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Enhancement of Physico-chemical and Biological Activities of Antibiotic Cephradine by Gamma Irradiation

Saleem Raza[a]*, Sikandar Khan[b], Ata Ur Rahman[c], Muslim Raza[d] and Fazal Wahid[e]

[a]College of Chemistry and Environmental Engineering, Shenzhen University, PR China
[b]Department of Biotechnology, Shaheed Benazir Bhutto University, Sheringal, Khyber Pakhtunkhwa, Pakistan
[c]Institute of Chemical Sciences, University of Peshawar, Peshawar-25120, Pakistan
[d]Shenzhen Key Laboratory of Synthetic Genomics, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhn, PR China
[e]Department of Chemistry, Government College Peshawar, Higher Education, Khyber Pakhtunkhwa, Pakistan

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Abstract. Radiation based sterilization is a common tool for microbial inactivation in different products on a commercial scale. The objective of the current study was to determine the effect of gamma radiation on cephradine antibiotic in the solid-state to enhance its biological response. Cephradine drug in powder form was treated with different doses (25, 50, 75, 100 and 125 kGy) of the cobalt-60 source in a Gamma cell-220 at a current rate of 8.5 gray/h. The effect of radiation doses on antibiotic was assessed with the help of different analytical techniques such as FT-IR, UV, XRD, SEM and HPLC. The UV spectra of radiated cephradine show some changes in the absorption peak by increasing the intensity of radiations while only slight changes were observed in the other peaks. The crystallinity of antibiotics was tested by the XRD and SEM, it shows a little morphological change. The FT-IR data disclosed significant changes in the absorption bands. The HPLC analysis showed reports an insignificant change which revealed that the radiolytic products are not formed. The radiated cephradine exposes a remarkable antibacterial activity against bacteria; indicating the enhancement of a biological response. In summary, a slight change was observed in cephradine drug with the radiation but the drug was chemically stable.

Keywords: cephradine, gamma radiations, antibacterial assay

Introduction

Cephradine is a first-generation cephalosporin antibiotic which is semisynthetic (Elander, 2003) and its chemical name is 7-[D-2-amino-(1,4-cyclohexadiene-1-yl) acetamido]-3-methyl-8-oxo-5-thia-1-azyabicyclo-octa-2-ene-2 carboxylic acid (El-Shaboury *et al*., 2007) (Fig. 1). Mostly it is found in hydrated form having 3-6% of water molecules in their crystal structure but actually these water molecules are not the stoichiometric hydrates and can be exchanged freely with their crystal lattice structure (Sultana *et al*., 2005).

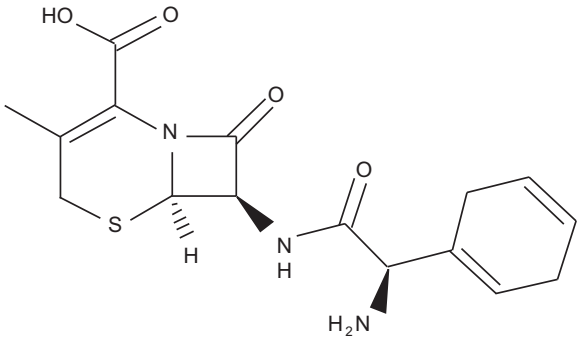

Fig. 1. The basic structure of cephradine.

Cephradine dihydrate (Xie *et al*., 2012) which crystallizes from aqueous solution under controlled conditions, is very stable and resistant to oxidation (Ray *et al*., 2002). However, on dehydration, it is easily dehydrating and becomes very unstable. Cephradine monohydrate recrystallized from acetonitrile (Nie *et al*., 2018) and water is a true hydrate, whereas cephradine recrystallized from anhydrous methanol also appears to be a true monohydrate, although another one-half mole of unbound water was also present (Robin, 2012). The color of cephradine is a white crystalline powder that has a molecular weight of 349 g/mol in anhydrous form, 367.43 g/mol for monohydrate form and 385.45g/mol for dihydrate. While, the melting point is 183-185 °C

*Author for correspondence; E-mail: s.raza47@yahoo.com

for cephradine dihydrate (Chang *et al*., 2008). Moreover, the cephradine has similar antibiotic activity as that of cephalexin, with only slight variations in their biological response (Sabir *et al*., 2014). Cephradine is slightly resistant to cephalothin, but is inactivated by Staphylococcal ß-lactamase (Hamidian *et al*., 2012; Sáez-Llorens and McCracken Jr, 2003). Cephradine antibiotic is mostly active against gram-positive bacteria including *Staphylococcus epidermis, Streptococcus pyogenes, Streptococcus pneumoniae* and *Streptococcus viridans*. However, the inhibitory action of cephradine and cephalexin is quite similar against gram-positive bacteria, while both of these drugs are not effective against gram-negative bacteria (Al Momani *et al*., 2013). Consequently, it is a need of the modern world to make the drugs more efficient and active against bacteria and other pathogens.

