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Green Synthesis, Characterization and Anticancer Activity of Polyacetal/Chitosan Doped with Gold Nanoparticles

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ABSTRACT

The reaction of poly vinyl alcohol (PVA) with salicyladehyde has produced polyacetal. The technique of solution casting used to prepare the polymer blends of poly acetal/Chitosan. The gold nanoparticles (AuNPs) were synthesized using the reducing agent onion peel extract. The nanocomposites were prepared by blending poly acetal /Chitosan /gold nano particles in various ratios. The nano particles were analyzed using X-ray Diffraction XRD analysis and Field Emission Scanning Electron Microscope FESEM microscopy. Fourier Transform Infrared Spectroscopy FTIR, FESEM, Differential Scanning Calorimetry DSC, and Thermogravimetric Analysis TGA were used to investigate the polymer blends and nano composites. When polyacetal was studied using FTIR, a new absorption band for the (O-C-O) functional group appeared at 1105 cm^{-1}, validating the synthesis of the compound. The developed polymer blends and nanocomposites thermal stability is confirmed using (TGA) Thermogravimetric Analysis and (DSC) Differential Scanning Calorimetry compared to the blends, the nanocomposites have demonstrated greater effectiveness in suppressing the prostate cancer cell line.

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INTRODUCTION

Nanotechnology is an emerging field in biomedical research with the potential to bring new settling for the diagnosis and treatment of diseases. Of all the nanomaterials, gold nanoparticles (AuNPs) stand out due to their exceptional optical, chemical, and biological properties that make them excellent prospects for drug delivery, imaging, and cancer therapy [1].

Polyacetal is a multilateral polymer known for its high strength, durability, and chemical resistance. In biomedical applications, polyacetal is utilized for its biocompatibility and stability, making it proper for use in medical devices, prosthetics, and drug delivery systems. Its ability to provide structural integrity while being non-toxic to biological systems highlights its importance in advanced biomedical research [2,3].

Chitosan, a naturally occurring polysaccharide from chitin, has attracted great interest owing to its exceptional properties, including biodegradability, biocompatibility, and antimicrobial properties [4,5]. It is being exploited intensively in various biomedical applications like drug delivery systems, tissue engineering, and wound healing [6]. Chitosan's ability to form stable complexes with biomolecules and its inherent bioactivity make

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it an excellent candidate for use in combination with other materials to enhance their therapeutic potential [7,8].

Nanocomposites, which combine polymers such as polyacetal and chitosan with nanoparticles, offer synergistic properties that significantly enhance their biomedical applications. These nanocomposites are increasingly being explored for their ability to improve drug delivery, provide targeted therapy, and enhance imaging capabilities. When doped with gold nanoparticles, the resulting material exhibits enhanced stability, biocompatibility, and therapeutic efficacy, making it a promising candidate for cancer diagnosis and treatment [9,10,11]. This research aims to establish an eco-friendly, cost-effective, and efficient candidate for anticancer therapy, advancing the integral of green chemistry and nanotechnology in medical science. Despite its fast progress, nanomedicine is encountered with hurdles for its use for cancer therapy, such as high toxicity, low biocompatibility, and high production cost. The present research offers a polyacetal/chitosan/gold nanoparticles nanocomposite that integrates current nanotechnology and green chemistry concepts to combat these hurdles.

MATERIALS AND METHODS

Materials

Poly vinyl alcohol M.Wt 67000 gm/mole, concentrated sulfuric acid and Dimethyl Sulphoxide (DMSO) were purchased from CDH (Delhi,India). Salicyladehyde was purchased from Sigma Aldrich (Massachusetts, United States), Chitosan was purchased from HIMEDIA (Mumbai, India), Sodium Hydroxide was purchased from ACS CHEMICALS (Gujarat, India), Acetic acid was purchased from BDH (Dubai, United Arab Emirates), Gold (III) chloride tri hydrate was purchased from Fluka (Darmstadt, Germany), Absolute ethanol was purchased from CHEM – LAB (Zedelgem, Belgium), Fetal bovine serum, RPMI 1640, Trypsin/EDTA were purchased from Capricorn (Ebsdorfergrund, Germany), Onion peels was purchased from local market in (Baghdad, Iraq).

