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Bioresponsive Chitosan-PVA Hydrogels Encapsulating Ginsenosides-Rich *Panax ginseng* for Triple Action Therapeutics: Integrating Smart Drug Release with Anti-Cancer, Anti-Diabetic and Wound Healing Applications

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KEYWORDS

Chitosan-PVA hydrogel, *Panax ginseng*, HT-29 colon cancer, anti-inflammatory, antidiabetic, drug delivery system

ABSTRACT:

The integration of natural phytochemicals into advanced biomaterials presents a transformative approach to managing complex chronic diseases. In this study, we developed a multifunctional, biodegradable hydrogel composed of chitosan and polyvinyl alcohol (PVA), encapsulating *Panax ginseng* extract, and evaluated its *in vitro* therapeutic potential across five biomedical domains anticancer, antioxidant, anti-inflammatory, antidiabetic, and wound healing. SEM analyses confirmed successful ginsenoside incorporation, revealing a porous, bioactive matrix suitable for sustained release. The hydrogel exhibited potent cytotoxicity against HT-29 colon cancer cells, achieving an IC_{50} of 25 µg/mL and inducing apoptosis. The MTT assay revealed a marked decrease in HT-29 cell viability at 25 µg/ml concentration of chitosan-PVA hydrogel containing *Panax ginseng*, with viability reduced to 48.3 ± 1.6%, indicating significant anticancer potential at this dose. Strong radical scavenging (78% DPPH inhibition) and protein stabilization (70% inhibition in egg albumin denaturation assay) demonstrated its dual oxidative and inflammatory suppression. The hydrogel also inhibited α-amylase and α-glucosidase by up to 75%, confirming metabolic regulatory potential. A biphasic release profile delivered nearly 100% cumulative release within 48 hours. Most notably, in a wound scratch assay using *Danio rerio* gill cells, the hydrogel achieved complete wound closure within 42 hours significantly faster than the 72-hour healing observed in comparable systems highlighting its regenerative efficacy. Compared to hydrogels loaded with honey or synthetic agents, our *P. ginseng*-based formulation demonstrated superior multifunctionality and biocompatibility. These results establish this smart hydrogel as a robust platform for integrated chronic disease management and regenerative medicine.

1. Introduction

The rising global burden of chronic diseases including cancer, diabetes mellitus, and inflammatory disorders has driven the need for advanced, sustainable, and multi-targeted therapeutic strategies (1). Colorectal cancer (CRC) is one of the leading causes of cancer related morbidity and mortality worldwide. Despite progress in chemotherapy and immunotherapy, treatment outcomes are often hampered by systemic toxicity, drug resistance, and lack of targeted delivery mechanisms (2). Similarly, diabetes mellitus continues to affect over 500 million individuals globally, placing enormous strain on healthcare systems. Conventional antidiabetic drugs are effective but frequently cause adverse effects, and they rarely address the multifactorial nature of metabolic dysfunction, inflammation, and oxidative stress that underlies disease progression (3). Inflammation, in particular plays a central role in the pathogenesis of both cancer and diabetes, suggesting that therapeutics capable of modulating multiple pathways may offer synergistic benefits (4). Natural compounds derived from traditional medicinal plants have received considerable attention as

Journal of Chemical Health Risks
www.jchr.org
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alternative or complementary agents for chronic disease management. Among these, *P. ginseng*, a basis of traditional East Asian medicine has demonstrated a broad range of pharmacological activities (5). Its bioactive constituents, particularly ginsenosides, have shown the ability to modulate key signaling pathways involved in apoptosis, oxidative stress, inflammation, and glucose metabolism. Ginsenosides have been reported to induce cell cycle arrest and apoptosis in cancer cells, scavenge reactive oxygen species (ROS), inhibit pro-inflammatory cytokines, and improve insulin sensitivity (6). However, the clinical application of *P. ginseng* is limited due to its poor aqueous solubility, low bioavailability, and rapid metabolism in the gastrointestinal tract. Therefore, an efficient and biocompatible delivery system is essential to enhance its stability, protect it from enzymatic degradation, and ensure sustained and localized release (7). Hydrogels, particularly those made from natural and synthetic polymers, have emerged as promising drug delivery platforms. Chitosan, a naturally derived polysaccharide from crustacean shells is widely recognized for its biocompatibility, biodegradability, antimicrobial activity, and mucoadhesive properties (8). Polyvinyl alcohol (PVA), a synthetic but non-toxic polymer, contributes excellent mechanical strength and film-forming capability (9). The combination of chitosan and PVA results in a hybrid hydrogel with improved physicochemical properties, suitable for encapsulating hydrophilic and hydrophobic bioactive. Importantly, such hydrogels enable controlled and sustained release, allowing enhanced cellular uptake and prolonged therapeutic action (10) (11). This research aims to formulate and evaluate a novel chitosan-PVA hydrogel encapsulating *P. ginseng* extract for multifunctional biomedical applications. By integrating plant-based therapeutics into a biopolymeric hydrogel system, this study explores the potential of this platform in four key therapeutic areas anticancer efficacy against HT-29 colon cancer cells, antioxidant activity to mitigate oxidative stress, anti-inflammatory effects relevant to chronic wounds and immune modulation, and antidiabetic activity targeting carbohydrate-digesting enzymes. The novelty of this work lies in combining the bio functional properties of ginsenosides with the structural and delivery advantages of chitosan-PVA hydrogels, thereby proposing an innovative approach for holistic management of chronic diseases. Through comprehensive *in vitro* evaluation and mechanistic insights, this study sets the foundation for developing an integrated phytochemical-based therapeutic strategy using smart polymeric biomaterials.

