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Effect of Gold Nanocomposite on the Cytotoxicity of Human Cancer Cell Lines

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Abstract: Gold nanoparticles (AuNPs) have a biological property that have attracted significant attention in this decade, especially in the field of biomedical application with great therapeutic potential. Additionally, the chitosan molecule appears to be a suitable polymeric complex used in this field. The aim of the current study is to explores the cytotoxicity and the IC_{50} value of Au-doped chitosan-poly (vinyl alcohol) (Cs/PVA/Au) nanocomposite that was developed by gamma irradiation with promising anticancer activity. The anti-cancer activity of the prepared nanocomposites was demonstrated in human liver cancer cell line HEPG2 and MCF7 breast cancer cell lines. It has significant effects against both cancer cell line. Concluding that, the future nanomedicine will be impacted greatly by the collaboration of biomedical research and developing nanoparticle therapy in the right directions, which will improve the outcome of cancer patients. It is expected that the next decade will reveal real potential for metal nanoparticles to cross the regulatory barrier into clinical use as effective therapeutics.

Key words: Chitosan nanocomposite, gold nanoparticles, liver and breast cell line.

1. Introduction

Nanoscience/nanotechnology has emerged as one the fastest growing fields of science and its application in various fields of science has earned a great concern this decade. Cancer is a worldwide public health problem and has a high fatality rate. The finding and developing new therapies and techniques was needed for possible treatment of cancer. The nanoparticles of noble metals with advancement of new materials have been successfully developed for different purposes in engineering and biological sciences [1, 2]. The intelligently designed nanoparticles are considered as exceptionally promising cancer therapy agents [3]. They are valuable in therapeutics because of their nano sizes and surface to volume ratios, which significantly improve their chemical and physical properties [1]. Furthermore, gold nanoparticles (Au-NPs) have been trialed as a vehicle for nanomaterial-based therapeutics with some unique chemical and physical properties [4, 5]. It is likely to provide an attractive platform for combining a variety of biophysicochemical properties like the inertness and biocompatibility that make them very promising for specific applications such as medical imaging, gene and drug delivery, and molecular sensing [6-8]. Additionally, AuNPs are useful because its synthesis is simple, it has a high affinity of binding and its conjugation with biomolecules such as DNA, protein, and receptors [1, 9, 10]. It can be used in biomedical applications as antimicrobial agents in surgically implanted catheters to reduce the infections caused during surgery and are proposed to possess anti-fungal, anti-inflammatory, anti-angiogenic and anti-permeability activities [11-13]. Indeed, gold nanoparticles have been broadly used as contrast agents, cancer drug vehicles, transfection agents, antineoplastic agents, etc. [14]. On the other hand, metallic gold as a biocompatible material is used in many applications, ranging from

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dental surgery to treatments for arthritis [15].

Polymeric materials with different functional groups have been reported as template materials for different metal nanoparticle to overcome the major problems such as aggregation, oxidation, or inactivation of metal nanoparticles [16]. Polyvinyl alcohol (PVA) is an artificial polymer that has been used during the first half of the 20th century worldwide. This polymer is widely used by blending with other polymer compounds, especially, in biomedical applications for its compatibility, nontoxicity, non-carcinogenic, swelling properties, and bio-adhesive characteristics [17, 18]. In biomedical fields and application, PVA composites, such as PVA gels, are used in the manufacturing of contact lenses, artificial heart surgery, drug delivery systems, and wound dressings [18]. Additionally, chitosan possess antitumor activity was tested both *in vitro* and *in vivo* [19]. Chitosan based hydrogels have been used for breast cancer, brain tumor, localized solid tumors, primary and secondary osteosarcoma, osteolysis and lung metastasis [20]. *In vitro* chitosan possessed a vigorous cytotoxicity against a colon cancer cell line (Calo320), gastric cancer cell line (BGC823), and liver cancer cell line (BEL7402) and HepG2. It showed a significant dose- and size-dependent antitumor activity against sarcoma-180 and hepatoma H22 in mice [21].

The aim of the current work was to examine the cytotoxic effect of prepared (Cs/PVA) hydrogel and (Cs/PVA/Au) nanocomposite prepared by gamma irradiation in human liver cancer HEPG2 and MCF7 breast cancer cell lines.

2. Materials and Methods

2.1 Materials

Poly (vinyl alcohol) (PVA; Mw 15,000) and medium molecular weight chitosan (Cs) were purchased from Sigma-Aldrich Inc. and were used as received. The other chemicals were reagent grade and used without further purification. Other chemicals were purchased from El-Nasr Co. for Chemical Industries, Egypt and used without further purification.

2.2 Gamma Radiation Source

Irradiation of samples was carried out using a Co^{60} gamma source installed at the National Centre for Radiation Research and Technology (NCRRT), Egypt.