Preparation of the Poly acetal

The chemicals were purchased from CHEM-LAB, Fluka, HIMEDIA, Aldrich and CDH companies. To prepare polyacetal, 1 g of poly vinyl alcohol (PVA) was dissolved in 25 mL of di methyl sulfoxide (DMSO) and stirring at room temperature for 30 minutes. 1 g of salicyladehyde was dissolved in 20 mL of absolute ethanol with 3 drops of concentrated Sulphuric acid H_2SO_4 and stirring at 50 °C temperature for 30 minutes. The mixture was heated for nine hours with reflex at a temperature of 50°C while being magnetically swirled. A few drops of (1N) Sodium Hydroxide NaOH solution were added to the resultant combination to bring the pH level down to 7 and the product was subjected to several rigorous washings with distilled water in order to remove any adhering acid, base, or solvent. The product was filtered after cooling, and an oven at 50°C was used to dry it for 24 hours [12]. The synthesis of polyacetal (PA) is shown in (Fig. 1).

Polymer blend preparation

Solution casting method was applied to produce polymer blends, while dissolution was used to create polyacetal solution. One gram of poly acetal was dissolved in one hundred milliliters of DMSO while being stirred at 50 degrees Celsius. Five grams of Chitosan were dissolved in one hundred milliliters of 2% aqueous acetic acid solution to create a five weight percent solution of the polymer. The mixture solution was poured onto petri dishes and dried for 24 hours at 50 °C in the oven. Blends of PA/Ch (25% Ch -75%PA, 50% Ch -50%PA) were created by combining various volume ratios [13].

Green synthesis of gold Nanoparticles

Making crude extract from onion leafs to make the onion peels extract, 10 g of leaf powder was

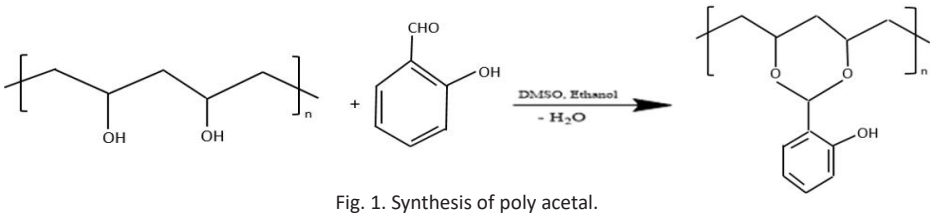

Fig. 1. Synthesis of poly acetal.

mixed with 100 mL of deionized water. The mixture was heated to 50 ° C for two hours while being agitated, and the end product was filtered and dried in an oven at that temperature. To get onion peel extract (100 ppm), 0.01 g of powder product were dissolved in 100 milliliters of deionized water. Fresh onion peel extract was used as a stabilizing and reducing agent [14].

For the preparation of $HAuCl_4.3H_2O$ Solution, stock solution was made by the following procedure: gold chloride trihydrate ($HAuCl_4.3H_2O$) (1 g) was dissolved in deionized water 100 mL. Then (2 mL) was taken from the solution and the remaining 100 mL was finished using successive dilution procedures to achieve (100 ppm). After that 10 mL of aqueous gold chloride solution, 3 mL of aqueous onion peel extract were added in that order, and the combined liquid was then stirred for 10 minutes at 25 °C. The yellow color of the gold changed to purple to show the development of AuNPs. The precipitate is removed, gathered, and thinned with deionized water during the nanoparticle separation process after centrifugation to extract them from the filtrate by a centrifuge (10000 ppm) [15].

Gold Nanocomposites preparation [16]

The nanocomposite film was created by adding 15 mL of poly acetal, 5 mL of Chitosan, 20 mL of concentrations (100 ppm) of AuNPs, in the correct order, and the mixture was stirred for two hours. The mixture was then put into Petri plates and kept at 50 ° C for 24 hours.

Cytotoxic Effect of polymer blend PA/Ch and PA/Ch-Au nanocomposite

The investigation of the effect of polymer blend PA/Ch and PA/Ch-Au nanocomposite on PC3 cell line was performed by in vitro method.

