Egypt. J. Rad. Sci. Applic. **Vol. 35,** No. 1-2, pp. 145-155 (2022)

Egyptian Journal of Radiation Sciences and Applications
http://ejrsa.journals.ekb.eg/

EGYPTIAN ATOMIC ENERGY AUTHORITY

The Novel Modified 5-Fluorouracil with Zinc Oxide Nano-Particles and its Combined Effect with Gamma Radiations on Human Cancer Cell Lines

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5-FLUOROURACIL (5-FU) is widely used in the treatment of cancer. The clinical use of 5-Flurouracil is constantly challenged by its poor selectivity and various side effects. The present study aims at modifying 5-fluorouracil with zinc oxide nanoparticles (NPs) and Cetrimonium bromide (CTAB) to enhance its antitumor activity. The nanocomposite synthesis was evident by Scanning Electron Microscope (SEM) analysis, Transmission Electron Microscopy (TEM) analysis, X-Ray Diffraction (XRD) analysis and Fourier Transforms Infrared (FT-IR) spectroscopy. In addition , the combined effect of ionizing gamma radiation with the modified 5-FU with Zinc oxide nanoparticles and Cetrimonium bromide (CTAB) was also investigated after 24 and 48h. The *in vitro* anticancer activity of nanocomposite (5-FU modified with zinc oxide nano particles and CTAB) on liver cancer cells lines (HepG2), (HUH-7) and breast cancer cells (MCF-7) was investigated using MTT (3-4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay after 24 and 48 hr. It was found that nanocomposite exerted better *in vitro* anticancer activity against the previous human cancer cell lines comparable to the activity of 5-FU alone after 24 and 48h. It could be concluded that the effectiveness of 5-FU modified with zinc oxide nanoparticles and CTAB as an antitumor drug is enhanced by the combination of chemotherapy and radiotherapy with nanotechnology.

Keywords: 5-Fluorouracil, Antitumor activity, Cetrimonium bromide, Human cancer cell lines, Zinc oxide nanoparticles.

Introduction

Cancer is a hyperproliferative disorder marked by metastasis into the vital organs of the body through invasion and angiogenesis (Sethy & Kundu, 2021).

Chemotherapy has been used in combination along with surgery or radiotherapy to treat many cancers**.** However, the toxic side effects of the conventional therapy are a disadvantage, which reduces the chances of remission. The therapeutic regimen mainly involves the surgical removal of the diseased area followed by adjuvant chemotherapy involving either cisplatin or 5-fluorouracil or both in combination with docetaxel or cetuximab along with radiation therapy (Dasgupta et al., 2020).

5-Fluorouracil (5-FU) is widely used in the treatment of cancer. Over the past 20 years, increased understanding of the mechanism of action of 5-FU led to the development of strategies that increase its anticancer activity. However, drug resistance remains a significant limitation to its clinical use. Emerging technologies, such as

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Received 13/02/2023; Accepted 05/04/2023
DOI: 10.21608/EJRSA.2023.193748.1150

DNA microarray profiling, have the potential to identify novel genes that are involved in mediating resistance to 5-FU. Such target genes might prove to be therapeutically valuable as new targets for chemotherapy, or as predictive biomarkers of response to 5-FU –based chemotherapy (Aghamiri et al., 2019).

Lack of specificity for tumor cells is a drawback, when combining radiotherapy with chemotherapy, since it is cytotoxic to both normal and tumor cells. Accordingly, the current study is designed to improve the properties of 5-fluorouracil so that it becomes selectively retained in tumor cells and not normal cells, so that when radiation therapy is applied, tumor cells are affected and normal tissue are less affected (Handali et al., 2019).

The first mechanism responsible for chemotherapy and radiation CT-RT interaction is the direct enhancement of the initial radiation damage, resulting from the incorporation of the chemotherapeutic drugs into DNA. The primary target for radiation injury is DNA, where halogenated pyrimidines such as 5-fluorouracil (5-FU) are incorporated, making the DNA more susceptible to RT (Handali et al., 2019).

Nanotechnology allows the manipulation of materials at nanoscale level (1-100 nm), which enables precision engineering to control nanoparticles (NPs') physicochemical properties, as well as their interactions with biological systems (Mohammed & Hanoon, 2021). Inorganic NPs, including metal oxides are promising materials for applications in medicine, such as cell imaging, biosensing drug, gene delivery and cancer therapy. Recently, ZnO NPs have received much attention for their implications in cancer therapy (Pavithra et al., 2020). Cetrimonium bromide (CTAB) belongs to a group of quaternary ammonium compounds, which also includes benzethonium chloride and dequalinium chloride, both of which have demonstrated anticancer properties *in vitro* and *in vivo* by targeting tumor mitochondria (Zhang, 2021).