2.3 Methods

2.3.1 Preparation of Cs/PVA Hydrogel

Cs/ PVA hydrogel was prepared according to Abaza et al. [22]. A stock solution of 1.0% (w/v) Cs was prepared by dissolving 1 g of Cs in acetic acid solution (1%) at 60 ^{o}C with continuously stirring for 6 h in water bath. An aqueous solution of 10% PVA (w/v) was prepared by dissolving 10 g of PVA at 70 °C in water bath with constant stirring for 6 h. After cooling down to room temperature two compositions of Cs/PVA were prepared in ratio Cs: PVA; 1:9 and 2:3 (V/V). The solutions were poured into test tubes (inner diameter 5 mm) and subjected to gamma-irradiation at irradiation dose 40 kGy. After irradiation the formed hydrogels were cut into nearly equal disks. The obtained hydrogels were extracted in distilled water at room temperature overnight to remove the non-cross-linked polymer, and then dried in air to constant weight.

2.3.2 Preparation of Cs/PVA/Au Nanocomposites

Cs/PVA/Au nanocomposite was prepared according to Abaza et al. [23]. A 0.1-g of dried Cs/PVA hydrogel was placed in 50 mL of Au ions solution of concentration 250 mg/L for 24 h to dope the Au ions in the hydrogel matrix. Au ions loaded hydrogels were placed in distilled water for another 24 h to remove unbound metal ions. The Au ions inside the hydrogels were reduced by transferring them into 50 mL of 5% NaOH for 6 h and then in 50 mL of 0.5 M $NaBH_4$ for another 6 h to complete reduction of Au. Soaking them in de-ionized water for 12 h and drying in oven at 40 °C. The average size of AuNp spherical particles was 36.3 ± 50.8 nm.

2.4 Human Tumor Cell Lines

Human tumor carcinoma liver HepG2 and breast MCF7 cell lines used in this study were obtained from the American Type Culture Collection (ATCC, Minisota, U.S.A.). The tumor cell lines were maintained at the National Cancer Institute, Cairo, Egypt, by serial sub-culturing. Samples were prepared by dissolving 1:1 Stock solution and stored at -20 °C in dimethylsulfoxide (DMSO) at 100 mM. Different concentrations of the drug (Cs/PVA and Cs/PVA/AuNP) were used 5, 12.5, 25, 50 μg/mL.

2.4.1 Cell Culture and Maintenance

RPMI-1640 medium was used for culturing and maintenance of the human tumor cell lines. The medium was supplied in a powder form. The working solution was prepared by dissolving 10.4 gm powder and 2 gm sodium bicarbonate dissolved in 1 L distilled water. The medium was then sterilized by filtration in a Millipore bacterial filter (0.22 μm). The prepared medium was kept in a refrigerator (4 °C). Before use the medium was warmed at 37 °C in a water bath and the supplemented with 1% penicillin/streptomycin and 10% fetal bovine serum. A cryotube containing frozen cells was taken out of the liquid nitrogen container and then thawed in a water bath at 37 °C. Then the cryotube was opened under strict aseptic conditions and its contents were supplied by 5 mL supplemented medium drop by drop in a 50 mL sterile falcon tubes. The tube was incubated for 2 hours then centrifuged at 1,200 rpm for 10 minutes and the supernatant was discarded, the cell pellet was suspended and seeded in 5 mL supplemented medium in T25 Nunclon sterile tissue culture flasks. The cell suspension was incubated and followed up daily the supplemented medium was replaced every 2-3 days. Incubation was continued until a confluent growth was achieved and the cells were freshly subcultured before each experiment to be in the exponential phase of growth.

2.4.2 *In Vitro* Cytotoxic Assay for HepG2 and MCF7 Cancer Cell

The cytotoxicity was carried out using Sulphorhodamine-B (SRB) assay following the method reported by Vichai and Kirtikara [24]. SRB is a bright pink aminoxanthrene dye with two sulphonic groups. It is a protein stain that binds to the amino groups of intracellular proteins under mildly acidic conditions to provide a sensitive index of cellular protein content. Cells were seeded in 96-well microtiter plates at initial concentration of 3×10^3 cell/well in a 150 μL fresh medium and left for 24 hours to attach to the plates. Different concentrations 0, 5, 12.5, 25, 50 μg/mL of drug were added. For each drug concentration, 3 wells were used. The plates were incubated for 48 hours. The cells were fixed with 50 μL cold trichloroacetic acid 10% final concentration for 1 hour at 4 °C. The plates were washed with distilled water using (automatic washer Tecan, Germany) and stained with 50 μL 0.4% SRB dissolved in 1% acetic acid for 30 minutes at room temperature. The plates were washed with 1% acetic acid and air-dried. The dye was solubilized with 100 μL/well of 10 M tris base (pH 10.5) and optical density (O.D.) of each well was measured spectrophotometrically at 570 nm with an ELISA microplate reader (Sunrise Tecan reader, Germany). The mean background absorbance was automatically subtracted and means value of each drug concentration was calculated. The experiment was repeated 3 times. The percentage of cell survival was calculated as follows:

Surviving fraction = O.D. (treated cells) / O.D. (control cells) The IC50 values (the half maximal inhibitory concentration value) is a measure the concentrations of drug required to produce 50% inhibition of cell growth) were also calculated

