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Effect of gamma irradiation on cytotoxicity, phenolics content and acute toxicity of *Cuscuta chinensis* L. extract

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ABSTRACT

Gamma irradiation is used as an efficient method for decontamination of some foodstuffs and herbal medicines. Several studies have shown that low to moderate doses of gamma irradiation do not alter the chemical composition and biological activities of herbal medicine; however, some changes in herbal composition, appearance or properties have been observed in another studies, and this remains controversial. The present study aimed to evaluate the effect of gamma irradiation on the biological activity and the content of bioactive compounds of *Cuscuta chinensis* L. extract. The dose of 5 kGy was studied for decontamination. The results of the *in vitro* study showed that the cytotoxic effects on A549 and HT-29 cell lines were improved without any alteration of cytotoxicity on normal cell line. Also, acute oral toxicity of the extract did not change according to *in vivo* study. However, due to the significant reduction of total phenol and flavonoids contents by 12% and 18% respectively, the improvement of cytotoxic properties maybe due to the formation of new active compounds, the identification of which requires further studies.

1. Introduction

The use of herbal medicine is on the rise worldwide and their microbiological contamination that may occur every stage of harvest, storage or herbal medicine preparation, is of serious concern (Kunleet al., 2012). Several sanitation methods have been developed to reduce microbial and fungal loads; however, they have some limitation. Fumigation with ethylene oxide is effective but is banned in the European Union because of carcinogenic and mutagenic effects (Steenlandet al., 2004). Steam treatment, microwave heating and radiofrequency heat treatment are other methods for decontamination. Irradiation, including electron beam, X-rays and gamma rays also has been used. There are also newer methods, including the use of ozone and plasma. Numerous studies have been conducted on the efficacy and effectiveness of these methods (Molnáret al., 2018). One of the most controversial methods is the use of gamma rays, which has been studied for effectiveness in decontamination and its effects on the herbal constituents and biological activities. Some of these studies have shown that no significant changes were observed in the content of bioactive compounds or in the physical properties of some herbal products, after irradiation (Choi et al., 2012; Gouvêa et al., 2018; Koseki et al., 2002; Kumar et al., 2010; Pereira et al., 2016), and some have even claimed an increase on some bioactive compounds (Khattaket al., 2010; Jamshidi et al., 2014; Pereira et al., 2017; Khawory et al., 2020). In some cases, changes in chemical composition, reduction of active ingredients, or discoloration have been reported (Bashir et al., 2017; Bhatti et al., 2013; Molnár et al., 2017; Kyung et al., 2018). Considering the extensive use of gamma radiation due to its ease, relative speed and high efficiency, it is very important to ensure that the biological effects of the herbal products have not been changed, since otherwise the herbals not only will not have the expected performance, but also it may cause unknown side effects in the consumer.

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Cuscuta chinensis L. which belongs to *Cuscutaceae* family, is a parasitic plant that is commonly used as a traditional medicine in several countries as an anti-aging, anti-inflammatory agent, pain reliever and aphroditic. Many isolated phytochemical compounds has been isolated from *C. chinensis* including at least 18 flavonoids; 10 volatile oils; 13 phenolic acids; 22 lignans; 2 steroids; 9 polysaccharides; 2 resin glycosides; 16 fatty acids and 1 hydroquinone. Also, various pharmacological properties have been reported, including antioxidant (Baoet al., 2002), antidepressant (Firoozeeiet al., 2020), anti-aging (Sun et al., 2014), anti-abortion (Wu et al., 2018), hepatoprotective (Yen et al., 2007), etc. Cytotoxic effect against several cancer cell lines has been shown as one of the main biological effects of this herbal medicine (Doonapeeet al., 2014; Ghazanfari et al., 2013; Jafarian et al., 2014).

The current study intends to investigate the hypothesis that radiation does not significantly change the quality of plant products. Evaluating cytotoxic effect of *Cuscuta chinensis* L. extract (CCE) on three cell lines, determining the total phenol and flavonoid contents as well as assessing its acute oral toxicity, will be performed in this regard before and after gamma irradiation to find probable alteration of its biological activities or active compounds. Regarding that flavonoids and phenolic compounds are well-known as antioxidant and other important bioactive agents which have preventive and therapeutic effects, their content in herbal medicine is a general criterion for assessing its bio-activity and usefulness for human health (Tungmunnithumet al., 2018).