The present study was aims at evaluating the potential therapeutic effect of a novel Nanocomposite (5-fluorouracil+zinc Oxide NPs) modified with CTAB on different human cancer cell lines. It also aims at studying the potential therapeutic effect of a novel Nanocomposite combined with ionizing gamma radiation.

Materials and Methods

Materials

5-FU (5-fluorouracil), MTT(3-4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), CTAB (Cetrimonium bromide), Trypan blue 0.4% and ZnO nanocomposite were kindly provided from Sigma-Aldrich Chemicals Co. DMEM media supplemented with L-glutamine, Trypsin Versene (EDTA) 200 mg/L and PEN-STREP 10.000 U Penicillin /mL 10.000 U Streptomycin/ml were purchased from Lonza chemicals Co. Ammonium Hydroxide (El-Gomhoria Chemicals Company). Gamma cell -40 Candian, Caesium137 at dose rate: 0.675 rad/second was provided from the Atomic Energy Authority.

Experimental design

1- Screening of the cytotoxic effect of nanocomposite against three human cancer cell lines (HepG2, MCF-7 and HUH-7) comparable to 5-fluorouracil, zinc oxide nanoparticles and cetrimonium bromide after 24 and 48 hr was carried out by MTT assay.

2- Screening of the cytotoxic effect of nanocomposite against normal cell line (WI-38) comparable to pure 5-fluorouracil after 24 and 48 hr was conducted by MTT assay.

3- Studying the effect of different doses of radiation (2, 6, 8, and 10 Gy) against nanocomposite was performed by using HepG2 to detect the effective dose that led to synergistic effect with nanocomposite against cancer cell lines and safe on normal cell line.

4- Studying the effect of 10 Gy radiation on the cytotoxicity effect of nanocomposite against different human cancer cell lines comparable to 5-fluorouracil, zinc oxide nanoparticles and cetrimonium bromide after 24 and 48 hr was conducted by MTT assay.

5- Screening of the cytotoxic effect of nanocomposite combined with 10 Gy against normal cell line (WI-38) comparable to pure nanocomposite after 24 and 48 hr was done by MTT assay.

Methods

The nanocomposite (5-fluorouracil modified with zinc oxide nanoparticles coated with

cetrimmonium bromide) was prepared. The resulted compound (75% of 5-fluorouracil + 23% of zinc oxide NPs coated with 2% Surfactant CTAB) was ball milled for 8 hours in the nanoscale to the corresponding nanocomposite (5-fluorouracil, zinc oxide nanoparticles and CTAB) according to Abou Zaid et al. (2015).

The size of the nanocomposite was analyzed using Transmission Electron Microscopy (TEM). Studies were carried out using Transmission Electron Microscopy (JEOL JEM 2100: Cx; Tokyo, Japan) at an accelerating voltage of 15 KV and 200 KV and total magnification of 30,000 KV that was used to qualitatively study the morphology of the nanocomposite.

Scanning electron microscope (SEM) analysis

SEM is useful for determining the particle shape and appropriate size distribution of the different compounds (Godbole et al., 2016).

X-Ray diffraction (XRD) analysis

X-ray diffraction studies provide information about the crystallinity of the sample, which is reflected by a characteristic fingerprint region in the diffraction pattern (Godbole et al., 2016).

Fourier transforms infrared (FT-IR) spectroscopy

FT-IR is one of the techniques that are used today for measuring the intensity of infrared radiation as a function of frequency or wavelength. Infrared radiation is invisible electromagnetic radiation just below the red color of the visible electromagnetic spectrum, with wavelength range from 700 nm to 1 mm (Singh et al., 2009).

Cell culture

Three Cancer cell lines of human origin are adopted, including HepG2(human hepatocellular carcinoma), HuH-7(a well differentiated hepatocyte-derived carcinoma cell line), MCF-7(a breast cancer cell line) and one normal cell line WI-38.

Cells were routinely cultured in DMEM media (Lonza), supplemented with 10% FBS (Lonza), 1% 100 u/mL penicillin and 100ug/ml streptomycin (Lonza) in a humidified incubator at 37°C with an atmosphere containing 5% CO_2. Each experiment was carried out in triplicate.

At 85% confluence cells were harvested using 0.25% trypsin and were subculture into 75 cm^2 and six –well plates or 96 –well plates according to selection of experiments. Cells were allowed to attach to the surface for 24 hr prior to treatment. Nanocomposite, 5-FU and Zn oxide NPs were dissolved in DMSO and diluted to appropriate concentrations: 5-FU alone (500, 250, 125, 62.5, 31.25, 15.625, 7.8 and 3.4 µg /mL), Nanocomposite (100, 50, 25, 12.5, 6.25 and 3.12 µg /mL) and ZnoxideNPs (100, 50, 25, 12.5, 6.25 and 3.12 µg/mL). CTAB was dissolved in culture medium at the concentrations: 25, 12.5, 6.25, 3.12, 1.6 and 0.8 µg /mL. Following each treatment, cells were harvested to determine cytotoxicity after 24 and 48 hr.

