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Original article

Development and evaluation of sildenafil/glycyrrhizin-loaded nanofibers as a potential novel buccal delivery system for erectile dysfunction

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ABSTRACT

Erectile dysfunction (ED) is a growing health condition that needs safe and effective therapy. One of the main common treatments is sildenafil which is used in clinics for managing erectile dysfunction by enhancing the blood supply to the penis. In the current study, sildenafil was formulated as nanofibers and mixed with the root extract of *Glycyrrhiza glabra* (glycyrrhizin) as a natural sweetener to be administrated in the buccal cavity for enhanced drug bioavailability, rapid drug absorption and improved patient compliance. The formulated dual-loaded nanofibers were evaluated by measuring diameter, disintegration, drug loading efficiency, drug release profile, and *in vitro* cell viability assessment. The results showed that the sildenafil/glycyrrhizin-loaded fibers had a diameter of 0.719 ± 0.177 μm and lacked any beads and pores formation on their surfaces. The drug loading and encapsulation efficiency for sildenafil were measured as 52 ± 7 μg/mg and 67 ± 9 %, respectively, while they were 290 ± 32 μg/mg and 94 ± 10 %, respectively, for glycyrrhizin. The release rate of sildenafil and glycyrrhizin demonstrated a burst release in the first minute, followed by a gradual increment until a complete release after 120 min. The *in vitro* cell viability evaluation exhibited that the application of sildenafil and glycyrrhizin is safe upon 24-hour treatment on human skin fibroblast cells at all used concentrations (i.e., ≤ 1,000 and 4,000 μg/mL, respectively). However, the application of sildenafil-glycyrrhizin combination (in a ratio of 1:4) demonstrated more than 80 % cell viability at concentrations of ≤ 250 and 1000 μg/mL, respectively, following 24-hour cell exposure. Therefore, sildenafil/glycyrrhizin dual-loaded PVP nanofibers showed a potential buccal therapeutic approach for erectile dysfunction management.

1. Introduction

Sildenafil is a phosphodiesterase type 5 (PDE5) that is commonly used to manage erectile dysfunction (ED) (Dilworth et al., 2013, Krishnappa et al., 2019). The global incidence of ED ranged from 3 % to 75 %, progression with a geriatric age (Kessler et al., 2019). This drug is reported to prevent the degradation of cyclic guanosine monophosphate (cGMP), which causes smooth muscle relaxation (Blount et al., 2004, Andersson, 2018). Some studies showed that sildenafil could be an intrauterine growth restriction (IUGR) treatment, which has been listed in the pregnancy data like gestational age (GA) at birth, birth weight, neonatal death, and stillbirth (El-Sayed et al., 2018, El-Shalakany et al., 2018). Sildenafil was approved in March 1998 by the FDA and in September 1998 by the European Medicines Agency (Dinsmore et al., 1999, Montorsi et al., 1999).

Sildenafil citrate, the active pharmaceutical ingredient in medications like Viagra®, exhibits variable solubility dependent on factors such as temperature and pH of the solvent. At room temperature, its solubility in water is moderately at around 4.1 ± 1.3 mg/mL, but slightly increases with higher temperatures (Pirhayati et al., 2017). Aldawsari *et al* have prepared solid dispersions of sildenafil using hydrophilic polymers such as Kollidon® 30, Kolliphor® P188, and Kollidon and demonstrated

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enhanced dissolution rate, and significant improvement in intercopulatory efficiency and copulatory efficiency in male rat models (Aldawsari et al., 2021). A previous study also explored the spray drying technology to prepare amorphous solid dispersion of tadalafil, another PDE5 completive inhibitor, using Kollidon® VA64, Koliphore 188, and Kollidon® 30 polymers to improve the ED (Ahmed et al., 2022).

Sildenafil was studied for its safety and effectiveness on more than 14,000 patients (Salonia et al., 2003, Laties and Sharlip, 2006), and data demonstrated that the dose of 50 or 100 mg was well tolerated and had rate good safety profile (Giuliano et al., 2010). Dosing varies based on patient needs and responses, typically taken before sexual activity for ED. However, its therapeutic benefits are accompanied by contraindications, potential side effects, and drug interactions, emphasizing the importance of informed medical guidance and adherence to prescribed dosages for safe and effective usage. It should not be used by individuals with a known hypersensitivity to the drug or its components and should never be combined with nitrates or nitric oxide donors due to the serious risk of severe hypotension as both drugs can independently promote vasodilation. Individuals with severe heart conditions, prior strokes or heart attacks should be cautious. Typical side effects like facial flushing, indigestion, nasal congestion, dizziness, headaches, and blurred vision are usually mild and transient. It is important to recognize the potential for rare yet serious side effects like priapism, sudden hearing loss, and vision changes, which require immediate medical intervention.

The use of natural excipients in the preparation of pharmaceutical dosage forms such as nanofibers has the advantages of lower cost, good stability and high compatibility (Chintamaneni et al., 2023). Glycyrrhizin, which is the root extract of *Glycyrrhiza glabra*, of the *Leguminosae* (*Fabaceae*) family, has been utilized as a natural sweetener and has commonly been applied by the food industry and herbal medicine (Wahab et al., 2021). It has a long history of use, dating back to ancient China, Egypt, and Japan. As an amphiphilic molecule, glycyrrhizin consists of a hydrophilic glucuronic acid residue and a hydrophobic glycyrrhizic acid residue (Bakr et al., 2022).

Glycyrrhizin has been also demonstrated to be a promising medication for curing several diseases such as viral infections (Fomenko et al., 2022). For instance, it can be effective against SARS-associated coronavirus by inhibiting the replication of SARS-CV and also preventing the cellular uptake of the virus (Cinatl et al., 2003). Moreover, it can be indicated from the mechanism of action of glycyrrhizin that it has activity against SARS-CoV-2 (COVID-19) by targeting a binding site of the SARS-CoV-2, angiotensin-converting enzyme II (ACE2) receptor, therefore inhibits the cellular uptake of SARS-CoV-2 (Luo et al., 2020). Also, glycyrrhizin can be a potent anti-inflammatory medication due to the ability of glycyrrhizin to eliminate reactive oxygen species (ROS) which is known to have many negative influences in regenerative diseases such as cancer (Račková et al., 2007).

Glycyrrhizin can be effective in drug delivery applications by enhancing the therapeutic activity of other drugs (Piao et al., 2022). Its amphiphilic properties enable it to form self-associates like dimers and micelles and bind to hydrophobic drugs by supramolecular complexes (Sun et al., 2023). This process significantly increases the solubility, stability, and bioavailability of these drugs, making glycyrrhizin a valuable tool in pharmaceutical formulations (Kim et al., 2021). The complexation of glycyrrhizin with nifedipine resulted in a remarkable antihypertensive therapeutic effect, allowing for a ten-fold reduction in the dosage of nifedipine (Selyutina and Polyakov, 2019). Additionally, a previous study has reported an enhanced drug solubility and bioavailability of tadalafil by using glycyrrhizin as a drug carrier (Muqtader Ahmed et al., 2020).

Recently, one of the reliable and functional emerging techniques that can be employed for formulating fibers in the submicron range is electrospinning. This technique works by using a high electric voltage through a metallic needle that ejects a steamed jet of the polymeric solution to produce a continuous stream of fibers (Subbiah et al., 2005, Agarwal et al., 2008). Electrospinning technology has attained much attention in biomedical research because of its flexibility, simplicity and biocompatibility, and currently employed intensively in the field of drug delivery (Agarwal et al., 2008, Rostamabadi et al., 2020).