2. Methodology

Materials

Medium molecular weight chitosan (CAS 9012-76-4) and polyvinyl alcohol (PVA; CAS 9002-89-5) were obtained from Sigma-Aldrich and SRL for hydrogel fabrication. Ferric chloride hexahydrate (CAS 10025-77-1) was purchased from HiMedia, whereas ferrous chloride tetrahydrate (CAS 13478-10-9) and glutaraldehyde (CAS 111-30-8) were supplied by SRL. Sodium hydroxide pellets (CAS 1310-73-2) and absolute ethanol (CAS 64-17-5) were procured from DRL and HiMedia. Analytical-grade acetic acid (CAS 64-19-7), used for chitosan solubilization, was sourced from SRL. HT-29 colon cancer cell lines were acquired from the National Centre for Cell Science (NCCS), Pune, for *in vitro* biological studies. Sterile tissue-culture flasks and assay plates were purchased from TPP through SRL distribution, and phosphate-buffered saline (PBS; HiMedia) was used throughout all rinsing and washing procedures. All glassware and disposable plasticware from Borosil and HiMedia were rigorously cleaned, oven-dried, and sterilized prior to experimental use. Fresh *Panax ginseng* roots were collected locally and processed for the extraction of *P. Ginseng.*

Extraction of *Panax ginseng* Leaves

Preparation of the Leaf Extract Fresh *Panax ginseng* plant material was collected and washed thoroughly under running tap water, followed by repeated rinsing with distilled water to eliminate surface dirt and impurities. The cleaned plant parts were cut into small segments using sterile scissors. For the preparation of extract a 1:10 (w/v) proportion was maintained, where 10 g of freshly cut *P. ginseng* pieces were transferred into a 250 mL beaker containing 100 mL of distilled water. The mixture was heated and allowed to gently boil for 15 minutes, during which the solution gradually developed a yellowish-brown coloration, indicating the release of water-soluble phytochemicals into the medium. After heating, the extract was left undisturbed to cool to room temperature and was then filtered through Whatman No. 1 filter paper to remove insoluble plant residues. The clear filtrate obtained was identified as the aqueous *P. ginseng* extract and was used in its fresh form for hydrogel loading and further experimental studies.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 3466-3477 | ISSN:2251-6727

Preparation of Chitosan (CS) Solution

Preparing Dilute Acetic Acid

A dilute acetic acid solution (1% v/v) was prepared by carefully adding 1 mL of glacial acetic acid to 99 mL of distilled water in a clean 100 mL volumetric flask. The acid was added slowly to the water with continuous stirring to ensure proper mixing and to avoid localized heating. The resulting solution was thoroughly mixed to obtain a clear and homogeneous dilute acetic acid solution. The freshly prepared solution was used for dissolving chitosan during hydrogel fabrication.

Dissolving Chitosan

Chitosan was dissolved using the freshly prepared 1% (v/v) dilute acetic acid solution. Briefly, 2 g of chitosan powder was slowly added to 100 mL of 1% acetic acid taken in a clean beaker while stirring continuously with a magnetic stirrer at room temperature. The mixture was stirred for 6-8 hours until a clear and uniform chitosan solution was obtained. Any undissolved particles were removed by filtration, and the resulting solution was allowed to degas to eliminate entrapped air bubbles. The prepared chitosan solution was used immediately for the fabrication of chitosan-PVA composite hydrogels.

Preparation of PVA Solution

A 5% (w/v) PVA stock solution was prepared by dissolving 5 g of PVA in 100 mL of distilled water. Initially, 80 mL of distilled water was heated to 80-90 °C on a magnetic stirrer, and PVA powder was added gradually with continuous stirring to avoid lump formation. The solution was maintained at this temperature and stirred for 4-6 hours until complete dissolution resulted in a clear and homogeneous solution. The final volume was adjusted to 100 mL using distilled water. After cooling to room temperature, the solution was briefly sonicated to remove entrapped air bubbles. The PVA solution was sterilized by autoclaving at 121 °C for 15-20 minutes and stored at 4 °C until further use.

Incorporation of *Panax ginseng* Extract into

CS/PVA Hydrogel

Preparation of Polymer Blend

To prepare the composite hydrogel matrix, the previously prepared chitosan and PVA solutions were combined in a fixed 1:4 ratio. For example, 10 mL of 0.5% chitosan solution was mixed with 40 mL of 5% PVA solution in a clean beaker. The mixture was stirred gently at low to moderate speed for 1-2 hours at room temperature. During this process, the solution gradually became more viscous, indicating proper blending and interaction between the two polymers.