Cell viability assay

The antitumor effect and inhibitory concentration 50 (IC50) of this novel nanocomposite will be investigated against MCF-7, HUH-7 and HePG2 in comparison with 5-FU, zinc Oxide Nps and CTAB and also the cytotoxic effect of nanocomposite was investigated against normal cell line (WI-38) in comparison with 5-FU. Briefly, $1x10^4$ cells/well were seeded in 96 well plates and Cells were allowed to attach to the surface for 24 hr prior to treatment then exposed to Nanocomposite, Zn oxide Nps, 5-FU and CTAB at the concentrations mentioned above for 24 and 48 hours and also blank, control cells without any treatment and cells treated with the solvent of the drugs (DMSO) in triplicate also (Tytus & Jurek, 2002).

At the end of exposure, culture medium was removed from each well and replaced with MTT solution (0.5 mg/mL PBS) and incubated for 3 hr at 37ºC until a purple –colored formazan product developed.

The resulting formazan product was dissolved in DMSO and left for 10 minutes. The absorbance was measured at 590 nm by a microplate reader (FLUOstar Omega, Cary, NC).

Gamma irradiation of cell culture and MTT assay

The effective dose of gamma radiation was 10 Gy for all used cell lines. The effective dose was selected according to preliminary study at different doses done on HepG2.

Procedure of MTT assay with gamma radiation

Viability of HepG2, HUH-7, MCF-7 and WI-

38 was assessed by the MTT assay as follows: The compounds were added at first in the 96 well plate after the formation of monolayer cells then irradiation of cells was conducted at Cesium unit and left for 24 or 48 hours and also blank, control cells without any treatment, cells treated with radiation and cells treated with the solvent of the drugs (DMSO) were done also in triplicate. The same procedure of MTT assay that was mentioned above was repeated and also the inhibiton rate percentage was calculated (Figs. 1 and 2).

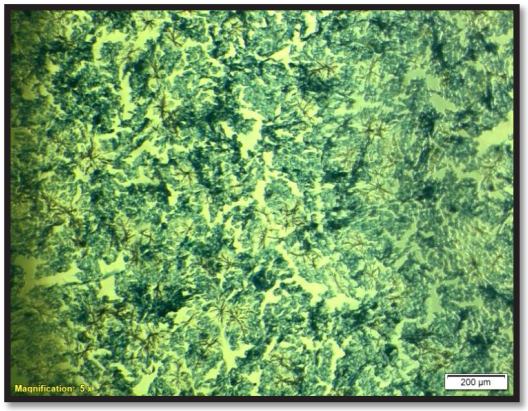

Fig. 1. HepG2 cell line control after MTT assay

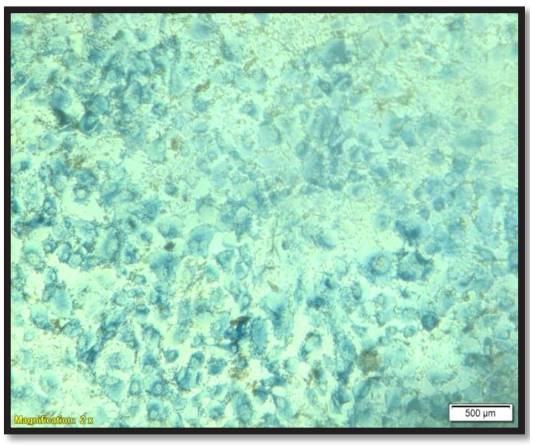

Fig. 2. Irradiated HepG2 with 10 Gy after MTT assay

Statistical analysis

The experiments were performed in triplicate (n= 3) and they are represented as mean ± standard error. Data were analyzed using one-way ANOVA followed by Tukey test. Significance was ascribed at $P<0.05$. All analyses were conducted using the Prism software package (GraphPad Software, Version5.0, GraphPad Software Inc., SanDiego, CA).

Results

Cytotoxicity assay by MTT method

The results obtained from Table 1 and Fig. **3** indicated that there is a significant difference between IC_{50} values (µg/mL) of nanocomposite on different human cancer cell lines and normal cell line after 24 hr. There is no significant difference between IC_{50} values (µg/ml) of 5-FU on HepG2 and HUH-7 but there is a significant difference between IC_{50} values of 5-FU on MCF-7 and WI-38 with HepG2 and HUH7. There is a significant difference between IC_{50} values (µg/ml) of zinc oxide NPs on different human cancer cell lines. There is no significant difference between IC_{50} values (µg/ml) of CTAB on HepG2 and MCF-7, but there is a significant difference between IC_{50} values (µg/ml) of CTAB on HUH-7 with HepG2 and MCF-7.