Electrospinning has transitioned from single-fluid systems to more intricate multi-fluid processes such as coaxial, tri-axial, and side-by-side electrospinning. These techniques enable the production of complex nanostructures, including core–shell, Janus fibers, and other multifunctional systems, which can be tailored for specific drug delivery applications. For instance, coaxial electrospinning can produce fibers with a core–shell structure that allows the encapsulation of active ingredients within a protective sheath, leading to controlled release and enhanced stability of the encapsulated drugs. This technique has been explored for various pharmaceutical applications, showing significant potential in improving drug dissolution rates and therapeutic efficacy (Huang et al., 2022). Tri-axial electrospinning introduces an additional layer or component, offering even more control over the release characteristics and enabling the combination of multiple active pharmaceutical ingredients within a single fiber. Such advancements have been demonstrated to effectively create fibers with tailored properties for specific therapeutic needs (Wang et al., 2020a). Side-by-side electrospinning allows for the fabrication of Janus fibers, which have distinct regions with different chemical or physical properties, facilitating dual drug loading and release profiles. This innovative approach can be particularly useful for combination therapies where the simultaneous delivery of two drugs with synergistic effects is desired (Yu and Zhou, 2024).

Polyvinylpyrrolidone (PVP) is one of the biocompatible polymers that is commonly used in the formation of nanofibers through electrospinning technology (Chen et al., 2022, Tan et al., 2022). This polymer offers several advantages in pharmaceutical dosage form production that make electrospun fibers good candidates for drug delivery applications. For instance, PVP can enhance the controlled release properties of the loaded drug, a structured drug release mechanism, and high encapsulation efficiency of the drug (Subbiah et al., 2005, Rostamabadi et al., 2020). Also, PVP has a mucoadhesive property when it is prepared by electrospinning (Surendranath et al., 2023). Therefore, PVP attracted much attention for the fabrication of drug-loaded fibers owing to its biocompatibility, biodegradability and drug-loading efficiency.

Many mucoadhesive pharmaceutical formulations that can dissolve quickly have been formulated for oral application and are commercially available, such as transdermal patches, tablets, gels, ointments and buccal patches (Arya et al., 2010, Joshua et al., 2016). However, these oral nanofilm dosage forms have a limitation of not having uniform film thickness which can interfere with the mechanical properties and the rate of the drug release (Tawfik et al., 2021). These limitations of the thin fiber could be eliminated by smart buccal delivery systems (Feitosa et al., 2019, Rohani Shirvan et al., 2019).

The fabrication of sildenafil drugs in a nanofiber dosage form can be exploited in the buccal delivery approach. There are several advantages of the buccal route including the ease of administration, faster absorbance rate of the drug and also it can overcome first-pass metabolism via the liver and low GI absorbance (Arya et al., 2010, Joshua et al., 2016). Optimizing the formulation of the smart buccal nanofibers by using advanced material will give optimal absorption and successful drug delivery (Feitosa et al., 2019, Rohani Shirvan et al., 2019). Using electrospun nanofibers as smart buccal deliveries has many advantages such as ultra-rapid disintegration, unique mechanical properties and fast release rates of the drug ($\leq$2 s) (Alkahtani et al., 2021).

Therefore, the buccal delivery of sildenafil/glycyrrhizin-loaded nanofibers presents a novel approach that could potentially bypass the first-pass metabolism, enhancing bioavailability, providing rapid drug absorption and a patient-friendly therapeutic approach for ED.

2. Materials and methods

2.1. Materials

PVP polymer with a molecular weight of (~1,300,000), HPLC grade dimethylformamide (DMF), ethanol (≥99.5 %), acetonitrile, phosphate buffer saline (PBS) tablets, and chloroform were purchased from Sigma-Aldrich (St. Louis, MO, USA).). The preparation of the PBS solution was performed by dissolving 5 tablets of PBS in 1L of distilled water and then adjusting the pH to 6.8 using 5 M HCl. Sildenafil citrate (CAS No. 139755–83-2), which will be referred to as sildenafil throughout the manuscript, was gifted from Jazeera Pharmaceutical Industry (JPI; Riyadh, Saudi Arabia),. Glycyrrhizin was bought from Azur Chemical and Pharmaceutical, (Hyderabad, India), while triethanolamine was obtained from Loba Chemie (Mumbai, India). Milli Q Millipore (Billerica, MA, USA) was used to generate distilled water.

2.2. Preparation of sildenafil- glycyrrhizin loaded PVP nanofibers

In this study, the Spraybase® electrospinning system (Dublin, Ireland), was utilized to fabricate PVP nanofibers loaded with sildenafil and glycyrrhizin, employing a modified electrospinning procedure (Alshaya et al., 2022). The preparation of the PVP polymer solution was performed by dissolving PVP powder in ethanol at 8 % (w/v) and stirring for 2 h until the complete dissolving of the PVP polymer. 4 % (w/v) of glycyrrhizin was added and kept under stirring for an extra hour. Subsequently, 1 % (w/v) of sildenafil was mixed with the PVP/glycyrrhizin solution to obtain a ratio of 8:4:1, representing the polymer, the sweetener and the drug, respectively. This mixture was further stirred for an hour to ensure uniform mixing of sildenafil with glycyrrhizin and PVP. PVP solution alone 8 % (w/v) was used as experimental control (i.e., blank). Electrospinning of the nanofibers was carried out under conditions of 30–45 % relative humidity and room temperature. The rate of flow of the electrospinning syringe pump was 800 μL/hour, while the needle and collector distance was kept at 15 cm. The inner needle diameter was set at 0.55 mm. A voltage of 8–9 kV was applied to establish a stable jet and to collect the fabricated nanofibers, the metallic collector's surface was coated with aluminum foil.

2.3. Diameter and morphology assessment of the electrospun nanofibers

Scanning electron microscopy (SEM) apparatus (JSM-IT500HR SEM, JEOL Inc., Peabody, MA, USA) was utilized for the assessment of surface structure and the morphology of both the drug-free and dual drug-loaded nanofibers, while ImageJ software (National Institute of Health, Bethesda, MD, USA) was used to analyze the diameter of the nanofibers. For each fibrous system, approximately 80 fibers were examined, and an accelerated voltage of 5 kV was applied. Aluminum foil was used to collect the fabricated nanofibers then the fabricated nanofibers were coated with a 2 nm layer of platinum using the JEC-3000FC auto fine coater.

2.4. Analysis of Fourier-Transform Infrared Spectroscopy (FTIR)

The Thermo smart ATR IS20 Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) was used for the analysis of the Fourier-Transform Infrared Spectroscopy (FTIR). The spectral resolution was configured at 4 cm^{-1}, and 32 scans were conducted for each sample, which was analyzed at a specific wavenumber range of 4000 to 600 cm^{-1}. The FTIR assessment included sildenafil, PVP, glycyrrhizin the physical mixture (PM, containing the same proportions of the raw materials used in the dual drug-loaded fibers), and the blank and dual drug-loaded fibers. Samples were placed on the sampling spot one sample at a time in small quantities (around 5 mg). The FTIR results were plotted utilizing the OriginPro® 2021 software (OriginLab Corporation, Northampton, MA, USA). The chemical structures for sildenafil and glycyrrhizin are presented in the Supplementary Materials Section – Figure S1.

