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Preparation and Applications of Drug Loaded Albumin/Pectin Cross Linking Nanoparticles

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ABSTRACT

A polymeric material obtained naturally is known as a natural polymer. Protein is provided by egg albumin. It is non-toxic, biodegradable, biocompatible, and readily available. In these investigations, egg albumin is employed. These nanoparticles are made using the Nano Precipitation technique. For drug delivery, albumin and pectin nanoparticles are synthesized. Glutaraldehyde is a cross-linking agent, while insulin and curcumin are used as drugs to manufacture these polymeric nanoparticles. Fourier transform infrared spectroscopy (FTIR), Ultra Violet (UV), Transmission electron micrographs (TEM), and X-ray diffraction are used to characterize the synthesized nanoparticles (XRD). The drug-loaded glutaraldehyde albumin pectin nanoparticles are amorphous according to XRD data, and the nanoparticles are spherical according to TEM images. These drug-loaded polymeric nanoparticles were also tested for anti-cancer and anti-diabetic properties. As a result, the medication was successfully encased in biopolymer nanoparticles.

Keyword: *Egg albumin, Pectin, Insulin, Curcumin, Glutaraldehyde, Polymeric Nanoparticles*

INTRODUCTION

Monomers are used to create polymers, which are long-chain molecules. Polymers are made up of many single structural units connected consistently by covalent bonds [1]. "Poly" means many, "mono" means one, and "Mers" means parts. Herbal polymers are compounds derived from plants or animals found in nature [2].

Albumin is a desirable macromolecular supplier because it produces a non-immunogenic harmless degradation product, is easy to purify, and is soluble in water. Protein-based nanoparticles, particularly albumin-based nanoparticles, have several advantages, including increased stability, nontoxicity, and antigenicity [3], biodegradability, ease of preparation, repeatability, and strong protein binding capability with diverse medicines [4]. Covalent derivatization of albumin nanoparticles with drug-targeting ligands is

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achievable due to functional groups such as amino and carboxylic acid groups [5,6]. Albumin nanoparticles can be used to deliver drugs. Albumin-based nanoparticles allow the electrostatic interaction of positively or negatively charged molecules without using other chemicals [7]. They have a fundamental structure and a high amount of charged amino acids.

Pectin is a polysaccharide that occurs naturally and is widely utilized in the food industry [8]. It contained methyl esterified carboxyl groups and was isolated from apple and orange peel [9,10]. Pectin is a significant constituent in therapeutic goods because of its gel formation, nontoxicity, and low cost [8]. Microbial enzymes contained in the content can break down pectin, allowing the encapsulated medicine to be released at the preferred place [11,12].

Crosslinking agent glutaraldehyde is used to crosslink native or regenerated collagen fibrils. Implants made of collagenous material that has been crosslinked with glutaraldehyde are vulnerable to biodegradation and low-grade immunological responses over time. These glutaraldehydes interacted with NH2 groups in collagen and bridged activated carboxyl groups with diamines [13].

Insulin cannot cure diabetes, although it does help to regulate blood sugar levels. Hormones are a type of drug that falls into this category. If people with diabetes continue to utilize insulin, they will be in good health. Human insulin replaces the insulin produced naturally by the body [14]. Curcumin is a brilliant yellow substance generated by the Curcuma longa genus of plants. Turmeric has a primary active ingredient called curcumin. It has more potent anti-inflammatory properties as well as significant antioxidant properties. It also boosts memory and makes you smarter, which makes sense given its impact on BDNF levels[15].

Materials and Methods

Materials

Egg albumin powder and pectin purchased from Sigma –Aldrich. Novolin R human insulin and curcumin drugs were obtained from Novo Nordisk. Glacial acetic acid (molecular weight 60.052 g/mol) was purchased from Merck. Glutaraldehyde was purchased from Sigma- Aldrich.

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Methods

Preparation of Albumin/ Pectin Nanoparticles

In 100 mL glacial acetic acid, 4 g pectin was dissolved. In 100 mL water, 5 g albumin powder was dissolved. With stirring, the dissolved pectin was added drop by drop to the dissolved albumin solution. As a cross-linking agent, 1 mL glutaraldehyde was added after 30 minutes. Stir this mixture for 3 hours until it is completely homogenous. Albumin/pectin nanoparticles (AP NPs) are now being formed.