The least IC_{50} value for nanocomposite is given by HepG2 cell line after 24hr of treatment (24.5 µg/dl) comparable with other cell lines MCF-7 (40.5 µg/mL) and HUH-7 (37.1 µg/mL)

Nanocomposite has IC_{50}= 80 µg/mL against WI-38 cell line after 24 hr comparable to 5-fluorouracil has IC_{50}= 250 µg/mL, the result showed that 5-fluorouracil has the similar results on both cancer and normal cell lines after 24 hr (300.8 µg/ml in case of HepG2, 289.2 µg/mL in case of HUH-7 and 455.1µg/mL in case of MCF-7)

The results obtained from Table 2 and Fig. 4 indicated that there is no significant difference between IC_{50} values(µg/mL) of nanocomposite on HepG2 with MCF-7, but there is a significant difference between IC_{50} values(µg/mL) of nanocomposite on HUH-7 and WI-38 with MCF-7 and HepG2. There is a significant difference between IC_{50} values (µg/mL) of 5-FU on different human cell lines but there is no significant difference between IC_{50} values (µg/mL) of 5-FU on HUH-7 and WI38.

There is a significant difference between IC_{50} values (µg/ml) of zinc oxide NPs on different human cancer cell lines. There is a significant difference between IC_{50} values (µg/mL) of CTAB on HUH-7 with MCF-7 and HepG2, but there is no significant difference between IC_{50} values (µg/mL) of CTAB on HepG2 withMCF-7.

TABLE 1. A comparison study of IC_{50} values (µg/mL) of nanocomposite, 5-FU, zinc oxide NPs and CTAB on different human cancer cell lines (HepG2, HUH-7 and MCF-7) and normal cell line (WI-38) after 24 hr

	HepG2	HUH-7	MCF-7	WI-38
IC_{50} of nanocomposite	24.5±0.11[a]	37.1±0.05[a]	40.53±0.12[a]	80±0.10[a]
IC_{50} of 5-FU	300.8±0.39	298.2±0.08	455.1±0.05[b]	245.6±0.5[b]
IC_{50} of zinc oxide NPs	66±0.5[c]	84.43±0.2[c]	38.17±0.12[c]	
IC_{50} of CTAB	4.9±0.08	1.2±0.14[d]	5±0.11	

All data were represented by Mean ± SE, P value<0.05.

a; refers to that there is a statistically significant difference of IC_{50} values(µg/mL) of nanocomposite on different cell lines.

b; refers to there is a statistically significant difference of IC_{50} values (µg/mL) of 5-FU on different cell lines.

c; refers to there is a statistically significant difference of IC_{50} values (µg/mL) of zinc oxide nanoparticles on different cell lines.

d; refers to there is a statistically significant difference of IC_{50} values (µg/mL) of CTAB on different cell lines.

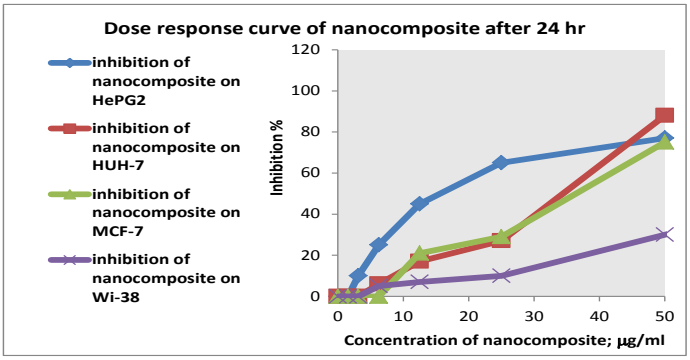

Fig. 3. The cytotoxicity effect of nanocomposite on different cell lines after 24 hr

TABLE 2. Comparison study of IC_{50} values (µg/ml) of nanocomposite, 5-FU, zinc oxide NPs and CTAB on different human cancer cell lines and normal cell line after 48 hr

	HepG2	HUH-7	MCF-7	WI-38
IC_{50} of nanocomposite	16.27±0.14	3±0.57[a]	17.33±0.20	60±0.3[a]
IC_{50} of 5-FU	95.63±0.32[b]	25.4±0.26[b]	6.167±0.12[b]	30±0.6[b]
IC_{50} of zinc oxide NPs	59.6±0.31[c]	69.27±0.21[c]	31.8±0.41[c]	
IC_{50} of CTAB	2.26±0.14	0.9±0.37[d]	3.13±0.18	

All data were represented by Mean ± SE, P value<0.05.

a; refers to that there is a statistically significant difference of IC_{50} values (µg/mL) of nanocomposite on different cell lines.

b; refers to there is a statistically significant difference of IC_{50} values (g/mL) of 5-FU on different cell lines.

c; refers to there is a statistically significant difference of IC_{50} values (µg/mL) of zinc oxide NPs on different cell lines.

d; refers to there is a statistically significant difference of IC_{50} values (g/mL) of CTAB on different cell lines.