Therefore, radiation sterilization of antibiotics through gamma radiation is a well-known technique (Darwish *et al*., 2018). There are numerous reports available on the sterilization of drugs by radiation (Zhang *et al*., 2019). Gamma radiation by cobalt-60 is mostly used to kill the living beings *via* irradiation (Wang and Wang, 2007). The gamma irradiation has a lot of applications in the medical field, including sterilizing medical equipments by killing micro-organisms and some other medically important objects (Huq *et al*., 2015). Gamma rays are also used to treat some types of cancer, despite their cancer-causing properties, since the rays kill cancer cells and this procedure is called gamma knife surgery (Chiang *et al*., 2018). In this method, multiple concentrated beams of gamma rays are directed on the infected area of the body to kill the cancerous cells (Mason *et al*., 2015). The beams are used at different angles to concentrate the radiation on the growth of cancerous cells, while minimizing damage to surrounding tissues. Gamma rays are also used for diagnostic purposes especially in nuclear medicine and nuclear imaging techniques (Volkringer *et al*., 2016; Nesse *et al*., 2010).

Recently the effect of 10 and 25 kGy γ-irradiation on penicillin G plus procaine and sodium ampicillin has been assessed by different chemical and microbiological analytical methods (Li *et al*., 2015; Marciniec *et al*., 2007). The results showed that the antibiotics caused a slight reduction in doses applied but the microbiological assays revealed that the activity of irradiated antibiotics was not reduced. Different microbiological studies were carried out to determine the minimum absorbed doses required to achieve a Sterility Assurance Level (SAL) of 10. However, lower irradiation doses seem to be useful for decontamination purposes (Chambers, 2006; Kemperman *et al*., 2001).

In the current study, cephradine antibiotic in the powder form was selected to investigate the effects of a Co-60 ray doses of 25 kGy to 125 kGy in order to study their degradation and variations effects on cephradine antibiotic. After the irradiation, different parameters were checked to study the dose rate, dose time, and total dose of cobalt irradiation and their antibacterial activities.

Material and Methods

Irradiation procedure. Analytical grade cephradine was purchased from a local enterprise Max Pharmaceutical Company, Islamabad Pakistan. Cephradine in the powder form was subjected to five different doses (25, 50, 75, 100, 125 kGy) of gamma radiation under standard conditions (25-30 °C, at room temperature) using a 60 °C gamma cell (2400 °C Hungary) providing a dose rate of 8.5 kGy/h as an ionizing radioactivity source at Nuclear Institute for Food and Agriculture (NIFA) Tarnab, Peshawar, Pakistan (Fig. 2).

Scanning electron microscopy (SEM). The morphology of irradiated crystal samples was carried out by scanning electron microscopy (SEM) JSM-5910 (Jeol Ltd, Tokyo, Japan) at Central Resources Laboratory (CRL), Physics Department University of Peshawar, Pakistan with a digital camera, at 2.0 kV accelerating voltage (Goldstein *et al*., 2017).

X-ray diffraction. The X-ray diffraction powder patterns of non-irradiated and irradiated cephradine were

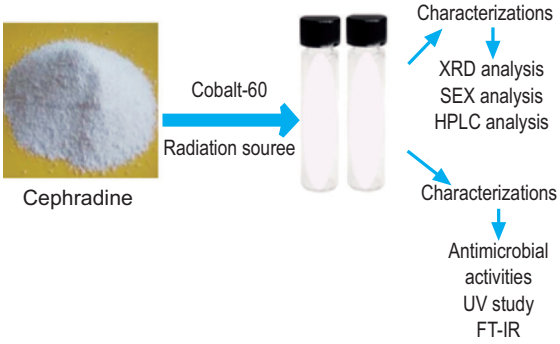

Fig. 2. Schematic diagram showing degradation of cephradine antibiotic in powder state.

performed by X-ray diffractometer system JDX-3532 (Ma *et al*., 2020) in the Central Resources Laboratory (CRL) at Physics Department University of Peshawar, Pakistan by using a Ni filtered CuK (a) radiation at a scanning rate of 20/m undercurrent of 30 mA and voltage of 40 kV. The diffractograms were noted from 5 to 40 degrees (2θ) at a time continual of 1.0 s and a step size of 0.040.