Loading of *Panax ginseng* Plant Extract

After obtaining a uniform CS/PVA polymer mixture, the freshly prepared aqueous *Panax ginseng* extract was added slowly under continuous gentle stirring. The volume of extract added was adjusted according to the required loading concentration. Stirring was continued until the extract was uniformly distributed throughout the polymer blend, resulting in a homogeneous drug-loaded CS/PVA pre-gel solution.

Crosslinking and Hydrogel Formation

To stabilize the polymer network, a 0.5% (v/v) glutaraldehyde solution was added dropwise to the CS/PVA- *Panax ginseng* extract mixture under constant stirring. After complete addition, the mixture was left undisturbed at room temperature for sufficient time until a physically stable hydrogel was formed. The formed hydrogel was then washed repeatedly with distilled water to remove any unreacted glutaraldehyde and to reduce surface acidity. The washed hydrogel was adjusted close to neutral pH, cut into required shapes, and finally exposed to UV light for surface sterilization before being used for further characterization and biological evaluation.

Scanning Electron Microscopy (SEM) Imaging

The surface appearance and structural characteristics of the chitosan-PVA hydrogel encasing *P. ginseng* were investigated by SEM analysis. Samples of freeze-dried hydrogel were photographed at different magnifications after being thinly coated with gold via sputtering. The SEM images revealed a porous and interconnected network structure, ideal for controlled drug release and efficient bioactive loading (12) (13).

Cell Culture Maintenance

Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 5% CO_2 was used to maintain HT-29 human colon cancer cells. The cells were then incubated at 37°C in a humidified environment. Cells were sub-cultured upon reaching 80–90% confluency using trypsin-EDTA and used for cytotoxicity and apoptosis assays to evaluate the anticancer efficacy of the chitosan-PVA-*Panax ginseng* hydrogel (14).

Journal of Chemical Health Risks

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JCHR (2026) 16(2), 3466-3477 | ISSN:2251-6727

Critique of Morphology

Morphological analysis of HT-29 cells treated with the chitosan-PVA-*Panax ginseng* hydrogel revealed significant structural changes compared to untreated controls. Under microscopy, treated cells exhibited classic apoptotic features such as cell shrinkage, membrane blebbing, and detachment, indicating cytotoxic effects. These changes suggest that the hydrogel formulation effectively induces apoptosis, supporting its potential as an anticancer agent (15).

Cytotoxicity Evaluation

The cytotoxic potential of the chitosan-PVA-*Panax ginseng* hydrogel was assessed using the MTT assay on HT-29 colon cancer cells. After being placed in 96-well plates, the cells were exposed to different hydrogel concentrations for a full day. MTT reagent was added after incubation, and the formazan crystals that resulted were dissolved in DMSO. Cell viability was computed in relation to untreated controls using absorbance measurements at 570 nm. The hydrogel showed a dose-dependent reduction in cell viability, confirming its anticancer efficacy (16).

Cell viability(%)=((OD of treated sample)/(OD of control)) x 100

Cell Death Analysis Using Fluorescent Microscopy

HT-29 colon cancer cells were studied for cell death using acridine orange/ethidium bromide (AO/EtBr) dual labeling. Cells were stained with AO/EtBr and examined under a fluorescence microscope following treatment with the chitosan-PVA-*Panax ginseng* hydrogel at an IC_{50} concentration. Green fluorescence was seen in live cells, yellow-orange nuclei were seen in early apoptotic cells, and red fluorescence was seen in late apoptotic or necrotic cells. The presence of apoptotic features confirmed that the hydrogel induces programmed cell death (17).

Antioxidant Activity

DPPH (2, 2-diphenyl-1-picrylhydrazyl) Assay

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay was used to evaluate the chitosan-PVA-*Panax ginseng* hydrogel's antioxidant capacity. After mixing different hydrogel concentrations with DPPH solution, the mixture was left to incubate for half an hour in the dark. A UV-Vis spectrophotometer was used to quantify the absorbance drop at 517 nm. Using ascorbic acid as a benchmark, the percentage of radical scavenging activity was computed. The hydrogel exhibited dose-dependent antioxidant activity, confirming its free radical scavenging potential (18).

Anti-Inflammatory Activity

Egg albumin denaturation assay

The anti-inflammatory effect of the chitosan-PVA-*Panax ginseng* hydrogel was evaluated using the egg albumin denaturation assay. Protein denaturation was induced by heating hydrogel samples at different concentrations to 70°C for 5 minutes after they had been treated with fresh egg albumin and phosphate-buffered saline (pH 6.4) at 37°C for 15 minutes. Absorbance at 660 nm was measured after cooling. Protein denaturation inhibition as a percentage was computed in comparison to a control. The hydrogel showed strong anti-inflammatory efficacy by inhibiting protein denaturation in a concentration-dependent manner (19).

% Inhibition-(Absorbance of Control-Absorbance of the sample)/(Absorbance of Control) x 100

***In vitro* Drug release Assay**

The drug release profile of the chitosan-PVA-*Panax ginseng* hydrogel was evaluated using a phosphate-buffered saline (PBS, pH 7.4) medium. Pre-weighed hydrogel samples were immersed in PBS at 37°C under constant shaking. At specific time intervals, aliquots were withdrawn and replaced with fresh PBS to maintain sink conditions. The amount of released P. ginseng was quantified using UV-Vis spectrophotometry at the λmax specific to the extract. Cumulative drug release (%) was calculated over a 48-hour period, revealing a biphasic release pattern an initial burst followed by sustained release indicating the hydrogel's potential for controlled drug delivery (20).