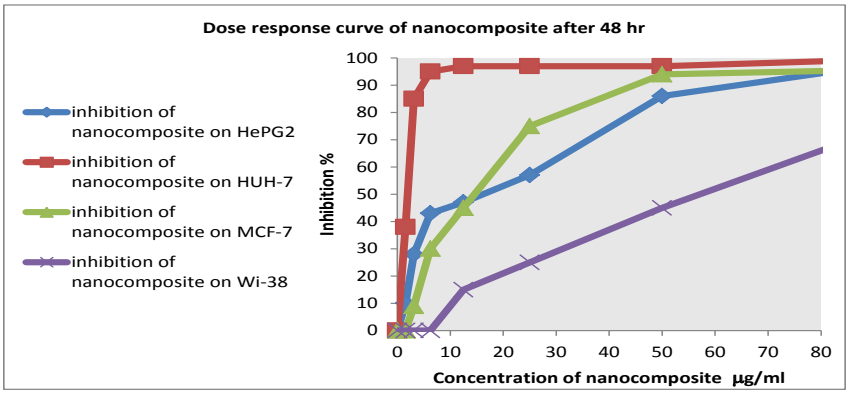

Fig. 4. The cytotoxicity effect of nanocomposite on different cell lines after 48 hr

The results showed that there is a significant difference between IC_{50} values (μg/mL) of nanocomposite, 5-FU, zinc oxide NPs, CTAB after 24 hr with IC_{50} values (μg/mL) of nanocomposite, 5-FU, zinc oxide NPs, CTAB after 48 hr on different human cancer cell lines and normal cell line and the nanocomposite is more targeted to cancer cell lines than normal cell line.

The least IC_{50} value for nanocomposite is given by HUH-7 cell line after 48 hr of treatment (3 μg/dl) comparable with other cell lines MCF-7(17.3μg/ml) and HepG2(16.2 μg/ml)

Nanocomposite has IC_{50}= 60 μg/mL against WI-38 cell line after 48 hr comparable to 5-fluorouracil has IC_{50} =30 μg/ml after 48 hr, so the nanocomposite is less toxic on normal cell line (WI-38) comparable to cancer cell lines (HePG2, MCF-7 and HUH-7)

Gamma irradiations of cell lines and cytotoxicity by MTT assay

The dose of radiation 10 Gy was detected from the preliminary study.

In the preliminary study; Different doses of radiation (2, 6, 8 and 10 Gy) were used with nanocomposite to show the effective dose of radiation that give the least IC50 (inhibitory concentration that kill 50% of cells The) with nanocomposite after 24 and 48 hr on HepG2 cell line. The effective dose was the 10 Gy after 48 hr. Whereas at 24 hr, there is no significant difference between IC50 of nanocomposite with and without radiation as in Table 3.

The results obtained from Table 4 and Fig. 5 indicated that there is a significant difference between IC_{50} values (μg/ml) of nanocomposite on different human cancer cell lines and normal cell line. There is a significant difference between IC_{50} values (μg/ml) of 5-FU on different human cell lines and normal cell line. There is a significant difference between IC_{50} values of zinc oxide NPs on MCF-7 with HUH-7 and HepG2, but there is no significant difference between IC_{50}values (μg/mL) of zinc oxide NPs on HepG2 with HUH-7. There is a significant difference between IC_{50} values (μg/ml) of CTAB on HUH-7 with HepG2 and MCF-7, but there is no significant difference between IC_{50} values (μg/ml) of CTAB on HepG2 with MCF-7. There is a significant difference between the inhibition effect of 10 Gy dose on HepG2 with WI-38 cell line .

The results obtained from Table 5 & Fig. 6 indicated that there is a significant difference between IC_{50} values (μg/ml) of nanocomposite on different human cancer cell lines and normal cell line. There is no significant difference between IC_{50} values μg/ml of 5-FU on WI-38 and HUH-7 with HepG2, but There is a significant difference between IC_{50} values μg/ml of 5-FU on MCF-7 with HepG2, HUH-7 and WI-38. There is a significant difference between IC_{50} values (μg/ml) of zinc oxide NPs on different human cancer cell lines. There is no significant difference between IC_{50} values (μg/mL) of CTAB on different cancer cell lines. There is a significant difference between the inhibition effect of 10 Gy dose on different cell lines.