To the best of our knowledge cytotoxic effect of *C. chinensis* on A-549 cell line has not been reported yet. On the other hand, gamma irradiation of herbal extract instead of intact plant and evaluating the change of its biological properties including toxicity both *in vitro* and *in vivo* may be considered as novelty of the present study.

2. Materials and methods

The current study was conducted under supervision of the Traditional Medicine Clinical Trial Research Center of Shahed University during November 2018 to December 2019. The protocol was approved by medical ethical committee of Shahed University (IR.SHAHED.REC.1399.058).

2.1. Medicinal plant

Cuscuta chinensis L. is the accepted name of a species in the genous *Cuscuta* and family *Cuscutaceae*. Dried herb of *C. chinensis* was obtained from local market of medicinal plants in Tehran, Iran. The plant was authenticated in the Herbarium of school of pharmacy, Tehran University of Medical Sciences and a voucher number (PMP-1311) was assigned to it.

2.1.1. Preparation of Cuscuta chinensis extract

In order to prepare *Cuscuta chinensis* extract (CCE), the dry medicinal plant was cleaned and slightly grinded, then it macerated in methanol: water (80:20) for 48 h. The extract was filtered through Whatman No.1 filter paper and the solvent was evaporated until drying. The dry extract was weighted and extraction yield was calculated. The CCE was stored in a dark glass vial in room temperature. The yield, is calculated by dividing "weight of the dry extract after solvents have been removed" on "weight of the dry plant before extraction"; and expressed as a percentage.

2.2. Irradiation of CCE

2.2.1. Dose calculation

Required dose of gamma ray was calculated according to the equation according to D_{10}-value of determinant bacterial contamination:

$$D10 = \frac{\mathrm{d}}{\log N0 - logN}$$

Where N0 stands for initial total aerobic count plate, N stands for 500 CFU (a conventional allowed contamination for this kind of medicinal herbs which was supposed to be used in cancer patients), *D10* which is defined as the radiation dose (kGy) required to reduce the number of initial aerobic count by 10-fold (one log cycle) or required to kill 90% of the total number and "d" stands for required gamma ray dose (Binte Atiqueet al., 2013; Kortei et al., 2018).

2.2.2. Gamma irradiation

The samples of CCE packed in dark glass vials, were irradiated at ^{60}Co source with the total activity of 5.720 kCi, at AEOI (Atomic Energy Organization of Iran), Tehran in July 2019. The average dose rate was 134 Gy/s, as determined with a Fricke dosimeter. The studied dose was 5 kGy to bring the contamination to an acceptable level and was the minimum dose which could be applied practically in IR-136 irradiation facility.

The irradiation was carried out at room temperature and the irradiated samples were stored at room temperature until further analyses were performed.

2.3. Evaluation of cytotoxic properties

2.3.1. Cell lines

HT-29 (Human colon cancer cell line), A549 (Adenocarcinoma human alveolar basal epithelial cells) and MRC-5 (Normal fibroblasts derived from lung tissue) cell lines were obtained from the National Cell Bank of Iran (NCBI) at Pasteur Institute. The cells were maintained, cultured and incubated in RPMI $_{1540}$ medium (Gibco-BRL, Australia) with 100 U/ml penicillin G (Hayan, Iran), 10% FBS (GibcoBRL, Australia), 100 μg/ml streptomycin (Hayan, Iran) at temperature of at 37 °C, a CO2 of 5%, and a humidity of 90% throughout the study. The cells viability was assessed by the trypan Blue dye exclusion test (Strober 2015).