2.5. Analysis of X-ray diffraction (XRD)

The Rigaku Miniflex 300/600 instrument (Tokyo, Japan) was used for the analysis of X-ray diffraction (XRD). This instrument was equipped with Cu Kα radiation which was 1.5148 227 Å. The operational voltage was 40 kV, whereas the applied current was 15 mA. The XRD examination encompassed sildenafil, PVP, glycyrrhizin the physical mixture (PM, containing the same proportions of the raw materials used in the dual drug-loaded fibers), and the dual drug-loaded and blank fibers. Nanofiber samples were positioned on glass holders and subjected to scanning within the 2θ range of 2° to 50°. The speed of scanning is set at 5°/min. The XRD outcomes were graphed utilizing the OriginPro® 2021 software (OriginLab Corporation, Northampton, MA, USA).

2.6. Disintegration test of electrospun nanofibers

The disintegration of dual drug-loaded and blank nanofibers was evaluated in a petri dish following a modified method (Alshaya et al., 2022). The experiment was performed using a shaking incubator (Excella E24 Incubator Shaker Series, New Brunswick Scientific Co., Enfield, CT, USA) and square portions of the nanofiber (3 mg) were positioned inside a petri dish containing pre-warmed PBS (37 °C) with the pH adjusted to 6.8, which represent the human oral cavity. The complete fibrous mat's detachment was recorded and measured in different time intervals. Results were determined as the average ± standard deviation (SD) of independent triplicates.

2.7. Quantification of sildenafil and glycyrrhizin using High-Performance liquid chromatography (HPLC)

Waters e2695 HPLC system that contains a Waters 600 binary pump, Waters® 717 plus autosampler, and Waters 2489 UV/detector (Waters Technologies Corporation, Milford, MA, USA). In addition, the Xselect HSS cyano column (250 mm × 4.6 mm, 5 μm) was used for the quantification of both sildenafil and glycyrrhizin. A mobile phase consisting of triethanolamine (1 %, pH was adjusted to 3.9 by formic acid), acetonitrile, and water at a gradient elution starting from 60 %, 40 % and 0 %, respectively, from 0 to 2 min, followed by 0 %, 40 %, 60 %, respectively, from 2 to 5 min and finally, 60 %, 40 % and 0 %, respectively, from 0 to 2 min was used for the separation of the loaded materials. The flow rate was set as 1.5 mL/min, the temperature was adjusted at 21 °C, the volume of injection was 10 μL, and the detection wavelength was 254 nm. Both sildenafil and glycyrrhizin were dissolved in 85 % (v/v) PBS and 15 % (v/v) ethanol to prepare the stock solution. The calibration curve was determined by using the above method in which the serial dilution was used in the range between 144 and 0.5 μg/mL. The retention times (Rt) of glycyrrhizin and sildenafil were determined at 2.3 and 6.2 min, respectively, as shown in the Supplementary Materials Section – Figure S2. The data analysis and the calibration curve were plotted by OriginPro® 2021 software (OriginLab Corporation, Northampton, MA, USA).

2.8. Quantification of sildenafil and glycyrrhizin drug loading (DL), encapsulation efficiency (EE%) and fiber's yield (Y)

For the quantification of the DL and EE% of sildenafil/glycyrrhizin-loaded nanofibers, at least 3 portions of the nanofibers (4.1 ± 0.1 mg) were dissolved in 20 mL of 85 % (v/v) PBS (pH 6.8) and 15 % (v/v) ethanol, and each solution was left for 7 h at room temperature to achieve full dissolution of the tested nanofibers. The HPLC-developed method of detection was used to quantify the concentration and amount of loaded materials, and the following equation was applied to quantify the EE% and DL:

$$EE\% = \frac{Actualentrappeddrugamount}{Theoreticalentrappeddrugamount} \times 100 \quad (1)$$

$$DL = \frac{Entrappeddrugamount}{Actualnanofibersamount} \quad (2)$$

The Y of the dual drug-loaded fibers were calculated using the following equation:

$$Y\% = \frac{Actualnanofibersamount}{Theoreticalnanofibersamount} \times 100 \quad (3)$$

The theoretical nanofibers' amount was quantified based on the solid materials amount presented in the spinning solution. The results are determined as the average ± SD of independent triplicates.

2.9. Quantification of sildenafil-glycyrrhizin drug release

For the determination of the release profile of sildenafil/glycyrrhizin-loaded nanofibers, at least three portions of the fibrous mat (4 ± 0.1 mg) were dissolved in a pre-warmed solvent mixture (20 mL) that consists of 85 % (v/v) PBS (pH 6.8) and 15 % (v/v) ethanol contained in glass vials. Sildenafil and glycyrrhizin release profile from the fabricated nanofibers was identified by using the shaking incubator (Excella E24 Incubator Shaker Series, New Brunswick Scientific Co., Enfield, CT, USA). The shaking rate was adjusted to 100 RPM and the temperature was optimized to be 37 °C. One mL of the prepared solution was collected at specified time points that range from 1 to 180 min, and replaced with an equal volume of the solvent mix. Sildenafil and glycyrrhizin amount was determined using the HPLC-developed method. The percentage of cumulative release was calculated as a function of time by using the following equation:

$$Cumulative\ Release\ \% = \frac{Cumulative\ drug\ amount}{Theoretical\ drug\ amount} \times 100 \quad (4)$$

The results are presented as the average ± SD of independent triplicates. The data analysis and the plot of the release profile of sildenafil and glycyrrhizin were determined by OriginPro® 2021 software (OriginLab Corporation, Northampton, MA, USA).

2.10. In vitro evaluation of sildenafil and glycyrrhizin cell viability

The assessment of *in vitro* cellular viability of sildenafil and glycyrrhizin was achieved through the colorimetric MTS assay (cell Titer 96® Aqueous one solution cell proliferation assay, Promega, Southampton, UK) following a 24-hour cell exposure to the human fibroblast HFF-1 cells (ATCC-SCRC-1401). This is an important step towards the determination of the optimum concentration of applied sildenafil, glycyrrhizin and the combination that is performing a therapeutic effect on living tissue while being safe on the treated cells. The evaluation of the metabolic activity of sildenafil and glycyrrhizin was conducted by applying the modified method (Alamer et al., 2023). HFF-1 cells were normally sub-cultured in Dulbecco's modified eagle medium (DMEM), containing 10 % FBS (v/v), 100 µg/mL of streptomycin, and 100 U/mL of penicillin.

Following the confluency of the cells, trypsin solution was applied and incubated for several minutes, and then the trypan blue exclusion test was used after HFF-1 cells detachment for counting cells. The collected cells were then seeded into 96-well plates with a seeding density of 1.5×10^4 cells per well. The seeded cells were then incubated overnight in a cell culture incubator at 37 °C and 5 % CO_2. A 100 µL of a serial dilution of sildenafil (from 1000 to 8 µg/mL), glycyrrhizin (from 4000 to 32 µg/mL), and the combination of sildenafil to glycyrrhizin with a ratio of 1:4 was added to the HFF-1 cells for 24 h. The negative control was cells incubated with only DMEM, while the positive control was cells incubated with 0.2 % Triton X-100. Aspiration of medium from each well was applied and then each well was washed gently with PBS. Next, fresh DMEM (100 µL) was added to the wells, and then MTS reagent (20 µL) was added. Thereafter, cells were incubated for 3 h in the incubator. The absorbance of formazan color produced from the living cells was measured at 492 nm by a microplate reader (Cytation 3, BIOTEK Instruments Inc., Winooski, VT, USA). The percentage of the viability of the cells was determined by the following equation (Aburayan et al., 2022):

$$Cell Viability\ \% = \frac{(S - T)}{(H - T)} \times 100 \quad (5)$$

S is the absorbance of the cells that are treated with tested samples, T is the absorbance of positive control, and H is the absorbance of negative control. The results were shown as the average ± SD of independent triplicate measurements.

