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Evaluation of the Silver Graphene Quantum Dot Effect when Combined with Resveratrol and Radiation in Colorectal Cancer Cells

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Abstract

Background: Colorectal cancer (CRC) is one of the major leading causes of cancer related death throughout the world. Among various therapeutic strategies for CRC, neoadjuvant radiotherapy, targeted therapy, and chemotherapy are the main techniques to destroy cancerous cells.

Objectives: The main purpose of this study was to evaluate the efficacy of radiotherapy in combination with silver graphene quantum dot (SQD) and Resveratrol (Res) on the HCT-116 colorectal cancer (CRC) cells.

Methods: Minimally cytotoxic concentrations of SQD (50μm) with Res (0.5×IC50 and 0.25×IC50) and radiation (2Gy of x-ray radiation) were selected for double and triple treatments. The Acridine Orange/Ethidium Bromide staining was used to detect apoptosis. The Caspase-3 mRNA expression level was measured by the real-time PCR method. In addition, cyclooxygenase 2 (COX-2) protein expression was studied by the western blot technique. Similarly, the superoxide dismutase (SOD), glutathione peroxidase (GPX) enzyme activities, and malondialdehyde (MDA) levels were determined in this study.

Results: In this study, after 24 h, triple combined cases showed decreased cell viability versus double and single treatments ($P<0.05$). Moreover, cellular viability was decreased in the SQD-radiation and SQD-Res treated cells, compared to the related single treatments ($P<0.05$). The increased apoptotic cells were observed in the triple combination group, compared to the double and single treated cases. Triple combination treatment exhibited decreased GPX activity versus single treatments ($P<0.05$). The triple combined case showed reduced SOD activity than the radiation and SQD single treatments. A remarkable increase in the MDA concentration was observed in the triple combination versus single treatments. According to the results, in double and triple treatments, upregulation of Caspase-3 mRNA was presented versus double and single treatments. There was a decreased COX-2 protein expression level in triple combinatorial cases versus double and single treated cells.

Conclusion: These findings proposed that SQD in combination with Res and radiation showed significant anti-cancer effects on CRC in vitro.

Keywords: Apoptosis, HCT-116 cells, Radiation, Resveratrol

1. Background

Colorectal cancer (CRC) is one of the major leading causes of cancer related death throughout the world (1). Among various therapeutic strategies for CRC, neoadjuvant radiotherapy, targeted therapy, and chemotherapy are the main techniques to destroy cancerous cells (2). Though a partially successful response toward radiotherapy is attained in cancer treatments, radiation resistance is inevitable (3). Therapeutic resistance mechanisms of CRC and radiation resistance have been under extreme investigation. Numerous mechanisms have been suggested to be related to radiation resistance.

Various mechanisms, including cell cycle alterations, apoptosis inhibition, and cancer stem cells have been associated with radiation resistance (4). The investigation of methods in radiation oncology that may increase the radiosensitizing effects is developed (3, 5). In this regard, radiosensitizing effects of Resveratrol (Res) on some cancer cells were reported (6, 7). As a polyphenolic compound, Res has diverse pharmacologic properties comprising cancer prevention and anti-inflammation effects. Moreover, numerous studies showed that Res with radiotherapy approaches or chemotherapies could be used as an effective therapeutic regimen in cancer treatment (8, 9). Res has antioxidant activity, and this effect is mostly related to its capability in the inhibition of free radical production, modulation of antioxidant-related enzymes, and lipid peroxidation. Superoxide dismutase (SOD) is one of the main antioxidant enzymes. Res decreases the SOD expression, and consequently, contributes to the inhibition of reactive oxygen species (ROS) (9).

With a significant progress in nanotechnology, numerous types of metal or metal oxide nanomaterial have been utilized in order to increase the efficiency of radiotherapy. In this regard, silver nanoparticles (AgNPs) have attracted attention owing to their considerable radiosensitizing effects (10). Cancerous cells treated with AgNPs showed size- and concentration-dependent cytotoxicity at comparatively harmless radiation doses (11, 12). Furthermore, the increased radiation impacts of silver nanomaterials were detected in various cancer cell lines, comprising glioma, gastric, and breast cancers (11, 13, 14).