UV-visible spectroscopy. UV-visible spectroscopy was carried out by spectrometer model M-350 at the Institute of Chemical Sciences, University of Peshawar, Pakistan. Spectra were obtained in the wavelength range of 200-700 nm using 0.5 g of the solid drugs dissolved in 10 mL of distilled water and all the data was repeated in triplicate.

FT-IR spectroscopy. FT-IR spectra of cephradine were obtained by using the instrument BIO-RAD FT-IR, model FTS-165 (Friese and Banerjee, 1995).

High-performance liquid chromatography (HPLC) analysis. The HPLC system model 7455 Merck-Hitachi (DAD), with a Diode Array Detector and pump model 7100 were used for the separation of the radiated product (Narayana *et al*., 2013). For all samples used, 20 mg of each sample was dissolved in 100 mL methanol and then used for analysis. Reverse phase, C-18 analytical column (25, 3.9 mm diameter), methyl alcohol as mobile phase and 200-300 nm as detection wavelength were employed. About 40 µL sample was injected for separation, purity evaluation and recording degradation product profile of all the doses. The times consumed are 10 min and the current used in miliampere.

Microbiological assay. The antibacterial activities of irradiated and non-irradiated samples were performed using the agar diffusion technique (Bonev *et al*., 2008). The media used for antibacterial assessment was nutrient agar medium and was prepared as per the manufacturer details and transferred in to 20×20 cm sterile Petri plates (approximately 80 mL/plate). The final agar width was 0.4 cm on each Petri-plate. The Petri-plates were kept to solidify by cooling overnight.

Results and Discussion

Sterilization of pharmaceutical products with irradiation of gamma rays is one of the most efficient techniques. In the current study, the effect of gamma radiation on solid cephradine particles was characterized so, that the drug could be used in the formulation of injections and eye preparations which require sterilization (Galante *et al*., 2018). The effect of gamma irradiation was tested at different radiation doses in a wide range between low (25 kGy) up to high radiation dose (125 kGy) in order to fully describe the effect of radiation on the properties of the solid drug cephradine.

The SEM images (Fig. 3) show the morphology of cephradine in the non-irradiated powders crystals compared with crystals of radiated powders. The SEM data of the samples are presented with different textural morphology characteristics from 25 kGy to 125 kGy dose irradiated cephradine antibiotic. At 75 kGy to 125 kGy dose, the morphology of the antibiotic was a little different as the crystal is very small which shows the effects of gamma radiation (Fig. 3d, 3e and 3f). The analysis and comparison of SEM micrographs reveal that the irradiated antibiotics samples are highly