Cell Line Maintenance [17]

A layer of cells is created in a vessel, following these steps:

Subsequent to the removal of the growing medium, the cell sheet was subjected to a rinse with phosphate-buffered saline (PBS). Following this, 2-3 ml of Trypsin/versine solution was introduced to the cells, and the vessel was gently agitated to ensure coverage of the entire cellular layer. The vessel was then incubated at 37°C for a duration of 1 to 2 minutes to facilitate the detachment of the cells from the surface. Fresh complete RPMI medium (15-20 ml) was added, and the cells were pipetted into the growth medium. The cells were subsequently transferred to appropriate culture containers, flasks, or plates at suitable concentrations and incubated at 37°C in a 5% CO2 environment. Lastly, cell concentration was determined by counting the cells using a hemocytometer. The formula employed for the total cell count per milliliter is as follows: cell count × dilution factor × 10^4.

MTT Assay

This study aimed to evaluate the cytotoxicity effectiveness of different concentrations (25- 400 µg/mL) of green synthetic nanoparticles loaded on onion peel extract. The MTT ready-to-use kit was used for this purpose, which includes MTT solution in 10 vials of 1ml each and solubilization solution in 2 bottles of 50 ml each.

Assay

Tumor cells were cultured in well micro-titer plates, with complete culture medium (200 µL) per well and a concentration range of 1x104–1x106 cells/ml. Sterilized parafilm covered the microplate and shaken gently. Then plates were incubated for 24 hrs at 37°C and 5% CO_2.

Polymer blend PA/Ch and PA/Ch-Au nanocomposite were subjected to intermediate elution, and then diluted concentrations ranging from 25 to 400 µg/mL were added to the wells. Triplicates were used for each concentration, including serum-free medium treated cells (controls). The plates were then incubated at 37°C and 5% CO for a selected exposure time of 24 hours. Next, added solution of 10 µL of MTT to each well, and the plates were incubated further at 37°C and 5% CO for 4hrs. The media was removed after incubation, and 100 µL of dissolution solution to each well was added for 5min. Finally, an ELISA reader was used for measuring the optical density at a wavelength of 575nm. The data analyzed statistically the concentration determination of the compounds required to cause a 50% cell viability reduction for each cell line.

RESULTS AND DISCUSSION

X-ray diffraction (XRD) Analysis

X-ray Powder Diffraction (XRD) (Malvern Panalytical) was used to estimate the crystallinity of the prepared gold nanorods as shown in (Fig. 2) The fact that dried AuNRs exhibited peaks at 38°,

44°, and 49° that matched Braggs planes (111), (200), and (220), respectively, demonstrated that the created AuNRs had a nano rod structure. Their corresponding peaks align with the given JCPDS card No. 04-0784 for gold and confirm the synthesized FCC crystalline structure for the obtained AuNRs. Earlier research presented similar XRD results. For instance, researcher presented the same diffraction peaks for the gold nanorods showing the face-centered cubic (FCC) structure and the crystalline nature of the nanorods [18].

Scanning Electron Microscopy (SEM)

SEM microscopy, an analytical technique, may

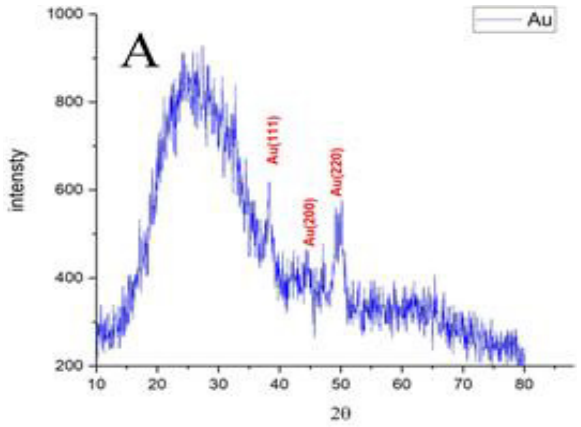

Fig. 2. XRD Patterns of (A-AuNPs).