Preparation of Insulin loaded nanoparticles

100 mL albumin pectin nanoparticles mixture was separated, and 1 mL insulin was added dropwise to the mixture while continuously stirring. The mixture was stirred continuously for 10 hours, forming insulin-loaded nanoparticles (APIG NPs). This mixture is centrifuged for 45 minutes at 8000 rpm. Wash the polymeric nanoparticles in water and store them as a powder for further investigation.

Preparation of Curcumin loaded nanoparticles

As a transparent solution, 5 milligrams of curcumin were dissolved in 50 mL ethanol. This curcumin solution was added to 100 mL of albumin pectin nanoparticles in a dropwise manner. After stirring for 8 hours, the nanoparticles (APCG NPs) were removed from the suspension by centrifuging at 7,000 rpm for 60 minutes and washing with water. For further research, keep the sample powdered.

Characterisation

UV-Visible spectroscopy is used to characterize the drug-loaded APIG and APCG nanoparticles, recorded using a Camspec-M350 double beam spectrophotometer. FT-IR spectroscopy with a Bruker FTIR spectrometer is used to predict the functional group. SEM-EDX was used to examine the nanoparticle's morphology. Transmission electron microscopy (TEM) was used to explore the particle state and surface morphology using a Philips-CM120 at a 120 KV accelerating voltage. Powder x-ray diffraction analysis was performed using a Rigaku D/max rA X-ray diffractometer.

CYTOTOXICITY ASSAY

Trypsinized L292 cell lines were suspended in a growth medium at 5 104 cells/ml density and plated in 96-well plates (100 l/well). Drug-loaded APF and APCG nanoparticle stock solutions were produced in dimethyl sulfoxide (DMSO) at a 1.0 g/L. Five different concentrations of drug-loaded polymeric nanoparticles are constructed and applied to the wells, then incubated at 37 °C in a 5% CO2 in air atmosphere. The cell viability was assessed using the Neutral red staining technique after 24 or 48 hours. Each well was filled with 150 µl of neutral red staining solution, which was incubated for 2 hours before being rinsed with a solution. 150 µl of solubilization solution was added, and the plate was shaken for 15 minutes at room temperature. The absorbance was readied at 540 nm.

% of cell viability= x-y/x×100.

Whereas,

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x=control

y=test sample

The regression graph from different concentrations is plotted against the % of cell viability.

INVITRO ANTI-DIABETIC ASSAY

Inhibition of α- amylase assay

In 500µl of 0.20 mM phosphate buffer (pH6.9) containing - amylase solution, various concentrations of Insulin-loaded APIG nanoparticles and standard medication were introduced and incubated at 25°C for 10 minutes. 500µl of starch solution in phosphate buffer was added to the mixture, again incubated at 25°C for 10 minutes. The reaction was halted by adding 1 mL of 3,5 dinitrosalicylic acid colour reagent to the mixture. It was incubated in boiling water and then allowed to cool at room temperature. After diluting the reaction mixture with water, the absorbance was measured at 520nm against a positive control.

Inhibition of α- glucosidase assay

To determine the inhibition of α- glucosidase activity, 1 ml of starch solution was incubated with various concentrations of APIG nanoparticles for 5 minutes at 37°C. Add 200µl of α- glucosidase (0.1U/ml) incubated for 5 mints at 37°C. The mixture was heated with boiling water for 2 minutes to stop the reaction. The amount of liberated glucose was measured using the glucose peroxidase method.

Calculation of IC50 value

Inhibition % = (A a – A b) / A a X 100

Whereas,

A a =Absorbance of control reaction

A b =Absorbance, in the presence of sample of drug-loaded nanoparticles

Using this formula, the IC50 value for both inhibition of α- amylase and Inhibition of α- glucosidase was calculated.

