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RESEARCH PAPER

Comparison of Radioprotective Effects of Colloidal Synthesis of Selenium Nanoparticles in Aqueous Rosemary Extract and Rosemary in Chinese Hamster Ovary (CHO) Cells

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ABSTRACT

Radiotherapy has a profound impact on ovarian function, leading to depletion of the primordial follicle reserve, premature amenorrhea, and loss of fertility during or shortly after completion of irradiation. The radioprotectors are compounds that can reduce the effects of ionizing radiation (IR). Stabilized selenium nanoparticles and extracts, including rosemary, demonstrated high antioxidant activity. The aim of this study is the evaluation of rosemary extract and selenium nanoparticles ability to scavenge free radicals. After rosemary extract was prepared, nanoparticles were made in their presence, and then CHO cells were cultured in vitro. They were then divided into different groups and treated with different concentrations of nanoparticles and rosemary, and irradiated with ionizing radiation at doses of 0.5, 1, and 2. Finally, the survival rate was measured by MTT assay. The results of the MTT test showed that the effect of radiation protection on nanoparticles is close to rosemary. Furthermore, the protective effect of the two is not significantly different from each other (P>0.05). Contrary to expectations, nanoparticles did not show a synergistic effect compared to rosemary.

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INTRODUCTION

Radiotherapy (RT) is one of the most common and most effective cancer treatment methods. it causes the death of cancer cells and damages normal tissues by produce ROS and free radicals[1].

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Free radicals destruct DNA and other cell constituents. Single- or double-strand breaks (DSB), base damage, and DNA–DNA or DNA–protein crosslinks are Lesions formed in DNA that DSBs are critical of them. DSBs have the prominent

role in cell killing [2]. The radiation damage to healthy cells can limit radiation therapy, preventing the dose needed to treat the tumor from being irradiated [2]. If RT use for Pelvis or in females create side effect for ovaries including premature ovarian, infertility, early menopause, and ovarian hormones are not secreted [3]. The patients have lower than 45 years for these reasons, infertility and damage to the ovary may decrease quality of life [4]. For lower side effects of radiotherapy in normal cells can use substances with protection properties against radiation.

Radioprotectors are compounds that are used pre-irradiation to reduce the amount of radiation damage to natural tissues [5]. This compound has many antioxidants and radical scavenger properties [6]. Radioprotectors by scavenging free radicals species such as hydroxyl radical (HO.) protects vital cell structures from damage[7]. Because of the possible lesion that can be developed by hydroxyl radicals, a number of cellular antioxidant defenses have been developed and some antioxidants are known, especially in food.

However, many chemical radioprotectives have inherent toxicity and high cost. That is why it is preferred to use a suitable natural component and plant [8].

Rosemary (Rosmarinus officinalis L.) is one of the herbal plants that have antioxidant and free radical scavenger properties and have one effective radioprotective [9-11]. Rosemary contains carnosic acid and carnosol ,with high antioxidant properties [9]. Also, rosemary plays a suitable role in apoptosis induction and inhibition of proliferation of ovarian cancer cells [12].

Selenium, like rosemary, has high antioxidant properties, but receiving more than 3 to 5 micrograms of this element is toxic to the body [13]. This element shows unique properties in nanophase, the most important of which is to reduce toxicity [14]. To measure the radioprotective effect of these compounds on the ovary responses, CHO cell was used, which shows the effect of radiation well due to its radiation sensitivity [15]. The aim of the study was to compare radioprotective effects of rosemary and a novel nano-scale composite of selenium nanoparticles in aqueous rosemary extract on the normal cell of Chinese Hamster Ovary (CHO).

MATERIALS AND METHODS

Rosemary (Rosmarinus officinalis L.) extract (19.7mgr/ml). Colloidal synthesis of selenium nanoparticles in rosemary extract aqueous. Because healthy ovarian cells are irradiated in women undergoing pelvic radiotherapy, this poses a risk to women in fertility ages. For this purpose, this study was performed on CHO cells as the only available healthy ovarian cell that is derived from the ovary of the Chinese hamster. The radioprotective effect of rosemary and CSSNANO defined by viability assay in vitro after four different dosages of radiation (0Gy, 0.5Gy, 1Gy, and 2Gy).