2.3.2. Performing MTT (methyl tetrazolium bromide cytotoxicity) assay

HT-29, A-549 or MRC-5 cells ($n = 10 \times 10^4$ cells) were seeded into the 96-well plates, and incubated with 0.5 ml medium per each well for 24 h in 37 °C and 5% CO_2. The CCE dried extract was dissolved in hydro-alcoholic (80:20) solution, while the effect of this solution on the mentioned cell lines had been previously tested without any significant lethal effect. Then the cell lines were exposed to the different concentrations (0, 0.1, 1, 10, 25 and 50 μg/ml) of the plant extract (irradiated or non-irradiated CCE) for 24 h. After the incubation of the cells with the extract, each vial of MTT reagent (Sigma, USA) was reconstituted with 3 ml of phosphate buffer solution (PBS) with a pH = 8, and added in an equal volume to 10% of the culture medium. The cells were returned to incubator for 4 h. Then, the plates were removed from the incubator and the resulting formazan crystals were dissolved by adding MTT solubilization solution in equal volume of the original culture medium. MTT formazan crystals were completely dissolved by pipetting up and down. The absorbance was measured at the wavelength of 570 nm, using UV–vis spectrophotometer (Spectronic Genesys, USA). The maximum cells' viability was determined according to the optical density (OD) of the wells which contained no extract. The inhibitory concentration 50% (IC50) was defined as the minimum concentration of the extract that reduced viability of the incubated cells after 24 and 48 h by 50%.

2.4. Determination of total phenolic and total flavonoid contents

2.4.1. Total phenolic content

The total phenolic content was determined by the Folin–Ciocalteu colorimetric method (Araj-Khodaeiet al., 2020). Folin-Ciocalteu as reagent and Gallic acid as standard phenolic compound were used. In this method, phenols react with phosphomolybdic–phosphotungustic components in the Folin– Ciocalteu reagent and produce a blue colored

complex which is measured at 765 nm. Several dilutions of Gallic acid in ethanol (at the concentrations 10, 20, 40,60, 80, 100 μg/ml) were prepared then, 1 ml of every dilution was thoroughly mixed with 5 ml of Folin-Ciocalteu reagent (diluted 1/10) and incubated in room temperature for 10 min. In the next step, 4 ml of the sodium carbonate solution (75 mg/ml) was added and the mixture was incubated at ambient temperature (26 ± 2 °C) for 30 min. The absorbance of the reaction mixture was measured and the result was expressed as Gallic acid equivalents (mg GAE/g) of the sample.

Then a linear calibration curve (absorbance versus concentration) was developed and a linear plot was obtained. The samples (herbal extract before and after irradiation) were tested with same procedure. 1 ml of the samples at the concentration of 500 μg/ml were used. The total phenolic content for each sample (as μg Gallic acid equivalent/mg of the extract) was calculated according to the following formula:

$TPC(\mu g/ml) = C \times V/M$,where C, V, and M were the concentration of Gallic acid obtained from the calibration curve (μg/ml), the volume of the extract (ml), and the weight of the extract (mg), respectively.

2.4.2. Total flavonoid content

The flavonoid content was determined through spectrophotometry by the aluminum chloride colorimetric method (Araj-Khodaeiet al., 2020). Aluminum chloride forms pink-colored complexes with flavonoids which are measured at 425 nm. In a separate set of experiments, a 2.5 ml of the Rutin solution in ethanol (at the concentrations 10, 20, 40, 60,80 and 100 μg/ml) was mixed with 2.5 ml Aluminum chloride reagent (20 mg/ml) and incubated in room temperature for 40 min. Then, the absorbance was measured at 415 nm. The test repeated for every dilution of Rutin three times, and then a standard curve (absorbance versus concentration) was developed and a linear plot was obtained. The total flavonoid content for each sample (as μg rutin equivalent/mg of the extract) was calculated according to the following equation:

$$TFC(\mu g/ml) = C \times V/M,$$

where C, V and M were the concentration of Rutin obtained from the calibration curve (μg/ml), the volume of the extract (ml), and the weight of the extract (mg), respectively.

2.5. Determination of microbiological contamination

2.5.1. Total aerobic plate count and total yeast and mold count

Total Aerobic Plate Count plate (TAPC) and Total Yeast and Mold Count (TYMC) were determined. Four sample solutions were prepared by diluting 1 g of dry samples in 10 ml of peptone water as 10^{-1} dilution, and made a 10^{-2} dilution, by using 1 ml of the diluents in 9 ml peptone water. One ml of each dilution was cultivated in Plate Count Agar (PCA) and Sabouraud Dextrose Agar (SDA) to determine aerobic mesophilic bacteria and total yeasts and molds respectively. Total aerobic mesophilic bacteria, yeasts and molds were counted after 5-day incubation of PCA and SDA plates at a 37 ± 1 °C and 30 ± 1 °C respectively. All the experiments were done before and after gamma irradiation and in duplicates. Determinant bacteria were evaluated with gram staining.