3. Result and Discussion

When Cs/PVA blended mixture is exposed to gamma irradiation, cross-linking of PVA and degradation of Cs to shorter chains take place simultaneously, at the same time occur to the formation of a three-dimensional network of hydrogel structure. It is well known that in aqueous solution, the indirect

effect of radiation is the main interaction mode, i.e. the primary reactions occur with water, producing powerful oxidizing species, such as hydroxyl radicals OH, that can attack the glycosidic bonds of chitosan [25]. Hence, the radiation processing of chitosan in presence of water would reduce significantly its molecular weight. It was reported that, when an aqueous solution of PVA containing polysaccharides is exposed to radiation, OH, H radicals and hydrated electrons are produced, as a major part of the energy is absorbed by the solvent [26]. OH radicals are mostly responsible for crosslinking of PVA and degradation of polysaccharides, and the rates of OH radical reaction with PVA and polysaccharides are similar. Therefore, besides crosslinking of PVA, a fraction of radicals would also degrade the polysaccharides in proportion to their concentration in aqueous PVA solution [27]. The formation of free radicals along the PVA chains leads to the formation of networks [28]. AuNPs were incorporated in Cs/PVA hydrogel to form Cs/PVA/Au nano composites by $NaBH_4$ reduction. The functional groups such as $–NH_2$ and –OH that exist on the Cs/PVA hydrogel network are responsible for metal ion absorption from their solutions. As all the sites are filled with these ions on the hydrogel network, the polymer network is also physically woven via $M–NH_2–$ electrostatic interactions between the metal ions and the $–NH_2–$ and –OH groups. Upon contacting these metal ions loaded hydrogels with a reducing agent such as $NaBH_4$, Au nanoparticles can be formed *in situ* within the hydrogel. The utilization of the hydrogel also provides a stabilizing effect on the produced metal nanoparticles [29]. Fig. 1 illustrates the schematic presentation of metal loading onto the Cs/PVA

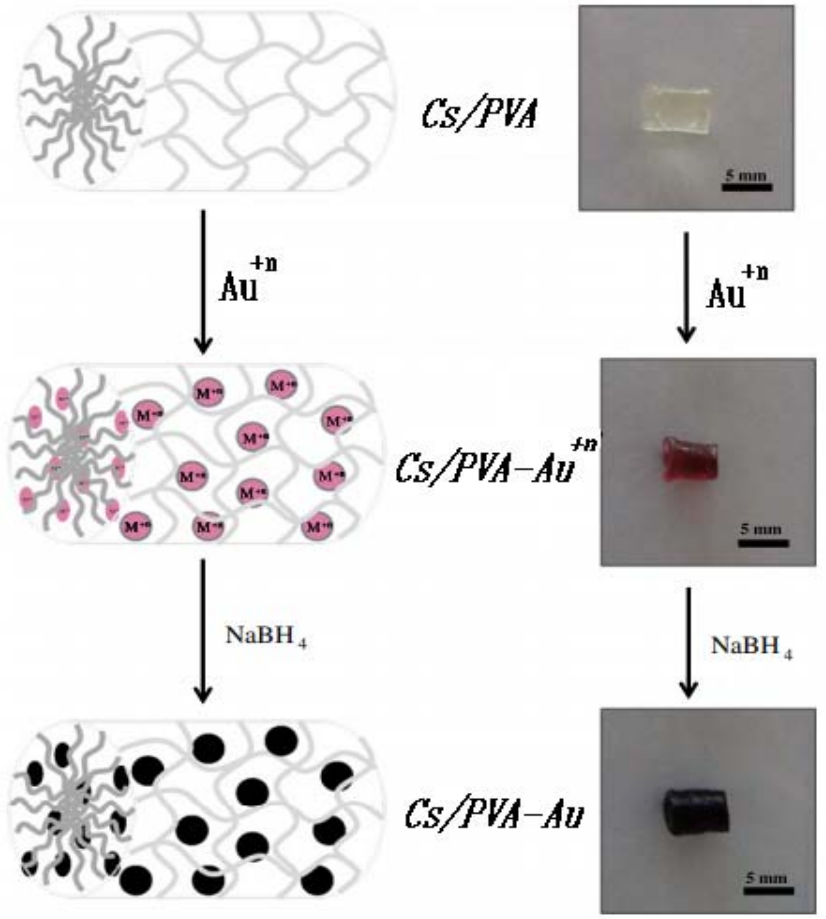

Fig. 1 Schematic presentation of metal loading onto the Cs/PVA hydrogel and their reduction within the hydrogel matrices along with digital camera images.

hydrogel and their reduction within the hydrogel matrices along with digital camera images. Polymerization and crosslinking take place simultaneously as illustrated in Fig. 2.

