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Preparation of liposomal hydrogel containing Calendula and application as a wound dressing

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ABSTRACT

Burn wound is one of the major medical and Hydrogels are one of the best wound dressings for burns. Herein, chitosan/*Aloe vera* hydrogel was prepared and cross-linked by genipin. The nano-liposomes of soy lecithin as a phospholipid containing calendula were added to the hydrogel. The surface morphology and functional groups were evaluated by SEM and FTIR methods, respectively. The average hydrodynamic diameter was calculated by the dynamic light scattering. Also, the nanoliposomes hydrogel containing calendula has a suitable swelling and vapor permeability. The encapsulation rate of calendula was 83 % which indicates a high load of calendula. In vivo release study of hydrogel containing calendula was achieved by the French diffusion cell. Finally, the cytotoxicity (MTT) test, the proliferation and viability of fibroblast cells (L929) were investigated and the results show no cytotoxicity of the hydrogel. for in vitro study, the passage of calendula-containing liposomes through the skin was investigated. Rat abdominal skin was used as a natural membrane. France diffusion cell was used as a two-compartment model to measure the amount of passage. The skin absorption of the calendula begins with a gentle slope and in 24 h approximately 90% of skin absorption has taken place.

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Introduction

Burns are one of the most common and destructive injuries. Incomplete healing of burn wounds, long-term treatments, as well as secondary complications of burns, necessitate research to accelerate the wound healing process in burn patients. One of the important principles of treatment is wound care and prevention of infection at the wound site. Wound infection is one of the most important and serious complications that will occur in the acute phase of injury, in which Pseudomonas infection is very common and is resistant to most antibiotics (1). One way to control infection and keep the wound clean is to use a wound dressing. Since the dressing is in contact with the wound, the use of suitable wound dressing is very important. Different types of wound dressings such as wet and dry dressings are used for various wounds that need to be drained, cleaned, and disinfected. Hydrogels are one of the types of wound dressings that provide the basic needs of a wound dressing including maintaining wound moisture or absorbing excess water, covering without sticking to sensitive subcutaneous tissue, reducing pain and increasing wound healing potential. Hydrogels are 3D network polymers and highly hydrophilic polymers that can absorb water several times its weight and even hold it under pressure (2,3). Hydrogels are more similar to living tissues in terms of soft and rubber surfaces, structure and physicochemical properties, so the technology of using hydrogels in biomedicine and medicine has been developed (4). Different types of natural materials are used to make hydrogels. Chitosan, as a natural polysaccharide, is similar in structure to cellulose and is obtained by the desolation of chitin. Chitin is the second most abundant biopolymer in nature after cellulose. It is made by many living things and has the major constituent of the fungal cell wall, and exoskeleton of arthropods such as crabs, shrimp and insects. Main biochemical activities of chitin and chitosan in wound healing (5,6). Pot marigold or calendula (Calendula officinal is L.) belongs to the Asteraceae family and is native to southern Europe. It grows as a bedding plant, cut flower, or potted plant. The petals color is different from yellow to orange and aromatic scent. Calendula is a sun plant that and grow also under half-shade conditions (7). In chemical studies of marigold extract, the main elements and compounds including triterpenoids, flavonoids, coumarins, sapiens, essential oils, carotenoids and amino acids have been identified. The saponins secretion of plasma into the tissues by reducing capillary permeability and reducing histamine and stops the growth of bacteria and fungi (8). Marigold has anti-inflammatory properties in the treatment of wounds, burns, bruises, skin rashes and inflammatory lesions (9). Marigold extract reduces the serum level of mar-