3. Results

3.1. Diameter and morphology assessment of the electrospun nanofibers

The SEM images revealed that the surface of fabricated nanofibers was smooth and lacked any beads and pores, as presented in Fig. 1a and 1b. The diameter measurement exhibited average nanofiber diameters of 0.597 ± 0.205 µm for blank fibers and 0.719 ± 0.177 µm for the dual drug-loaded fibers (Fig. 1).

3.2. Analysis of (FTIR)

The FTIR results in Fig. 2 demonstrated the distinctive peaks for sildenafil at 3298 cm^{-1} (NH symmetric and asymmetric stretching), 1580 cm^{-1} (symmetric stretching frequency of COOH groups of the citrate-ion), 1361 cm^{-1} (asymmetric SO_2), and 1171.5 cm^{-1} (symmetric SO_2) with multiple sharp peaks between 1107 and 619 cm^{-1}. At the region of 1700 cm^{-1}, there is an asymmetric stretching frequency of the COOH group that might overlap with the isolated carbonyl (C—O) group band. Glycyrrhizin FTIR spectrum exhibited a broad stretching between 3500 and 3000 cm^{-1} suggesting the free OH groups. Other characteristic peaks of glycyrrhizin were shown at 1663 cm^{-1} (C = O), 1395 cm^{-1} (CH3), and 1035 cm^{-1} (secondary cyclic alcohols). The PVP IR spectrum exhibited a stretching band at 1646.9 cm^{-1} (C = O), 1421 cm^{-1} which represents the CH deformation modes from the CH2 group and 1577–1455 cm^{-1}, which indicates the C–H deformations. Two other peaks at 1278.5 cm^{-1} and 956 cm^{-1} attributed to the C–N stretching. It is noted that amines and hydroxyl bands between 3500 and 3400 cm^{-1} were not observed. The PM FTIR peaks demonstrated a mix of the distinctive peaks of the three raw materials (PVP, glycyrrhizin and sildenafil), particularly at 1667 cm^{-1}, 1490 cm^{-1}, 1361 cm^{-1}, 1030 cm^{-1}, 804 cm^{-1}, 736 cm^{-1}, which were also observed in the dual drug-loaded fibers, unlike the blank fibers that had a similar spectrum to the raw PVP polymer.

3.3. Analysis of X-ray diffraction (XRD)

Fig. 3 exhibits intense reflections of sildenafil at 2θ: 7.43°, 8.13°, 10.36°, 14.46°, 17.77°, 19.97°, 20.84°, 23.04°, and 28.58°, with several less intensity peaks ranging from 30.59° to 44.93°. The PVP polymer and the glycyrrhizin demonstrated broad-halo XRD patterns. The PM showed distinctive peaks at 2θ: 7.97°, 10.34°, 14.28°, 19.88°, 22.81° and 28.57°, indicating the presence of the sildenafil within the polymer mixture. The XRD pattern for the dual drug-loaded and blank nanofibers had a similar broad-halo shape to the PVP and glycyrrhizin.

3.4. Disintegration test of electrospun nanofibers

Fig. 4 shows the ultra-rapid disintegration of both nanofibrous

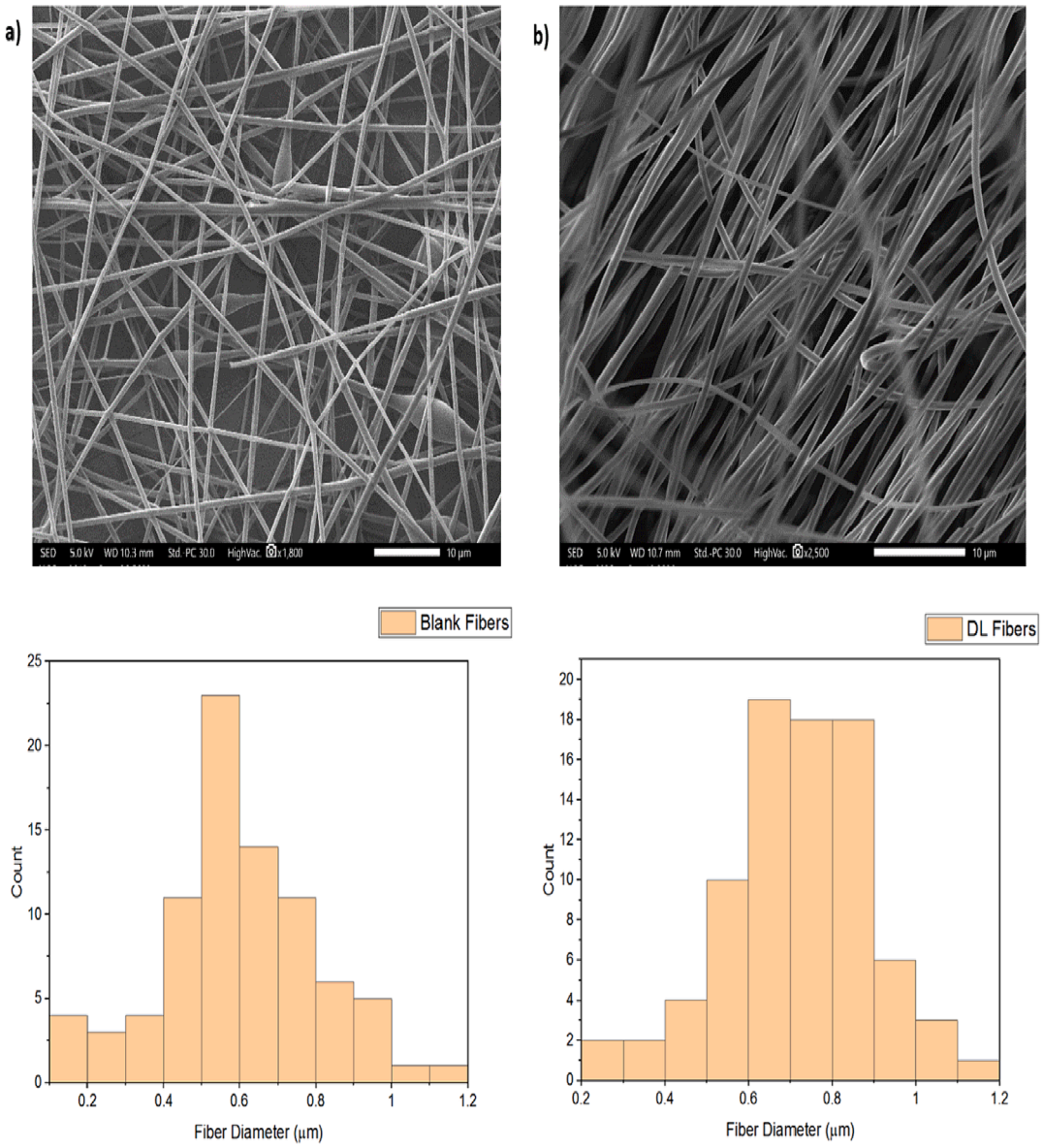

Fig. 1. The SEM images showed the lack of beads and pores of the (a) blank nanofibers and (b) dual drug-loaded nanofibers, with average fiber diameters of 0.597 ± 0.205 μm and 0.719 ± 0.177 μm, respectively (n = 80). DL, drug-loaded.

systems in PBS, which were measured as 2 ± 1 s and 4 ± 1 s for the blank and dual drug-loaded nanofibers, respectively.