It has been reported that apoptosis is associated

with radiation therapy that induced cancer cell death (11). On the other hand, in normal cells, redox systems are under tight regulations although in cancer cells, these systems are dysregulated to enable carcinogenesis. Notably, these dysregulations are related to radioresistance. In this regard, redox protein-targeted therapy is considered the potential approach to elevate the radiotherapy efficiency (15).

To increase the nano-radiosensitizers development, the radiosensitizing efficiency needs to be confirmed.

2. Objectives

This study aimed to evaluate the in vitro cancer-targeting property and radiosensitizing effects of silver graphene quantum dots (SQD) and Res on CRC cells at megavoltage energies and examine the possible underlying mechanisms of radiosensitivity effects of these agents.

3. Methods

3.1. Silver-Graphene Quantum Dots

A mixture of the same volume of sulfuric acid, nitric acid, and 5 ml of graphene oxide solution was sealed, then heated for 12 h at 200°C, and centrifuged. Following that, the supernatant containing graphene quantum dots (GQDs) was isolated. Indeed, GQDs in the supernatant were obtained using a rotary evaporator. To prepare GQD/AgNP hybrids, ammonium hydroxide was added to GQD solution and followed by probe sonication. Under vigorous stirring, Tollens's reagent with silver nitrate was added to the GQD solution. Then, the mixture was stirred for 30 min and refluxed. The hydrodynamic diameter of the synthesized SQD was evaluated by the DLS study, and Zeta potential was also determined at pH7.

3.2. Cell culture, treatments, and cell viability assay

HCT-116 cells were purchased from the cell bank of Pasteur institute (Iran) and cultured in Dulbecco's Modified Eagle Medium. Media were supplemented with 1% penicillin-streptomycin and 10% fetal bovine serum. This cell line was adherent and proliferated in a humidified incubator at 37°C with a 5% CO2 atmosphere. 1×104 HCT-116 cells were seeded into 96-well plates 24 h before treatments. CRC cells were treated with various concentrations (5, 10, 20, 30, and 40 µm) of Res and SQD (50,100, 200, 250, and 270) at a 24-time point.

After 24 h, the cellular viability was evaluated using the MTT cell viability (Kia zist, Iran) technique. Then, dose-response curves were drawn for SQD and Res. The IC50 value of Res was collected (16). The dose-response curve of SQD showed that at a concentration of 50 µM, the minimal toxic effect was observed versus other concentrations. Therefore, in all further experimental analyses, this concentration (50 µM) was used for SQD treatments.

1×104 HCT-116 cells were exposed to x-ray radiation at a single dose rate of 2 Gy in a field size of 10 cm×15 cm and source-to-surface distance of 100 cm with a clinical linear accelerator machine (Elekta Compact 6 MV) at the Radiotherapy Unit, Imam Khomeini Hospital, Urmia, Iran. The cell viability of the post-treated cells (after 24 h) was studied by the MTT method.

In combined cases, the concentrations lower than IC50 of Res (0.5×IC50 and 0.25×IC50 in double and triple combinations, respectively) with SQD at 50 µM were selected and then treated with x-ray irradiation (2 Gy of 6 MV x-ray radiation). These selected doses were utilized for all tests.

3.3. Cell morphology study by Acridine Orange/Ethidium Bromide Staining

HCT-116 cells were cultured 24 h before treatments and then treated with x-ray, SQD, and Res in various single and combined treatments as mentioned above. Similarly, untreated control cells without any treatments were collected. Subsequently, the HCT-116 cells were fixed (with 4% formaldehyde), and acridine orange/ethidium bromide (AO/EB) solution (10 µL) was added for 2-5 min. The cells were then studied by a fluorescence microscope.

3.4. Western blotting assay

1×107 HCT-116 cells were cultured and then treated as described above. Afterward, the cells from cultures were lysed via incubation with RIPA lysis buffer (Bio-Rad, USA) with protease inhibitor cocktail (Sigma, USA). In the next stage, the protein content was measured by protein assay kit (Bio-Rad) using bovine serum albumin (Sigma-Aldrich) as a standard. Proteins were separated by (SDS)-polyacrylamide gel electrophoresis through a Bio-Rad apparatus, and they were transferred into a polyvinylidene difluoride membrane. The membranes were blocked with skim milk (5%) at room temperature. After washing with TBST, the membranes were incubated overnight with primary anti-cyclooxygenase 2(COX-2) and β-Actin mouse monoclonal antibodies. After washing with TBST, the membranes were incubated for 4 h with secondary antibodies. The protein bands were visualized by the ECL detection system, photographed, and then studied with a gel imaging system.