2.5.2. E. coli detection

One ml of each sample solution was inoculated in lactose broth containing durham tubes. Turbidity and gas production due to fermentation of sugar was evaluated after 24 h of incubation in 37 ± 1 °C and the presence of *E. coli* was confirmed by metallic growth on Endo C agar.

2.6. Acute oral toxicity study

2.6.1. Animals

A total of 10 female adult rats of Wistar strain, 8–9 weeks old and in the weight range of 180–200 gr were obtained from the laboratory animal house of Pasteur Institute of Iran. They were maintained in standard condition include 25 °C temperature, 12 h light/dark cycle, and free access to water and food ad libitum and allowed to acclimatize for 10 days before the procedure.

2.6.2. Acute oral toxicity study

The LD 50 value of extracts were estimated using the limit dose test procedure according to the Organization for Economic Cooperation and Development (OECD) test guidelines −425 for Acute Oral Toxicity. The protocol was approved by medical ethical committee of Shahed University (Session no. 111, 2012-1-)

According to this protocol, one female rat is orally administered a single dose of 2000 mg/kg of the extract by gavage, and the animal is monitored for 48 h. If the first rat survives, four additional rats receive the same dose of 2000 mg/kg by gavage, sequentially, if three or more animals survive, the LD50 value is estimated to be over 2000 mg/kg, and the test substance is considered to be safe. In the present study, rats fasted overnight before treatment; then, one female rat received a single dose of 2000 mg/kg of irradiated or non-irradiated CCE through gavage after that rats are monitored for any mortality, morbidity or any signs of toxicity for 48 h. Because death was not observed during 48 h, additional four rats in each group were administered the same dose of extract. All animals were monitored for 14days. The observation interval was hourly during the first 4 h and every 6 h until 48 h.then animals were observed once a day for 12 consecutive days. The following signs were monitored: tremor, convulsion, change in fur and skin, autonomic signs such as lacrimation, salvation, diarrhea, heart and respiratory rate, and any abnormal movement. All animals were weighed daily. The extracts solution was prepared in 5% tween 80 in water; therefore, 5% tween 80 aqueous solution was administered to five rats as a control group.

2.7. Statistical analysis

Data were analyzed using SPSS software version 19 and MLwin software version 2.2. In the descriptive section, the mean and standard deviation were used to describe the data, and in the quantitative section, independent, dependent and mixed model T-test tests were used.

Independent *t*-test for comparing the mean difference between two groups, a T-paired test was used to compare the mean of a variable before and after the intervention and the mixed method analyze was used to evaluate the process of changing variables over time. Also, linear regression method was used to evaluate the standard curve in this study. In order to check the normality of the data, the Kolmogorov-Smirnov test was used and due to the small volume of data, Bayesian estimation method was used. Statistical significance was declared at $p^{<}$ 0.05.

3. Results

3.1. Extraction yield

Extraction yield of hydro alcoholic extract was 14.75%.

3.2. Irradiation dose and microbial contamination before and after irradiation

Total aerobic mesophilic bacteria, yeasts and molds were reported as Colony Forming Unit (CFU) after 5 days of incubation. Determinant bacteria were consisted of spore forming bacilli. According to reported *D10* value of maximum 3.2 kGy for aerobic spore formers (*Bacillus cereus* spores*)* in dry state (Munirand Federighi2020) and the mentioned equation, *N* is considered as 500 (a conventional allowed contamination), *N0* as average total aerobic mesophilic bacteria (1.8×10^3) the required gamma dose (d) which was described as minimum dose was calculated as 1.8 kGy. The studied dose was 5 kGy due to the dose which could be applied practically in IR-136 irradiation facility. The results of microbial contamination in CCE samples before and after irradiation are shown in Table 1.

Table 1
Microbial contamination of *C. chinensis* extract (CCE) samples before and after irradiation.

	Before irradiation CFU±SD	After irradiation CFU±SD	Standard CFU
Total aerobic count plate	$1.8 \times 10^3 \pm 8 \times 10^2$	$5 \times 10^2 \pm 2 \times 10^2$	500
Total yeast and mold count	$2.5 \pm 5 \times 10^{-1}$	0	10
E. coli	-	-	-

3.3. Effect of irradiation on CCE cytotoxicity

As shown in Fig. 1, the viability of HT-29 cell line after treatment with irradiated CCE is significantly lower than when treated with non-irradiated extract. Therefore, the cytotoxic effect of the irradiated extract was significantly higher than the non-irradiated extract. Also, viability of A-549 cell line decreased after treatment with irradiated CCE as shown in Fig. 2, indicating higher cytotoxicity of irradiated extract compared to non-irradiated one. On the other hand, as shown in Fig. 3, irradiated CCE compared to non-irradiated extract had no significant alteration on cytotoxicity on normal cell line (MRC 5).