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kers released from the liver and strengthens the defense mechanisms in burn injuries. The mentioned markers are tissue-destroying enzymes from flavonoids in marigolds that release histamine and produce prostaglandins that cause allergies, pain and swelling (10) Miller barbadensis Aloe has a cactus-like, succulent, succulent appearance and leaves with a gel-like texture. Numerous studies have been performed on its restorative and antibacterial effects of it. Its restorative properties are mostly due to a compound called glumanan which is rich in polysaccharides such as mannose. Glumanan affects fibroblast growth receptors and stimulates their activity and proliferation, which in turn increases collagen production (11). *Aloe vera* gel not only increases the amount of wound collagen, but also changes the collagen composition, increases the collagen cross-links, and as a result, accelerates wound healing (12-14). In chitosan wounds, the presence of *Aloe vera* increased water absorption and keeps the wound moist. It also absorbs wound secretions over time (15). It also absorbs wound secretions over time. Therefore, by placing anionic groups in chitosan hydrogels, hydrogels with the ability to swell at different pHs are prepared (16). Hydrogels must be cross-linked for better strength and stability. Natural compounds are better alternatives to crosslink due to their lower toxic effects. Genipin is a good candidate for combination with the chitosan amine group due to its OH group (17,18) Nowadays, the application of nanotechnology in medical fields has been developed and one of these fields is drug delivery. Dermal delivery using Nanocarriers is highly desirable due to their very small diameter. Liposomes have attracted significant attention as a class of antimicrobial delivery vehicles owing to their unique features including biocompatible lipid materials, a bilayer structure capable of fusing with microbial membrane, readily modifiable formulation properties, and high drug loading capacity (19). Due to the fact that spontaneous fusion of liposomes, especially with sizes less than 100 nm, their stability is weakened It causes liposomes to agglomerate (20,21). Lipid-based systems often face the problem of instability. They show higher biocompatibility compared to polymer systems because they are considered natural components of the human body. However, since polymer-based systems are more stable than lipids, the use of lipids with lipids can increase their resistance to degradation (22).In this study, nanoliposomes cross-linked with Genipin were prepared (Scheme 1) for the controlled release of calendula. Nano-liposomes containing calendula were loaded into the chitosan/*Aloe vera* hydrogel. Finally, hydrogels containing nanoliposomes were evaluated by different experiments, characterization and comparison.

Materials and Methods

Chitosan was purchased from Sigma-Aldrich. Co., genipin was obtained from the Challenge Bio Products Co., Ltd. Soy lecithin was obtained from the German grail., calendula (CAS number: 84776-23-8) and *Aloe vera* (CAS number: 85507-69-3) form plant were purchased from a local store. (Chloroform, ethanol, acetic acid) used by the German Mark.

Calendula Extract

5 g of calendula powder was added to 100 ml of 0.70 ethanol and poured into a bottle. It was placed on a strainer for 24 h and sonicated for 15 min. afterward it passed through filter paper twice and evaporated in a rotary at 50 °C. The rotation speed was set at 150 rpm for 30 min. The obtained extract was freeze-dried to dry completely.

Preparation of liposome containing Calendula

Nanoliposomes containing Calendula were prepared by thin layer hydration and ultrasound. First, 20 mg of lecithin was dissolved in 1 ml of chloroform and dried by evaporator at 50 °C, 150 rpm, for 30 min. 6 mg of Calendula was dissolved at 50 mL distilled water at 50 °C and added to a balloon containing a thin lipid film and mixed. The obtained suspension contains multilayered Calendula liposomes. To remove excess layers and reduce the size of the liposome, probe sonication was repeated three times at five-minute intervals. The suspension was sonicated 15 minutes in a bath at 50 °C. The samples were centrifuged for 20 min at 1500 rpm at 4 °C. The sediments containing liposomes were dissolved in 1 mL of distilled water.

Extraction of Aloe vera

The outer part of the plant leaves was washed with distilled water. The gel of the plant was separated from the green outer part and turned into a completely paste-like mixture by a mixer. In order to separate the gel part from the fibrous components, the resulting paste was placed in a centrifuge at 1000 rpm for 30 min. The pure gel was freeze-dried to dry completely.

Synthesis of hydrogels containing nanoliposomes

For the preparation of hydrogels containing nanoliposomes**,** first 0.02 % V/V of hydrogel, chitosan was dissolved in 15ml distilled on a stirrer for 6 h. Then 0.10 weight of the polymer was added to *Aloe vera* and stirred for 6 h. Liposomes containing calendula were added and stirred for 1 h. Then 0.0075 g of genipin as a cross-linker was added to 15 mL of hydrogel solution was poured into a plate and dried completely.