3.5. Quantification of sildenafil and glycyrrhizin using high-performance liquid chromatography (HPLC)

A developed HPLC method which was described previously was implemented for the quantification of sildenafil and glycyrrhizin, where the regression equation and the coefficient of determination (R^2) were determined as y = 9365.8x-11923 (R^2 = 0.9991), and y = 4194.8x-3519.2 (R^2 = 0.9998), respectively. The calibration curve exhibited a good linearity of this developed method and its successful separation of both materials (Fig. 5).

3.6. Quantification of sildenafil and glycyrrhizin drug loading (DL), encapsulation efficiency (EE%) and fiber's yield (Y)

The previously mentioned HPLC method was used to calculate the DL and EE% of the nanofibers loaded with sildenafil and glycyrrhizin as 52 ± 7 μg/mg and 67 ± 9 %, respectively, for sildenafil and 290 ± 32 μg/mg and 94 ± 10 % for glycyrrhizin, whereas the Y of the dual drug-loaded fibers was measured as 75 ± 4 %.

3.7. Quantification of sildenafil and glycyrrhizin drug release

The developed HPLC method was also applied to measure the sildenafil and glycyrrhizin release from nanofibers, which demonstrated an ultra-rapid drug release during the 3-hour study, as shown in Fig. 6. The release rate of sildenafil and glycyrrhizin demonstrated an initial burst release in the first minute, followed by a gradual increment until a complete release after 120 min.

3.8. In vitro evaluation of sildenafil and glycyrrhizin cell viability

Increased concentrations of sildenafil alone, glycyrrhizin alone and sildenafil-glycyrrhizin combination with a 1:4 ratio against human dermal fibroblasts (HFF-1) were tested using the MTS assay following a 24-hour incubation time. The results presented in Fig. 7 demonstrated

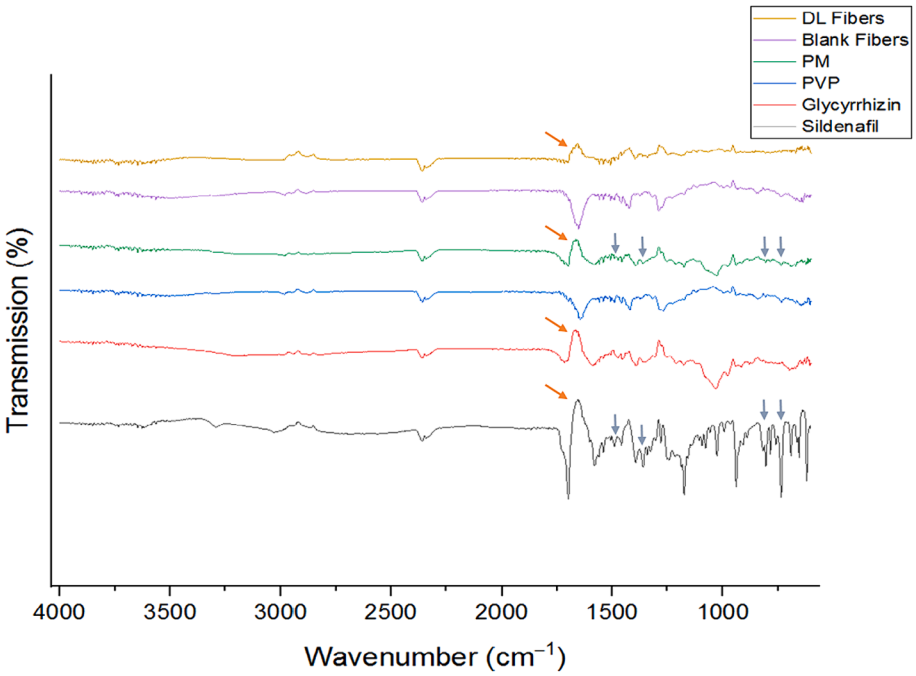

Fig. 2. FTIR spectra of sildenafil, glycyrrhizin, PVP, PM, dual drug-loaded, and blank nanofibers showed the distinctive peaks of glycyrrhizin (1667 cm^{-1} and 1030 cm^{-1}) overlapped with sildenafil in the PM and the dual drug-loaded fibers and slightly sifted, which suggest their presence in the drug-loaded fibers and their intermolecular interaction with PVP; hence, good compatibility and stability. DL, drug-loaded; PM, physical mixture.

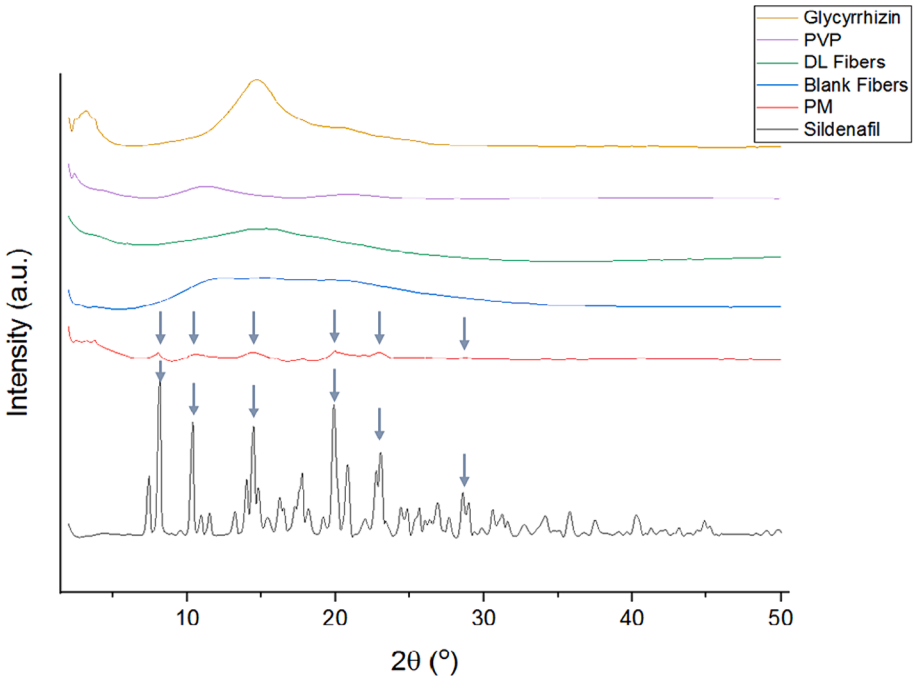

Fig. 3. XRD patterns of sildenafil, PVP, glycyrrhizin, PM, dual drug-loaded, and blank nanofibers showed that sildenafil was in the crystalline form as a pure drug and the PM. The dual drug-loaded nanofibers were in a broad halo pattern which suggests the molecular dispersion of the sildenafil compared to the XRD patterns of the raw drug and PM. DL, drug-loaded; PM, physical mixture.

that all tested concentrations of sildenafil (1,000 to 8 μg/mL) - Fig. 7a - and glycyrrhizin (4,000 to 32 μg/mL) - Fig. 7b - exhibited high metabolic activity of HFF-1 cells and high cellular viability of > 80 %, following the incubation for 24-hour.

In addition to the assessment of sildenafil and glycyrrhizin effects on HFF-1 cells, the effect of incubation of both compounds as a combination in a ratio of 1:4 of sildenafil and glycyrrhizin for 24 h was explored following the below table: See (Table 1).

Results showed that the incubation of sildenafil and glycyrrhizin combined with HFF-1 cells for 24 h demonstrated high cellular viability (> 80 %) in all applied concentrations that is ≤ 250:1000 μg/mL (i.e., concentration points A-F). However, higher concentrations of sildenafil and glycyrrhizin (i.e., G and H) exhibited lower metabolic activity, as demonstrated in Fig. 8.