(Drug Released at Time (t) / Total Drug Content) × 100.

Anti-Diabetic Activity

α-amylase and α-glucosidase inhibition assay

The antidiabetic potential of the chitosan-PVA-*Panax ginseng* hydrogel was assessed through *in vitro* α-amylase and α-glucosidase inhibition assays. For the α-amylase assay, hydrogel samples were incubated with α-amylase enzyme and starch substrate, followed by the addition of DNS reagent. The absorbance was measured

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 3466-3477 | ISSN:2251-6727

at 540 nm. In the α-glucosidase assay, samples were mixed with α-glucosidase enzyme and p-nitrophenyl-α-D-glucopyranoside (pNPG) as a substrate, the reaction was terminated with Na_2CO_3, and absorbance was recorded at 405 nm. The percentage of enzyme inhibition was calculated using a standard formula. Results showed a concentration-dependent inhibition, demonstrating that the hydrogel effectively suppresses carbohydrate digesting enzymes, indicating promising antidiabetic activity (21).

Cell line

Danio rerio (DrG) gill cell lines were obtained from the National Repository of Fish Cell Lines at the Aquatic Animal Health Laboratory, C. Abdul Hakeem College in Melvisharam, Tamil Nadu, India, as reported by Nathiga Nambi et al. (2017). In this work, a wound-healing scratch experiment was performed using DrG cells. Leibovitz's L-15 media supplemented with 10% fetal bovine serum (FBS) was used to cultivate the cell lines (22).

Wound healing activity of chitosan/PVA-*Panax ginseng* hydrogel

At a density of 3.8×10^5 cells per milliliter, DrG cells were plated in six-well plates and incubated for twenty-four hours at 28 °C. According to Linga et al. (2007), a sterile 200 µL pipette tip was used to make a scratch in the cell monolayer. To get rid of any debris, PBS was used to wash the wells. 15 mm hydrogel discs made of chitosan/PVA-*Panax ginseng* hydrogel (50 µg/mL) were applied to the scratched areas in L-15 media in order to measure the *in vitro* wound-healing activities. As the positive control, hydrogen peroxide (4 µM H_2O_2) was used. To track the healing process, micrographs were obtained under 100× magnification at different intervals (23).

3. Results

Surface morphology using Scanning Electron Microscopy

SEM analysis of the chitosan-PVA-*Panax ginseng* hydrogel revealed a porous and interconnected network structure with uniform surface distribution. The micrographs showed well-defined pores and smooth polymeric surfaces, which are essential for effective drug encapsulation and sustained release (Figure.1). The porous morphology also facilitates the diffusion of bioactive compounds, indicating that the hydrogel structure is well-suited for biomedical applications such as wound healing, drug delivery, and tissue engineering.

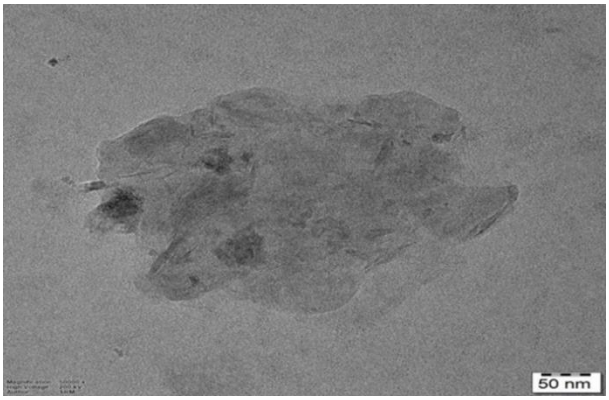

Figure.1. Scanning Electron Microscopy (SEM) characterization of the synthesized nanomaterial reveals a thin, wrinkled, and sheet-like morphology with aggregated structures, indicating a layered nanoscale architecture; the scale bar represents 50 nm.

Cytotoxicity Assay

The MTT assay demonstrated that the chitosan-PVA-*Panax ginseng* hydrogel exhibited significant, dose-dependent cytotoxicity against HT-29 colon cancer cells. Cell viability decreased notably with increasing concentrations of the hydrogel, with the IC_{50} value determined at approximately 25 µg/mL. Treated cells showed reduced metabolic activity, indicating effective inhibition of cell proliferation (Figure.2). These findings suggest that the hydrogel formulation enhances the anticancer efficacy of P. ginseng through improved delivery and bioavailability.

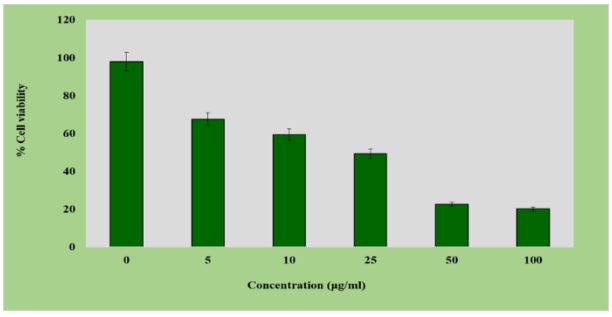

Figure.2. MTT assay results show a dose-dependent decrease in cell viability upon treatment with the synthesized nanomaterial. At 25 µg/mL, cell viability reduced to approximately 50%, indicating marked cytotoxicity.

