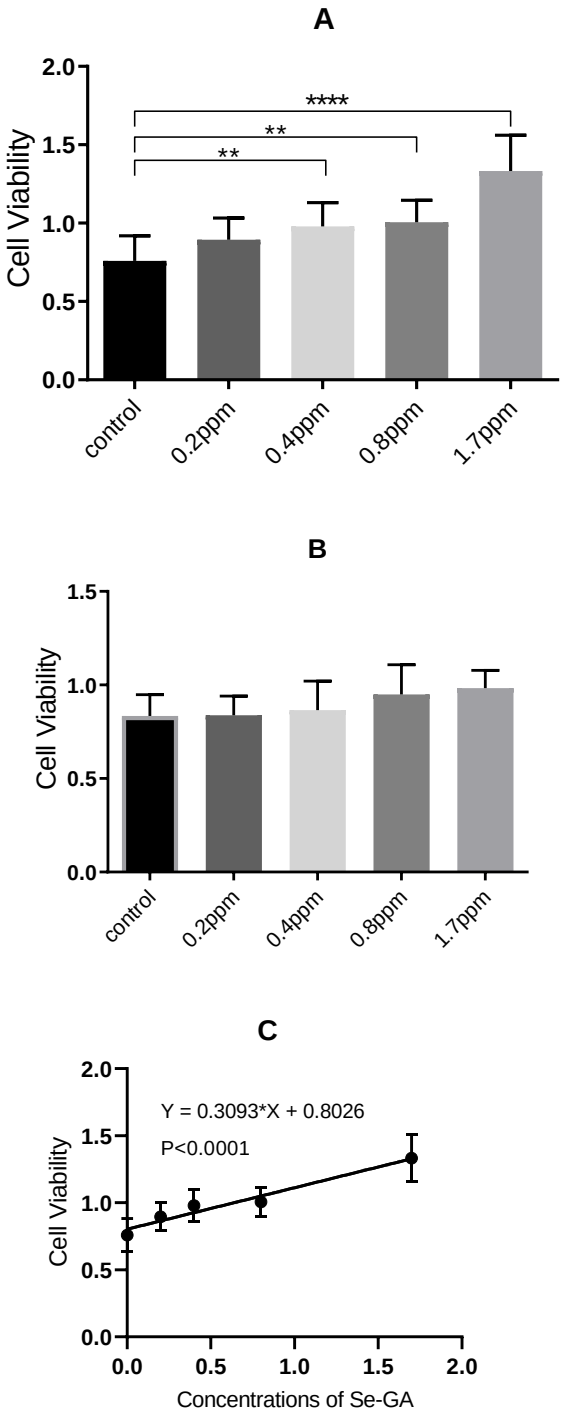


**Figure 3.** Viability of CHO cells irradiated 24 hours after receiving the different concentrations of NPs (A) Se-GA (B) Se-PAC and C) Simple linear regression analysis of cell viability as a function of Se-GA concentrations

**Table 1**. Different behaviors of SeNPs on modulating the response to IR in recent in vitro and in vivo studies

| Reference | Cell or tumor Type | Concentration | Size of Nano | Effects | Conclusion |
|---|---|---|---|---|---|
| Current study | Chinese hamster ovary (CHO) cells | 1.73 ppm | 20-30 nm | Increase cell viability for Se-GA | Adjunctive use Se-GA can decrease adverse effects of radiation in normal tissue |
| Gudkov *et al.* [31] | Mice | 5 mg / kg of weight body | 50 nm | Prevent oxidative stress caused by ionizing radiation<br>Protect animals from radiation-induced death.<br>Decrease hepatotoxicity | Radioprotective effect |
| Fahmy *et al.* [32] | Liver of Rat | 0.5 mg/kg of weight body rat | 49.6 nm | Normalized level of: IL6<br>Angiotensinogen xanthine oxidase (XO) NF-κB<br>(Nrf2) protein | Radioprotective effect |
| Karami *et al.* [11] | Blood and renal tissue of mice | 0.1 mg/kg | 20-50 nm | Protect kidneys against gamma radiation<br>Decrease oxidative damages | Radioprotective effect |
| Zhang *et al.* [33] | Lung cancer cells (A549) | 60 -100 µg/ml | 50–60 nm | Killed the cancer cells<br>Decreased the cell viability<br>Higher apoptosis | Radiosensitive effect |
| Yu *et al.* [34] | cancer cell lines HeLa cervical cancer cells and mouse embryonic fibroblast NIH3T3 | 20 ppm | 500 and 200 nm | Enhanced the cells growth inhibition<br>Cell apoptosis<br>Intracellular ROS generation | Radiosensitive effect |
| Chen *et al.* [12] | breast cancer cells (MCF-7) | 0, .15 or.3 µg/ml | from 900 to 200 nm | Cell death induction<br>Led to autophagy<br>Cell cycle arrest at the G2/M phase<br>Increase ROS | Adjunctive use SeNPs can increase the effectiveness of radiotherapy in breast cancer treatment as a radiosensitizer |
| Yang *et al.*[35] | breast cancer cells (MCF-7) | 0.42 µM - 32µM | 192 nm | Increase synergistically by radioactive 125I seeds to realize anticancer efficacy<br>Prevent colony formation ability<br>Increasing ROS | Acts as a radiation sensitizer for 125I |