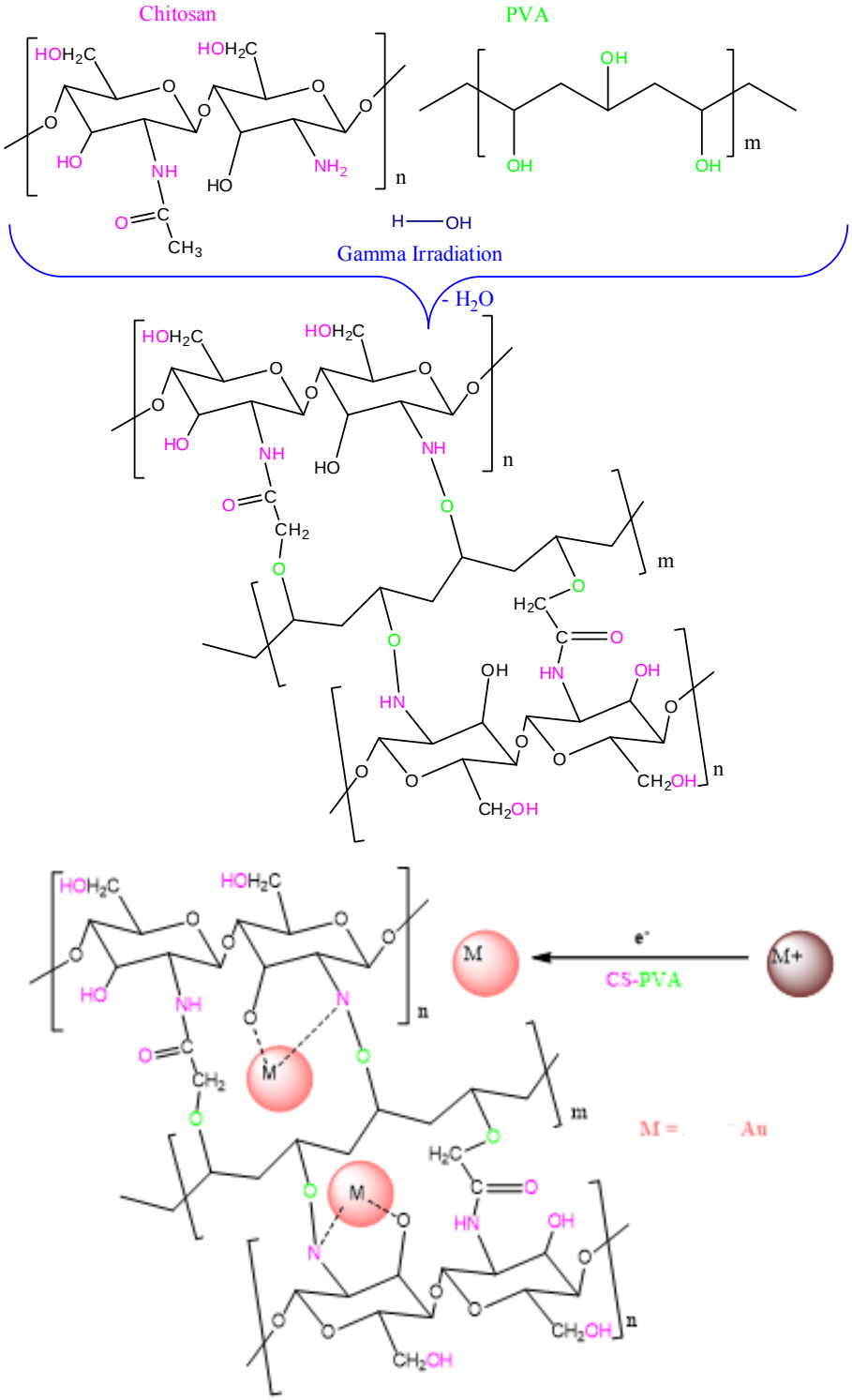

Fig. 2 Proposed scheme for preparation of CS/PVA/Au nanocomposite.

$$H_2O \rightarrow e_{aq}^{-}, OH^{\cdot}, H^{\cdot}, H_3O^{+}, H_2, H_2O_2 \quad Eq.1$$

$$[C_6H_{11}O_4N]_n + OH^{\cdot} \rightarrow [C_6H_{11}O_4N]_{n-1}\,[C_6H_{11}O_4N]^{\cdot} + H_2O \quad Eq.2$$

$$PVA(H) + OH^{\cdot} \rightarrow PVA^{\cdot} + H_2O \quad Eq.3$$

$$PVA^{\cdot} + [C_6H_{11}O_4N]_{n-1}\,[C_6H_{11}O_4N]^{\cdot} \rightarrow [C_6H_{11}O_4N]_{n-1}\,[C_6H_{11}O_4N] - PVA \quad Eq.4$$

$$4Au^{+} + BH_4^{-} + 4OH^{-} \rightarrow 4Au + BH_2(OH)_2^{-} + 2H_2O \quad Eq.5$$

$$4Au^{+} + BH_2(OH)_2^{-} + 4OH^{-} \rightarrow 4Au + BO_2^{-} + 2H_2O \quad Eq.6$$

$$[C_6H_{11}O_4N]_{n-1}\,[C_6H_{11}O_4N] - PVA + Au \rightarrow [C_6H_{11}O_4N]_{n-1}\,[C_6H_{11}O_4N] - PVA{:}\,Au \quad Eq.7$$