3.4. Effect of irradiation on total phenolic and flavonoid contents of CCE

The standard curves calculated and plotted based on Gallic acid and Rutin. The results of evaluating the total phenolic and total flavonoid content of the CCE before and after irradiation can be seen in Table 2.

3.5. Acute oral toxicity study

The LD50 was estimated by the limit test method according to the OECD protocol. No mortality or morbidity was observed in both irradiated and non-irradiated CCE treated groups at 2000 mg/kg dose. During the 14 days of the observation period, animals in both groups did not show any monitored signs of toxicity. The mean daily body weight is indicated in Fig. 4. Significant differences between extract-treated and control groups were not observed, and all rats had healthy weight gain during the observation period. According to these findings, the LD50 value of both irradiated and non-irradiated CCE is estimated to be higher than 2000 mg/kg; therefore, they are categorized as non-toxic and are considered safe.

4. Discussion

Due to extensive use of gamma irradiation as an efficient method to decontaminate and increase the shelf life of herbal products and foodstuffs, it is clear that assessing safety and its potential effects on the biological activity of natural compounds is very important (Dal Molim et al., 2016). The present study after measuring the microbial load of CCE, determined the required dose of decontaminant irradiation as 5 kGy. This result is mostly consistent with a study on almond oil that determined 6 kGy as a sufficient dose to eliminate all microbial contaminations (Bhatti et al., 2013). However, the results of a study showed that a dose range of 4–30 kGy is needed to eliminate contamination of botanical materials, the exact amount of which depends on the plant type and its microbial load (Ražem and Katušin- Ražem, 2002). Some other studies, determined the optimal dose for decontamination of herbals as10 kGy (Kumar et al., 2010; Pereira et al., 2016). The effect of gamma irradiation on phenolic compounds in the present study showed that irradiation at a dose of 5 kGy reduced total phenol and total flavonoid contents by 12% and 18%, respectively. This result is in line with a study reporting reduction of phenolics content after irradiation with doses more than 2 kGy (Shabanaet al., 2017). The result is semi-consistent with Janiak's study showing that 5 kGy irradiation altered the phenolic compounds profile of thyme extract; also, their more detailed assays revealed that some phenolic compounds have increased and some have decreased (Janiaket al., 2017). Some other studies reported unchanged or increased total phenolics content by irradiation up to dose of 15 kGy, are inconsistent to the current results (Khattakand Simpson, 2010; Pereira et al., 2017).

The difference in the effect of radiation on total phenolic content may be due to plant type, its phenolic content composition, method of extraction, dose of gamma irradiation, etc. The exact mechanism of change in the phenolic content of plants after gamma irradiation is not well understood. Some suggested explanations, such as increased extractability of these compounds from the plant, may be possible reasons for this increment after irradiation. Decreased phenolic content can also be considered due to the degradation of these compounds.
(Khattakand Simpson, 2010; Gumus, 2011).

The effect of irradiation on biological activity was evaluated *in vitro* and *in vivo* in the current study. *In vitro* cytotoxicity of the CCE before