Several studies have revealed that the adverse effects of radiation on the liver and kidney's function are decreased in the presence of GA [22, 23]. Also, recent evidence suggests that the presence of GA in some NPs, including gold [24], selenium [25], silver [25], magnesium orotate [26], and curcumin [27] can cause an antioxidant effect. Therefore, the hypothesis of this study was confirmed as our results well demonstrated that selenium and GA act as antioxidants and their synergistic effect can increase the radioprotective effects, as shown in (Figure 3), and irradiated cells in the presence of Se-GA NPs showed more remarkable survival compared to the irradiated cells in the control group. However, contrary to the

expectations, the same NPs synthesized in the presence of the PAC did not provide significant radioprotective effects for the cells (Figure 3). The increase in survival observed in the irradiated cells in the presence of PAC is due to the inherent protective effect of SeNPs, and the PAC could not have a synergistic effect on it.

These observations can be justified in this way: unlike GA, no protective or antioxidant effects have been reported for PAC.

Karl Buch *et al.* (2012) showed that MTT and colony assay was well-related, so that MTT assay could be a good alternative [28]. This test shows survival rate and radiation damage 24 hrs. after irradiation. For this reason, the same time interval was used in this study, and the survival rate was well estimated [29, 30]. Concentrations of less than 1.7 ppm of NPs did not show a significant protective effect, perhaps because these concentrations were too low to influence cells.

## 5. Conclusion

Natural products can be used as natural radioprotectors in cancer treatments in combination with RT while reducing IR damage to normal cells/tissues. Selenium-Gum Arabic nanoparticles showed promising results in this study and may be able to play the role of a radioprotector in the future. Due to the radioprotective effect seen against oxidative stress generated by low-LET ionizing radiation and the non-toxicity of 1.7 ppm of the biosynthesized Se-GA NPs to the cells. In this study, Se-PAC nanoparticle was also investigated, which showed less protective ability. This is probably due to the antioxidant effect of gum arabic and this substance has been able to synergistically increase the antioxidant effect of selenium nanoparticles. Since antioxidants are free radical scavengers, substances that have stronger antioxidant properties are more successful in scavenging these radicals or upregulating antioxidative systems in cells. Therefore, they can better protect cells from damage caused by ionizing radiation. In this regard, our future studies would be addressed on the possible use of Se-GA NPs nanoparticles for radioprotection. The observed mitigative effects in the present study need to be validated in humans by more detailed experiments.

## Acknowledgments

This study was approved by the Mashhad University of Medical Sciences Institutional Review Board (IR.MUMS.MEDICAL.REC.1398.330).

## References

1- Jong Ha Hwang *et al.*, "Association between the location of transposed ovary and ovarian function in patients with uterine cervical cancer treated with (postoperative or primary) pelvic radiotherapy." *Fertility and sterility*, Vol. 97 (No. 6), pp. 1387-93 .e2, (2012).

2- Casey M Cosgrove and Ritu Salani, "Ovarian effects of radiation and cytotoxic chemotherapy damage." *Best Practice & Research Clinical Obstetrics & Gynaecology*, Vol. 55pp. 37-48, (2019).

3- C Haie-Meder *et al.*, "Radiotherapy after ovarian transposition: ovarian function and fertility preservation." *International Journal of Radiation Oncology* Biology* Physics*, Vol. 25 (No. 3), pp. 419-24, (1993).

4- Qinqi Wang *et al.*, "Radioprotective Effect of Flavonoids on Ionizing Radiation-Induced Brain Damage." *Molecules*, Vol. 25 (No. 23), p. 5719 , (2020).