Nanoparticle synthesize

Colloidal synthesis of selenium nanoparticles in aqueous rosemary extract was done as follows: First rosemary (Rosmarinus officinalis L.) plant with herbarium code: E1131-FUMH was extracted by Hosseini et al. method]17 [, Then a 100ml solution of 10 mM selenium salt solution is prepared, and

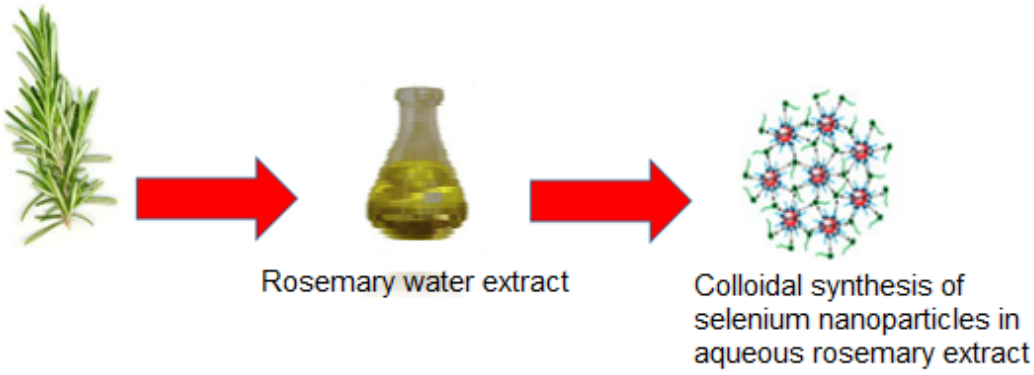

Fig. 1. Preparation of Rosemary and colloidal synthesis of selenium nanoparticles.

20ml of the extract is added drop by drop to the salt solution. The solution is stirred for one hour at 80 °C and then for 24 hours at ambient temperature. The resulting solution is then centrifuged (10000 rpm) and the precipitate is placed in the freezer at -80 °C and dried by the freezer dryer for 48 hours.

Characterization of colloidal synthesis of selenium nanoparticles (CSSNANO)

The size and shape of the NPs are obtained by Transmission Electron Microscopy (TEM). The absorption spectra of these NPs are recorded by a UV–Vis spectrophotometer (UNICO UV-2100, USA). (Fig. 3)

Toxicity for rosemary and CSSNANO nanoparticles

In plate 96 well, different concentrations of rosemary and CSSNANO nanoparticle in every well was medium including CHO cell for 6, 12, and 24 hours with different cells. Then cells were cultured in RPMI 1640 containing 10% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 µg/ml). The cells were incubated at 37°C in an atmosphere of 5% CO2. These cells were seeded into 96-well plates in 200 µl of 10% medium for 24 h containing 200 µl of 3% medium in a CO2 incubator. Then after 24, 12 and 6 h, empty medium and add 100 µl fresh medium with 20 µl of MTT solution (5 mg/ml) in an environment without light after use of aluminum foil for covered plate because MTT is very sensitive to light. Plate put in an incubator, past of 4 h Remove from the incubator and out medium with MTT and added 200 µl of DMSO to Every plate. Plate 10 minutes put on Rotor for dissolving DMSO in the plate. After it, by Epoch plate reader devise and wavelength of 570 and 630, the absorption peak was read. Analyzing the result of the MTT assay was realized that 6000 cells in every well with 0.21625 µgr/ml concentration for rosemary and CSSNANO relative with the normal group and these concentrations without toxicity for CHO cells.

In vitro experiments

After the optimum concentration of rosemary and nanoparticle was determined, use of that concentration in four plates (control, radiation 0.5 Gy, 1 Gy, 2 Gy) that every plate include three groups normal, rosemary, and CSSnano.