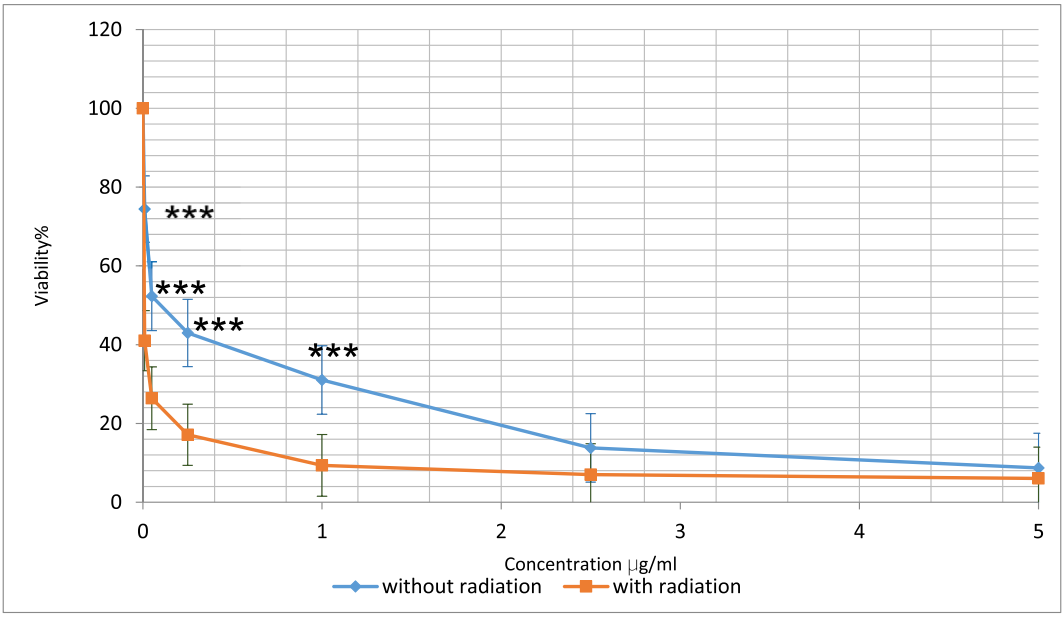

Fig. 1. Comparison of cytotoxicity of *C. chinensis* extract (CCE) on HT-29 before and after irradiation.

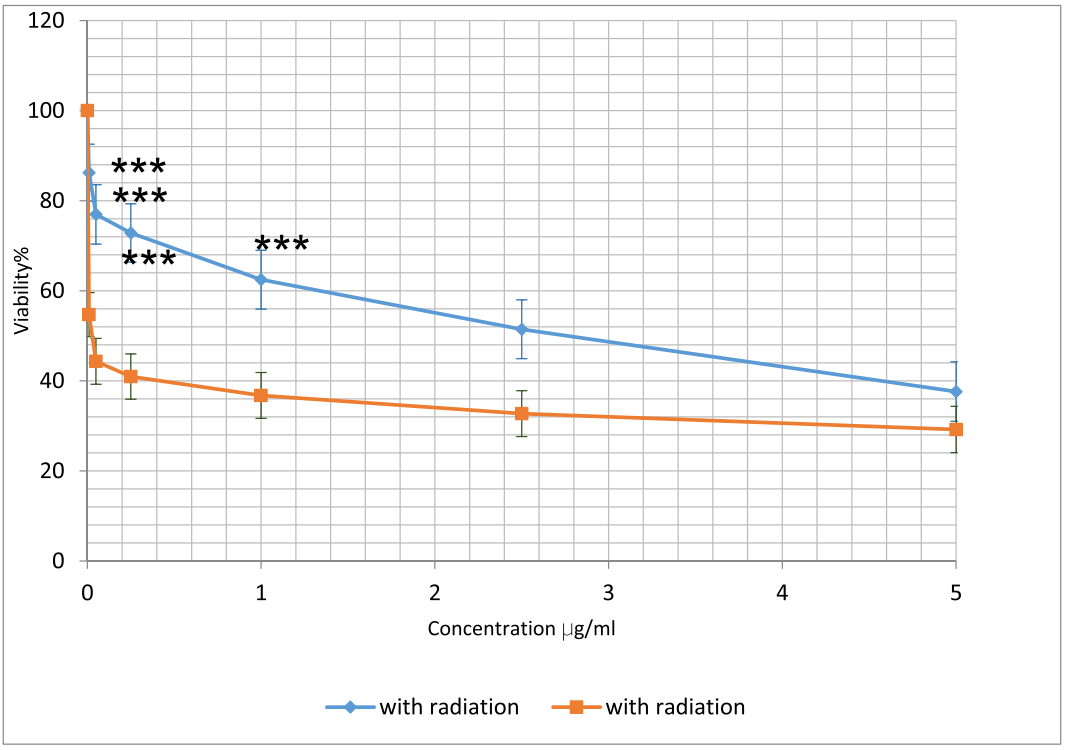

Fig. 2. Comparison of cytotoxicity of *C. chinensis* extract (CCE) on A-549 before and after irradiation.