5- Qiongge Hu, Yunxiang Zhou, Shijie Wu, Wei Wu, Yongchuan Deng, and Anwen Shao, "Molecular hydrogen: A potential radioprotective agent." *Biomedicine & Pharmacotherapy*, Vol. 130p. 110589, (2020).

6- H Bagheri *et al.*, "Radioprotective Effects of Zinc and Selenium on Mice Spermatogenesis." *Journal of Biomedical Physics & Engineering*, Vol. 10 (No. 6), p. 707, (2020).

7- Richard J Lobb, Gregory M Jacobson, Ray T Cursons, and Michael B Jameson, "The interaction of selenium with chemotherapy and radiation on normal and malignant human mononuclear blood cells." *International journal of molecular sciences*, Vol. 19 (No. 10), p. 3167, (2018).

8- JF Weiss, V Srinivasan, KS Kumar, and MR Landauer, "Radioprotection by metals: selenium." *Advances in Space Research*, Vol. 12 (No. 2-3), pp. 223-31, (1992).

9- 1006 "poster Influence of selenium on the radiosensitivity of glioma cells." *Radiotherapy and Oncology*, Vol. 73, pp. S423-S24, 2004/10/01 /(2004).

10- Bagher Farhood *et al.*, "Selenium as an adjuvant for modification of radiation response." *Journal of cellular biochemistry*, Vol. 120 (No. 11), pp. 18559-71 , (2019).

11- Masoumeh Karami, Siamak Asri-Rezaei, Banafshe Dormanesh, and Ali Nazarizadeh, "Comparative study of radioprotective effects of selenium nanoparticles and sodium selenite in irradiation-induced nephropathy of mice model." *International journal of radiation biology*, Vol. 94 (No. 1), pp. 17-27, (2018).

12- Feng Chen, Xiao Hong Zhang, Xiao Dan Hu, Pei Dang Liu, and Hai Qian Zhang, "The effects of combined selenium nanoparticles and radiation therapy on breast cancer cells in vitro." *Artificial cells, nanomedicine, and biotechnology*, Vol. 46 (No. 5), pp. 937-48 , (2018).

13- Yanhui Zhou *et al.*, "Green synthesis of Se/Ru alloy nanoparticles using gallic acid and evaluation of theiranti-invasive effects in HeLa cells." *Colloids and Surfaces B: Biointerfaces*, Vol. 144pp. 118-24, (2016).

14- S. K. Torres *et al.*, "Biosynthesis of selenium nanoparticles by Pantoea agglomerans and their antioxidant activity." *Journal of Nanoparticle Research*, Vol. 14 (No. 11), p. 1236, 2012/10/20 (2012).

15- Mahin Velayati, Hassan Hassani, Zahra Sabouri, Asma Mostafapour, and Majid Darroudi, "Biosynthesis of Se-Nanorods using Gum Arabic (GA) and investigation of their photocatalytic and cytotoxicity effects." *Inorganic Chemistry Communications*, Vol. 128p. 108589, 2021/06/01/(2021).

16- Mahin Velayati, Hassan Hassani, and Majid Darroudi, "Green synthesis of Se-Nanorods using Poly Anionic Cellulose (PAC) and examination of their photocatalytic and cytotoxicity effects." *Inorganic Chemistry Communications*, Vol. 133p. 108935, 2021/11/01/(2021).

17- SK Torres *et al.*, "Biosynthesis of selenium nanoparticles by Pantoea agglomerans and their antioxidant activity." *Journal of Nanoparticle Research*, Vol. 14 (No. 11), p. 1236 , (2012).

18- Bo Huang, Jinsong Zhang, Jingwu Hou, and Chang Chen, "Free radical scavenging efficiency of Nano-Se in vitro." *Free Radical Biology and Medicine*, Vol ٣٥ .(No. 7), pp. 805-13 ,(2003).

19- Huali Wang, Jinsong Zhang, and Hanqing Yu, "Elemental selenium at nano size possesses lower toxicity without compromising the fundamental effect on selenoenzymes: Comparison with selenomethionine in mice." *Free Radical Biology and Medicine*, Vol. 42 (No. 10), pp. 1524-33, 2007/05/15 /(2007).

20- SK Torres *et al.*, "Biosynthesis of selenium nanoparticles by Pantoea agglomerans and their antioxidant activity." *Journal of Nanoparticle Research*, Vol. 14 (No. 11), pp. 1-9, (2012).