Nanocomposite combined with 10 Gy exerted better *in vitro* anticancer activity against the previous human cancer cell lines comparable to the activity of pure5-FU+10 Gy alone after 24 and 48 hr, except in case of MCF-7, there is no significant difference between the effect of nanocomposite and 5-fluorouracil after 48 hr.

TABLE 3. IC_{50} values (g/mL) of nanocomposite on HepG2 either without and with different doses of radiation after 24 hr and 48 hr

		With Radiation			
	Without radiation	**2 Gy**	**6 Gy**	**8 Gy**	**10 Gy**
IC_{50} of nanocomposite after 24 hr	24.1±0.11	31.19±0.567	29.9±0.86	30.6±0.293	25.73±0.286^{ns}
IC_{50} of nanocomposite after 48hr	16.70.14	29.2±0.673	24.7±0.327	23.89±0.234	6.7±0.088*

All data were represented by Mean ± SE, P value<0.05.

***** Refers to there is a significant difference between IC_{50} value of nanocomposite alone and with 10 Gy after 48 hr.

ns refers to there is no significant difference between IC_{50} value of nanocomposite alone and with 10 Gy after 24 hr.

TABLE 4. Comparison study of IC_{50} values (µg/ ml) of nanocomposite, 5-FU, zinc oxide NPs and CTAB combined with 10 Gy radiation on different human cancer cell lines and normal cell line after 24 hr

	HePG2	HUH-7	MCF-7	WI-38
IC_{50} of nanocomposite +10Gy.	25.18±0.28[a]	39.1±0.20[a]	49.19±0.14[a]	67±1.76[a]
IC_{50} of 5-FU + 10Gy.	261.7±0.37[b]	395.4±0.26[b]	440.1±0.031[b]	200±2.88[b]
IC_{50} of zinc oxide NPs + 10Gy	64.5±0.28	67.23±0.18	79.3±0.25[c]	
IC_{50} of CTAB + 10Gy	4.133±0.08	0.567±0.12[d]	5.2±0.11	
10 Gy	20%±1.528[e]	15%±0.57	10%±0.57	5%±0.28[e]

All data were represented by Mean ± SE

a; refers to that there is statistically significant difference of IC_{50} values(µg/ml)of nanocomposite combined with 10 Gy on different cell lines, p value <0.05.

b; refers to there is statistically significant difference of IC_{50} values **(** µg/ml) of 5-FU combined with 10Gy on different cell lines, p value <0.05.

c; refers to there is statistically significant difference of IC_{50} values(µg/ml) of zinc oxide NP combined with 10Gy on different cell lines, p value <0.05.

d; refers to there is statistically significant difference of IC_{50} values **(** µg/ml) of CTAB combined with 10Gy on different cell lines, P value<0.05.

e; refers to there is statistically significant difference of inhibition percentage of 10Gy on different cell lines, P value<0.05.

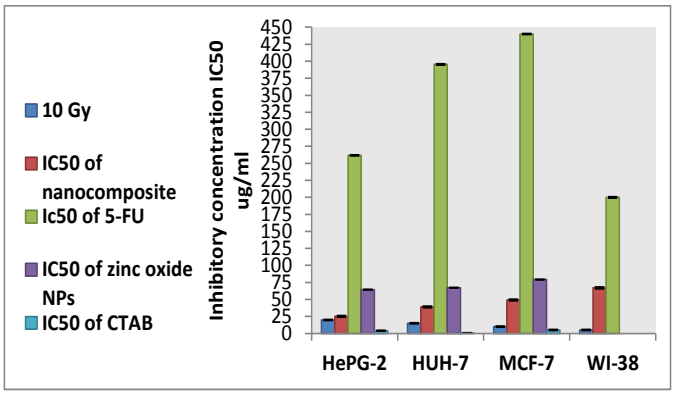

Fig. 5. The comparison study of IC50 values(µg/mL) of nanocomposite in comparison with 5-fluorouracil , CTAB and zinc oxide nanoparticles all of them combined with 10 Gy and 10 Gy alone on different cancer cell lines and also on normal cell line in comparison to 5-fluorouracil combined with 10 Gy and 10 Gy alone after 24 hr

TABLE 5. Comparison study of IC_{50} values (µg/ml) of nanocomposite, 5-FU, zinc oxide NPs and CTAB combined with 10 Gy radiation on different human cancer cell lines and normal cell line after 48 hr

	HePG2	HUH-7	MCF-7	WI-38
IC_{50} of nanocomposite +10 Gy	6.13±0.08[a]	15±0.11[a]	9.08±0.04[a]	56±0.82[a]
IC_{50} of 5-FU + 10Gy	29.43±0.29	30.75±0.38	10.13±0.08[b]	25±1.52
IC_{50} of zinc oxide NPs + 10Gy	38.97±0.08[c]	65.33±0.24[c]	30.2±0.11[c]	
IC_{50} of CTAB + 10Gy	1.06±0.12[ns]	0.2±0.05[ns]	0.916±0.04[ns]	
10 Gy	50%±1.52[e]	40%±0.88[e]	45%±1.15[e]	15%±1.45[e]