AuNPs have become an important biomedical tool for scientists in cancer research because of the several advantages of it like high loading efficiency for the target substance, enhanced ability to cross various physiological barriers, and low systemic side effects [30-32]. AuNPs are a novel agent in cancer therapy and show aggregation and size-dependent cytotoxic activity against different cancer cells. Its use minimizes the risk of side effects and limits the damage to normal (noncancerous) cells [1]. However, it has been reported that soluble CS and CS microspheres show some degree of toxicity towards certain cell lines like the murine melanoma cell line and human gastric carcinoma MGC803 cell line suggesting their application as antitumor drugs [21, 33]. On the other hand, primary liver cancer is the third highest cause of death worldwide and the fifth most commonly diagnosed cancer. Additionally, hepatocellular carcinoma (HCC) represents around 85% of all primary liver cancer and its incidence is increasing all around the world despite of declining an incidence of some cancers [34-36]. Furthermore, human breast cancer is the second most common cause of cancer-related deaths in women and the incidence of breast cancer has increased worldwide in the last few years [37]. For efficient breast cancer treatment, there is requirement of developing novel nanomaterials capable of reacting to the local tumor environment [38]. Therefore, in the current study, the cytotoxic effect of various concentrations of Cs/PVA and Cs/PVA/AuNP (5, 12.5, 25 and 50 µg/mL) was assessed in HepG2 and MCF7 cell cultures using SRB colorimetric assay at 48 h time intervals. The combination of AuNP and Cs/PVA hydrogel was found to be more effective against HepG2 and MCF7 cells than the activity of Cs/PVA hydrogel individually as shown in Tables 1-4 and Figs. 2-4. So, by exploring the cytotoxic effect of Cs/PVA on HepG2 cell line, the survival fraction was found to be high (70.2%) in Cs/PVA (1:9) than Cs/PVA (4:6) concentration (56.1%). This means that the cell inhibition ratio was more (43.9%) in Cs/PVA (4:6) concentration but with no statistical significant effects (p-value = 0.368). Also, there was no effect on the IC_{50} value. (Table 1). However, the physicochemical properties, and surface modifications of CS play a crucial role in the cytotoxic profile and targeting of cancers that are characterized by rapid division and aggressive growth [21, 23]. Furthermore, by assessing the *in-vitro* cytotoxic effect of various concentrations of the Cs/PVA/AuNP, against HepG2 cell lines, the survival fraction was found to be 55.8% and 66.7% in hydrogel concentration of (1:9) and (4:6) with 250 ppm AuNP at 50 µg/mL. The inhibition ratio was better in (4:6) concentration (33.3%), but the IC_{50} value was negative in both. Meaning that, there was a negative effect on the HepG2 cell cultures by adding 250 ppm AuNP to both concentration. However, after increasing ppm AuNP to 1,000, the survival fraction was significantly improved to 27% with inhibition ratio 73% (p-value = 0.046). The IC_{50} value was found to be 21.1 µg/mL (Table 2, Fig. 3). There are limited studies on the cytotoxic effects of AuNPs against different cancer cells. Patil et al. [1] in 2017 determined the effects of AuNPs on the different cancer cell proliferation including Hep3B (Hepatocellular carcinoma) cell lines. They recorded an increase of cytotoxicity in a dose dependent manner against cancer cells. This result was in accordance with the results of

Table 1 Surviving fraction % and inhibition ratios % of liver cancer cell line (HepG2) at different concentrations of Cs/PVA hydrogel at concentrations range from 5 to 50 µg/mL.

Concentration (µg/mL)	Cs/PVA (1:9)		Cs/PVA (4:6)		*p*-Value
	Surviving fraction %	Inhibition ratio %	Surviving fraction %	Inhibition ratio %	
0.0	100	0	100	0	
5.00	98.2	1.8	94.7	5.3	
12.5	87.7	12.3	94.7	5.3	0.368
25.00	91.2	8.8	73.7	26.3	
50.00	70.2	29.8	56.1	43.9	
IC_{50} (µg/mL)	None		None		

* *p*-Value was always two-tailed and is significant $p \leq 0.05$.

Table 2 Surviving fraction % and inhibition ratios % of liver cancer cell line (HepG2) with different concentrations of Cs/PVA/AuNP composite at concentrations range from 5 to 50 µg/mL.

Concentration (µg/mL)	Cs/PVA/AuNP (1:9) (250 ppm)		Cs/PVA/AuNP (1:9) (1,000 ppm)		Cs/PVA/AuNP (4:6) (250 ppm)		*p*-Value
	Surviving fraction %	Inhibition ratio %	Surviving fraction %	Inhibition ratio %	Surviving fraction %	Inhibition ratio %	
0.0	100	0	100	0	100	0	
5.00	99.6	0.4	91.6	8.4	100	0	
12.5	75.9	24.1	76.1	23.9	96.5	3.5	0.046
25.00	51.6	48.4	38.8	61.2	87.7	12.3	
50.00	55.8	44.2	27	73	66.7	33.3	
IC_{50} (µg/mL)	None		21.1		None		

* *p*-value was always two-tailed and is significant $p \leq 0.05$.