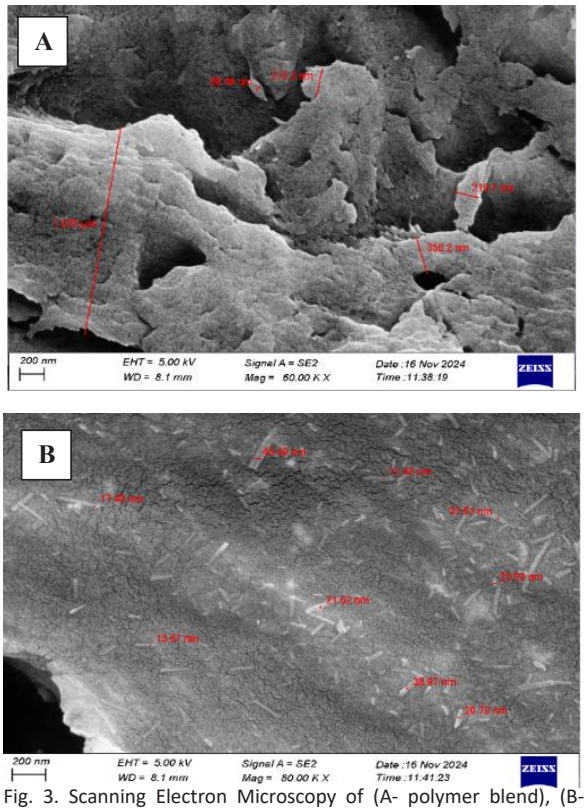

Fig. 3. Scanning Electron Microscopy of (A- polymer blend), (B- Nanocomposite (PA/ Ch -AuNPs).

be used to identify the typical size and shape of nanoparticles in the test material [19]. The changes of morphology surface for the prepared PA/Ch polymer blend, and Au nanocomposite were studied using SEM technique as shown in (Fig. 3) The images of the SEM revealed that the distribution of the nanoparticles is homogeneous over the matrix's surface. In nanocomposite film the morphology of the particles were found with almost in nanorods and the average particles nano size is ranged between 13-46 nm for gold nanoparticles.

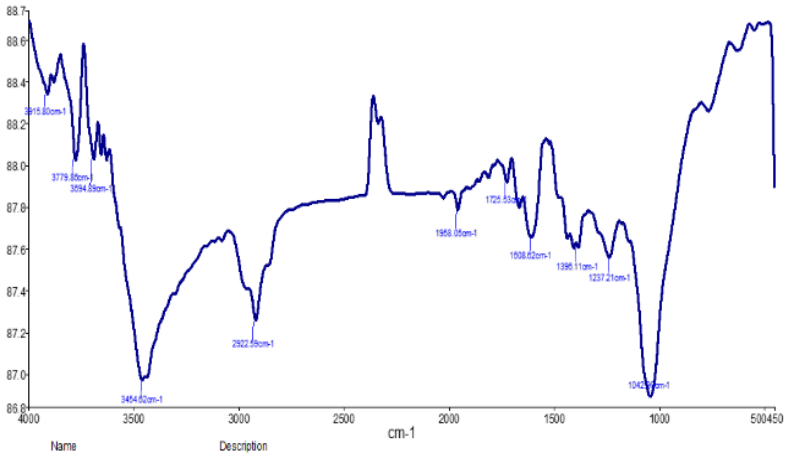

Fig. 4. FTIR Spectrum of Polyacetal.