Physical properties of liposomes

The hydrodynamic diameter of particles was done by Dynamic Light Scattering (Malvern Instruments Ltd. Worcestershire, UK). After the synthesis of nano-liposomes, 1 ml of the solution was poured into 1.5 ml of the cuvette and placed in the sample reading place inside the device. For determination zeta potential of nanoliposomes containing Genipin, 1 ml of the solution was poured into 1.5 mL of the cuvette and placed in the sample reading place inside the device scanning electron microscopy (SEM) (TESCAN-Vega3 co Czech) performed to observe the microstructure morphology of the liposomal sample and to examine the size and dispersion. The solution was diluted 10 times with distilled water, and placed in a sonic bath for 5 min until the solution was uniform, and then 10 µL of the solution was poured onto a lamel and placed in a desiccator for 24 hours to dry. The entire surface of the lamel was covered with a very thin layer of gold and examined with an SEM at a magnification of 1 µm. To study the morphology of the surface of the hydrogels, films with dimensions of 1 × 1 cm were prepared and covered with thin layers of gold

The functional groups and formed bonds in the samples were evaluated by Fourier transform infrared spectroscopy (FTIR). The samples were ground and formed into thin

tablets. IR absorption peaks were observed in wavelengths in the range of -400 cm^{-1} to 4000 cm^{-1} and the type of bonds was determined by examining the absorption band.

Evaluation of Hydrogel swelling

An inflation test was performed to estimate the adsorption capacity and swelling capability of chitosan /*Aloe vera* hydrogel canting liposome. First, the weight of the prototype was measured. Afterward, the hydrogel film was placed in phosphate buffer saline (PBS) solution at 37 ℃ for 5 min. the inflation rate of the sample was calculated in time intervals) 5, 10, 15, 30 and 60) min. After reaching equilibrium, the sample was weighed. The water absorption rate or swelling rate of samples was calculated.

Water vapor passes through the hydrogel

Frist control, a sample without hydrogel, and a disk with a diameter of 3 cm from the samples (Hydrogel - *Aloe vera*, hydrogel + *Aloe vera*, hydrogel + *Aloe vera* + liposome) was fixed on the mouth of a test tube with a 3 cm diameter of containing 25 ml of double distilled water, separately. In this test, the tube containing 25 ml of double distilled water without cap was considered as a control. The first tubes were weighed with water and a proven sample. The samples were then incubated for 24 h at 37 ℃ and 0.040 humidity. After 24 h the samples were weighed again. The amount of water vapor boron was calculated from the following equation) In this regard W_i initial weight of the system) test tube, Water and polymer film (, W_f The final weight of the system after reduction and A The area of the mouth of the bottle((23).

$$WVTR\ (g/(m)^2/h) = \frac{Wi - Wf\ \times 10^6}{24\ \times A}$$

Investigation of biodegradability

In order to calculate the degradation rate of samples, pieces of film with dimensions of 1. 1 cm were prepared. The samples were weighed and placed in Falcon containing 10 ml of PBS solution and stored in a CO_2 incubator at 37 °C. Samples were removed from PBS in 1, 2, 5, 7, 14, 21 and 27 days and placed in the oven for 2 h and weighed again. The amount of weight loss and swelling rate was calculated from the following equation) In this formula W_i the initial dry weight of the sample and W_f the dry weight of the sample is after degradation (24).

$$WL\ (\%) = \left(\frac{\mathrm{Wi} - \mathrm{Wf}}{\mathrm{Wi}}\right) \times 100$$

Investigation of the strength of hydrogel samples

Samples were prepared based on the ASTM D00882 standard in dimensions of 1× 6 cm. In this test, the tensile speed of the films was 50 mm/min and the distance between the two jaws of the device was about 3 cm. The percentage of elongation and elastic modulus of the films were calculated through the stress-strain diagram and the tensile strength by the following formula) In this regard "A" the initial area of the film and "F_{max}" the most forceful possible tolerance is before failure ((14).