Therefore, in plate 96 well seeded 6000 cells in every well then all plates added 100µl fresh medium (17%) and incubated at 37°C in an atmosphere of 5% CO_2. after 24 h. all groups were out of medium and replaced by rosemary and nanoparticle solved in the medium and normal group without intervention. Pass 24 h out medium and twice washed plate with PBS then replaced by 100µl new medium (3 % FBS). Plates irradiated with 0.5,1, 2 Gy with 6 MV X-rays (Elektra, Stockholm, Sweden), but the control plate was

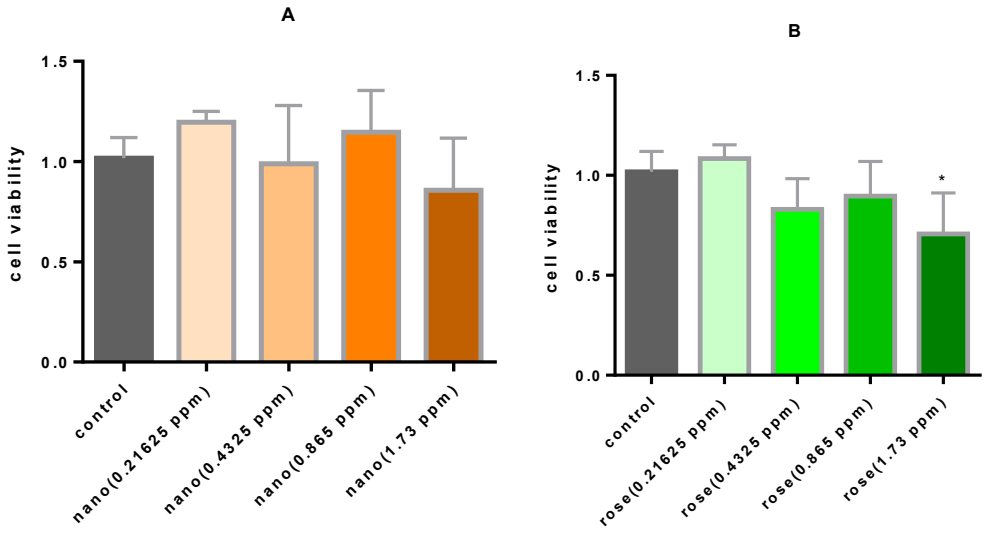

Fig. 2. Cell viability of the CHO cells incubated with different concentrations of Rosemary (A diagram) and CSSNANO nanoparticles (B diagram). * indicates p value <0.05.

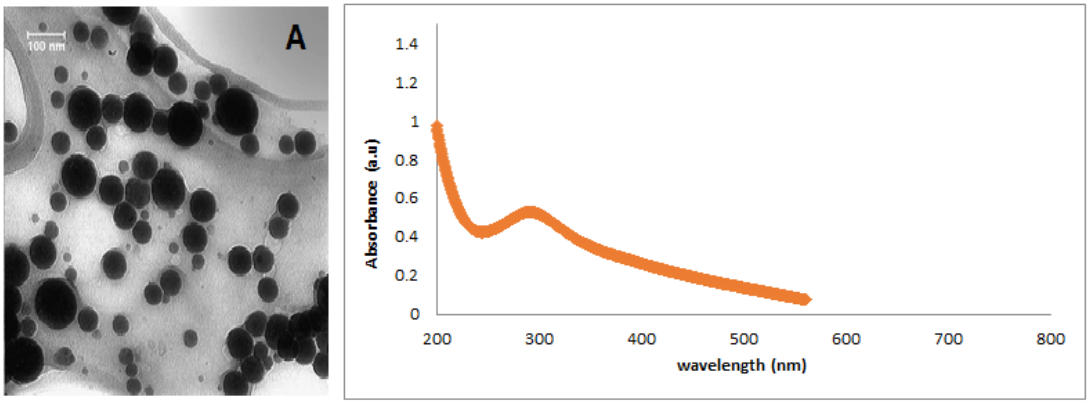

Fig. 3. A) TEM of spherical shaped colloidal synthesis of selenium nanoparticles in aqueous rosemary extract (CSSNANO). B) UV–visible absorption spectrum of the CSSNANO suspension

without irradiated in similar conditions. Then, 100µl fresh medium (17%) was added to all plates. When plates were incubated for 24 h, we used an MTT assay to study the viability in cells.

Radiation design

Plates radiation in 25*25 field and on a 1.5 cm phantom with SSD 100 by 6 MV energy of X-ray Electa device.

Statistical Analysis

In this study, due to the normality of the data One-way ANOVA test was used, and all statistical analyzes were performed using Graphpad Prism 6 software.