ANTI-CANCER ASSAY

Eagles' Minimum Essential Medium was used to cultivate human osteosarcoma cells. The growth media for the HOS (GRL 1543) cell line contains 10% fetal bovine serum. APCG nanoparticle samples in various concentrations were introduced to these cells and cultured at 37°C with 5% CO2 in a CO2 incubator. The medium is sucked out after 24 hours, and then 5 mg/ml MTT in PBS solutions

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is added and incubated for 3 hours. The medium was then solubilized, and the absorbance was measured at 570nm with an ELISA reader from Denver Jasco Model 7800 UV/VIS Spectrophotometer, Tokyo, Japan.

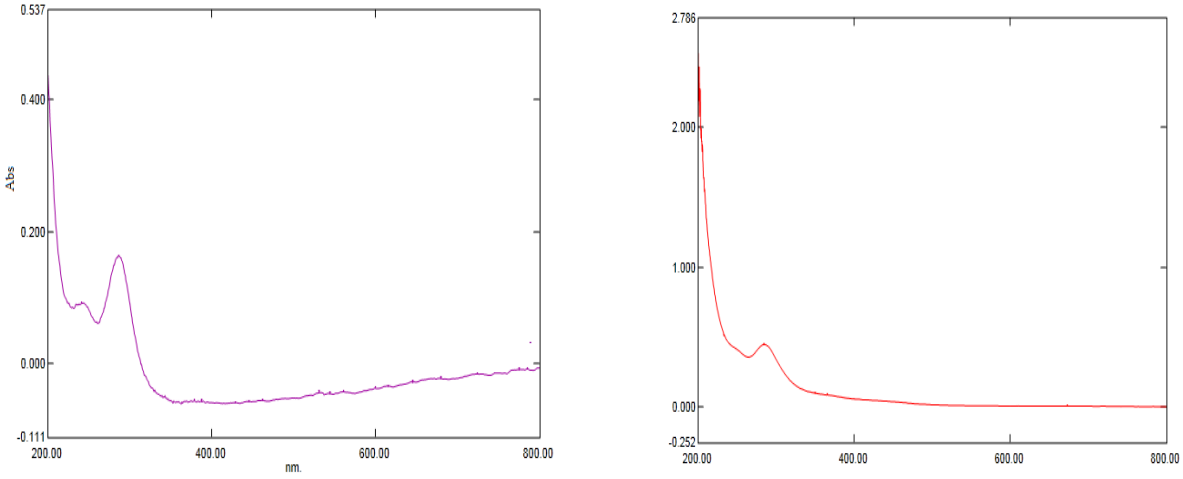
RESULTS AND DISCUSSION

Formation of drug loaded APIG and APCG nanoparticles

The electrostatic interaction between opposite charges of the amino group in albumin and carboxyl group in pectin on the nanoparticle surface was used to generate drug-loaded APIG and APCG nanoparticles. Because the amino group of albumin will be protonated at pH 5-6, this range is ideal for the formulation of drug-loaded albumin pectin nanoparticles.

UV-Visible spectrum

A UV-Vis spectrophotometer with a wavelength range of 200 to 800 nm was used to confirm the synthesis of drug-loaded APIG and APCG nanoparticles. The peak appears at a wavelength of 286 nm in the absorption spectra of APIG (Fig. a). The absorption peak for APCG NPs occurs at a wavelength of 284 nm. (Fig. b).



(a) **(b)**

Fig: 1. UV spectrum of APIG (a), APCG (b)

FTIR spectrum

In the spectrum of egg albumin (Fig. a), the peak at 1537 cm-1 is absorbed. This peak is amide II bending vibration of albumin. In the pectin spectrum, the peak at 1625 cm-1 is absorbed due to the carboxyl group of pectin. The spectrum of insulin-loaded APIG nanoparticles shows a change