21- Badreldin H Ali, Amal Ziada, and Gerald Blunden, "Biological effects of gum arabic: a review of some recent research." *Food and chemical Toxicology*, Vol. 47 (No. 1), pp. 1-8, (2009).

22- EM Hussein, "Modulatory role of arabic gum in gamma rays-induced damages in rats." *J. Rad. Res. Appl. Sci*, Vol. 1 (No. 2), pp. 427-41, (2008).

23- Heba A Mohamed, Ahmed S Nada, Neamat Hanafi, Hala F Zaki, and Sanaa A Kenawy, "The reno protective effect of gum arabic in gamma-irradiated and cisplatin treated rats ". *International Journal of Scientific and Research Publications*, Vol. 5 (No. 6) , (2015).

24- JS Djajadisastra, Pony Purnamasari, and ANUNG Pujiyanto, "Antioxidant activity of gold nanoparticles using gum arabic as a stabilizing agent." *Int J Pharm Pharm Sci*, Vol. 6 (No. 7), pp. 462-5, (2014).

25- Huiling Kong, Jixin Yang, Yifeng Zhang, Yapeng Fang, Katsuyoshi Nishinari, and Glyn O Phillips, "Synthesis and antioxidant properties of gum arabic-stabilized selenium nanoparticles." *International journal of biological macromolecules*, Vol. 65pp. 155-62 ,(2014).

26- A Hassani, SA Hussain, N Abdullah, S Kamarudin, and R Rozita, "Preparation, antioxidant potential and angiotensin converting enzyme (ACE) inhibitory activity of gum arabic-stabilised magnesium orotate nanoparticles." *International Food Research Journal*, Vol. 27 (No. 1) ,(2020).

27- A Hassani *et al.*, "Formulation and antioxidant properties of curcumin gum Arabic nanoparticles for delivery to cancer cells." in *IOP Conference Series: Materials Science and Engineering*, (2020), Vol. 991 (No. 1): *IOP Publishing*, p. 012101.

28- Karl Buch, Tanja Peters, Thomas Nawroth, Markus Sänger, Heinz Schmidberger, and Peter Langguth, "Determination of cell survival after irradiation via clonogenic assay versus multiple MTT Assay-A comparative study." *Radiation oncology*, Vol. 7 (No. 1), pp. 1-6,(2012).

29- Zohre Rezaee, Ali Yadollahpour, and Vahid Bayati, "Single intense microsecond electric pulse induces radiosensitization to ionizing radiation: effects of time intervals between electric pulse and Ionizing Irradiation." *Frontiers in oncology*, Vol. 8p. 418 ,(2018).

30- Zohre Rezaee, Ali Yadollahpour, Vahid Bayati, and Fereshteh Negad Dehbashi, "Gold nanoparticles and electroporation impose both separate and synergistic radiosensitizing effects in HT-29 tumor cells: an in vitro study." *International Journal of Nanomedicine*, Vol. 12p. 1431 ,(2017).

31- Sergey Gudkov *et al.*, "Production and application of selenium nanoparticles to prevent ionizing radiation-induced oxidative stress." in *IOP Conference Series: Earth and Environmental Science*, (2019), Vol. 390 (No. 1): *IOP Publishing*, p. 012031.

32- Hanan A Fahmy, Afrag Sh Abd El Azim, and Ola A Gharib, "Protective effects of omega-3 fatty acids and/or nano-selenium on cisplatin and ionizing radiation induced liver toxicity in rats." *Indian Journal of Pharmaceutical Education and Research*, Vol. 50 (No. 4), pp. 649-56 , (2016).

33- Hejia Zhang, Qingjia Sun, Lingling Tong, Yanru Hao, and Tianyu Yu, "Synergistic combination of PEGylated selenium nanoparticles and X-ray-induced radiotherapy for enhanced anticancer effect in human lung carcinoma." *Biomedicine & Pharmacotherapy*, Vol. 107pp. 1135-41 , (2018).

34- Bo Yu, Ting Liu, Yanxin Du, Zuandi Luo, Wenjie Zheng, and Tianfeng Chen" ,X-ray-responsive selenium

nanoparticles for enhanced cancer chemo-radiotherapy." *Colloids and Surfaces B: Biointerfaces*, Vol. 139pp. 180-89 , (2016).

35- Yahui Yang *et al.*, "Functionalized selenium nanosystem as radiation sensitizer of 125I seeds for precise cancer therapy." *ACS applied materials & interfaces*, Vol. 9 (No. 31), pp. 25857-69 ,(2017).