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RESEARCH ARTICLE

In vitro and In vivo assessment of Gelatin nanoparticles loaded doxocetal scaffolds

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ABSTRACT

Aim: The aim of this study to evaluates the physiochemical properties, drug loading, in vitro release, antibacterial and wound healing activity. Gelatin is a common natural polymer or protein which is normally produced by denaturing collagen. It has been used in pharmaceutical and medical applications due to its outstanding properties such as biodegradability, biocompatibility, and low antigenicity. **Materials and Methods:** Docetaxel -loaded gelatin nanoparticles by using UV-Vis spectroscopy, XRD, Particle size and size distribution, Scanning electron microscopy (SEM), Drug entrapment efficiency, Differential scanning caloriemetry (DSC) and EDX were characterized. Wound healing activity was determined on Wistar rats using excision wound models. **Results:** Solubility, crystallinity, and the crystal properties of an active pharmaceutical ingredient (API) play a critical role in the value chain of pharmaceutical development, manufacturing, and formulation.. The rate of drug release for formulation stored at 45 ± 1^0C was increased as compared with the fresh formulation; it might be due to the formation of more pores in the nanoparticles due to evaporation of residual amount of solvent. Studies on docetaxel-loaded gelatin nanoparticles-impregnated ointment for its wound healing property on excised wound showed significant results in terms of epithelialization period and wound contraction and was close to the standard (Povidone iodine ointment) used. **Conclusion:** The antibacterial activities and sorption capacities of the Docetaxel -loaded gelatin nanoparticles are strong indicators to their in vivo functionalities as wound dressing.

Keywords: Docetaxel, gelatin, wound dressing, Antioxidant, Bacteria.

INTRODUCTION

Gelatin is also a natural polymer derived from collagen of animal skin and bones. It is biocompatible, hydrophilic, and biodegradable under normal physiological conditions. Gelatin nanoparticles are effective in enhancing the growth induced tissue regeneration and various biomedical applications [1]. Gelatin has been widely used for the encapsulation of dug materials to treat various diseases. Drug bound to gelatin matrix are released as gelatin degrades enzymatic ally and therefore the release profile can be tailored by controlling the cross linking density and surface to volume ratio [2, 3]. Gelatin is known to prevent fluid loss due to exudation, resulting in enhancement of its wound healing properties [4, 5]. Wounds have a tremendous impact on the healing healthcare economy [6]. A major problem with wounds is the high risk of infection; hence, if an agent active against these microorganisms causing the infection is used in the healing process, it will then help to reduce the risk of infection and the overall time for wound healing can be reduced significantly [7]. For example, it is very easy for bacteria to enter through the broken skin and penetrate the rest of the body. Bacteria colonize wounds within 48 hours after injury and bacteria such as Staphylococcus aureus, Pseudomonas aeruginosa, and Streptococcus spp may cause infection and this may prolong inflammatory phase of wound healing [8]. Therefore suitable antimicrobial agents can be used either topically or systematically to prevent infection of wounds and speed up wound healing process. The process of inflammation normally leads to the release of biologically active mediators to attract neutrophils, leucocytes and monocytes, to the wound area and these attack foreign debris and microorganisms through phagocytosis. This then leads to the production of oxygen-free radicals such as hydrogen peroxide, superoxide anion, and hydroxyl anion and excess of these agents causes tissue damage in man or animal if they overwhelm the natural antioxidants of the host such as

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catalase, superoxide dismutase, and glutathione peroxidase [9]. Therefore, antioxidants prevent the activity of the free radicals and thereby prevent the damage to cells and tissues, providing protection to human and animal subjects, and also enhance healing of infected and non-infected wounds [10]. The microencapsulation of either antibacterial agents or biotherapeutic molecules such as growth factors provides to overcome various limitations in the controlled release formulation of wound care system such as low solubility, high potency, and/or poor stability of many of these drugs [11]. The docetaxel -based drug delivery can impact efficacy and potential for commercialization as much as the nature of the drug itself. Moreover, the microencapsulation facilitates biocompatible could provide high bioavailability at the site of injury and are capable of sustained release for long periods into the wound environment [12]. There are certain limitations available in the existing wound care system such as lack of controlled delivery, microbial degradation of collagen-based wound dressings by wound pathogens at the wound site [13]. To overcome these limitations, an effective wound care system is developed based on nanoparticle-based controlled delivery of a potent and broad spectrum antimicrobial agent into an infected wound environment. In this novel wound care system, the gelatin nanoparticles provides to control effectively the infection at the wound site and fast the wound regeneration including dermis and epidermis [14]. The regeneration of connective tissue at the injured soft tissue is still exigent. The presence of bacterial pathogens at the wound site delays wound closure and regeneration of connective tissue as well as degrades various extracellular matrix like collagen and elastin and also produce high inflammation matrix at the wound site by its native enzymes such as microbial collagenase and elastase [15]. As the drug is known to possess superior antibacterial activity against a wide range of microorganisms, a topical drug delivery system localizing the drug at the skin will be much effective for the treatment of skin infections [16]. Hence in the present work topical controlled drugs are prepared by incorporating the prepared gelatin nanoparticles to control the release rate of the drug over a period of time, so that the frequency of application of the formulation can be reduced, which also enhances patient compliance with better wound healing activity.