$$TS = \frac{Fmax}{A}$$

Drug release tests

Determination of entrapment efficiency and Loading Efficiency

UV-*vis* spectrometer was used for evaluating the entrapment efficiency of Calendula in Nanoliposomes. The concentration of Calendula in nanoparticles was calculated by the calibration curve. The calibration curve was achieved from the UV absorbance of different standard Calendula aqueous solutions. The suspensions of freshly prepared nanoparticles were centrifuged at 14800 rpm for 30 min. The supernatants were then eliminated for the determination of free Calendula in the suspensions. Calculation of EE was performed by utilizing of formula presented below:

$$\text{Entrapment Efficiency \%} = \frac{\text{Actual amount of drug loaded in liposomes}}{\text{Actual amount of drug used for liposomal preparation}} \times 100$$

Drug release investigation

In vitro release study was done by the diffusion technique. The hydrogel containing nanoliposomes was placed in a dialysis bag (cut off 12 kDa) and placed in 50 mL of phosphate buffer saline (pH 7.4). It is maintained at room temperature with constant stirring at 550 rpm. At timely (0, 2, 4, 6, 8, 10, 12, 24, 36, 72) intervals, a 2 mL aliquot of the release medium was removed, and the same volume of fresh phosphate buffer saline solution was added into the system. The concentration of Calendula in the release medium was calculated from the Calendula absorption at 232 nm.

Skin absorption of Calendula

After synthesizing hydrogel, the in vitro study was done based on the use of natural membranes to evaluate the passage of liposomes containing calendula through the skin**.** The rats used in this research were Sprague Dawley (weight range of 200 to 150 g). Animals were kept in the same conditions before the experiment. Storage conditions included polycarbonate cages, uniform food and water, and 12-hour light-dark periods. The animal was anesthetized with chloroform and then killed. The entire abdomen of the animal was completely dissected. The skin of this area was completely removed. The fat under the skin was thoroughly removed and kept in a normal saline solution until use. To be closer to the body conditions and to prevent changes in the physicochemical and biological properties of the skin due to long-term storage, the skin was freshly prepared at each time. To investigate the passage of liposome containing Calendula, the French diffusion cell was used as a two-compartment. The cell was filled with 30 mL of phosphate buffer 0.05 M (pH = 7.4) as the receptor phase. For similarity to the body condition, the temperature of the diffusion cell was kept constant at 37 °C. The Hydrogels corporation liposome containing Calendula was applied separately on the skin. At timely (0, 2, 4, 6, 8, 10, 12, 24, 36, 72) intervals, a 2 mL aliquot of the release medium was removed, and the same volume of fresh phosphate buffer saline solution was added into the system. The concentration of Calendula in the release medium was calculated from the Calendula absorption calibration curves at 232 nm.

Cell Culture

L929 cells were plated as a monolayer in 25 cm^2 culture flask (Orange Scientific, Belgium) and in DMEM cell culture medium (5% fetal calf serum), 100 UmL^{-1} peni-

cillins and 100 µgmL^{-1} streptomycins and were incubated (37°C, 5% CO_2 and 95 % humid air).

Cell viability (%) (MTT assay)

MTT assay was used for cell viability measurement. 1×10^4 cells were incubated for 48 hours in a 96-well culture plate in different treatment media. Using a microplate reader (EL800; USA) at 570 and 630 nm, the optical density of each well was measured (0/0001, 0/05 0/005, 0/5 mg/ml of calendula).

Results and Discussion

Investigation morphology

According to Figure 1[A] the zeta potential in liposomes is (-31.4), The zeta potential is very important for the deposition of nanoliposomes and important factor in the stability of nanoparticles (26). The dynamic light scattering method is a physical, non-destructive and fast method that is used to determine the size distribution of particles in solution and suspension. If the PDI value is less than 0.05, the particles Uniform distributions are excellent. As shown in Figure 1[B] liposome containing Calendula with an average size of (108.9 nm) and a PDI (0.297).

Also, as shown in Figure 2[A], the presence of liposomes containing calendula on the surface of the hydrogel is observed, which are evenly distributed throughout the surface without agglomeration and without change size (27). During the SEM examination, the hydrogel sample was completely dried and the water in the hydrogel was removed. therefore, the cavities of nanoliposomes are not clear in the electron microscope image and few cavities are seen in the microscopic image shown in Figure 2[B] (28).