All data were represented by Mean ± SE

a; refers to that there is a statistically significant difference of IC_{50} values (µg/ml) of nanocomposite combined with 10 Gy on different cell lines, P value <0.05.

b; refers to there is a statistically significant difference of IC_{50} values (µg/ml) of 5-FU combined with 10Gy on different cell lines, P value <0.05.

c; refers to there is a statistically significant difference of IC_{50} values (µg/ml) of zinc oxide NPs combined with 10Gy on different cell lines, P value <0.05.

ns; refers to there is no statistically significant difference of IC_{50} values (µg/ml) of CTAB combined with 10Gy on different cell lines, P value<0.05.

e; refers to to there is a statistically significant difference of inhibition percentage of 10Gy on different cell lines, P value<0.05.

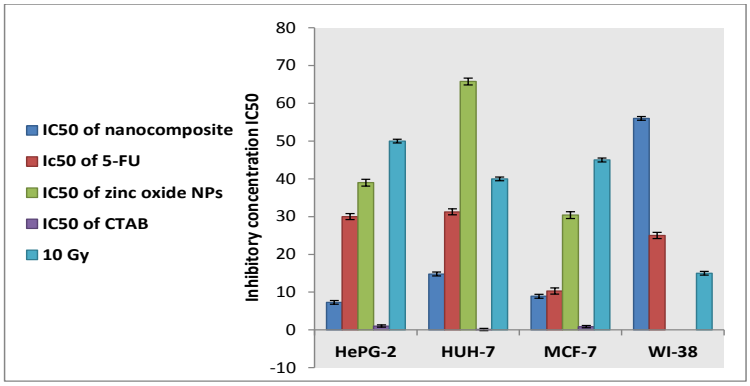

Fig. 6. The comparison study of IC50 values (μg/mL) of nanocomposite in comparison with 5-fluorouracil , CTAB and zinc oxide nanoparticles all of them combined with 10 Gy and 10 Gy alone on different cancer cell lines and also on normal cell line in comparison to 5-fluorouracil combined with 10 Gy and 10 Gy alone after 48 hr

Nanocomposite combined with 10 Gy exerted better *in vitro* anticancer activity against HepG2 comparable to the activity of nanocomposite alone after 48 hr.

The least IC_{50} value for nanocomposite combined with 10 Gy is given by HepG2 cell line after 24 hr and 48 hr of treatment as in Tables 4, 5 and Figs. 5, 6.

Nanocomposite combined with 10 Gy has IC_{50}= 67 μg/mL against WI-38 cell line after 24 hr comparable to nanocomposite alone has IC_{50} = 80 μg/mL due to the effect of radiation on normal cell line.

Nanocomposite combined with 10 Gy has IC_{50}= 56 μg/mL against WI-38 cell line after 48hr comparable to nanocomposite alone has IC_{50}= 60 μg/mL. So, there is no significant difference between the IC_{50} (μg/mL) value of nanocomposite on WI-38 with and without radiation after 48 hr. 10 Gy radiation exerted more inhibition effect on different cancer cell lines and normal cell line after 48 hr than 24 hr due to the time exposure.

Discussion

The use of a nanodrug delivery system has been used for transporting anticancer drugs to cancer cells, while reducing undesired drug distribution in healthy tissues and decreases the toxicity (Ma et al., 2017).

In comparison to conventional drug delivery, nanoparticles (NPs) are used to protect cancer drugs from first-pass metabolism, enzymatic degradation in the stomach and small intestine, which leads to an increase in the amount of drug available for localized delivery within the cancer cells and therapeutic effectiveness of several anticancer agents (Díaz & Vivas-Mejia, 2013).

For the last 70 years, the 5-fluorouracil (5-FU) has been used as first line chemotherapy in the treatment of many cancers such as colorectal, head, neck and breast cancers. 5-fluorouracil is an antimetabolite drug, it acts as a thymidylate synthase inhibitor, interferes with DNA and RNA synthesis. 5-fluorouracil causes severe toxic effects on the gastrointestinal tract and also hematological, cardiac and dermatological reactions. The response rates of 5-FU for advanced cancers is less than 15% and the bioavailability is also limited (Chandran et al., 2017).

Several strategies have been adopted to improve the 5-FU therapy. These include; adjunct therapy of 5-FU with other chemotherapeutic drugs or with targeted therapy (Zhao et al., 2019), and natural compounds that improve the targeted delivery of 5-FU and significantly reduce the associated side effects (Pariente et al., 2018), or nano drug delivery strategy of 5-fluorouracil for the treatment of cancer (Chandran et al., 2017).