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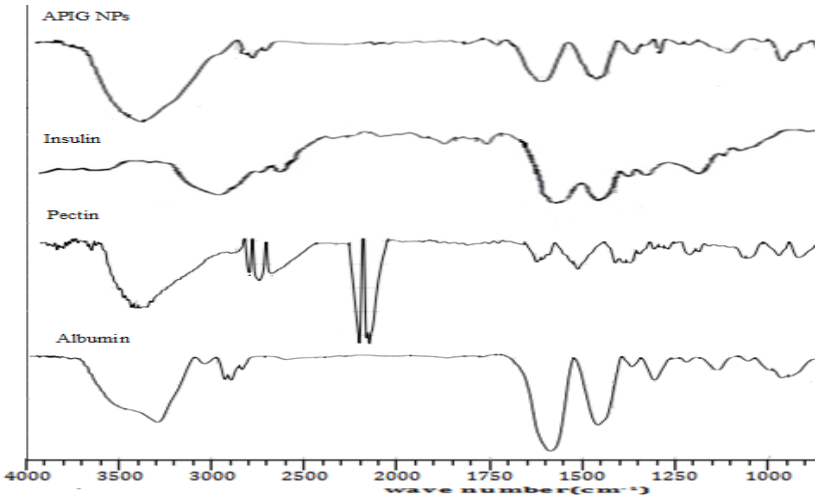
in the number wave. The peak at 1537 cm-1 shifted to 1526 cm-1. The peak at 1635 cm-1 of pectin is disappearing. In APCG spectrum, the peak at 1537 cm-1 is shifted to 1530 cm-1. This shows that there is an interaction between the charges. Because the interaction of the positive charge of albumin and negative charge of pectin forms the drug-loaded albumin pectin nanoparticles. The insulin-loaded nanoparticle formation indicates the peak at 1654cm-1 in (Fig: a), and the curcumin-loaded nanoparticles in 1649cm-1 (Fig: b). The peaks at 1654cm-1 and 1649cm-1 indicated an interaction between albumin and glutaraldehyde in drug-loaded nanoparticles. Therefore the drug-loaded nanoparticles were confirmed by the FTIR spectrum.

(a)



(b)

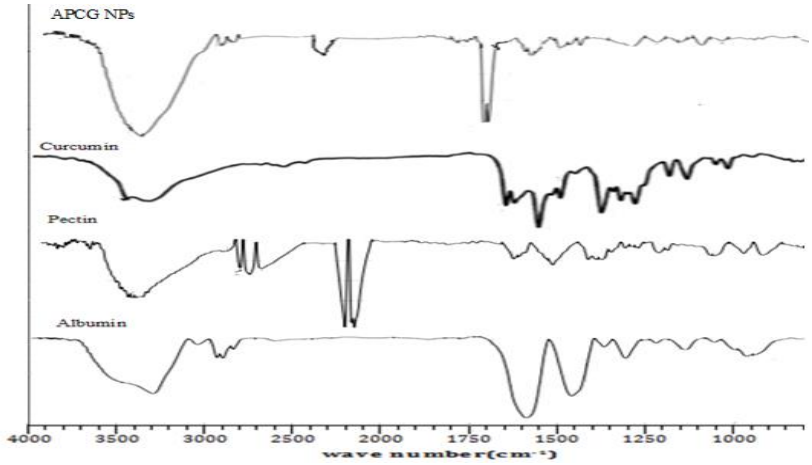


Fig: 2. FT-IR spectrum of APIG (a), APCG (b)

X-ray Diffraction Studies (XRD)

APIG and APCG nanoparticles were studied using X-ray diffraction. APIG and APCG nanoparticles with drugs exhibit broad peaks. APIG and APCG nanoparticles have a two theta value