Investigation of water absorption and Steam passage

The ideal wound dressing should be absorbed large amounts of secretions in the wound quickly and in the shortest possible time and prevent the growth and accumulation of bacteria. Figure 3 [A] shows the swelling power of the hydrogel in the PBS solution. Due to the slope of the graph, in the first five minutes, the slope of the graph is very high and the swelling rate has reached approximately 2000 %. This is due to the formation of hydrogen bonds between the sample and the solution and the rapid penetration of the solution into the hydrogel sample. Adsorption of the solution and swelling of the hydrogel continue so that the weight of the sample reaches 3500 % after 15 minutes, then the percentage of swelling of the sample is fixed. Therefore, the results indicate the excellent swelling of the sample due to the gradual breaking of the compact structure of the hydrogel sample and the penetration of the solution into these parts and the filling of these parts (23). The water vapor transmission rate is a distinct factor that shows the potential of hydrogel in the transmission of body liquid or wound exudates. Creating proper vapor permeability and oxygen permeability speeds up wound healing and reduces the formation of residual scar tissue on the wound surface. If the vapor permeability of the wound is low, the possibility of evaporation and extra secretions from inside the wound will be reduced, so the accumulation of infection and accumulation of bacteria in the wound will be reduced and the wound will heal quickly (28). Figure 3 [B] shows that the presence of *Aloe vera* increases the number of hydrophilic groups in the hydrogel samples and increases the absorption of water vapor through the surface in contact with water and its passage through the surface in contact with air. On the other hand, the presence of liposomes in the hydrogel increases the porosity at the surface of the samples, which increases the passage rate Water vapor is effective.

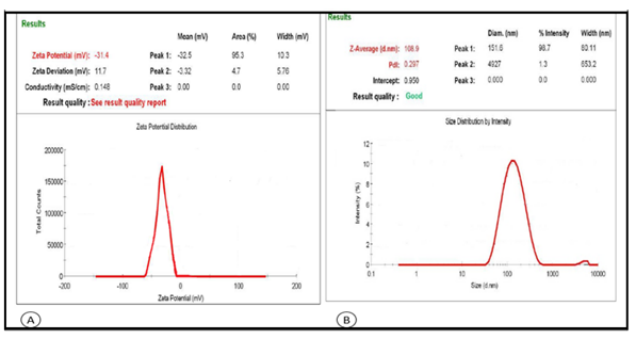

Figure 1. Zeta potential of nanoliposomes, hydrodynamic diameter of nanoliposomes by Dynamic Light Scattering.

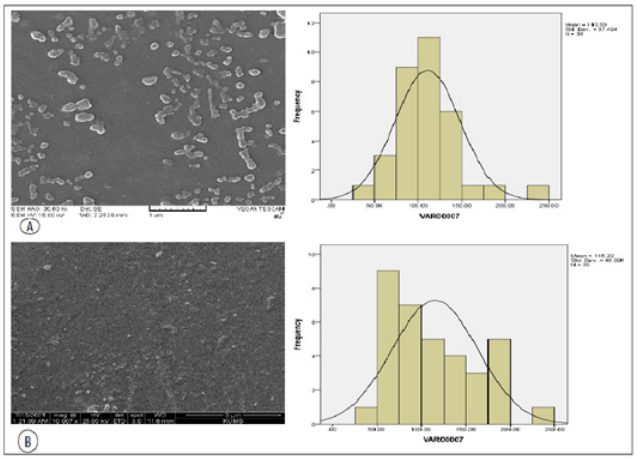

Figure 2. Morphology and size of nanoliposomes containing Calendula by Scanning electron microscopy (SEM), Hydrogels corporation liposome containing Calendula.

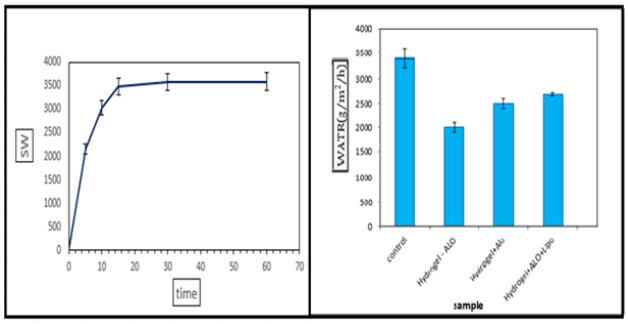

Figure 3. (A) Percentage water absorption of hydrogels corporation liposome containing Calendula (B). Vapor permeability of the hydrogel sample (control, Hydrogel-*Aloe vera*, hydrogel + *Aloe vera*, hydrogel + *Aloe vera* + liposome).