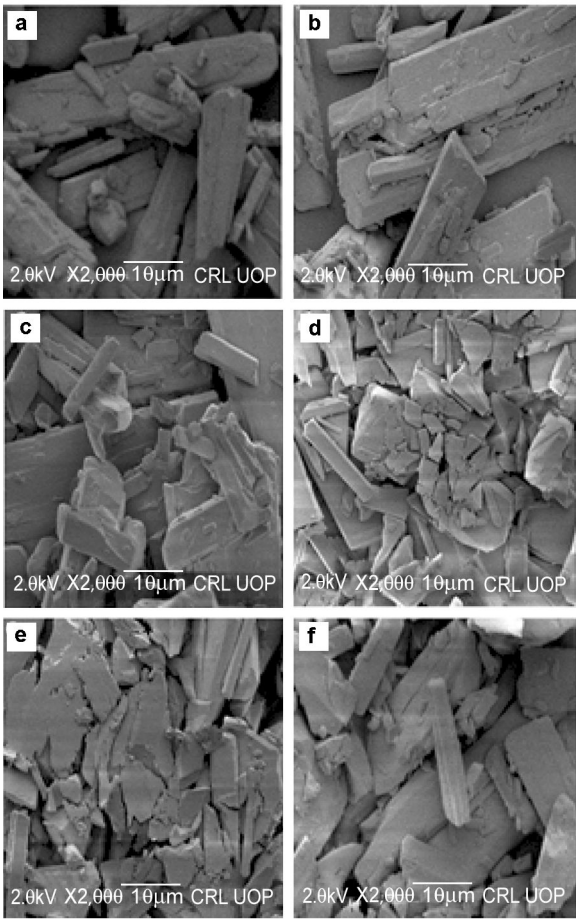

Fig. 3. SEM images of cephradine **(a)** un-irradiated sample **(b)** 25 kGy dose **(c)** 50 kGy dose **(d)** 75 kGy dose **(e)** 100 kGy **(f)** 125 kGy dose.

crystalline but the crystallinity of un-irradiated and irradiated cephradine antibiotic is not largely different.

The same results were obtained by UV-visible spectroscopy. The UV spectra were recorded by dissolving un-irradiated and irradiated powder of cephradine in double distilled water (Fig. 4). The UV-spectra of different irradiated cephradine drugs showed peaks at the wavelength range of 300, 330, 330, 310 and 310 nm, while the un-radiated sample shows wavelength spectra at 300 nm. The peaks become enlarged and these changes indicate that there is a clear difference before and after irradiations which indicate that the physico-chemical changes were observed in the samples. These physico-chemical changes as reflected in the UV-spectra might be the effect of increasing dose of cobalt-60 (such as 25 kGy to 125 kGy).

Furthermore, the X-ray diffraction patterns were investigated to study the crystallinity and amorphous form of the solid mixtures of non-irradiated and radiated drugs as shown in Fig. 5. During the application of different irradiation doses, some peaks become enlarge with the appearance of new peaks as shown in Fig. 5b and 5c). Moreover, with the increase of irradiation doses, the peaks are very intense and sharp. The new peaks indicate their crystallinity. However, these new peaks were detected in XRD patterns which show that with an increase of radiation doses from 25 kGy to 125 kGy slight changes were observed but it would not affect the crystal structure of the drug cephradine.

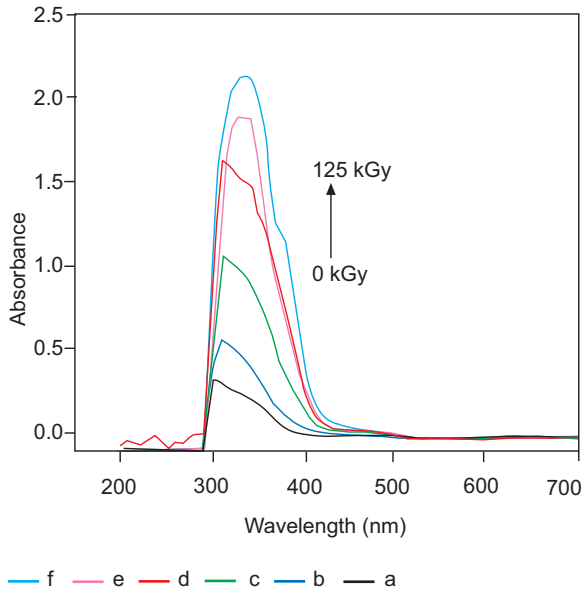

Fig. 4. UV spectra of cephradine **(a)** un-irradiated **(b)** 25 kGy dose **(c)** 50 kGy dose **(d)** 75 kGy dose **(e)** 100 kGy dose **(f)** 125 kGy dose.

Furthermore, the FT-IR spectra of the drug molecules after and before irradiation at different doses of cephradine which shown the characteristic absorptions band for the un-irradiated and radiated drug. The lactam (C=O) band at 1750/cm and the amide (C-N) band at 1687/cm in the spectrum of cephradine is also found in the spectra of the irradiated drug as shown in Table 1, (Xie *et al*., 2012). The oxygen carbonyl asymmetry stretching band at 1519/cm for non-radiated drug, while the same peak for the irradiated cephradine drug become enhance and changes were observed in the peak intensity as shown in Table 1 and COO symmetry stretching band in non-radiated drug at 1352/cm the same characteristic peaks appeared in the spectra of radiated drugs (doses from 25 to 125 kGy) but with a little increase/decrease in value. Thus, the FT-IR spectra indicate that there are no significant changes occurred in the vibrational characteristic peaks between radiated and non-radiated drugs and no radiolytic products were detected in the FT-IR spectra but some new peaks are observed in the spectra with the passage of radiation. However, the drug was structurally and chemically stable.