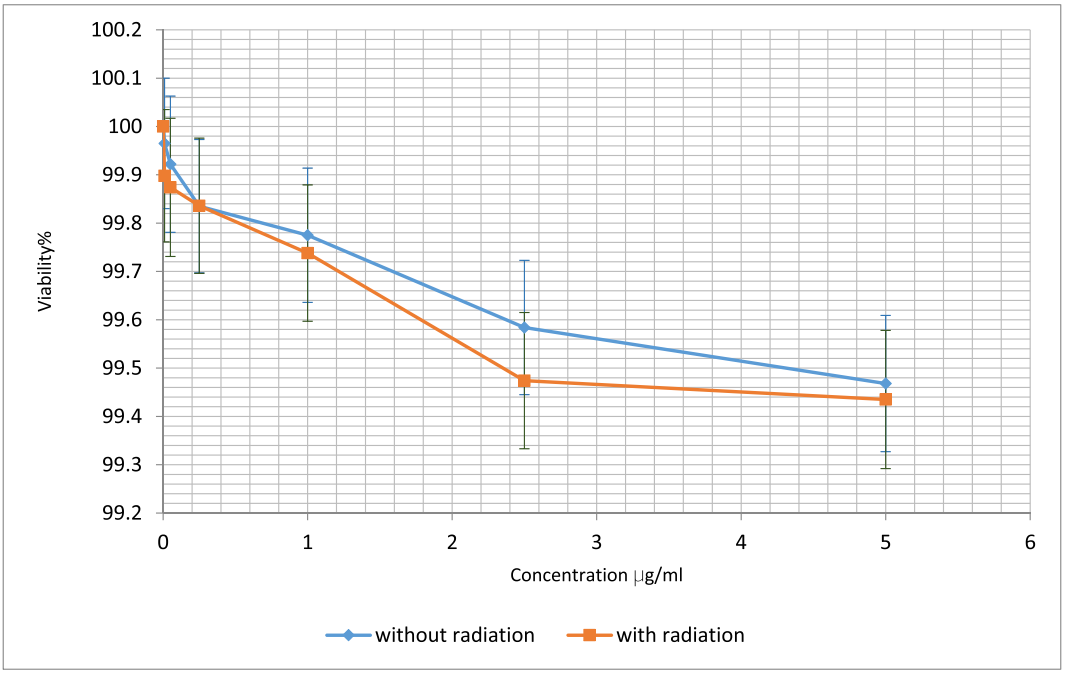

Fig. 3. Comparison of cytotoxicity of *C. chinensis* extract (CCE) on MRC-5 before and after irradiation.

Table 2
Total phenolic and flavonoid contents (µg Gallic acid/ml and µg Rutin/ml resp.) of irradiated and non-irradiated CCE.

Tests	Non-irradiated CCE			Irradiated CCE			T-value	P- value
	Mean	SD	%	Mean	SD	%		
Total phenolic content	23.21	0.08	4.64	20.32	0.68	4.06	7.8	0.016
Total flavonoid content	21.26	0.73	4.25	17.35	0.49	3.47	11.13	0.008

P-value is based on paired sample *t*-test.

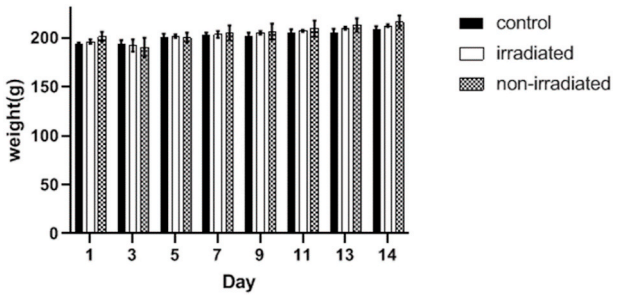

Fig. 4. Daily weight in rats receiving irradiated and non-irradiated CCE in comparison with control group.

and after irradiation on A-549, MRC-5 and HT-29 cell lines showed some considerable changes. Although cytotoxic effects of CCE were increased on lung cancer (A-549) and colon cancer (HT-29) cell lines, but it was unchanged on normal lung cell line (MRC-5). Increased cytotoxic effect of the extract after irradiation is in line with Pereira study that reported increase in cytotoxicity of some herbal extracts after exposure to 10 kGy radiation (Pereira et al., 2018). It is also consistent with the results of a study reporting that 10 kGy irradiation increased the toxicity of thyme and mint extracts, while radiation at doses of 2 and 5 kGy did not affect it (Pereira et al., 2016).