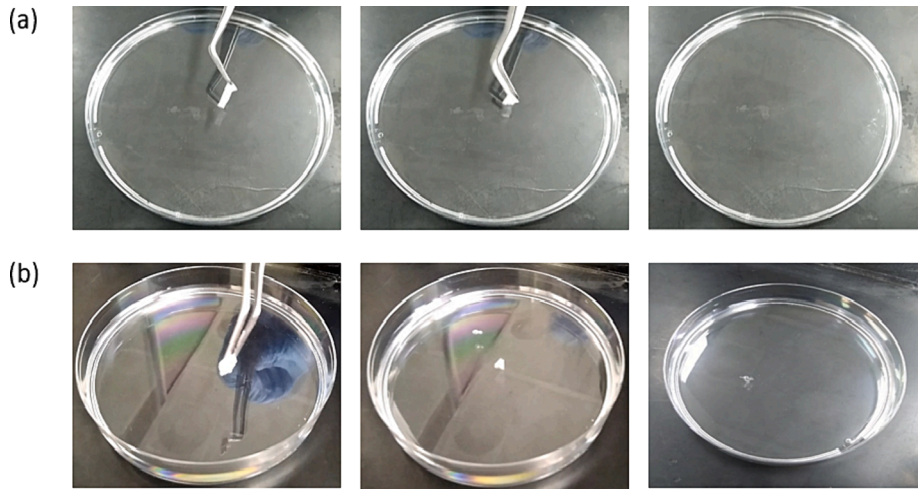

Fig. 4. The disintegration of (a) blank nanofibers and (b) dual drug-loaded nanofibers shows an ultra-rapid dissolving of both nanofibrous systems ($\leq 4 \pm 1$ s, n = 3).

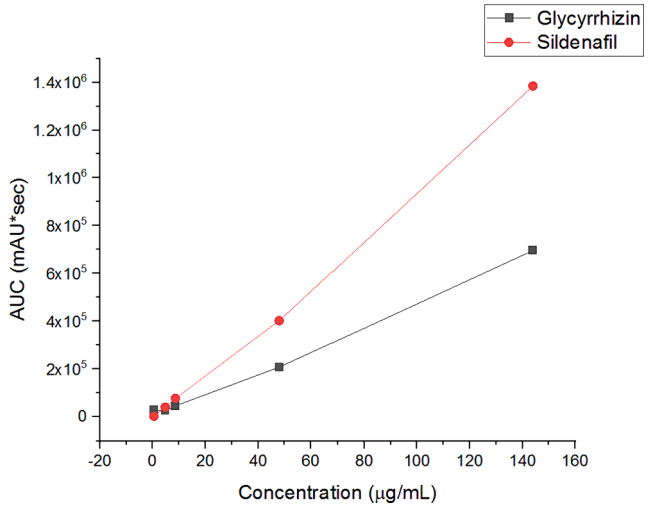

Fig. 5. Calibration curves of sildenafil and glycyrrhizin from the developed HPLC method, show good linearity for both materials.

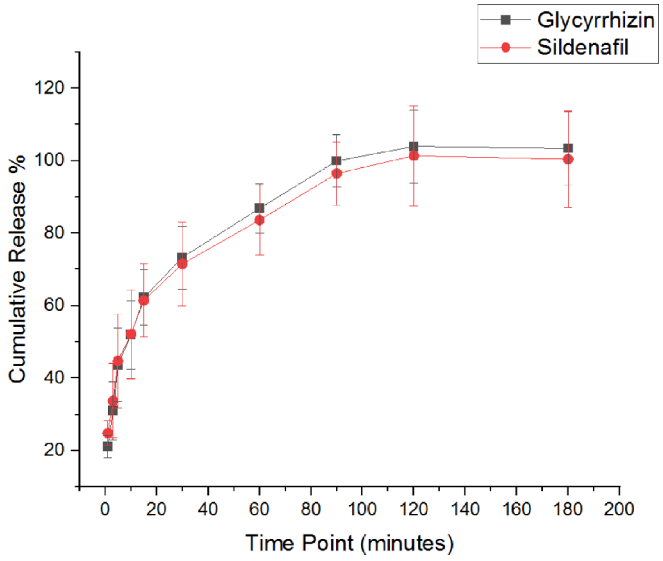

Fig. 6. The cumulative release (%) of dual drug-loaded nanofibers over 3 h. Complete drug release was observed after 120 min for both sildenafil and glycyrrhizin. The results are shown as the average ± SD (n = 3).

4. Discussion

PVP has emerged as a polymer of significant interest in pharmaceutical applications due to its exceptional properties, including biocompatibility, biodegradability, and drug-loading efficiency. In the context of our study, PVP serves as an excellent matrix for sildenafil/glycyrrhizin-loaded nanofibers due to its water solubility and ability to form mucoadhesive bonds, enhancing the delivery of active pharmaceutical ingredients through the buccal mucosa. The versatility of PVP is also evident in its use in electro-sprayed particles, which offers a different approach to creating nanoparticulate drug delivery systems. This technology, as shown in the study by Pharmaceutics, 2023 (Xu et al., 2023), allows for the production of particles with remarkable control over drug release kinetics, which can complement the nanofiber-based delivery systems that were developed in this current study.

Furthermore, when comparing PVP with other soluble polymers like Hydroxypropyl Methylcellulose (HPMC), each polymer offers distinct advantages in drug formulation and release. HPMC is renowned for its controlled-release properties, which have been extensively explored (Chen et al., 2023). However, the rapid dissolution and mucoadhesion of PVP are particularly beneficial for buccal delivery systems where immediate drug release is desired. The findings in this current study are in alignment with the properties of PVP, which demonstrate an accelerated dissolution rate that could potentially enhance the bioavailability of sildenafil and glycyrrhizin, offering an ultra-rapid onset of action for conditions such as erectile dysfunction.

The blank nanofibers and the dual drug-loaded PVP nanofibers morphology were evaluated as per the modified method (Alamer et al., 2023). The SEM images revealed that the fabricated nanofibers were successfully prepared, with a successful preparatory criterion of smooth surface and the lack of beads and pores formation. The average fibers diameters were measured as 0.719 ± 0.177 µm and 0.597 ± 0.205 µm for the sildenafil/glycyrrhizin-loaded and the blank nanofibers, respectively (Fig. 1). The variation in nanofibers' average diameter between the two measured formulations might be attributed to the concentration of loaded materials that causes a slight rise in the size of dual drug-loaded nanofibers. Moreover, this increase indicated the successful encapsulation of the drug. There are no detected drug crystals on the surfaces of dual drug-loaded nanofibers. It is suggested from morphological structures that the preparatory criteria were successful which was consistent with previous nanofiber systems (Alkahtani et al., 2021, Aburayan et al., 2022, Alshaya et al., 2022, Alamer et al., 2023).