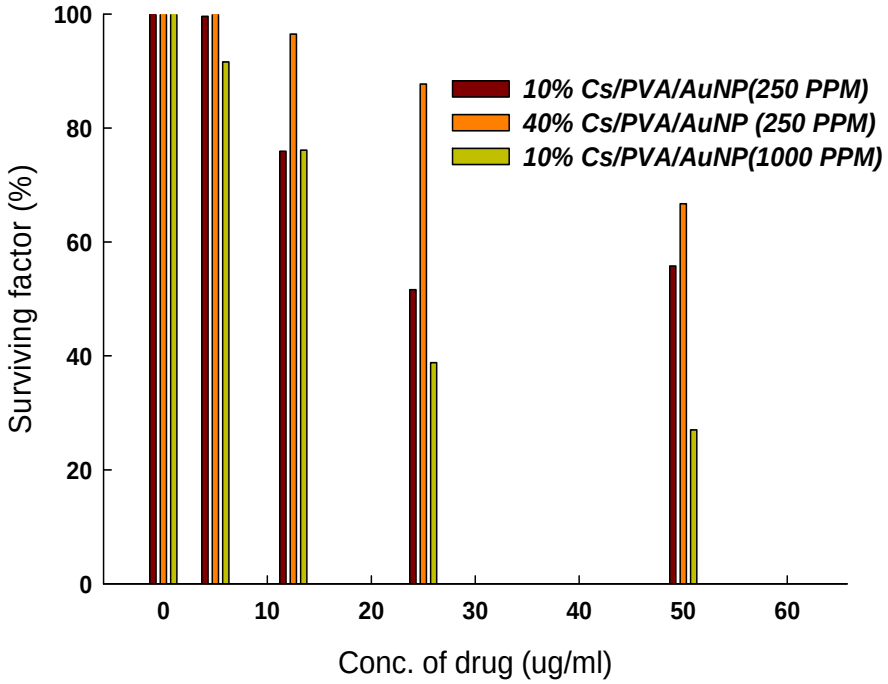

Fig. 3 Surviving factor (%) of HepG2 cells after 48-h treatment with different concentrations of CS/PVA/AuNP, as calculated from the SRB assay.

the present study, that observed a high cytotoxic activity with increasing the concentration of AuNP to 100 μg/mL, and the concentration of 50 μg/mL was not very active. Patil and his colleague [1] also, observed 50% of cell inhibition at AuNPs concentration of approximately 150 μg/mL and the 200 μg/mL sample reduced all cells to around 20% viability. On the other hand, Abaza et al. [22] in 2018 reported that the silver-doped chitosan-poly (vinyl alcohol) (Cs/PVA/AgNP) inhibition fraction of HepG2 cell proliferation at 50 ug/mL in concentration of (4:6) and (1:9) was 54.4% and 40.8 respectively. The IC_{50} value of concentration (4:6) was 43.7 μg/mL. There is a negative IC_{50} value in concentration of (1:9). However, AgNPs and Ag ions are known to have higher antitumor ability. Previously, Roa et al. [39] in 2009 also reported the toxicity of AuNPs alone in prostatic cancer, it was 10% with cell proliferation assays. Additionally, Abaza et al. [23] in 2018, assessed the cytotoxic effect of various concentrations of Au-doped Chitosan/poly (vinyl alcohol) (Cs/PVA/AuNP) in prostatic cancer (PC3) cell cultures. The survival fraction was 24.8% and 23.8 % in concentration of (4:6) and (1:9) with 250 ppm AuNP. The IC_{50} was the same in both. There is a significant improvement in the survival fraction to 18.1% with increasing ppm AuNP to 1,000. However, metal nanoparticles have shown a good experimental success in the field of nanomedicine especially in cancer treatment, which has always been an area of high concern. The collaboration of biomedical research in the identification and characterization of biomedical strategies using the interesting metal nanocomposite will impact the future nanomedicine greatly.

The *in vitro* cytotoxic assay of different concentrations of the Cs/PVA and Cs/PVA/AuNP, was assessed against MCF7 cell lines. The survival fraction of the Cs/PVA was found to be nearly the same in both hydrogel concentration of (1:9) and (4:6) with 250 ppm at 50 μg/mL. The IC_{50} value was found to be 69.2 μg/mL in (4:6) hydrogel concentration with a negative effect in other concentration (Table 3). Then, by adding different concentrations of AuNP, and after increasing ppm AuNP to 1,000, the survival fraction with the concentration of 50 μg/mL was not very active; however, high cytotoxic activity was observed when the 100 μg/mL sample was employed. The survival fraction was significantly improved in all concentration of (1:9) and (4:6) with 250 ppm and 1,000 ppm with highly statistically significance (p-value ≤ 0.001). The IC_{50} value was better in concentration of (4:6) (67 μg/mL). It was 55.7 μg/mL at concentration of (1:9) (250 ppm) and 54.7 μg/mL at concentration of (1:9) (1,000 ppm) (Table 4, Fig. 4). Recent studies that demonstrate the toxic effects of nanoparticles have created controversy [1]. Joshi et al. [40] in 2012 reported that AuNP had no effect on cell viability of MCF-7 cells, but they observed an enhanced cytotoxicity of chloroquine-gold nanoparticle conjugates (GNP-Chl) against MCF-7 cells and the IC50 value was 30 ± 5 μg/mL. It exhibited concentration-dependent cytotoxicity in MCF-7 breast