Investigation of biodegradability of hydrogels

Biodegradability of hydrogel was done and the results showed that the highest rate of destruction of the samples is observed on the first day, but from the end of the first day to the twenty-seventh day, the destruction continues at a lower rate. Figure [4] shows that because the maxi-

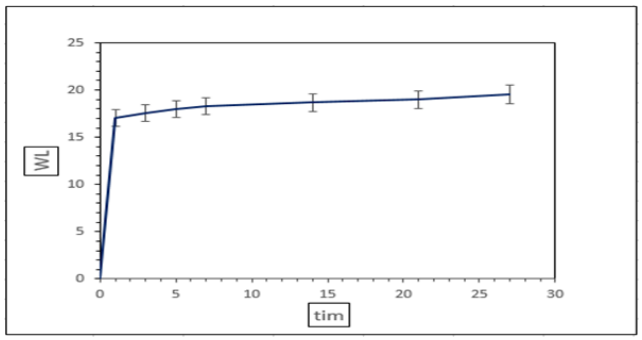

Figure 4. Percentage of degradability of hydrogels corporation liposome containing Calendula.

mum amount of water absorption is in the early hours and increasing the amount of water absorption increases the rate of destruction, so the rate of destruction is greater on the first day (29).

Investigation Bonds formed in hydrogels

The FT-IR spectrum of the extract of Calendula and a liposome containing the extract was shown in Figure [5] the liposome showed a vibrating band with a distinct peak for the OH group at 3375 cm $^{-1}$. Peaks at 2924 cm $^{-1}$ and 2856 cm^{-1} were attributed to the asymmetric and symmetric tensions of the CH_2 group of phospholipids. Carbonyl functional groups showed characteristic peaks at 1739 cm^{-1} and 1620 cm^{-1}. It was suggested that the terminal methyl group showed a vibration frequency of 1376 cm $^{-1}$ (30). Hydroxyl and carbonyl functional groups showed clear vibration bands in different chemical compounds extracted from calendula extract in the adsorption band 1616 cm^{-1} and 3377 cm^{-1} respectively. Liposomes containing calendula extract showed small changes in the CH_2 band at 2922 and 2852 cm^{-1}. C = O functional groups were observed with slight changes in the absorption and intensity of peaks in the area of 1735 and 1616 cm^{-1}. Hydrogen bonding is possible for hydroxyl functional groups and was attributed to 33581 cm^{-1} due to a change in the band. Figure 5 shows the FT-IR spectra of aloe vera, Genipin, and chitosan, along with samples of genotype cross-linked chitosan, cross-linked chitosan containing more than 10 % *Aloe vera*, chitosan with aloe vera, and marigold liposome extract. *Aloe vera* is a complex combination of polysaccharides, proteins and other compounds that lead to the complex spectrum of FTIR. *Aloe vera* showed an adsorption band of about 3398 cm $^{-1}$, which may be characteristic of amino acids due to the presence of hydrogen-bonded N - H. The absorption band at 12922 cm^{-1} is symmetric and asymmetric due to the C - H tension of the CH2− group. This band is also characteristic of the presence of aliphatic groups (CH−) in these compounds. The absorption band at 1720 cm^{-1} is characteristic of C=O bending and indicates the presence of carbonyl groups. The strong absorption band at 1598 cm^{-1} is due to the C = C tension, which indicates the presence of vinyl ether and the composition of Alaina. The absorption band at 1236 cm $^{-1}$ is due to the tensile vibrations of the C-O groups of esters and phenols. The absorption band at 1880 cm^{-1} is off-plane due to the C-H deformation. The non-reactive genipin spectrum is characterized by three bands at 989, 1085, and 1618 cm^{-1}, which are bent into the C-H ring outside the plate (31). Adsorption at 1085 cm^{-1} may also include the C-O tensile state of the primary alcohol on the genipin molecule. In addition, the asymmetric tensile strength of C-O-C and bending of CH_3 methyl ester were observed at 1300 and 1442 cm^{-1}, respectively. Adsorption at 1105 cm^{-1} is assigned to cyclic ether vibrations. The FT-IR spectrum of the chitosan adsorbed band at 34341 cm^{-1} is due to the partial overlap of the tensile vibrations of the amine and hydroxyl groups and the peak characteristic of the strong amino proton at about 1598 cm^{-1}. The adsorption band was characteristic of amide adsorption at 1680 cm^{-1}. 896 cm^{-1} and 1155 cm $^{-1}$ peaks were assigned to vibrational states associated with chitosan saccharide units. The adsorption band at 1257 cm^{-1} can be attributed to the hydroxyl group and at 1026 and 1087 cm^{-1} was the result of C-O and C-N tensile. The chitosan spectrum cross-linked with genipin showed the intensity and displacement of the peaks. The elongated C–O band at 1053 cm^{-1} and the weak absorption band at 1400 cm^{-1} were related to the ring tension of the genipin molecule.