The FTIR analysis was conducted to evaluate to interactions that

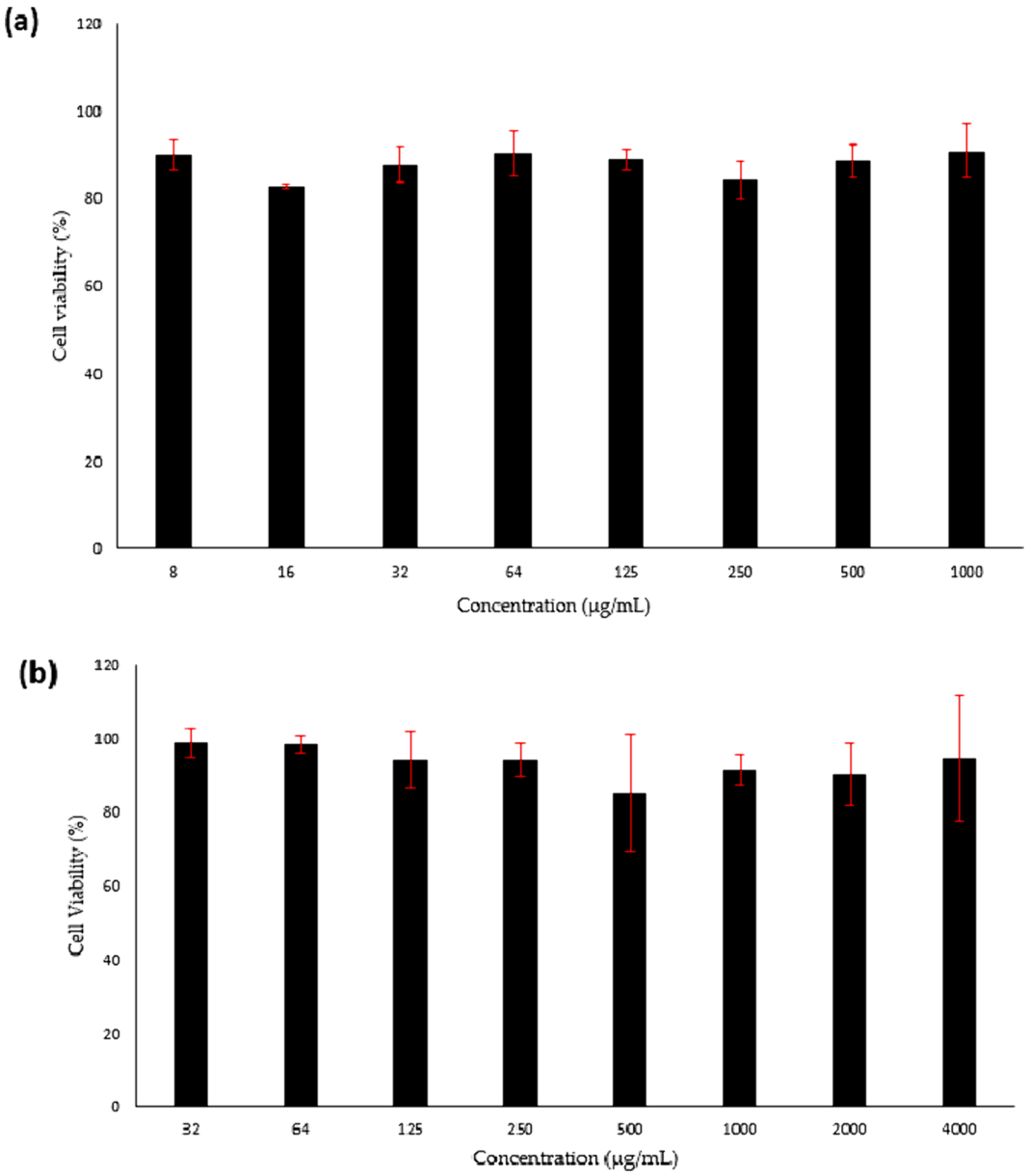

Fig. 7. Cell viability of (a) sildenafil and (b) glycyrrhizin after 24-hour HFF-1 cell exposure. The results revealed that sildenafil and glycyrrhizin are safe at ≤ 1,000 µg/mL and ≤ 4,000 µg/mL, respectively. The data was expressed as cellular viability (%) and presented as the average ± SD (n = 3).

Table 1
Different concentrations of sildenafil and glycyrrhizin in a ratio of 1:4.

Concentration Point	Sildenafil (µg/mL)	Glycyrrhizin (µg/mL)
A	8	32
B	16	64
C	32	125
D	64	250
E	125	500
F	250	1000
G	500	2000
H	1000	4000

might indicate for the compatibility between the active compounds (sildenafil and glycyrrhizin) and the polymer (PVP) within the fibers as an indicator for high quality and stability that lack solid-phase separation (Alkahtani et al., 2021, Aburayan et al., 2022, and Alshaya et al., 2022). The sildenafil FTIR spectrum (Fig. 2) was consistent with Melnikov *et al*, who reported the FTIR spectra of sildenafil base and sildenafil citrate (Melnikov et al., 2003), while the spectrum of glycyrrhizin (Fig. 2) was in agreement with the reported FTIR spectra of Zu *et al* (Zu et al., 2013) and Danaboina *et al.* (Danaboina et al., 2023) Previous studies by Mireles *et al.* (Mireles et al., 2020) and Safo *et al.* (Safo et al., 2019) reported similar distinctive bands that are shown in the PVP IR spectrum for this current study. Sildenafil characteristic peaks at 1490 cm^{-1}, 1361 cm^{-1} 804 cm^{-1}, and 736 cm^{-1} were present in the PM spectrum but in a weak intensity corresponding to the PVP:glycyrrhizin: sildenafil ratio (8:4:1) which indicate for this drug presence in the PM (Fig. 2). Glycyrrhizin's distinctive peak at 1663 cm^{-1} (C = O) overlapped with sildenafil peak at 1658 cm^{-1} in the spectrum of the PM creating a slight shift to this peak to 1667 cm^{-1}. Another peak of glycyrrhizin, i.e., secondary cyclic alcohols, was slightly shifted from 1035 cm^{-1} to 1030 cm^{-1}. These shifts may indicate intermolecular interaction, such as the formation of hydrogen bonding between the active compounds and PVP, which suggests a good compatibility between the fiber components, as reported in the previous studies of Alkahtani et al., 2021 and Aburayan et al., 2022. The FTIR spectra for the blank and dual

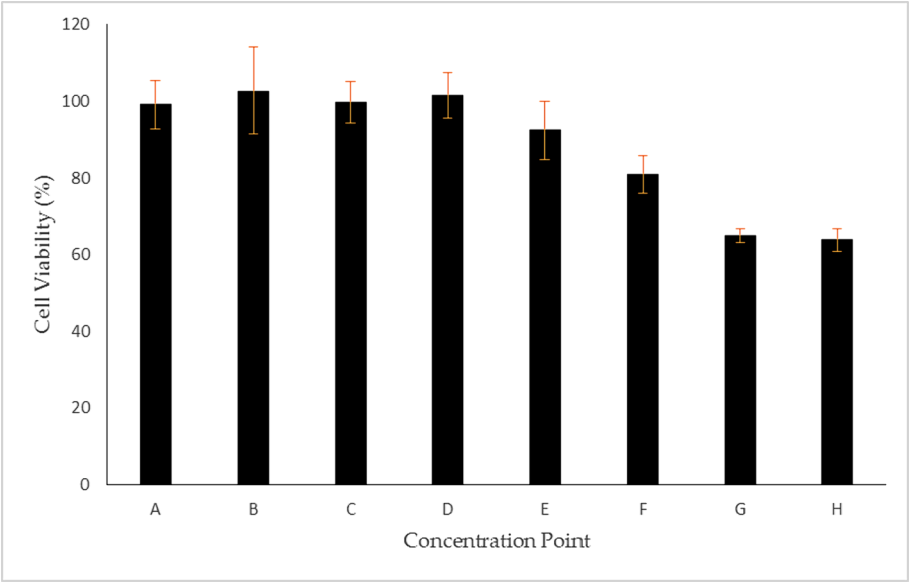

Fig. 8. Cell viability of sildenafil-glycyrrhizin combination with 1:4 ratio after 24-hour HFF-1 cells exposure. The results revealed that sildenafil-glycyrrhizin is safe at ≤ 250:1,000 μg/mL. The data was expressed as cellular viability (%) and presented as the average ± SD (n = 3).

drug-loaded fibers demonstrated similar spectra to the PVP and the PM, respectively, which is expected owing to the lack of both sildenafil and glycyrrhizin in the former fibers compared to the later fibers. This observation was also seen in previous electrospun fibers of Alkahtani et al., 2021, Aburayan et al., 2022 and Alshaya et al., 2022.