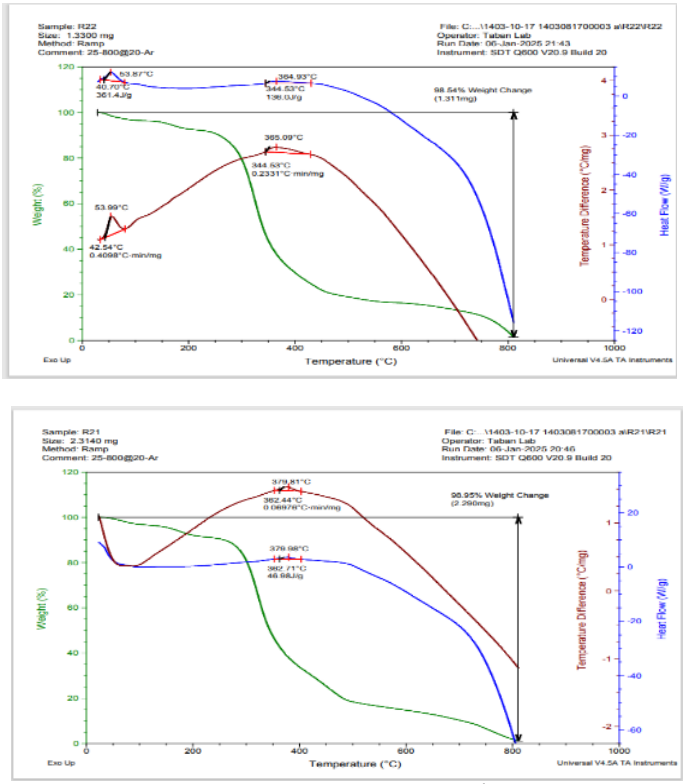

Fig. 5. Thermal analysis (TGA, DSC), (A- Polymer blend PA/Ch), (B-Nanocomposite (PA / Ch -AuNPs).

Thermal Analysis (TGA, DSC)

At the range of temperatures from 25°C to 1000°C at a constant rate of 10°C per minute, the thermogravimetric analysis and differential scanning calorimeter (TGA, DSC) has been utilized to study PA/Ch polymer blend, and PA/Ch- Au nanocomposites. From the thermogravimetric curves (Fig. 5) The PA/Ch blend and the PA/Ch-Au nanocomposites have a relatively good thermal stability, as can be seen from the absence of significant weight loss up to 300°C. Above this temperature range, the polymer blend and the nanocomposite under investigation decompose very quickly to reach a weight loss of nearly 98 wt% by the time they reach the 800°C value [21,22].

The broad band is (3454) cm^{-1} for the (OH stretching vibration), 2922 cm^{-1} for the (C-H symmetric stretch), 1608 cm^{-1} and 1396 cm^{-1} for the (C=C), 1042 cm^{-1} for the (C-O-C) bending vibration. These assignments

are compatible with prior polyacetal experiments, which confirmed the existence of the predicted functional groups [20].

Anticancer cell line

Polymer blend and nanocomposite cytotoxic impact on cancer cells was investigated. To evaluate the blend's anticancer effectiveness, nanocomposites were tested for their ability to inhibit the development of the prostate