Journal of Chemical Health Risks
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JCHR (2026) 16(2), 3466-3477 | ISSN:2251-6727

Cell Morphology Analysis

Microscopic examination of HT-29 cells after treatment with the chitosan-PVA-*Panax ginseng* hydrogel revealed distinct morphological changes characteristic of apoptosis. Figure.3a is compared to the control group, which exhibited normal, polygonal-shaped cells with intact membranes, treated cells showed rounding, shrinkage, membrane blebbing, and detachment from the surface. These alterations, especially at the IC_{50} concentration (25 µg/mL), confirm that the hydrogel induces morphological hallmarks of programmed cell death, supporting its potential as an effective anticancer agent (Figure.3b).

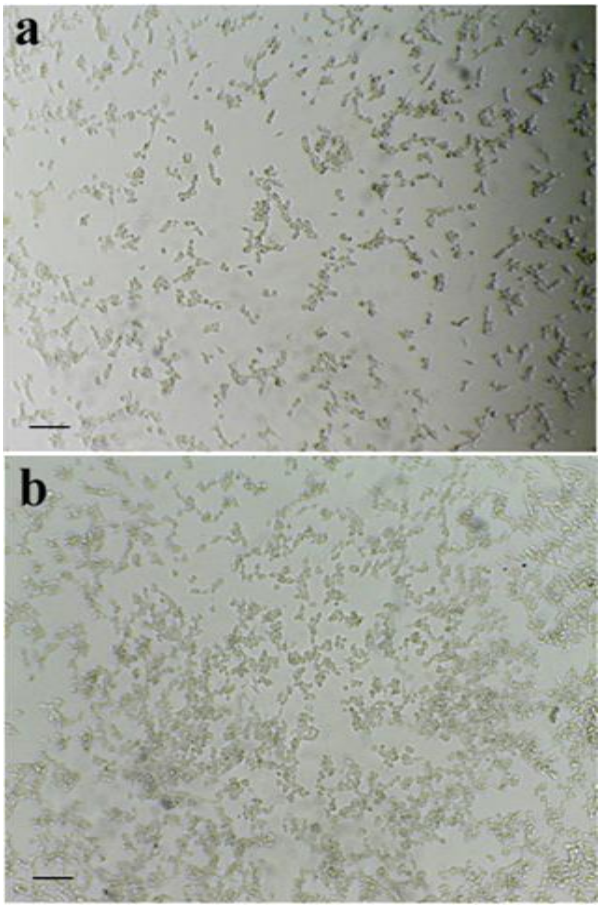

Figure.3. Morphological changes in HT-29 cells after Cs/PVA- PG Hydrogel treatment: (A) Control cells showing normal morphology. (B) IC50-treated cells (25µg/mL) displaying shrinkage, membrane blebbing, and detachment.

Cell Death Analysis Using Fluorescent Microscopy

Fluorescent microscopy using acridine orange/ethidium bromide (AO/EtBr) staining revealed clear evidence of apoptosis in HT-29 cells treated with the chitosan-PVA-*Panax ginseng* hydrogel. Figure.4a displayed uniform green fluorescence, indicating live, healthy nuclei. In contrast, treated cells exhibited orange to red fluorescence with condensed or fragmented nuclei, indicative of early and late apoptotic stages. These observations confirm that the hydrogel induces apoptosis-mediated cell death, correlating with the cytotoxicity assay results (Figure.4b).

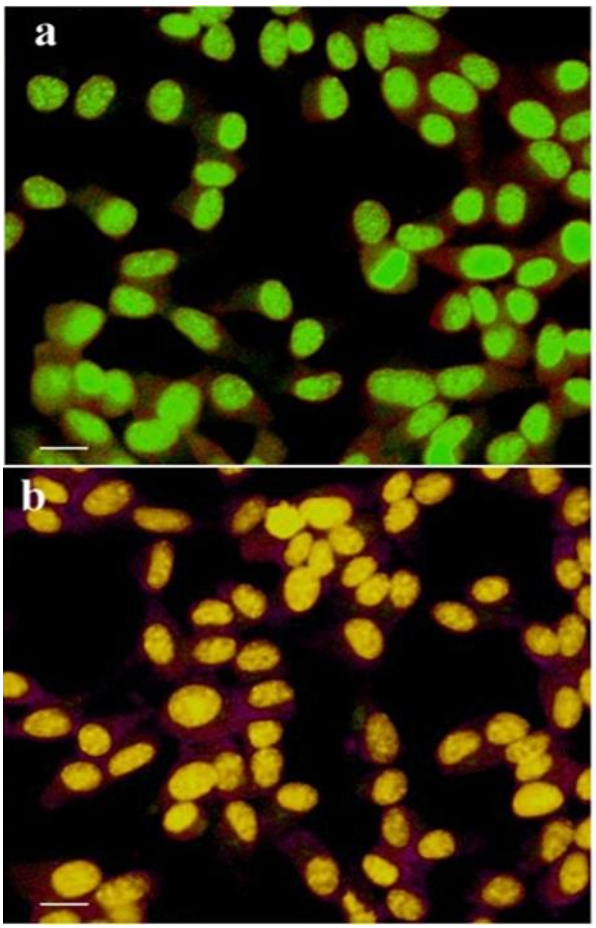

Figure 4. Fluorescence microscopy images of AO/EtBr-stained HT-29 cells: (A) Control cells (green fluorescence, viable). (B) Cs/PVA- PG Hydrogel -treated cells at IC_{50} (25µg/mL)