The effects of radiation on the various biological activities of plants are also different. Irradiation by gamma-ray as an ionizing radiation can lead to a variety of chemical changes in molecules, including degradation on the one hand or the formation of new bonds between atoms and formation of new chemical structures on the other. The type of these reactions depends on many factors such as the intensity of the radiation and the type of primary molecules. The result of these changes in chemical structures is a change in biological effects. Therefore, the results of gamma radiation are not the same for different plants that have different compounds. For example, as reported by Pereira, irradiation of thyme at a 10 kGy dose, increased the proliferative activity in MCF-7, HeLa and HepG2 cell lines; while for peppermint, it reduced the cytotoxic activity in MCF-7 and HeLa cell lines, and did not change it in HepG2 cell line (Pereira et al., 2018).

Despite of the significant reduction of total phenol and flavonoid contents of irradiated CCE in the current study, its cytotoxic property improvement maybe due to the formation of some novel active compounds. As, the enhanced biological activity of a new compound generated by gamma irradiation of rosmarinic acid, is reported in a recent study (Jeonget al., 2018).

There have been several studies on the changes that radiation can make in medicinal herbs or herbal extracts' biological activity. Enhancement of some nutraceutical properties is reported for black soybean extract (Krishnan et al., 2018), also gamma irradiation may Improve food safety by degradation of mycotoxins based on the results of two studies on wheat flour and pepper (Caladoet al., 2018; Jalili et al., 2012).

Also, change of some other biological properties after irradiation has been reported, including increased antioxidant activity (Khattakand Simpson, 2010; Kumar et al., 2010) and modified hypoglycemic activity (Hussain et al., 2018). However, another studies reported unchanged antioxidant, cytotoxic and genotoxic activities as well as coumarin content of the plant (Ražem and Katušin- Ražem, 2002; Gouvêa et al., 2018). Also decreased antioxidant activity and tocopherol content after irradiation were reported (Bhatti et al., 2013).

In the current results, evaluation of the acute oral toxicity of this extract and the estimated dose of LD_{50} in rats showed that 5 kGy radiation did not significantly change them. Any similar study has not been performed to evaluate the acute toxicity of herbal extracts after irradiation.

In vitro cytotoxicity of various extracts of *C. chinensis* have been reported on some human cancer cell lines including CCRF-CEM (Acute lymphocytic leukemia), JM (B cell lymphoma), Hela (Cervical cancer), HT-29 (human colon cancer), MDA-MB-468 (Mammary gland breast cancer), Raij (Burkitt's lymphoma) and SK-MEL-3 (Malignant melanoma) (Ghazanfariet al., 2013; Jafarian et al., 2014; Zeraati et al., 2010). But the effectiveness of hydro-alcoholic extract of *C. chinensis* on A 549 cell line (Adenocarcinomic human alveolar basal epithelial cells) has not been studied so far.

5. Conclusion

It can be concluded that CCE irradiation at a dose of 5 kGy could reduce its microbial contaminations to an acceptable level. Although the phenolics content of the plant has been reduced by this process, its cytotoxic effects have been significantly improved selectively without increased toxicity on normal cells. *In vivo* assessment also showed that radiation did not increase the acute oral toxicity of the extract. These results, of course, are preliminary findings and complementary studies are needed for definitive conclusions. Considering the current results, the hypothesis that radiation is ineffective on the phenolics contents and cytotoxicity of CCE is rejected.

The increase of cytotoxicity despite of the phenolic compounds reduction in response to radiation, may be due to the formation of new active compounds that exert their cytotoxic effects specifically on cancer cells without having any side effects on healthy cells. This result may be considered as a novel finding of the current study. Further research to identify probable new active compounds is recommended.

CRediT author statement

Fatemeh Alijaniha: Conceptualization, Methodology, Writing - Original Draft and editing. **Fatemeh Emadi:**Writing - Review & Editing. **Mohsen Naseri:** Supervision.**Mohammad Kamalinejad:** Supervision. **Elahe Motevaseli:** Methodology ,Formal analysis.**Malihe soodi:**Methodology, Investigation.**Roya Karimi:**Investigation.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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