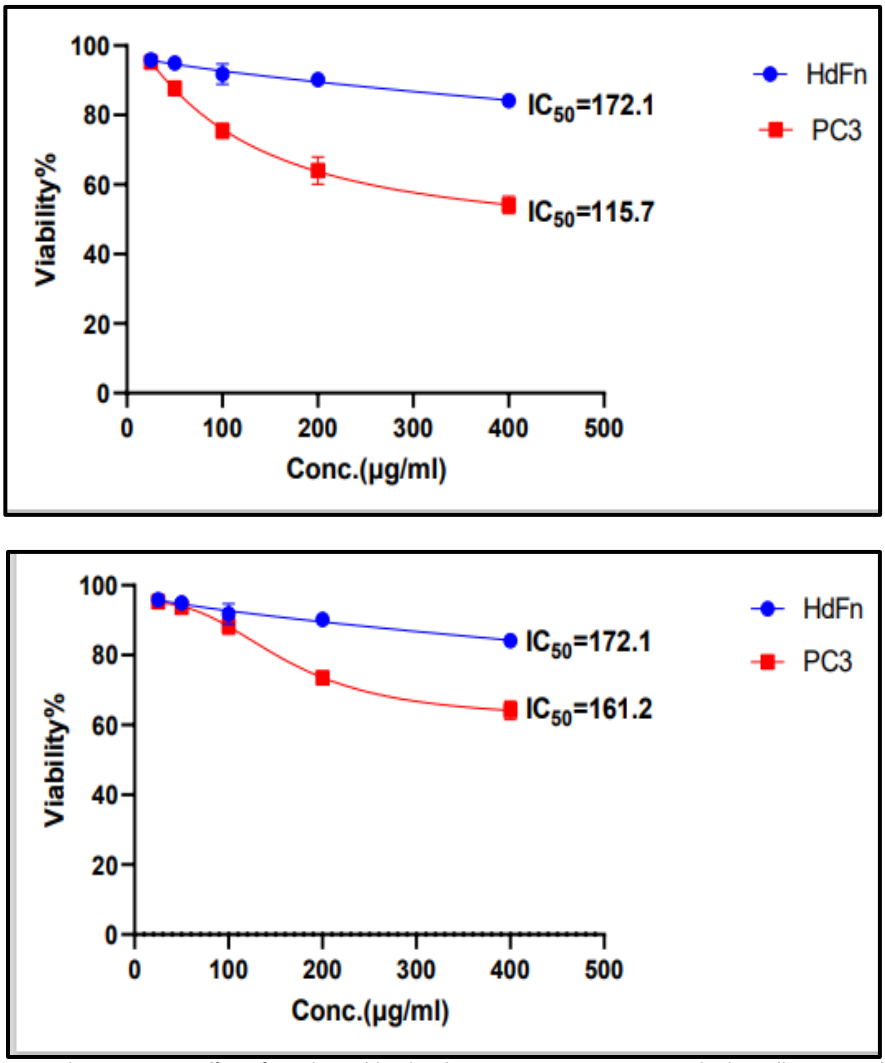

Fig. 6. The cytotoxicity effect of A-polymer blend and B-nanocomposite on PC3 and HdFn cells measured after a 24-hour incubation at 37°C.

cancer cell line PC3. The findings of this study showed that blend. As shown in (Fig. 7) against human cancer cell lines and the nanocomposites exhibited significant cytotoxic impact. The results demonstrate that blends and nanocomposites can prevent cell line growth, and that this effect is concentration dependent [23].

Through aggregation and trapping, the nanoparticles' NPs direct their attention toward the tumor cells. Another characteristic of the

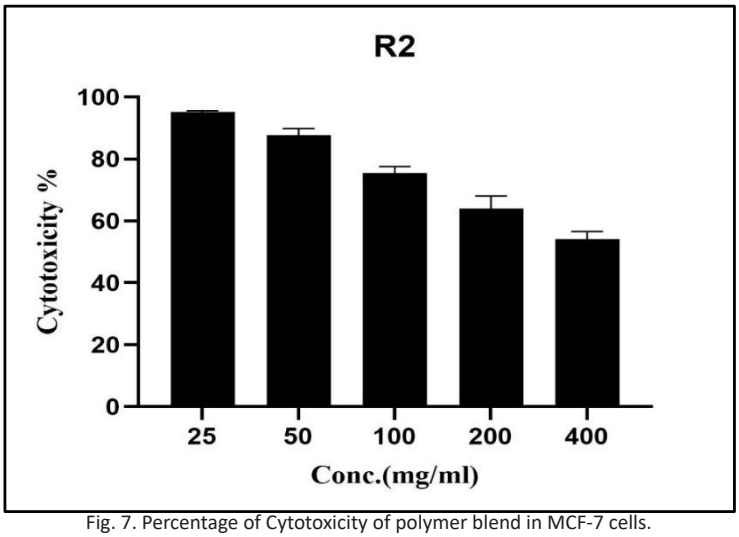

Fig. 7. Percentage of Cytotoxicity of polymer blend in MCF-7 cells.

Table 1. displays the cytotoxicity effects of polymer blend on PC3 and HdFn cells after (24 hrs.) of incubation at 37°C.

R22	HdFn		PC3	
Conc.	mean	SD	mean	SD
400	84.105	0.571066	64.19733	2.655186
200	90.23933	0.467819	73.53367	1.269836
100	91.78233	2.891034	88.31033	2.349419
50	94.94567	0.928419	93.67267	0.98457
25	95.87233	0.534626	95.25467	0.834408

Table 2. displays the cytotoxicity effects of nanocomposite on PC3 and HdFn cells after (24 hrs.) of incubation at 37°C.