Investigation of the strength of hydrogel samples

The results of the mechanical analysis of the film are summarized in Table 1. All mechanical properties, tensile strength (TS, in GPa) Indicates the maximum tensile stress that the film can withstand, the increase in fracture length (E, in%) as the maximum change during a test film before breaking, and the elastic modulus (EM, in MPa) which is the measure of film stiffness (32).

The addition of *Aloe vera* to the film significantly reduced the tensile strength, increased the film modulus and also increased the fracture length. The ideal wound dressing does not have a high modulus or tensile strength but is soft, flexible and easily portable. It should also be stretched according to the patient's movements and return to its first dimensions (33). The addition of liposome na-

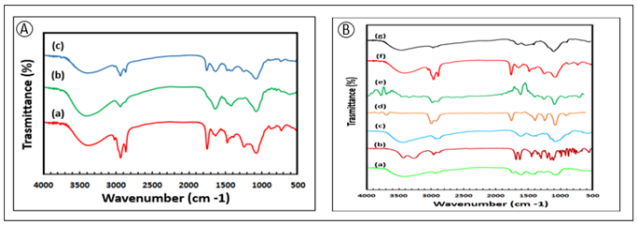

Figure 5. (A): FTIR analysis of (a) Blank liposomal formulation, (b) pure Calendula (c) liposomal containing Calendula and Fig 5(B): a) *Aloe vera*, b) genipin, c) Chitosan, d) Chitosan /genipin, e) Chitosan / *Aloe vera*, f) liposomal containing Calendula, g) Chitosan / *Aloe vera* / liposomal.

Table 1. Investigation of the strength of hydrogel samples.

Film	Tensile (GPa)	Elongation at break (%)	Young Modulus (GPa)
Chitosan	6±0.56	0.92±0.003	0.1933±0.009
Chitosan + *Aloe vera*	0.98±0.007	1.97±0.027	0.5±0.042
Chitosan + *Aloe vera* + Liposome	2.93±0.03	1.38±0.034	0.0587±0.0021

noliposomes can help reduce the modulus and soften the system in favor of the mechanical properties expected for wound dressing.

Release measurement

Figure 6 [A] shows the percentage of calendula release at different times from the nanoliposomes. As can be seen, the release of calendula begins slowly, and within 24 h, approximately 90 % release is observed due to the destruction of liposomes following the phenomenon of PBS penetration. After 72 h, the release rate remains constant 73 %. Also, the amount of Calendula EE in liposomes was 83 % this indicates a high percentage of calendula loading in the liposome. Therefore, the resulting hydrogel has a controlled release, so it is an ideal wound dressing for wound healing.

After synthesizing calendula-containing liposomes and inserting them into the hydrogel, the in vitro study method, based on the use of natural membranes, was used to investigate the passage of calendula-containing liposomes through the skin. Rat abdominal skin was used as a natural membrane. France diffusion cell was used as a two-compartment model to measure the amount of passage. According to the results obtained in Figure 6 [B] the skin absorption of calendula begins with a gentle slope and in 24 h approximately 90 % skin absorption has taken place 70 %. Therefore, the resulting hydrogel has controlled skin absorption, so Wounds are ideal for healing burns.