3.5. Real-time polymerase chain reaction analysis

In order to assay the Caspase-3 gene expression level, about 107 cells were seeded for 24 h in 6-well plates. After 24 h of treatments, untreated and treated HCT-116 cells were collected, and total RNAs were separated by RNA extraction kit (GeneAll, South Korea) according to the kit protocol.

RNA purity was evaluated by determining the ratio of optical density at 260 nm to that at 280 nm. cDNA was synthesized through a cDNA Synthesis Kit

(TAKARA Bio Inc, Japan). Following that, real-time PCR was carried out in a total volume of 26 µL using SYBR® Premix Ex Taq™ (Takara Bio Inc, Japan). Real time-PCR using cDNAs and specific primers of Caspase-3 and β-Actin (as housekeeping gene), as well as thermal cycling were run (annealing for 20 sec at 59°C). The results were determined using the 2-ΔΔCT method.

3.6. Assessment of GPXA activity, SOD, and Malondialdehyde

In order to assay the GPX and SOD enzyme activities based on the colorimetric method, after various treatments as described, cell culture supernatants were collected and then centrifuged. The GPX activity was measured based on the manufacturer's protocol (ZellBio GmbH, Germany). The absorbance was read with an ELISA reader at 412 nm, and GPX activity in all cases was calculated using the following formula:

GPX activity (U/mL)=(ODcontrol-ODsample)/(ODstandard-ODblank)×6000

In addition, the SOD activity in cell supernatants was measured based on the manufacturer's instruction (ZellBio GmbH, Germany). The well absorbance at 0 and 2 min were read by ELISA reader at 420 nm. The SOD activity was calculated in all samples based on the following formula:

SOD Activity (U/mL)=(Vp-Vc)/(Vp)×60
Vp=OD sample 2 min-OD blank 2min
Vc=OD sample 0min-OD blank 0min

MDA levels in the supernatant of the untreated (control) and treated (single, double, and triple combinations) samples were detected by malondialdehyde (MDA) assay kit based on *the manufacturer's assay protocol* (ZellBio GmbH) after 24 h. The absorbance was read at 535 nm. MDA levels in all examined samples were calculated based on the standard curve, which was drawn using standard points' absorbance.

4. Results

This study evaluated the cytotoxic effects of SQD in combination with irradiation and Res in the HCT-116 CRC cell line. In this regard, the SQDs were prepared, and then the hydrodynamic diameter of the synthesized SQD was evaluated by the DLS study, which showed the highest frequency around 50 nm. In addition, SQD Zeta potential at pH7 was -12mV. HCT-116 cells were exposed to different concentrations of Res and SQD for 24 h. The cytotoxicity of Res and SQD was examined by the MTT assay (Figure 1). The SQD induced a meaningful decrease in cell viability in a dose-dependent manner (Figure1). For combinatorial cases in all experimental tests, a concentration lower than IC50 was selected. In addition, SQD at a concentration of 50 µM was selected as the optimal concentration in all tests since this concentration showed minimal cytotoxicity. The MTT assay was used for the examination of cell viability after various combinatorial treatments (Figure 2). Statistical analysis showed that the cytotoxic effects of Res (in single treatments at IC50 concentration) were higher than those in the untreated controls ($P<0.05$). In addition, radiation and SQD in single treatments decreased cellular viability, compared to the controls ($P<0.05$). Similarly, there was higher cell toxicity in all double and triple combination cases, compared to the untreated controls ($P<0.05$).

Furthermore, triple combined cases showed decreased cell viability versus double and single treatments ($P<0.05$). Similarly, cellular viability was decreased in the SQD-radiation and SQD-Res, compared to the related single treatments ($P<0.05$). In the case of Res-radiation, a lower cell viability rate was presented, compared to the SQD monotherapy ($P<0.05$).