R2	HdFn		PC3	
Conc.	mean	SD	mean	SD
400	84.105	0.571066	54.08967	2.56831
200	90.23933	0.467819	64.08167	3.985002
100	91.78233	2.891034	75.463	2.047671
50	94.94567	0.928419	87.73167	2.093137
25	95.87233	0.534626	95.17733	0.438024

process is the retention and penetration impact that abnormal lymphatic flow and angiogenic vessels have on malignant cells as a result, as compared to normal cells, these NPs accumulate more or more specifically inside malignant cells [24]. The findings indicated that Nanocomposites

Table 3. cytotoxic effect of polymer blend & nanocomposite on human lung cancer cell line (A549) with human dermal fibroblasts (HdFn).

R2				R22			
Conc. (µg/mL)	Cell viability %		P value	Conc. (µg/mL)	Cell viability %		P value
	HdFn	A549			HdFn	A549	
	Mean±SD	Mean±SD			Mean±SD	Mean±SD	
400	84.105 ±0.571	54.090 ±2.568	< 0.01 → **	400	84.105 ±0.571	64.197 ±2.655	< 0.01 → **
200	90.239 ±0.468	64.082 ±3.985	< 0.01 → **	200	90.239 ±0.468	73.534 ±1.270	< 0.01 → **
100	91.782 ±2.891	75.463 ±2.048	< 0.01 → **	100	91.782 ±2.891	88.310 ±2.349	< 0.05 → *
50	94.946 ±0.928	87.732 ±2.093	< 0.05 → *	50	94.946 ±0.928	93.673 ±0.985	< 0.05 → *
25	95.872 ±0.535	95.177 0.438	ns → non-significant	25	95.872 ±0.535	95.255 ±0.834	ns → non-significant

Table 4. The significant differences (Tukey test) of polymer blend.

R2 A549						
The relationship between concentrations	N	Mean difference	√MSW/n	HSD (q calc)	q crit	significant
25-50	3	7.445	1.443183	5.158737	4.37	S
25-100	3	19.714	1.443183	13.66009	4.37	S
25-200	3	31.095	1.443183	21.54613	4.37	S
25-400	3	41.087	1.443183	28.46971	4.37	S
50-100	3	12.269	1.443183	8.501349	4.37	S
50-200	3	23.65	1.443183	16.38739	4.37	S
50-400	3	33.642	1.443183	23.31098	4.37	S
100-200	3	11.381	1.443183	7.886042	4.37	S
100-400	3	21.373	1.443183	14.80963	4.37	S
200-400	3	9.992	1.443183	6.923586	4.37	S

Table 5. The significant differences (Tukey test) of Nanocomposite.

R22 A549						
The relationship between concentrations	N	Mean difference	√MSW/n	HSD (q calc)	q crit	significant
25-50	3	1.582	1.027797	1.539214	4.37	NS
25-100	3	6.945	1.027797	6.757169	4.37	S
25-200	3	21.721	1.027797	21.13355	4.37	S
25-400	3	31.058	1.027797	30.21802	4.37	S
50-100	3	5.363	1.027797	5.217955	4.37	S
50-200	3	20.139	1.027797	19.59433	4.37	S
50-400	3	29.476	1.027797	28.67881	4.37	S
100-200	3	14.776	1.027797	14.37638	4.37	S
100-400	3	24.113	1.027797	23.46085	4.37	S
200-400	3	9.337	1.027797	9.084477	4.37	S