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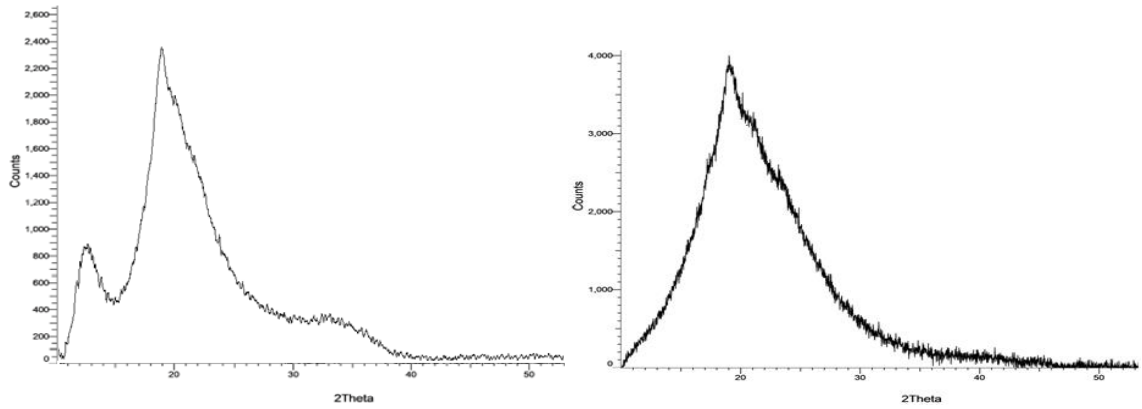
of 18.980° and 19.120°, respectively. Broad peaks can be found in these drug-loaded polymeric nanoparticles. This broad peak in drug-loaded polymeric nanoparticles indicates that APIG and APCG nanoparticles are amorphous. These peaks show that the pharmaceuticals are contained within the nanoparticles and that the drugs in the polymeric nanoparticles were molecularly distributed. This amorphous drug has all of the properties of a drug in nanoparticles.



(a) **(b)**

Fig: 3. XRD data of APIG (a), APCG (b)

Transmission Electron Microscopy (TEM) Analysis:

The spherical morphology of drug-loaded APIG and APCG nanoparticles can be seen in TEM images. APIG nanoparticles have a spherical form at 10 nm, while APCG nanoparticles have a spherical shape at 20 nm. The medicine has been successfully loaded, as evidenced by this image.

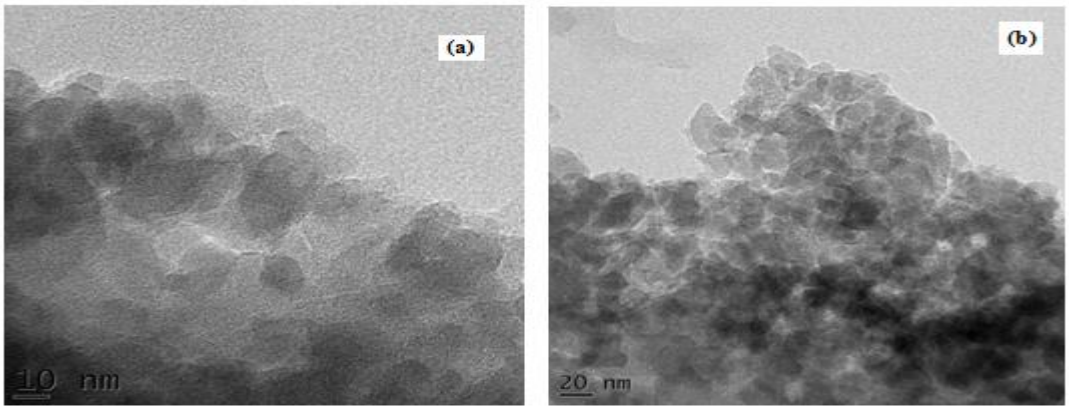


Fig: 4. TEM image of APIG (a), APCG (b).

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CYTOTOXICITY ASSAY

Cytotoxicity assay of APIG nanoparticles

Using L292 cells and a neutral red staining assay technique, the cytotoxicity of APIG nanoparticles is investigated (Tab-1 and Gra-1). The cell line plate is given five different concentrations. APIG nanoparticles at a concentration of 10 µl had a cell viability of 99.89 percentages. The percentage of cell viability was 99.33, 99.89, and 98.88 at 20, 40, and 60 µl concentrations, respectively. The percentage of cell viability of L292 cells was 98.67 and 97.7 for APIG concentrations of 80 and 100 µl, respectively. Positive control had a cell viability rate of 5.10. Until a concentration of 100 µl, the drug-loaded APIG nanoparticles have no cytotoxicity.