Investigation of toxicity

Figure [7] shows the MTT test results for different concentrations of samples. The proliferation of L929 cells in low concentrations at (0.0001, 0.005 mg/ml) no toxicity was observed and we see an increase in cell viability in the vicinity of these concentrations due to the presence of calendula. However, no significant difference was observed between these samples and the control. With increasing concentration) 0. 05, 0. 5 mg/m (, the survival rate and cell proliferation increased significantly, indicating a significant difference between these samples and the control sample. it is due to increased bioavailability and controlled release of calendula during the two days of testing. Therefore, no toxicity was observed in the samples, even in high concentrations.

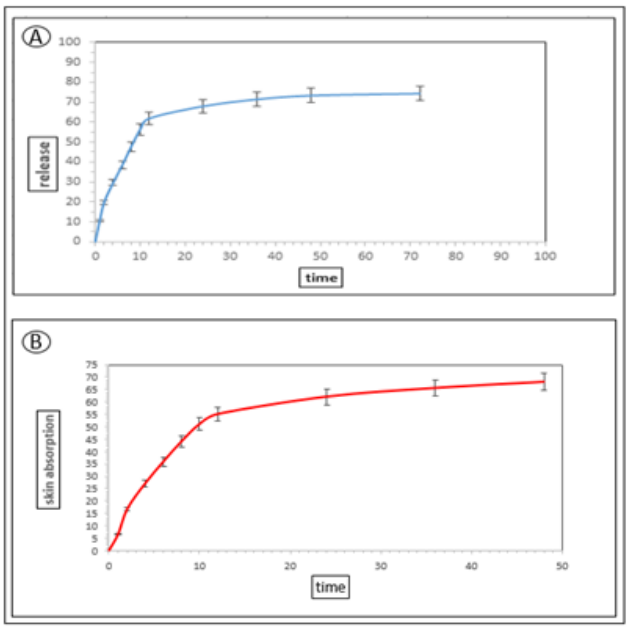

Figure 6. (A): Percentage of release from Hydrogels comprising nanoliposomes containing Calendula, (B) Dermal absorption of Calendula.

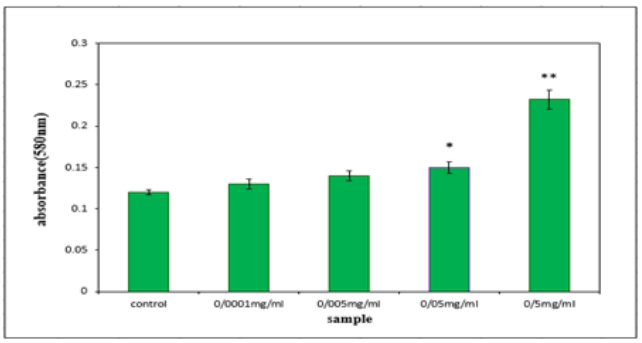

Figure 4. Cell viability L929 in contact with nanoliposomes at different concentrations for two days of cell culture (* Increase in cell viability in the sample with concentration 0.05 mg / ml and 0.5 mg/ml compared to the other three samples was significant ($p< 0.05$).

Conclusion

In this study, a new Chitosan/*Aloe vera* hydrogel wound dressing containing calendula nanoliposomes was developed for use in wound healing. In hydrogel dressings, a combination of chitosan and *Aloe vera* was used as a substrate with the ability to absorb liquids and suitable vapor permeation conditions. Calendula was also used as a natural agent to speed up the wound-healing process. To increase bioavailability and release control, calendula was loaded into the liposome. The results confirm that the addition of liposomes to the hydrogel increases the ability to absorb and create better vapor permeability in the area of granular tissue created on the wound surface. The results of the release and dermal absorption studies showed a slow and controlled release and absorption. Cellular studies also showed no cytotoxicity in the nanoliposomes, even at high concentrations. Therefore, Nano-containing hydrogel dressings can be used as a suitable choice for rapid wound healing.

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Conflict of interest

The authors declare that they have no conflict of interest

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