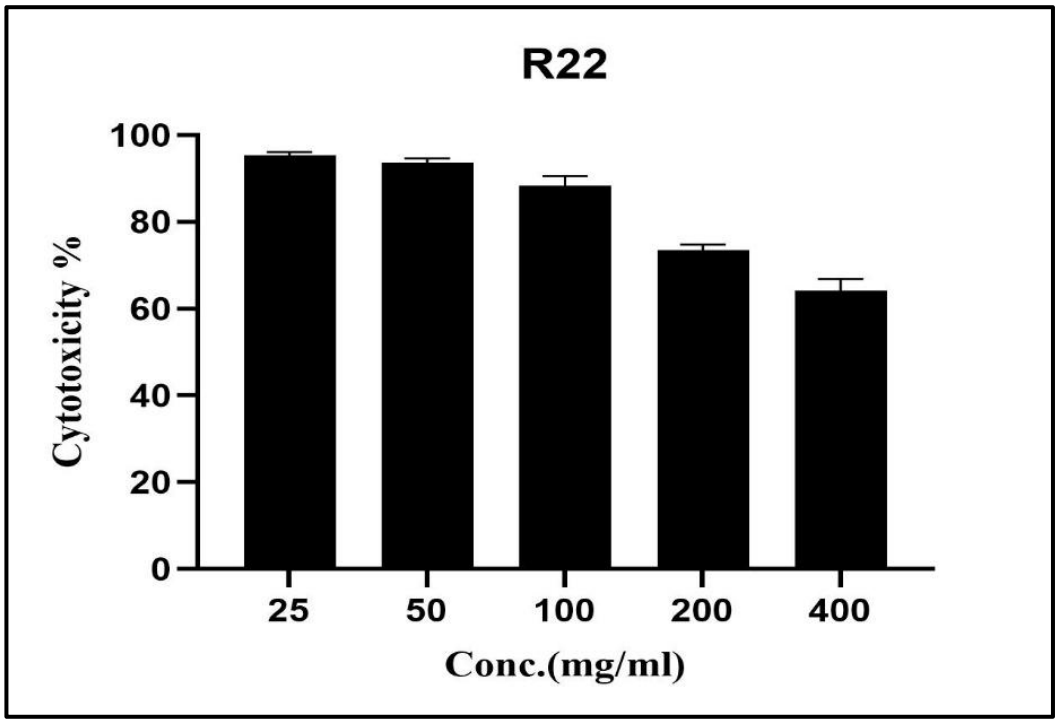

Fig. 8. Percentage of Cytotoxicity of nanocomposite in MCF-7 cells.

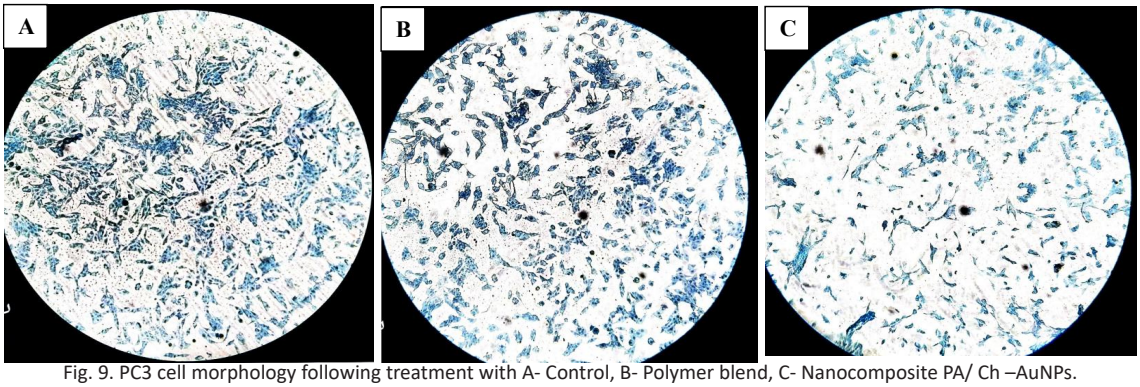

Fig. 9. PC3 cell morphology following treatment with A- Control, B- Polymer blend, C- Nanocomposite PA/ Ch –AuNPs.

have more inhibitor than the polymer blend.

The results indicated that PC3 cells were cytotoxic, with IC_{50} values of 161.2 µg/mL for polymer blend and 115.7 µg/mL for nanocomposite as shown in (Fig. 9).

CONCLUSION

In the current study polyacetal PA, PA/Ch polymer blends, and PA/Ch-Au nanocomposites were prepared. Onion peels were utilized as a reducing and stabilizing ingredient while creating gold nanoparticles. The crystallinity of these synthesized nanoparticles was evaluated using XRD measurements. AuNPs were both face-centered cubic particles. Due to the presence of Au nanoparticles, which increase activity toward the PC3 prostate cancer cell line, nanocomposites have demonstrated more anticancer activity than polymer blends. Despite these promising results, more in-vivo testing must be conducted. With continued development and clinical authentication, such smart nanocomposites

may be embedded within drug delivery systems targeted at cancers for safer and more efficient methods for the cure of cancers.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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