Table 1

Percentage of viability of APIG and APCG nanoparticles

Sample Concentration (µg/mL)	Average OD of APIG	Percentage of Viability of APIG	Average OD of APCG	Percentage of Viability of APCG
10µl	1.918	99.89±0.27	1.917	99.86±0.21
20µl	1.907	99.33±0.42	1.914	99.7±0.5
40µl	1.918	99.89±1.18	1.910	99.47±0.76
60µl	1.899	98.88±0.91	1.898	98.83±0.39
80µl	1.883	98.05±0.5	1.885	98.19±0.47
100µl	1.885	98.15±0.71	1.876	97.72±0.56
Positive Control	0.098	5.10±0.38	0.098	5.10±0.38

Cytotoxicity assay of APCG nanoparticles

L292 cells are used to test the cytotoxicity of APCG nanoparticles using a neutral red staining assay technique (Tab-1 and Gra-2). The cell line plate is given five different concentrations. The cell viability of APCG nanoparticles at a concentration of 10 µl was 99.86. The percentage of cell viability was 99.70, 99.47, and 98.83 at 20, 40, and 60 µl concentrations, respectively. The percentage of cell viability of L292 cells was 98.190.47 and 97.72 at APF concentrations of 80 and 100 µl, respectively. Positive control had a cell viability rate of 5.10 %. Until a concentration of 100 µl, the drug-loaded APCG nanoparticles have no cytotoxicity.

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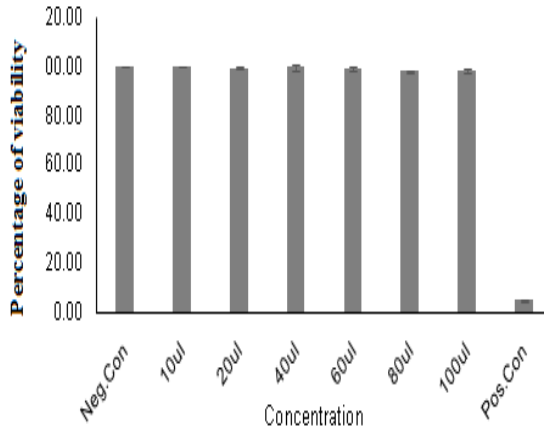
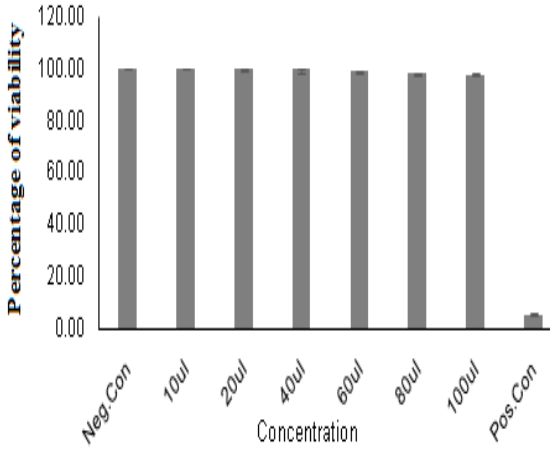


Graph-1: Cytotoxicity assay of APIG nanoparticles



Graph-2: Cytotoxicity assay of APCG nanoparticles

INVITRO ANTI-DIABETIC ASSAY

Invitro anti-diabetic activity was carried out in APIG polymeric nanoparticles using α-amylase and α- glucosidase enzymes because APIG is insulin loaded nanoparticle.

Inhibition of α- amylase activity

Insulin-loaded polymeric nanoparticles significantly impact the enzyme - amylase (Gra 3). The - amylase inhibitory assay tests different concentrations of drug-loaded APIG nanoparticles (100, 200, 400, 600, 800, 1000 µl /ml). In 1000 µl /ml, the highest inhibition value of 15% was obtained. The amylase inhibition assay has an IC50 value of 7.5 µl /ml.

Inhibition of α- glucosidase activity

Insulin-loaded polymeric nanoparticles significantly impact the enzyme - amylase (Gra 4). The - amylase inhibitory assay tests different concentrations of drug-loaded APIG nanoparticles (100, 200, 400, 600, 800, 1000 µl /ml). In 1000 µl /ml, the maximum inhibition value was 9.49 percentages. The amylase inhibition assay has an IC50 value of 4.74 µl /ml.