The XRD analysis was assessed to investigate the physical state of the active compounds (sildenafil and glycyrrhizin) after being electrospun, as it was previously reported that the electrospinning technique can form amorphous solid dispersions (Alkahtani et al., 2021). The raw materials (sildenafil, PVP polymer, glycyrrhizin and the PM), blank and dual drug-loaded nanofibers' physical state was evaluated by XRD and the solid dispersion of the sildenafil was confirmed due to electrospinning. An intense Bragg reflections pattern (distinctive, sharp and less diffused peaks) of sildenafil observed at 2θ: 7.43°, 8.13°, 10.36°, 14.46°, 17.77°, 19.97°, 20.84°, 23.04°, and 28.58°, with multiple less intensity peaks ranging from 30.59° to 44.93°, indicating the crystallinity nature of the pure sildenafil (Fig. 3). An almost similar XRD pattern for sildenafil has been reported previously by Canbay and DoĞAntÜRk, 2019. The XRD pattern of the PVP polymer showed a broad-halo shape pattern due to the amorphous character of this polymer, while the glycyrrhizin showed a similar pattern but with a broad peak at 14.65°. The PM characteristic pattern demonstrated distinctive peaks at 2θ: 7.97°, 10.34°, 14.28°, 19.88°, 22.81° and 28.57°, which indicates that the sildenafil in the polymer mixture was in the crystalline form (Fig. 3). The low intensity of sildenafil in the drug-polymer mixture (i.e., PM) might be attributed to the low ratio between the drug and the polymer that was used in the preparation of the nanofibers. In contrast, the sildenafil/glycyrrhizin-loaded nanofibers XRD results showed a broad-halo shape pattern, similar to the blank nanofibers, which may indicate the molecular dispersion of sildenafil which was caused by the process of electrospinning. This result was consistent with the XRD of previous studies using PVP polymer (Alkahtani et al., 2021).

One of the critical parameters for all dosage forms that are normally administrated via the oral cavity is the disintegration test. These dosage forms (i.e. buccal medications) should be absorbed rapidly (≤30 s), according to US FDA regulation (FDA-CDER, 2008). The disintegration of the nanofibrous systems in PBS was fast and started from the edge to the center leading to their complete dissolving after ≤ 4 s (Fig. 4), indicating the appropriateness of this nanofibrous system for buccal delivery. This ultra-rapid disintegration property was observed in previous studies utilizing PVP nanofibers (Tort et al., 2019, Alkahtani et al., 2021).

The HPLC-developed method was employed to determine the EE%, DL and release of sildenafil-glycyrrhizin-loaded nanofibers. The EE% and DL were measured as 52 ± 7 μg/mg and 67 ± 9 %, respectively, for sildenafil and 290 ± 32 μg/mg and 94 ± 10 % for glycyrrhizin. This EE% was similar to several drug-loaded PVP nanofibers studies for different applications (Alkahtani et al., 2021, Aburayan et al., 2022, Alshaya et al., 2022). The overall yield of the prepared dual drug-loaded nanofibers was 75 ± 4 %, while the remaining percentage might be lost during the deposition process as part of the nanofibers adhere to the chamber walls of the electrospinning instrument as well as during the removal of the mat from the aluminum foil. Furthermore, sildenafil and glycyrrhizin release were assessed by insertion of the dual drug-loaded nanofibers in glass vials containing a solvent mix of 85 % PBS (pH 6.8) and 15 % ethanol. The dual drug-loaded nanofibers showed a very rapid release behavior during 3 h (Fig. 6). After a minute, a burst release of both materials occurred, and then a gradual increment of the released material was shown until the full release of both sildenafil and glycyrrhizin at 120 min was obtained. This ultra-fast release is due to the fast release of the surface of sildenafil and glycyrrhizin molecules followed by steady release, where the release is controlled by both erosion and diffusion mechanisms. In addition, the result of sustained and controlled release is due to the unique structure of PVP, which enhances the release of drugs from nanofibers as reported by (Wang et al., 2020b). PVP is highly hygroscopic and water soluble and these characteristics which cause ensure the release of loaded drug instantly (fast first-phase release) in an erosion manner with the PVP matrix (Wang et al., 2020b). The release rate of sildenafil was very rapid despite using glycyrrhizin as a natural sweetener. The release study shows the promising result of selecting PVP as a polymer to control the release of the drug in the buccal environment.

The evaluation of *in vitro* cell viability of the tested drug on human living cells is an essential step to demonstrate its appropriateness for clinical applications (Alamer et al., 2023). In this assay, increased concentrations of sildenafil, glycyrrhizin, and sildenafil/glycyrrhizin combined were examined against the HFF-1 cell line to determine the optimal concentration of the drug that does not perform cytotoxicity on living cells and define 'safe' concentration for further applications. MTS result demonstrated the effect of applying different sildenafil concentrations on the HFF-1 cell's metabolic activity. This was investigated after the 24-hour incubation time of the cells by using an MTS assay

(Fig. 7a). Data indicated that a high level of cellular metabolic activity of the HFF-1 cell line was observed in all used concentrations, even at the highest used concentration (1,000 μg/mL). Furthermore, the findings exhibited a lack of effect at all used concentrations on the cell viability of HFF-1 (1,000 to 8 μg/mL). Hence, the *in vitro* assessment presented that sildenafil is safe in the concentrations used. For glycyrrhizin, the application of different doses (4,000 to 32 μg/mL) for 24 h showed high metabolic activity of HFF-1 cells in all applied concentrations as presented in Fig. 7b. The application of sildenafil and glycyrrhizin as a combination exhibited high metabolic activity (> 80 %) in all concentrations ≤ 250:1000 μg/mL of sildenafil and glycyrrhizin (concentration points A-F). However, the higher concentrations (i.e., G and H) exhibited lower metabolic activity as demonstrated in Fig. 8.

5. Conclusion

In summary, this study showed that sildenafil/glycyrrhizin-loaded PVP electrospun nanofibers can be successfully promoted as a buccal delivery approach for erectile dysfunction management. The nanofibers had smooth surfaces and lacked form beads and pores formation, with an average diameter of 0.719 ± 0.177 μm. The EE% and DL were measured as 52 ± 7 μg/mg and 67 ± 9 %, respectively, for sildenafil and 290 ± 32 μg/mg and 94 ± 10 %, for glycyrrhizin, whereas the Y% was measured as 75 ± 4 %. The disintegration of the dual drug-loaded nanofibers was demonstrated after 4 ± 1 s. The drug release of both sildenafil and glycyrrhizin was shown as a very rapid release profile, with a full release of both materials observed after 120 min. A novel delivery platform for sildenafil and glycyrrhizin nanofibers can be provided from this study which can be administered via the buccal route which offers a rapid delivery and sustained release of the loaded drug. Further studies are needed to validate and evaluate prepared nanofibers in relevant *in vivo* models.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2024.102038.

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