HPLC analysis was used to know the structure of cephradine antibiotic that whether the drug was stable or degraded into some other form with the passage of radiation doses. The sample of approximately 10 mg of irradiated and non-irradiated samples was dissolved in 50 mL methanol solution and then used for analysis. The phase used for the study is a reverse phase. The time consumed is 10 min and the current used in mili ampere. The cephradine HPLC irradiated and non-irradiated values investigate and then analyzed which

Table 1. FT-IR data of cephradine

Compound	(C=O) lactam	(C-N) amide	(COO) asymmetry	(COO) symmetry	(C-S)
0 kGy	1749	1687	1519	1352	960
25 kGy	1755	1640	1568	1364	956
50 kGy	1714	1612	1516	1354	1004
75 kGy	1755	1698	1562	1380	1038
100 kGy	1759	1658	1552	1380	698
125 kGy	1756	1619	1583	1338	1024

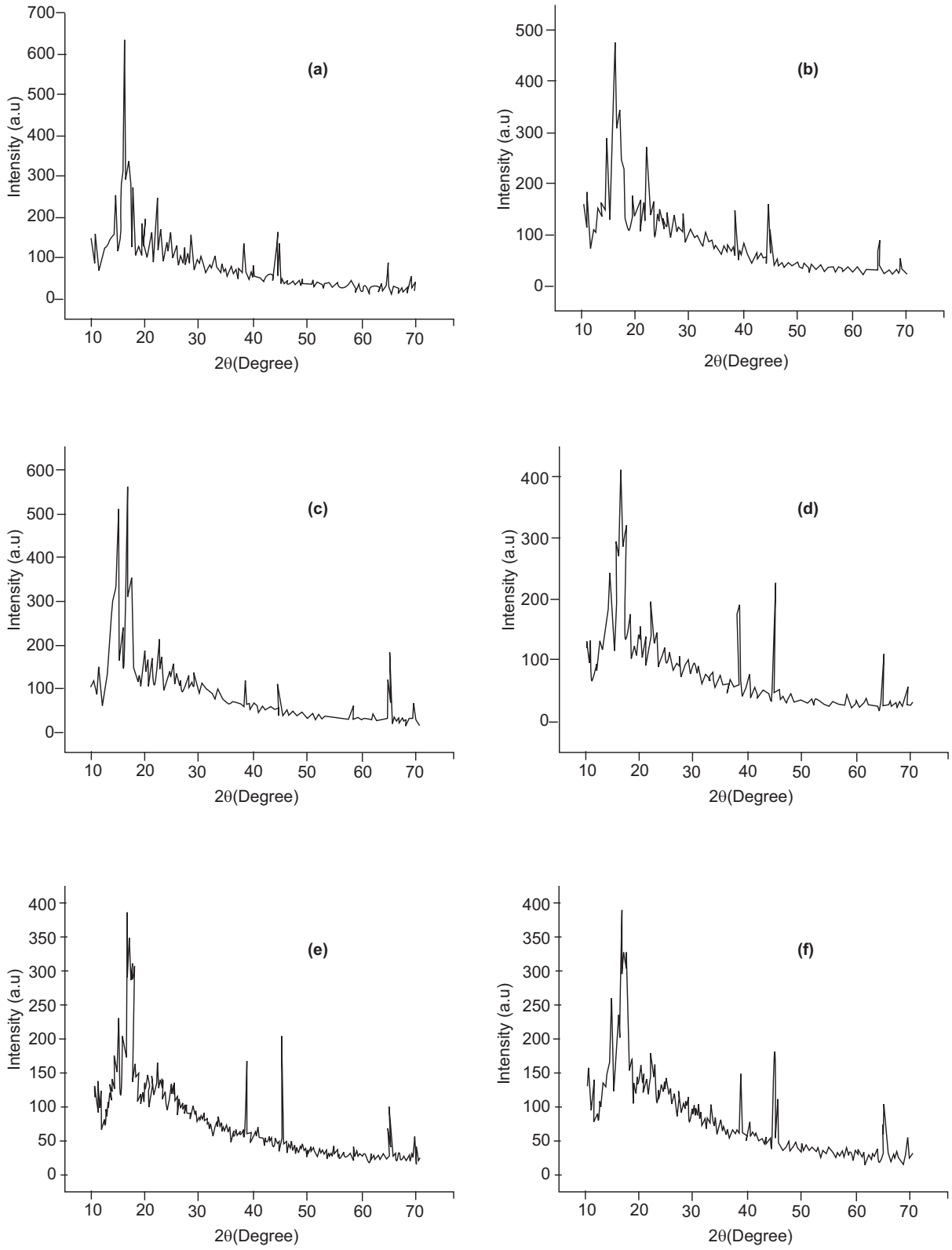

Fig. 5. X-rays graphs of cephradine **(a)** Un-irradiated **(b)** 25 kGy dose **(c)** 50 kGy dose **(d)** 75 kGy dose **(e)** 100 kGy dose **(f)** 125 kGy dose.

shows no clear separation or change with the passage of different irradiation doses. However, no new peaks observed in the HPLC spectra of irradiated and non-irradiated cephradine. Similar peaks with the same intensity were observed in all the spectra as shown in Fig. 6. In addition, no significant peaks were observed in the visible range indicating that no radiological intermediate was formed during the irradiation. Therefore, it is concluded that with irradiation the cephradine is quite stable.

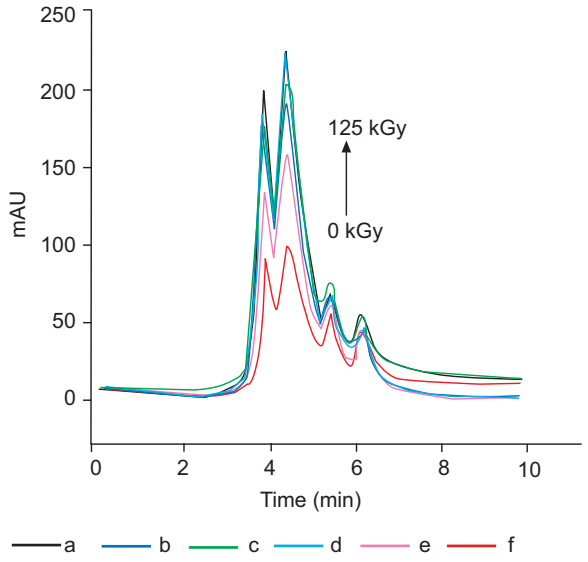

Fig. 6. HPLC spectra of cephradine **(a)** un-irradiated **(b)** 25 kGy dose **(c)** 50 kGy dose **(d)** 75 kGy dose **(e)** 100 kGy dose **(f)** 125 kGy dose.

Table 2. Antibacterial activities against cephradine with different bacteria species

Radiation strength (kGy)	*E. coli*	Staph	Pseudo	Citro	MRSA
	(mm)				
0	16	18	17	14	18
25	17	19	18	15	19