Table 3 Surviving fraction % and inhibition ratios % of breast cancer cell line (MCF7) at different concentrations of Cs/PVA hydrogel at concentrations range from 5 to 50 μg/mL.

Concentration (μg/mL)	Cs/PVA (1:9)		Cs/PVA (4:6)		p-Value
	Surviving fraction %	Inhibition ratio %	Surviving fraction %	Inhibition ratio %	
0.0	100	0	100	0	
5.00	90.7	9.3	97.9	2.1	
12.5	67.9	32.1	87.9	12.1	0.950
25.00	57.1	42.9	64.3	35.7	
50.00	57.1	42.9	60.7	39.3	
IC_{50} (μg/mL)	None		69.2		

* p-Value was always two-tailed and is significant $p \leq 0.05$.

Table 4 Surviving fraction % and inhibition ratios % of breast cancer cell line (MCF7) with different concentrations of Cs/PVA/AuNP composite at concentrations range from 5 to 100 µg/mL.

Concentration (µg/mL)	Cs/PVA/AuNP (1:9) (250 ppm)		Cs/PVA/AuNP (1:9) (1,000 ppm)		Cs/PVA/AuNP (4:6) (250 ppm)		*p*-Value
	Surviving fraction %	Inhibition ratio %	Surviving fraction %	Inhibition ratio %	Surviving fraction %	Inhibition ratio %	
0.0	100	0	100	0	100	0	<0.001
5.00	90	10	100	0	100	0	
12.5	71.4	28.6	89.3	10.7	88.6	11.4	
25.00	57.1	42.9	64.3	35.7	71.4	28.6	
50.00	53.6	46.4	53.6	46.4	69.3	30.7	
100.00	18.6	81.4	15	85	16	84	
IC_{50} (µg/mL)	55.7		54.7		67		

* *p*-Value was always two-tailed and is significant $p \leq 0.05$.

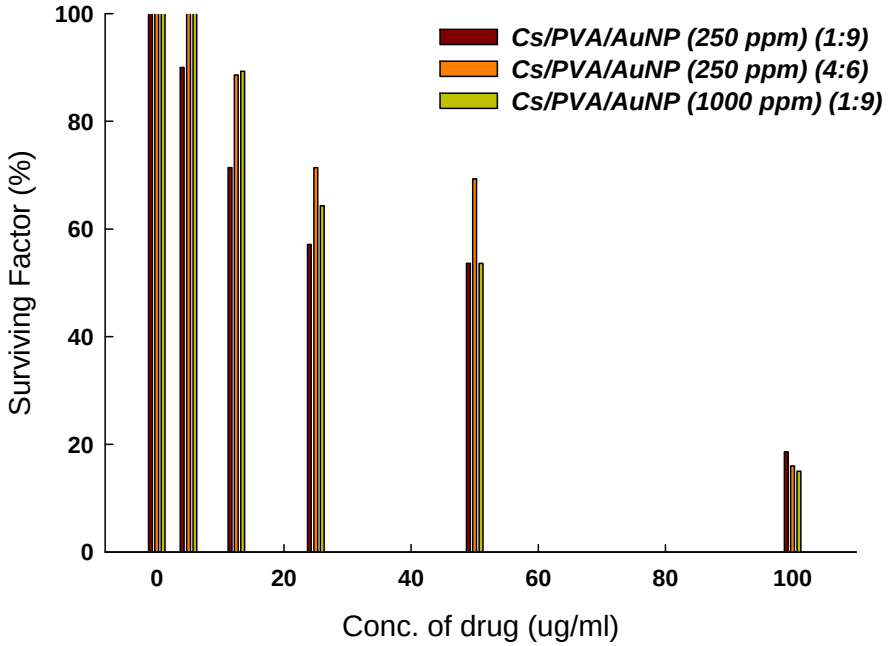

Fig. 4 Surviving factor (%) of MCF7 cells after 48 h of treatment with different CS/PVA/Au nanocomposites, as calculated from the SRB assay.