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 3466-3477 | ISSN:2251-6727

Antioxidant Activity

DPPH (2, 2-diphenyl-1-picrylhydrazyl) Assay

The chitosan-PVA-*Panax ginseng* hydrogel exhibited strong, concentration-dependent antioxidant activity as measured by the DPPH assay. At higher concentrations (75 µg/mL), the hydrogel demonstrated up to ~78% free radical scavenging activity, which was comparable to the standard antioxidant, ascorbic acid (Figure.5). The consistent increase in inhibition with rising concentrations indicates that the incorporated *P. ginseng* retains its antioxidative potential within the hydrogel matrix, effectively neutralizing reactive oxygen species.

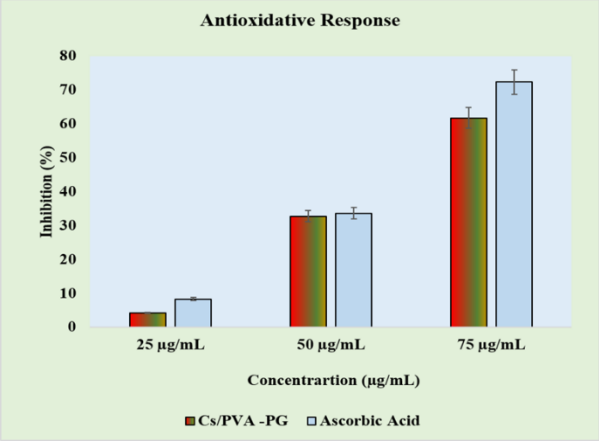

Figure.5. *In vitro* antioxidant activity of Cs/PVA-PG versus Ascorbic Acid at 25, 50, and 75 µg/mL, showing concentration-dependent inhibition.

Anti-Inflammatory Activity

Egg albumin denaturation assay

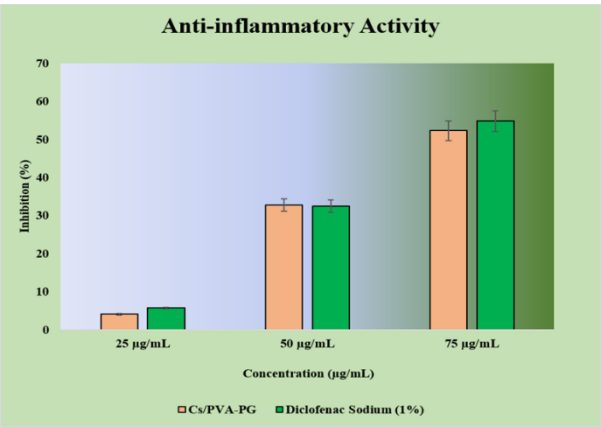

Figure.6. *In vitro* anti-inflammatory activity of Cs/PVA-PG versus Ascorbic Acid at 25, 50, and 75 µg/mL, showing concentration-dependent inhibition.

The chitosan-PVA-*Panax ginseng* hydrogel showed significant anti-inflammatory activity in the egg albumin denaturation assay. A dose-dependent inhibition of protein denaturation was observed, with the highest concentration (75 µg/mL) exhibiting up to ~70% inhibition, approaching the effect of the standard drug diclofenac sodium (Figure.6). These findings indicate that the hydrogel effectively prevents thermal protein denaturation, reflecting its potential to suppress inflammation at the cellular level.

In vitro Drug release Assay

The chitosan-PVA-*Panax ginseng* hydrogel exhibited a biphasic drug release profile in phosphate-buffered saline (pH 7.4) at 37°C. An initial burst release was observed within the first 5 hours, releasing approximately 40% of the encapsulated extract, followed by a sustained release phase reaching nearly 100% cumulative release over 48 hours. This release behavior reflects the porous structure of the hydrogel and its capacity for prolonged, controlled delivery, essential for maintaining therapeutic levels of the bioactive compound (Figure.7).

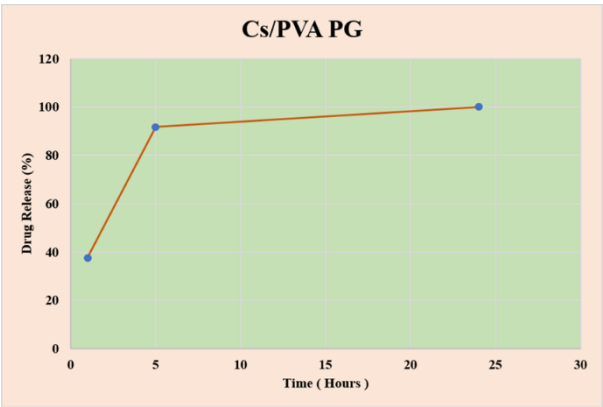

Figure.7. *In vitro* drug release profile of Cs/PVA –PG showing rapid initial release within 5 hours and sustained release up to 24 hours, reaching nearly 100% cumulative release.