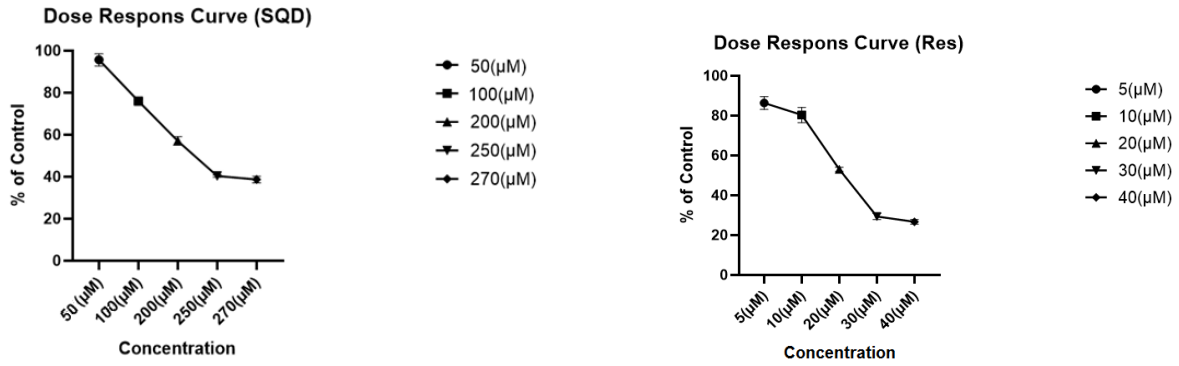

Figure 1. Results of cell viability assay test of Res and SQD at various concentrations in the HCT-116 cells after 24 h. Cytotoxic effects of Res and SQD in various concentrations were evaluated by MTT assay to plot dose-response curves. All experiments were replicated (n=3). Data are presented as mean±SD.
SQD; Silver-Graphene Quantum Dots.
Res; Resveratrol.

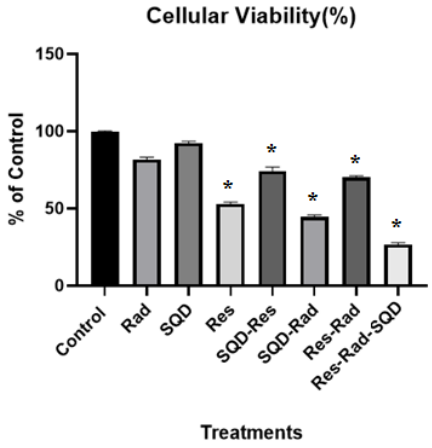

Figure 2. HCT-116 cell viabilities after radiation, SQD, and Res treatments (single and combined cases) using MTT assays. HCT-116 cells treated with SQD at the concentration of 50 uM, with 2Gy of radiation and Res (IC50, 0.5×IC50, and 0.25×IC50 for single, double, and triple combinations, respectively). Three independent experiments were carried out in triplicate, and all data are expressed as mean±SD *; P<0.05, significant differences, compared to controls.
SQD; Silver-Graphene Quantum Dots. Res; Resveratrol.

Among double combinations, higher cell toxicity was observed in the SQD-radiation, compared to the Res-radiation and SQD-Res (P<0.05). In order to evaluate the rate of necrotic and apoptotic cells after various treatments, the AO/EB staining was performed. Then, the HCT-116 cells were studied by fluorescent microscopy (Figure 3). Early-stage apoptotic cells that were crescent-shaped or granular yellow-green in the AO staining were observed (Figure 4). Moreover, late-stage apoptotic cells were identified through concentrated and asymmetrically localized orange nuclear EB staining. Necrotic cells increased in volume and displayed uneven orange-red fluorescence at their periphery.

Increased apoptotic cells were observed in the triple combination group, compared to the double and single treated cases. Furthermore, double combinations, including Res-radiation and SQD-radiation, had higher apoptotic cells versus related-single treatments. Likewise, there were increased apoptotic cells in all double and triple treatments to control cells. Res, SQD, and radiation in single-treated cases displayed a higher percentage of apoptotic cells