RESULTS AND DISCUSSION

As shown in Fig. 3-A the sensitized nanoparticles in this study have spherical shape with a 30 nm mean diameter. UV–visible (UV–vis) absorption spectra of these NPs were recorded at a wavelength range of 200-800 nm with broad peaks with intensity maxima at 300 nm (Fig. 3-B) at room temperature by using UNICO UV-2100 spectrophotometer. (UNICO UV-2100, USA).

MTT assay was used to evaluate toxicity, and it helps to find a concentration that is non-toxic to the cells because the substance to be studied for its protective properties should not be toxic to healthy cells [16]. Also, this test can measure the radiation-induced growth inhibition based on metabolic viability after receiving ionizing radiation and compare this rate in the intervention groups. Results of toxicity were showed differences between groups (Fig. 2). Therefore, when comparing the groups with the control group, it is observed that the 1.73 ppm concentration of rosemary has a significant decrease (p= 0.0027). For this reason, another concentration of rosemary and nanoparticle concentrations were used to measure survival and protective effect.

These non-toxic concentrations were used to compare radioprotective activity between rosemary and CSSNANO.

At 0.5 Gy in the concentration of 0.21625 ppm of rosemary, cell survival increased significantly ($P<0.05$), while at a concentration of 0.865 ppm nanoparticles for this radiation dose, a significant decrease in cell survival was observed ($P>0.05$). In the other words, nanoparticles in the concentration of 0.865 has caused toxicity and lethality.

At 1 Gy ، 0.865 ppm concentration of rosemary significant increase in cell viability was observed ($P<0.05$). At 2 Gy, rosemary in the concentration of 0.21625 ppm showed a significant increase in cell survival ($P<0.05$). However the protective effect of nanoparticles did not show any significant difference compared to control and rosemary.

Biological implications generated by ionizing radiation can cause cell death and necessity of protecting the normal cells from these lesions is not hidden from anyone [2]. An understanding of the role of antioxidants in reduction of radiation injury may be valuable in so far as it proposes possible protecting strategies that could be employed for normal tissues adjacent to irradiated tumors on radiotherapy. Rosemary is known as radioprotective for its antioxidant and free radical scavenging properties.[10, 17]. As demonstrated

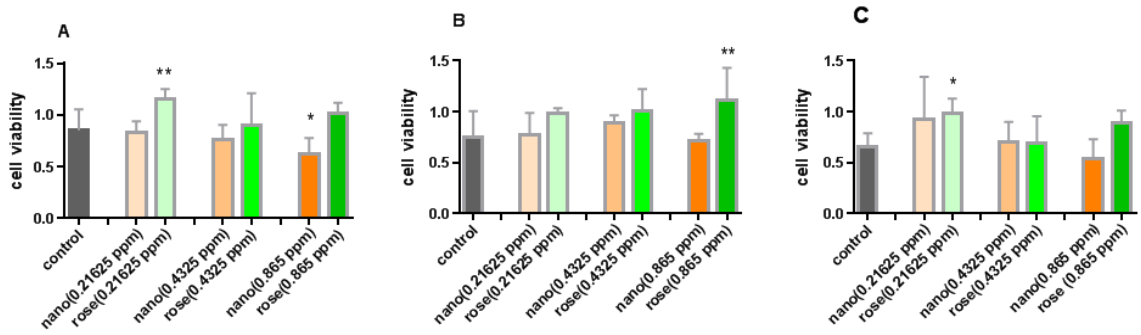

Fig. 4. In A diagram, the cell viability is shown after 0.5 Gy ، B diagram 1 Gy of radiation, and in C diagram 2 Gy of radiation. The symbol* indicates p-value <0.05 and ** indicates p-value <0.01. Each bar represents the mean for all repetitions, and each error bar represents the standard deviations.

in Fig. 2 rosemary in the concentration of 0.21625 ppm has no toxicity and enhanced cell viability in CHO cells at 0.5Gy and 2Gy of ionizing radiation (Fig. 4). As mentioned, viability was increased in treated samples with rosemary compared to controls. This is probably due to free radicals scavenging and the antioxidant properties in rosemary that was induced to protect cells. Incubated cells with nanoparticles before irradiation also showed higher survival after irradiation than control samples. Because Selenium nanoparticles and rosemary both have antioxidant properties, we expected to see a synergistic effect in complex of selenium nanoparticles and rosemary extract, but contrary to our expectations, this effect was not seen.