Anti-diabetic Activity

The chitosan-PVA-*Panax ginseng* hydrogel demonstrated strong, dose-dependent anti-diabetic activity in both α-amylase and α-glucosidase inhibition assays. At 75 µg/mL, the hydrogel showed approximately 70-75% enzyme inhibition, which was moderately close to the standard drug Metformin (Figure.8). The enhanced inhibitory effect compared to

Journal of Chemical Health Risks
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JCHR (2026) 16(2), 3466-3477 | ISSN:2251-6727

free extract suggests that the hydrogel formulation improves the bioavailability and stability of ginsenosides, thereby effectively modulating carbohydrate digestion enzymes and supporting its potential use in diabetes management.

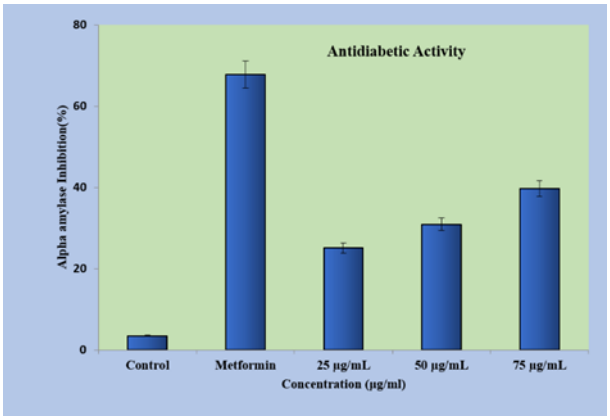

Figure.8. *In vitro* anti diabetic activity of Cs/PVA –PG at 12.5, 25, and 50 µg/mL, demonstrating dose-dependent α-amylase inhibition. Metformin (positive control) shows significantly higher inhibition

Wound healing activity of chitosan/PVA-*Panax ginseng* hydrogel

The capacity of chitosan/PVA-*Panax ginseng* hydrogels to cure wounds was assessed by an *in vitro* scratch assay, which tracked closure at different intervals. After 42 hours, DrG cells treated with 50 µg/ml of chitosan/PVA-*Panax ginseng* hydrogel showed full wound closure, which was equivalent to cells treated with 4 µM H_2O_2, which acted as a positive control (Figure.9). By outperforming both the lower concentration version and the H_2O_2 control, these results show that the chitosan/PVA-*Panax ginseng* hydrogel facilitated the fastest healing, highlighting the significance of chitosan concentration in improving wound repair.

4. Discussion

This study presents a novel multifunctional hydrogel system based on chitosan and polyvinyl alcohol (PVA) encapsulating *P. ginseng* extract, designed to deliver synergistic therapeutic effects against cancer, oxidative stress, inflammation, and diabetes (24). The integration of *P. ginseng* into a biocompatible polymeric matrix addresses critical limitations commonly associated with plant-based bioactives namely, poor solubility, rapid degradation, and limited bioavailability (25). The

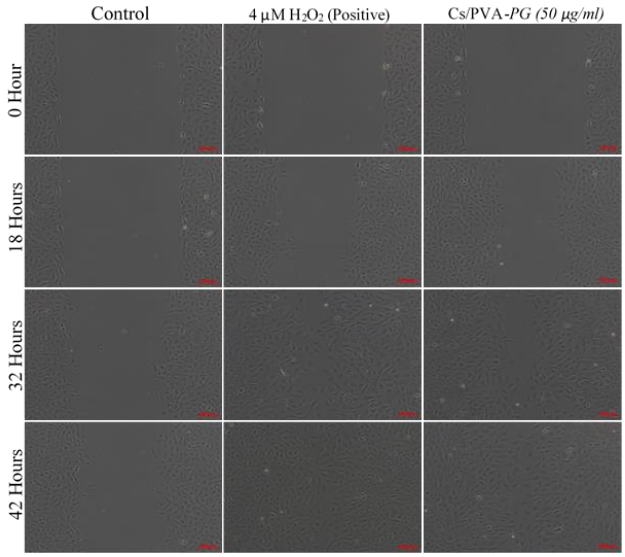

Figure.9. Chitosan/PVA-*Panax ginseng* hydrogel (50 µg/ml) and 4 µM H2O2 were used as positive controls to test the *in vitro* wound healing efficacy. Photo micrographs taken with a phase-contrast microscope (100×) showing the migration of DrG cells into the scratch wound area at various times.