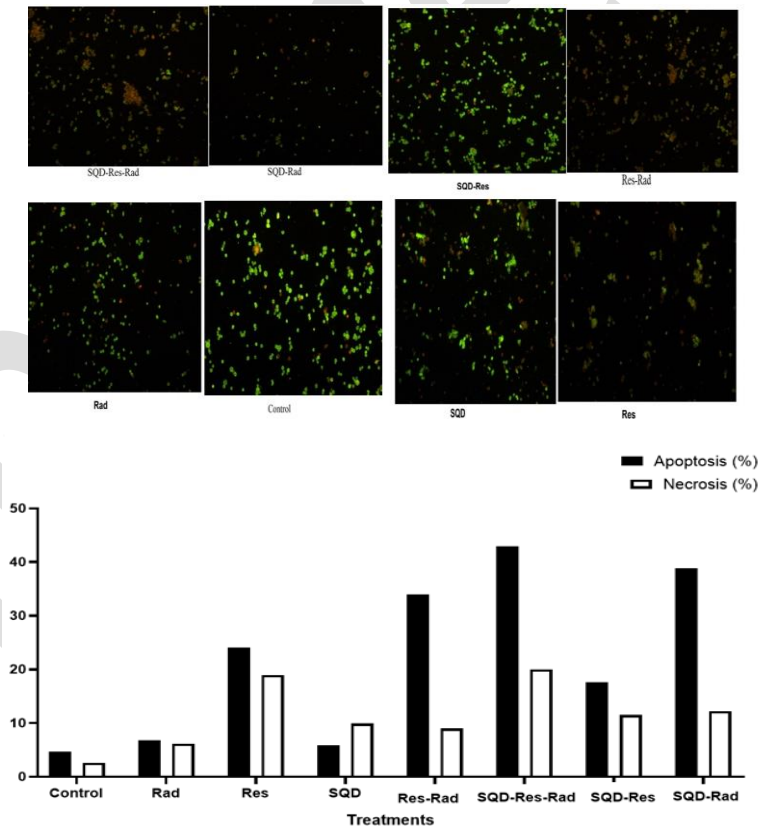

Figure 3. Dual acridine orange/ethidium bromide fluorescent staining of the HCT-116 cells. The cells were treated at IC50, 0.5×IC50, and 0.25×IC50 concentrations of Res in single and combined treatments with Res and SQD. Results indicate necrotic cells and apoptotic cell percent. The images were taken with a fluorescence microscope. AO/EB staining was used to evaluate the Res, radiation, and SQD apoptosis and necrosis induction in the HCT-116 cell line.
SQD; Silver-Graphene Quantum Dots.
Res; Resveratrol. AO/EB; acridine orange/ethidium bromide.

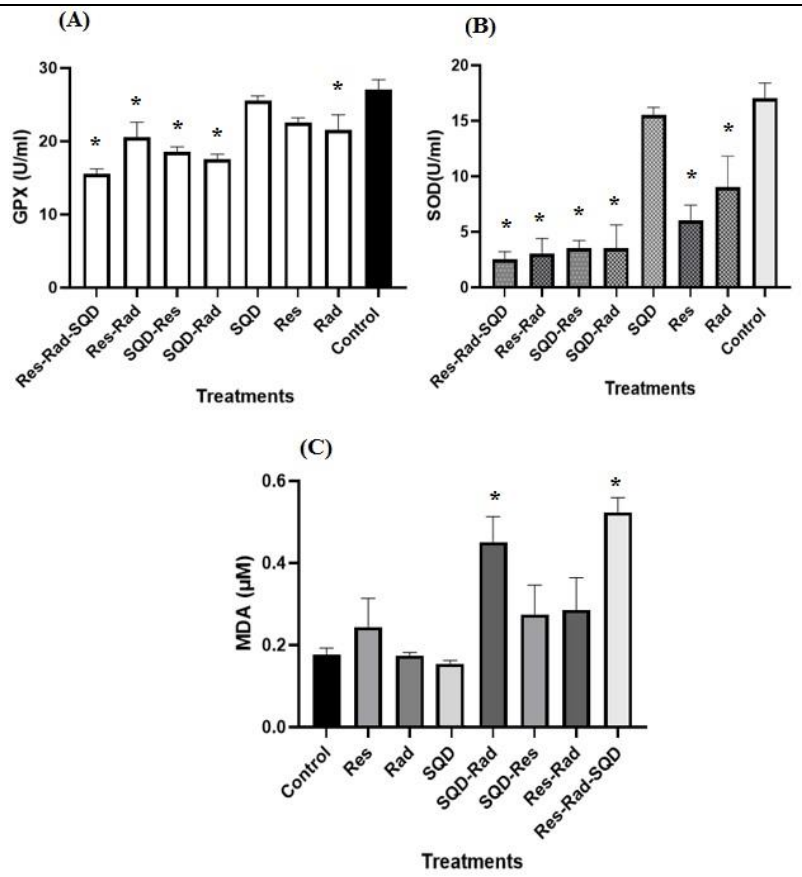

Figure 4. GPX activity (a), SOD activity (b), and MDA(c) level were evaluated by colorimetric assay. HCT-116 cell lines were treated with 2 Gy of radiation in combination with SQD and Res (IC50, 0.5×IC50, and 0.25×IC50 in single, double, and triple combined treatments, respectively). Data are presented as mean±SD.
*P<0.05, significant differences, compared to controls.
GPX: glutathione *peroxidase*; SOD: *superoxide dismutase*; *MDA*: Malondialdehyde; Res: Resveratrol; SQD: Silver-Graphene Quantum Dots.

than the untreated HCT-116 cells (Figure 4).