50	19	20	18	16	20
75	20	21	19	18	20
100	21	22	20	18	21
125	22	23	21	20	22

Note: E. coli = *Escherichia coli*; Staph = Staphylococcus aureus, Pseudo = *Pseudomonas aeruginosa*; **Citro** = *Citrobacter*; MRSA = *Methicillin-resistant Staphylococcus aureus*; mm = millimeter.

Antibacterial assay. The noteworthy results were obtained, when the drug-treated with different bacterial strains as shown in (Table 2). All the strains were significantly affected by the drug, their biological response was enhanced by increasing the irradiation doses. The highest inhibition zone were found at 125 kGy for irradiated cephradine against all five bacteria. In addition, the most significant zone was found against *Staphylococcus aureus* bacteria at 125 kGy radiation dose. Moreover, the inhibition zones increases for all bacteria suggesting that the biological activities of antibiotic against bacteria enhance with the increase of irradiation doses. In the future, this study may lead to the improvement of new antibiotics of diverse potencies.

Conclusion

It is concluded that after treatment with cobalt-60 radiation the cephradine drug is chemically stable, although with different doses of irradiations some minor changes occur which were observed by different analytical techniques (SEM, XRD, UV, FTIR, HPLC). Moreover, this study has discussed some effects of radiation on the biological response of the drug against different bacterial strains. The results disclosed that the biological activities of the drug are enhanced by increasing the radiation doses. In the future, these preliminary results obtained could provide information for the drug designer to use radiation therapy to enhance the biological activity of therapeutic compounds.

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Conflict of Interest. The authors have declared no conflict of interest.

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