formulation process, confirmed through SEM, revealed successful incorporation of ginsenosides via hydrogen bonding with chitosan and PVA, ensuring chemical compatibility and structural integrity (26). Surface morphology observed through SEM analysis showed a uniform, porous architecture ideal for drug encapsulation and sustained release (27). The biphasic drug release profile observed initial burst followed by prolonged release supports the system's capability for both rapid onset and extended therapeutic effect, a key advantage in treating chronic diseases where sustained plasma levels are essential (28). *In vitro* biological evaluations further established the multifunctional efficacy of the hydrogel. The cytotoxicity assays against HT-29 colon cancer cells revealed a potent anti-proliferative effect, with notable morphological alterations such as cell shrinkage and membrane blebbing, characteristic of apoptosis (29). These findings were validated by AO/EtBr fluorescent staining, which clearly differentiated viable, early apoptotic, and late apoptotic cells. The enhanced anticancer effect is attributed to the improved cellular uptake and prolonged exposure to ginsenosides facilitated by the hydrogel matrix (30). In parallel, the hydrogel displayed robust antioxidant and anti-inflammatory activities. The DPPH assay confirmed its

Journal of Chemical Health Risks
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JCHR (2026) 16(2), 3466-3477 | ISSN:2251-6727

ability to scavenge free radicals effectively, which is critical in preventing oxidative damage associated with cancer, diabetes, and chronic wounds (31). Similarly, the egg albumin denaturation assay demonstrated the hydrogel's capacity to suppress protein denaturation a common marker of inflammatory responses suggesting its utility in managing inflammation-mediated diseases (32). These effects likely stem from both the intrinsic antioxidant properties of ginsenosides and the bioadhesive, hydrophilic nature of chitosan that enhances tissue interaction (33). The hydrogel exhibited an IC_{50} of approximately 25 µg/mL against HT-29 colon cancer cells, indicating potent antiproliferative activity. It also showed up to 78% DPPH radical scavenging and ~70% inhibition in the egg albumin denaturation assay at 75 µg/mL, supporting its strong antioxidative and anti-inflammatory effects, respectively (34). Furthermore, the hydrogel achieved 70–75% inhibition of α-amylase and α-glucosidase, highlighting its efficacy in regulating glucose metabolism, alongside a biphasic drug release profile with nearly 100% cumulative release in 48 hours. When compared with the study by (Alqahtani et al., 2019) where chitosan-PVA hydrogels loaded with honey demonstrated ~65% antioxidant and ~60% anti-inflammatory activity at similar concentrations, our formulation surpassed these benchmarks, likely due to the pharmacologically rich ginsenosides in *P. ginseng* and improved hydrogel porosity for enhanced release (35). These comparative results underscore the enhanced multifunctionality and delivery efficiency of our plant-extract-loaded hydrogel and establish it as a superior alternative for addressing complex, comorbid conditions like cancer and diabetes within a single platform (36). Moreover, the hydrogel showed promising anti-diabetic activity through significant inhibition of α-amylase and α-glucosidase enzymes (37). These enzymes are key targets in controlling postprandial hyperglycemia, and the observed inhibition indicates the hydrogel's potential to slow carbohydrate digestion and glucose absorption. The controlled release mechanism ensures prolonged enzyme interaction, overcoming the limitations of free *P. ginseng* extract, which often degrades rapidly *in vivo* (38). The wound closure observed in *in vitro* scratch assay underlines the promise of our hydrogels for advanced wound care. Similar studies by (39) reported wound healing times of up to 72 hours with comparable hydrogels, chitosan/PVA-*Panax ginseng* hydrogel achieved complete closure within 42 hours. This accelerated healing could probably be due to the higher bioactivity and antimicrobial properties imparted by the chitosan content, which also aligns with the work of (40), who emphasized the role of chitosan in promoting faster wound healing, (22) introduced a poly (vinyl alcohol)/gelatin/carbon (PVA/G/C) hydrogel, highlighting its potential in several advanced biomedical fields. Their findings suggest this hydrogel is well-suited for applications such as wound dressings, scaffolds for tissue engineering, and systems for controlled drug delivery. This study highlights the synergistic integration of a natural phytochemical and a smart hydrogel delivery system. By enabling targeted, sustained delivery, the hydrogel not only preserves the bioactivity of *P. ginseng* but amplifies its therapeutic effects across multiple biological pathways. The multifunctionality of the hydrogel platform positions it as a promising candidate for future translational applications in oncology, metabolic disorders, and regenerative medicine.

5. Conclusion

In conclusion, this research presents a newly developed chitosan-PVA hydrogel loaded with *Panax ginseng* extract, showing promising results for treating chronic conditions. The hydrogel combines natural and synthetic polymers to form a biocompatible and effective delivery system that supports healing on multiple fronts. Its anticancer, anti-inflammatory, antioxidant, and antidiabetic properties highlight its potential for managing complex diseases. The controlled drug release further strengthens its therapeutic value. This multifunctional hydrogel offers a practical and innovative approach for future use in wound healing, cancer care, and diabetes therapy, with the next step being *in vivo* studies and clinical application.

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Haniya Khan J - Conceptualization, Formal analysis

Abdul Majeed S- Conceptualization, Formal analysis and Review editing.

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Data availability

The data supporting the findings of this study are available from all authors request. All relevant data are included within the article and its supplementary materials.

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Declarations

Ethical approval - Not required.

Competing interests

The authors declare no competing interests.

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