The results of the GPX activity assay (Fig 4 a) showed that monotherapies with SQD and Res decreased the GPX activity; however, these reductions did not reach a significant level (P>0.05). In a single treatment with radiation, GPX activity was decreased, compared to the controls (P<0.05). In all combinatorial cases, including radiation-Res, radiation-SQD, Res-SQD, and radiation-SQD-Res, a lower GPX activity was observed, compared to the untreated controls (P<0.05). In addition, the triple combination treatment exhibited decreased GPX activity, compared to the single treatments (P<0.05). Similarly, radiation-SQD and SQD-Res significantly decreased the GPX activity versus SQD single treatment (P<0.05). SOD activity in radiation and Res single treatments were lower than that in the controls, which had reached a significant level (Figure 4b; P<0.05). As presented in Figure 4 b, SQD-Res and SQD-radiation had lower SOD enzyme activity than the SQD monotherapy. In the same line, all double and triple combination treatments decreased the SOD enzyme activity versus controls (P<0.05). The triple combination cases showed reduced SOD activity, compared to the single radiation and SQD treatments (P<0.05), which had reached a significant level. Nonetheless, the triple combination did not show a significant difference, compared to other single and double treatments (Figure 4b). The results of the MDA assay showed that in the monotherapies with radiation, Res, and SQD, there were no significant differences, compared to the controls (Figure 4 c ; P<0.05). In addition, SQD-radiation and triple combinations showed increased MDA levels, compared to the untreated control (P<0.05). A remarkable increase in the MDA concentration was observed in the triple combinatorial cases versus single treatments (P<0.05). The MDA level in the SQD-radiation treatment was significantly increased, compared to the single treatments (Figure 4 c; P<0.05).

SQD-Res and radiation-Res treatments exhibited elevated levels of MDA versus single treatments that did not reach a significant level (P>0.05). In the single treatments, including Res and radiation, the Caspase-3 gene expression levels were significantly increased, compared to the controls (P<0.05). Moreover, there were increased Caspase-3 mRNAs in double and triple treatments, compared to the controls (P<0.05). In the same vein, in triple combination treatment,

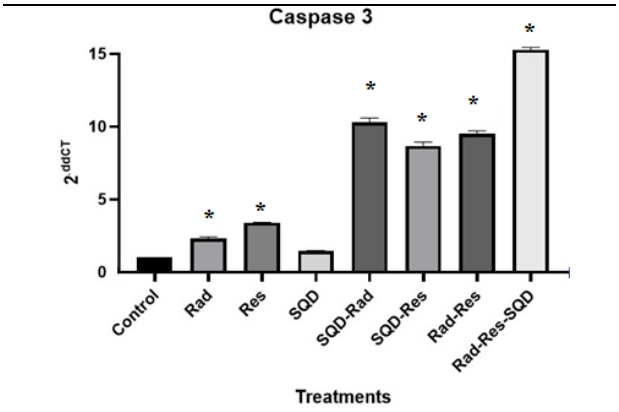

Figure 5. In order to evaluate the Caspase-3 gene expression levels in the treated and untreated cases in the HCT-116, real-time PCR method was used. HCT-116 cells were treated with 2Gy of Rad, SQD (50µm), and Res (IC50, 0.5×IC50, and 0.25×IC50 concentrations in monotherapy, double, and triple combinations, respectively). Data are presented as mean fold change±SD.
*P<0.05, significant differences, compared to controls.
SQD; Silver-Graphene Quantum Dots. Res; Resveratrol.

upregulation of Caspase-3 mRNA was observed versus double and single treatments. Furthermore, a statistically significant increase in Caspase-3 gene expression level was presented in radiation-Res, radiation-SQD, and SQD-Res double combinations versus single treatments (P<0.05). The COX-2 protein expression level was decreased in the triple combination of SQD-Radiation-Res, compared to the untreated control. In addition, there was a decline in COX-2 protein expression in the triple combinatorial case versus the single treatments, including radiation, SQD, and Res. Furthermore, COX-2 mRNA levels were decreased in the SQD-radiation-Res, compared to the double combinations of SQD-radiation, SQD-Res, and Res-radiation (Figure 6).