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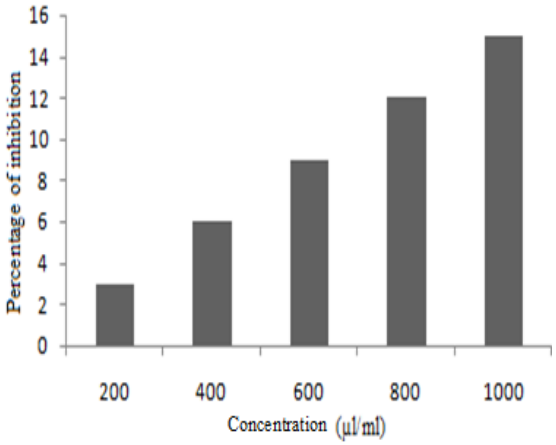
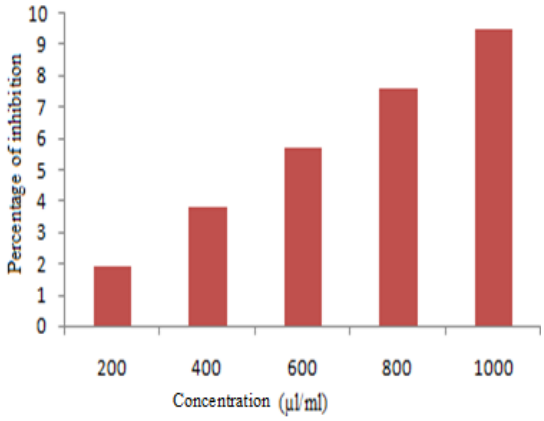


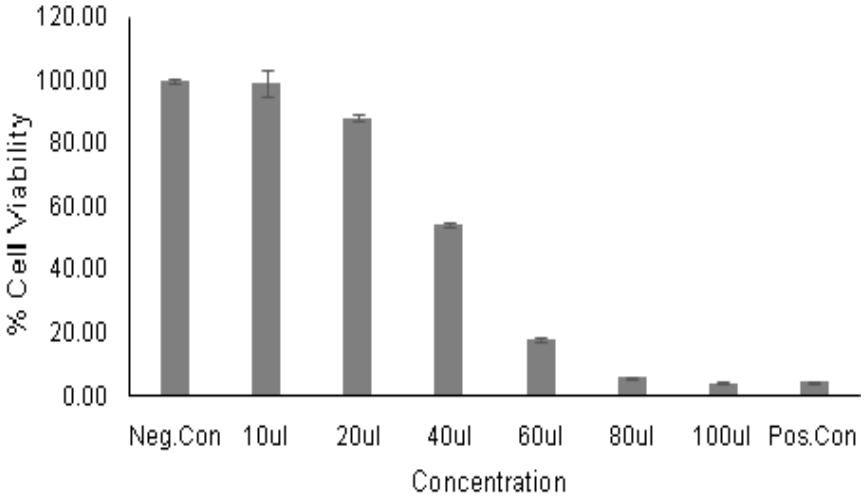
Graph-3: α- amylase activity of APIG nanoparticles



Graph-4: α- Glucosidase activity of APIG nanoparticles

ANTI-CANCER ASSAY

The MTT assay was used to examine curcumin-loaded APCG polymeric nanoparticles' anti-proliferative activity on human Osteosarcoma cells (Gra 5). Depending on the dose, these drug-loaded polymeric nanoparticles have high cytotoxicity against HOS cells. The cell viability percentage increases as the drug-loaded polymeric nanoparticles' concentration rise. 36.28 µl /ml is the IC 50 value.



Graph-5: Anti-cancer activity of APCG nanoparticles

CONCLUSION

The Nano precipitation technique makes drug-loaded albumin, pectin, and glutaraldehyde polymeric nanoparticles. Insulin and curcumin have been loaded successfully into albumin and pectin nanoparticles. FTIR, Transmission electron micrographs (TEM), and X-ray diffraction were used to

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characterize polymeric nanoparticles. The amorphous nature of the nanoparticles was validated by XRD data, and TEM images confirmed the shape of the nanoparticles. The drug-loaded APIG and APCG nanoparticles had a higher inhibitory effect against metabolic enzymes in a cytotoxicity assay, and these nanoparticles are used for drug delivery.

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Conflict of interest

The authors declare no conflict of interest.

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