Thus, there is a need to develop a potential drug delivery system for 5-FU to achieve better therapeutic efficacy with less side effects.

In the present study, the development of a potential drug delivery system of 5-FU with less side effects is carried out by the synthesis of the nanocomposite compound. The nanocomposite was prepared by loading 5-fluorouracil on zinc oxide NPS coated with cetrimonium bromide

(CTAB) as stabilizer to enhance the anticancer activity of 5-fluorouracil. Thus, we probed the activity of these three different materials (5-FU, zinc oxide NPs and CTAB) in a single composite-based formulation.

Zinc oxide nanoparticles are used in the nanocomposite preparation because zinc oxide NPs show relatively high biocompatibility, easy synthesis, *in vitro* selective cytotoxicity against cancerous cells compared with other nanoparticles and also can be further surface engineered to show increased selective cytotoxicity. The present data are in agreement with previous studies (Bai et al., 2017; Siddiqi et al., 2018).

CTAB is utilized in the nanocomposite preparation for many reasons because it is used as a stabilizer and surfactant to control the morphology and size of the nanoparticle and also it shows anticancer activity with high selectivity against cancerous cells in the *in vitro* condition.

The utilization of CTAB as stabilizer in nanocomposite agreed with the findings of Vahdat Vasei et al. (2019) who used CTAB with zinc oxide NPs to reduce its size.

One of the major challenges in cancer therapy is to improve the selectivity and efficacy of anticancer drugs and reduce their side effects to improve the quality of life for cancer patients (Wu et al., 2011).

The present study is purposed to inspect the possible therapeutic impact of a nanocomposite and also to examine whether ionizing radiation exposure with the nanocomposite could provide a better cancer cells therapy. It was observed that nanocomposites have distinct effects on mammalian cell viability via killing cancer cells (HepG2, MCF7, and HUH-7) comparable to the activity of 5-FU alone after 24 and 48 hr, while posing minimal impact on normal cells such as WI-38. It has been suggested that the anticancer activity of nanocomposites and the selective property towards cancer cells are due to the presence of zinc oxide nanoparticles and CTAB with 5-fluorouracil, as in Tables 1, 2. and Figs. 3, 4.

The results from the present study have shown that the use of nanocomposite in combination with gamma radiation leads to radio sensitization of tumor cells, in case of MCF-7 breast cancer cell line and HepG2 liver cancer cell line after 48 hr as in Table 5 and Fig. 6.

It was observed that there is no significant difference between the IC_{50} values (μg/mL) of nanocomposite, 5-FU, zinc oxide nanoparticles and CTAB alone and with 10 Gy radiation after 24 hr on HepG2, MCF-7, WI-38 and HUH-7, as in Table 4 and Fig. 5.

There was a significant difference between IC_{50} values (μg/ml) of nanocomposite alone and with 10 Gy radiation after 48 hr in case of HepG2 and MCF-7(Synergistic effect), but in case of HUH-7, there was no effect because HUH-7 is radioresistant where cancer cell membranes are known to be deficient in polyunsaturated fatty acids, which renders them radioresistant, as in Table 5 and Fig. 6.

In case of HepG2, the nanocomposite combined with 10 Gy, it exerted a better anticancer effect than the nanocomposite alone after 48 hr due to the presence of CTAB and zinc oxide NPs in the nanocomposite formulation that enhance radio sensitization effect, as in Table 5 and Fig. 6.

In the present study, there was a significant difference between the IC_{50} values (μg/mL) of CTAB alone and with 10 Gy radiation after 48 hr on different cancer cell lines, as in Tables 5 and Fig. 6.

The synergistic effect in case of HepG2, HUH-7 and MCF-7is due to the fact that CTAB is a radiosensitizer. The present results are in agreement with the clonogenic survival curves demonstrated that CTAB interacted additively with radiation in a dose and time -dependent manner (Ito et al., 2009).

In the present study, there was a significant difference in the inhibition effect of 10 Gy on different cell lines between after 24 hr and 48 hr, while posing minor effect on normal cell line (WI-38), as in Tables 4, 5 and Figs. 5, 6.

Conclusion

Based on the present results, it is possible to conclude that 5- FU modified with zinc oxide nanoparticles and CTAB exerted a better effect than that of 5-FU alone on different human cancer cell lines after 24 and 48 hr. Moreover, the 5- FU modified with zinc oxide nanoparticles

and CTAB with10Gy radiation exerted a better effect than that of 5- FU modified with zinc oxide nanoparticles and CTAB alone.

Finally, the effectiveness of 5-FU as an antitumor drug is enhanced by the combination of chemotherapy and radiotherapy with nanotechnology.

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