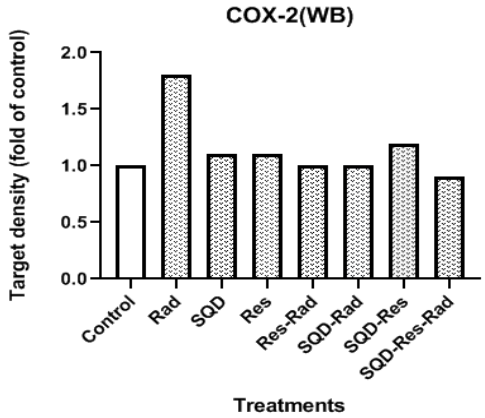

Figure 6. In order to investigate COX-2 protein expression level in the treated and untreated cases in the HCT-116, the western blotting method was used. The HCT-116 cells were treated with 2Gy of Rad, SQD (50µm), and Res (IC50, 0.5×IC50, and 0.25×IC50 concentrations in monotherapy, double, and triple combinations, respectively).
COX-2; cyclooxygenase-2
SQD: Silver-Graphene Quantum Dots; Res: Resveratrol.

5. Discussion

The present study aimed to investigate the efficiency of CRC treatment with Res, SQD, and radiation in a combinatorial therapy; moreover, it was attempted to evaluate the related mechanisms by targeting apoptosis induction and other associated markers. Previous studies have been initiated to investigate the use of AgNPs in combination with anticancer agents to treat different types of cancers (11, 13, 14). The promising evidence of anticancer effects showed the AgNPs combination with radiation to increase the radiation effect (11, 12, 14). Moreover, the combination of Res and radiation showed an encouraging antitumor efficiency (6). However, the mechanism by which radiation effects were increased when combined with Res and AgNP was somewhat more complex than that predicted in earlier studies. This study evaluated the effects of Res, radiation, and AgNP (SQD) in CRC. According to our results, it is suggested that the SQD and Res significantly increased the therapeutic efficiency of radiation by inhibiting cancer cell growth, apoptosis induction, and inhibiting antioxidant major enzymes that play the *major* roles in ROS scavenging in the human HCT-116 CRC cell line. SQD and Res combined with radiation significantly increased the apoptosis occurrence and upregulated Caspase-3 gene expression as the major mediator of apoptosis and enhanced the anti-cancer effects on CRC cancer cells by promoting apoptosis. To investigate whether SQD alters cell radiation sensitivity, a dose was selected, and when it was presented alone, it only exerted minimal cytotoxic effects on the HCT-116 cells, which was at about 50 uM concentration. It was found that at such a relatively harmless concentration, SQD enhanced radiation effect in HCT-116 cells, while the effect of Res at a low concentration combined with radiation was much weaker than SQD-radiation. Similar cytotoxic effects were observed in the triple combination. Taken together, our results exhibit that SQD could function to increase radiation-induced cytotoxicity of CRC cells.

The anti-cancer effects of AgNPs have been primarily associated with apoptosis induction and oxidative stress activation (17-20). Recently, the AgNPs' radiosensitizing mechanisms have not been fully understood. Consequently, various studies have suggested that the AgNPs' radiosensitizing mechanism may be correlated with the release of Ag+ cation from the Ag nanostructures inside cells. Ag+ cation has the capability to electron capture, and therefore, acts as an oxidative agent that could decrease the cellular content of ATP and promote ROS production (17). Liu et al. (21) reported that when AgNPs and radiotherapy were treated with

gliomas, they led to proapoptotic and antiproliferative effects. Similarly, in this study, SQD and radiation-induced apoptosis, and this combination case exerted antiproliferative effects.