cancer cells. Manivasagan et al. [38] in 2017 observed no significant cytotoxicity after 24 and 48 h incubation with any concentration of chitosan-polypyrrole nanocomposites (CS-PPy NCs). The viability of the cell population was more than 60%, even after 48 h exposure to the highest concentration of CS-PPy NCs (500 µg/mL), indicating a very low cytotoxicity and good biocompatibility for the CS-PPy NCs. Nivethaa et al. [41] in 2015 used chitosan/gold nanocomposite with encapsulation of 5-FU. They exhibit good antiproliferative activity towards MCF-7 cells while being non-toxic to the surrounding non-carcinogenic cells. The estimated half maximal inhibitory concentration (IC50) value was found to be 31.2 µg/mL. Abaza et al. [22] in 2018 reported the percentage of inhibiting fraction of Cs/PVA/AgNP in MCF7 cell line at 100 ug/mL was 61.8% and 80% with IC50 value of 60 and 52.5 µg/mL in (1:9) and (4:6) concentration respectively. The expected mechanism of AgNP induced toxicity being in the interactions between nanomaterials and cells [42]. However, the toxicity of AuNPs depends on the accumulation of

nanoparticles and the presence of different cell lines [43, 44]. Additionally, several mammalian cell lines showing induced cytotoxicity based on AuNPs aggregation and dependent size [45, 46]. Phytogenic synthesized AuNPs have exhibited a wide range of anticancer effects on several types of cell lines [1, 47, 48]. On the other hand, Adokoh et al. [49] in 2014 prepare glyconanoparticles and conjugated glyconanoparticles with the anticancer drug, gold(I) triphenylphosphine. The *in-vitro* cytotoxicity was tested in MCF7, HepG2 and normal cell lines. The glyconanoparticles and their Au(I)PPh3 conjugates were all active against both cancer cell lines, but galactose-functionalized glyconanoparticles {P(GMAEDAdtc) AuPPh3)-st-LAEMA)AuNP} were found to be the most cytotoxic to HepG2 cells (IC50 ~ 4.13 ± 0.73 μg/mL). The p(GMA-EDAdtc(AuPPh3)-st LAEMA) AuNP was found to be a 4-fold more potent antitumor agent in HepG2 cells. Additionally, the glyconanoparticles Au(I) conjugates are found to be significantly more effective at inducing apoptosis and inhibiting cellular proliferation compared to well-known anticancer chemotherapeutics reagents, such as cisplatin and cytarabine (IC50 = 30.11 and 320.07 μg/mL in HepG2, respectively). Yamada et al. [50] in 2015 observed that therapeutic metal nanoparticles, such as gold, are toxic and similar to chemotherapeutics agents thus, and can be used for therapeutic application. Furthermore, MCF-7 cells have been reported to have lower cytotoxicity results with AuNP-7 demonstrated a 2.5-fold decrease in toxicity (IC50 = 11.39 ± 0.41 μg/mL) compared to the HepG2 cell line [49, 51, 52]. Similarly, the free polymeric glyconanoparticles AuNP-3 and AuNP-6 also showed good toxicity (IC50 = 15.34 − 17.65 μg/mL) against MCF7. This may be attributed to the architecture of the polymers on the AuNPs surface that could enhance toxicity profile [49, 53-55]. Moreover, Wang et al. [9] in 2015 suggested that, the biological effects and the factors influencing the cellular effects of AuNPs will be crucial to reveal how these factors mediate these cellular effects. However, the cell membrane is an important barrier transporting and exchanging intracellular and extracellular substances. Physicochemical properties of AuNPs are closely related to their biological effects, including shape, size, aspect ratio, surface modification, and charges [56]. AuNPs in biological fluids can form NP–protein complexes that can be recognized by cell membrane receptors and uptaken by cells. It can be wrapped by the retracted cell membrane, directly transported into cells, and then can affect the cellular responses directly [57]. AuNPs can be internalized by cells receptor mediated endocytosis and phagocytosis pathways. The processes in both pathways include the formation of Au NP–protein complexes, the cell membrane receptors recognition, the cells engulfment into a vesicle, the penetration and transportation into cells, the signal pathways activation, the sequential trafficking inside cells, and the storage or elimination of AuNPs by cells [58, 9].

4. Conclusions

Metal nanoparticles are being used in a wide array of applications that reach far beyond therapeutics. However, in the therapeutic arena it is clear that, gold and silver nanomaterials are the most promising agents. These biosynthesized nanoparticles have determined solid cytotoxic impacts against many cancer cells such as MCF-7, A549, PC3 and Hep2 cells compared to normal cell lines, so it can be utilized as anticancer agents for the treatment of various cancer types. The aim of the current study is to explore the cytotoxicity and the IC_{50} value of Cs/PVA/Au nanocomposite that was developed in different ratios and synthesized by gamma radiation with promising anticancer activity. The anti-cancer activity of the prepared nanocomposites was demonstrated in human HEPG2 and MCF7 cell lines and was found to have a significant effect against both. Concluding that, the future nanomedicine can impact the outcome of cancer patients, which can be improved greatly by the

collaboration of biomedical research and developing nanoparticle therapy in the right directions. However, the need of some *in vivo* examination is necessary to find their role and mechanism of nanoparticles inside the human body. This may require complete study to bring out their role in anticancer medication. It is expected that the next decade will reveal real potential for metal nanoparticles to cross the regulatory barrier into clinical use as effective therapeutics.

Reference

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