Selenium nanoparticles behave differently in different sizes, and sometimes appears as radiosensitizer in tumor cells [18]. Se nanoparticles with antioxidant activity can cause

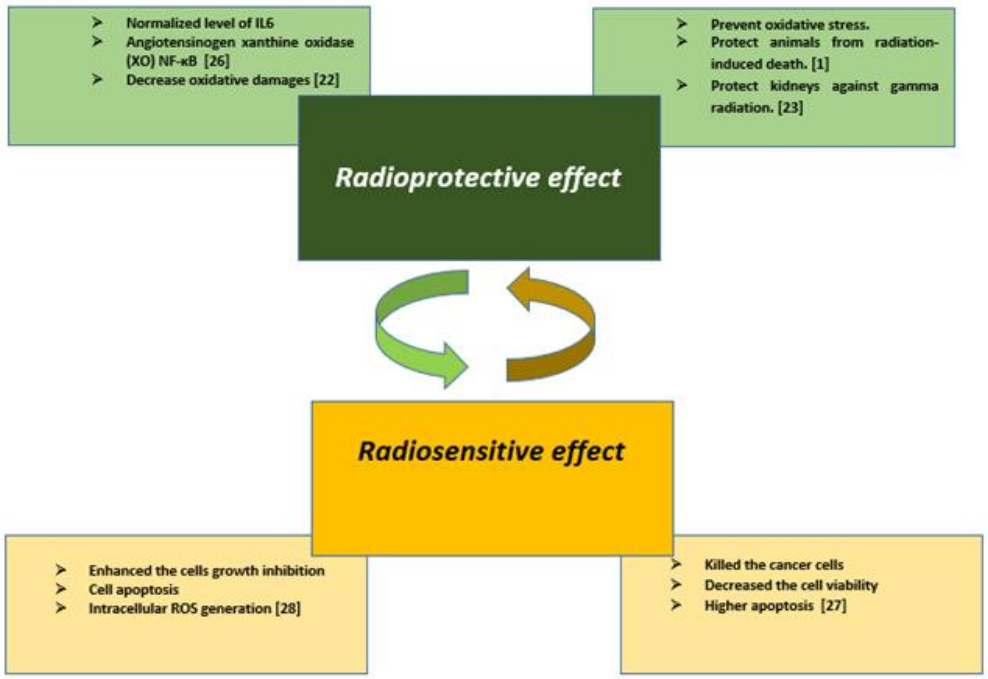

Fig. 5. The figure shows the dual property in selenium based on articles.

decreased radiation damage. May the surface of the selenium nanoparticles be coated with rosemary extract, or because the nanoparticles are floating in the rosemary extract, the two may form complementary compounds that prevent antioxidant and radiation protection properties.

Because small nanoparticles have a more significant antioxidant effect, nanoparticles made with a size of 30 nm were expected to have a high antioxidant effect and show more remarkable survival. This reinforces the hypothesis that rosemary extract inhibits the nanoparticle function.

Because a lower concentration of nanoparticles has a more significant protective effect (Fig. 4), perhaps if the concentration of rosemary decreases in the manufacture of subsequent nanoparticles samples, a more protective effect can be seen. Furthermore, this suggests that, contrary to our expectations, the two antioxidants in combination may not show a higher antioxidant effect or have a synergy, or that other mechanisms that are unknown to us, may be involved.

CONCLUSION

Free radical scavenging activity of antioxidants reduce the damage caused by ionizing radiation and acts as a radioprotector. Antioxidant properties of rosemary and small size selenium nanoparticles have been proven in previous studies. However, contrary to expectations, colloidal synthesis of selenium nanoparticles in aqueous rosemary extract did not show synergistic effect of radiation protection on CHO cells. Two main hypotheses can be proposed to explain the reason for lack of CSSNANO synergistic effect. First, covering the surface of selenium nanoparticles with rosemary extract. Second, formation of complementary compounds due to floating nanoparticles in rosemary extract. Further work is needed in order to elucidate the mechanism of antioxidant properties inhibition of selenium nanoparticles by rosemary.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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