Moreover, in this study, Res in the double treatment with irradiation exerted cytotoxic effects and induced apoptosis. Previous research displays that Res can be considered a potential radiation sensitizer. Certainly, increased radiation-induced apoptosis after Res treatment was presented in cancer cells (22) which is in line with our results. In addition, in this study, the COX-2 protein level was decreased in the triple combination of radiation-SQD-Res, compared to the untreated CRC cancer cells. Furthermore, in cancer cells, the COX-2 activation can increase the resistance of cancerous cells to radiotherapy. Therefore, it has been suggested that COX-2 inhibition improved the therapeutic response (23).

Similarly, Lu et al. (24) indicate that synthesized AgNPs exert noticeable x-ray irradiation enhancement on the breast adenocarcinoma cells. Moreover, nanosilver significantly increased the radiosensitivity of the HepG2 cells. In this case, increased expression of Caspase-3 led to apoptosis induction. In addition, decreased SOD and total GSH were related to the enhanced radiosensitivity of the HepG2 cells (25) which were consistent with our results.

In a parallel study by Elshawy et al., data showed that the combination of irradiation and AgNPs enhanced the gamma irradiation effects that might be related to the alteration of Caspase-3 gene expression and inhibition of proliferation (26). Definitely, the biological mechanisms for the AgNPs radiosensitization could be more complex (27). It has been indicated that the free radicals after radiation have an affinity to the lipid membrane that may lead to membrane damage (28). Radiation exerts lipid peroxidation by deactivating the antioxidant enzymes. Baranwal et al. (29) reported that in the anaplastic astrocytoma patients, there was a significant increase in the MDA after radiotherapy. In addition, another study exhibited that the lipid peroxidation product level elevated after radiotherapy (30). Definitely, increased ROS level can lead to apoptosis by elevating the cellular lipid peroxidation level by promoting mitochondrial membrane permeability (31, 32). In our study, the MDA levels were increased in the single radiation versus control; however, this difference did not reach a significant level. Nonetheless, in the double combinations of the radiation-SQD and triple combination, the MDA level was increased significantly, compared to the single treatments.

Therefore, our results suggest that the combinations of Res, SQD, and radiation might be effective in the treatment of CRC. Indeed, in these treatments, the low concentration of Res in combination with a minimal toxic concentration of SQD may be considered the potential effective radiosensitizers in the treatment of CRC cells. Certainly, the efficiency of the radiotherapy of CRC cells was increased by utilizing SQD and Res (in vitro). The results of the present study will help to provide a basis for the potential use of SQD as an effective nano-radiosensitizer in the targeted treatment of CRC.

Overall, the above results point to the involvement of some molecular mechanisms in the cell death induced by SQD, Res, and 2 Gy of irradiation. For the description of the observed phenomenon, further studies should be conducted to evaluate the interaction of the studied SQD and Res when combined with irradiation. Based on the obtained findings, it is proposed that the SQD in combination with radiation and Res increased the cell death of human CRC cells by the elevation in the MDA production and decreasing the SOD and GPX activity. Consequently, it led to apoptosis induction. Our study confirmed that SQD and Res in combination with radiation induced a marked anticancer activity. This fact pointed to the potential use of SQD and Res with CRC cancer radiotherapy.

6. Conclusion

It has been suggested that apoptosis induction is considered a possible radiosensitizing mechanism of nanosized materials (10). In this study, the apoptotic response of the HCT-116 cells to SQD and Res with 2 Gy X-ray irradiation was assessed by the AO assay. The Res-SQD in the combined cases increased the percentages of apoptotic cells versus the single treatment. SQD when combined with irradiation, upregulated Caspase-3 mRNA, compared to the single treatment. The apoptosis rate of the HCT-116 cells induced by the SQD and Res plus irradiation was higher than that of double and single treatments. Based on these results, it can be indicated that apoptosis contributed to the cell death induction by SQD or Res plus irradiation, and the better radiosensitizing effect of SQD versus Res might be related to the higher cellular apoptosis level.

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Footnotes

Authors' contribution: Saba Alidadi performed laboratory examination. Mahshid Mohammadian contributed to the all experimental tests in this study and was the advisor of the project. Reza Zohdiaghdam contributed to the laboratory examination. Kosar Esgandari performed data

analysis. Zhaleh Behrouzkia wrote the manuscript draft and was the project supervisor.

Ethical Approval: The study protocol was reviewed and approved by the Ethics Committee of Urmia University of Medical Sciences, Urmia, Iran.

Conflicts of Interests: None declared.

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Financial Disclosure: None declared.

Informed consent: Not applicable.

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