MATERIALS AND METHODS

Chemicals and Reagents

The chemicals used in all experiments were obtained from sigma (Bangalore, India) and Merck (Mumbai, India). Docetaxel, Gelatin (food grade, NF), lactic acid (90%), glutaraldehyde, Trypsin, and dimethyl sulfoxide (DMSO) (Sisco, Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich, Mumbai.

Docetaxel

Docetaxel drugs is belongs to antimetabolites, comes under plant alkaloids, drug was chosen for the study.

Preparation of gelatin nanoparticles

Gelatin nanoparticles were prepared using an overhead stirrer with a five-blade paddle (diameter 50 mm) (15). Five mL of gelatin solution (20%, m/V, in water) was preheated to 80 °C and added drop-wise to 70 mL of sesame oil (viscosity 43.4 m Pa s at 20 °C) containing 1% (m/m) Span 80 (with respect to the mass of the oil phase) warmed to the same temperature. The biphasic system was stirred under turbulent flow conditions using an overhead stirrer (RW20DZM.n, IKA Labortechnik, Germany) to form a w/o emulsion. Glutaraldehyde-saturated toluene was prepared by mixing equal volumes of glutaraldehyde and toluene in a decantation funnel. After shaking for 10 minutes, the mixture was allowed to separate. The upper toluene layer saturated with glutaraldehyde was separated and added to the w/o emulsion. The dispersion was mixed for various time intervals at an appropriate speed (1200 rpm). Nanoparticles were then separated by decantation and washed free of oil with 20mL of toluene for 2 min at 1500 rpm. The nanoparticles were then washed and dehydrated 3 times with 20 mL of acetone at 2000 rpm. Finally, nanoparticles were allowed to dry at room temperature (25 °C). Upon drying, a yellow to yellowish orange colored free flowing, fine powder was obtained. The gelatin nanoparticles were observed by both optical microscopy (B3050 Prior, Prior Scientific, UK) and scanning electron microscopy (Leica Manuf. Cambridge S 360, UK). Three different formulations with drug: polymer ratios (1:1, 1:2, and 1:3) are prepared and coded as F1, F2 and F3.

UV-Spectroscopy analysis

The first requirement of any pre-formulation study is the development of a simple analytical method for quantitative estimation in subsequent steps. Most of drugs have aromatic rings and/or double

bonds as part of their structure and absorb light in UV range, UV spectroscopy being a fairly accurate and simple method is a performed estimation technique at early pre-formulation stages. The absorption Co-efficient of the drug can be determined.

Scanning electron microscopy (SEM)

Scanning electron microscopy has been used to determine particle size distribution, surface topography, texture, and to examine the morphology of fractured or sectioned surface. SEM is probably the most commonly used method for characterizing drug delivery systems, owing in large to simplicity of sample preparation and ease of operation. SEM studies were carried out by using JEOL JSM T-330A scanning microscope (Japan). Dry Docetaxel nanoparticles were placed on an electron microscope brass stub and coated with in an ion sputter. Picture of LP nanoparticles were taken by random scanning of the stub [17].

Particle Size, Shape and Surface Area

Bulk flow, formulation homogeneity, and surface-area controlled processes such as dissolution and Surface morphology of the drug particles. In general, each new drug candidate should be tested during Preformulation with the smallest particle size as is practical to facilitate preparation of homogeneous samples and maximize the drug' s surface area for interactions [18]. Various chemical and physical properties of drug substances are affected by their particle size distribution and shapes. The effect is not only on the physical properties of solid drugs but also, in some instances, on their biopharmaceutical behavior. It is generally recognized that poorly soluble drugs showing a dissolution- rate limiting step in the absorption process will be more readily bio available when administered in a finely subdivided state rather than as a coarse material. In case of tablets, size and shape influence the flow and the mixing efficiency of powders and granules. Size can also be a factor in stability: fine materials are relatively more open to attack from atmospheric oxygen, the humidity, and interacting excipients than are course materials [19].

Particle size Determination

Though microscopy is the simplest technique of estimating size ranges and shapes, it is to slow for quantitative determination the material is best observed as a suspension in non dissolving fluid [20]. Sieving is less useful technique at pre-formulation storage due to lack of bulk material. Andreason pipette is based on the rate difference of sedimentation of different particles, but techniques like this are seldom used due to their tedious nature instruments based on light scattering, (Royco), light blockage (HIAC) and blockage of electrical conductivity path (coulter counter) are available.

Preparation of saturated solution of glutaraldehyde

Equal quantity of aqueous glutaraldehyde solution and toluene was taken in a separating funnel and shaken for 1 hour to allow the saturation of glutaraldehyde in toluene. Then the aqueous phase and toluene phase was separated. Thus, obtained toluene saturated with glutaraldehyde was used to cross-link gelatin nanoparticles.

Determination of drug content

The amount of Docetaxel presents in the gelatin nanoparticles was determined by digestion with 1M sodium hydroxide. Briefly, 100 mg of nanoparticles was dispersed in 100 ml of 1M sodium hydroxide in a 100 ml standard flask. And kept overnight for 12 h. It was then filtered, diluted and Docetaxel content was determined spectrophotometrically (Shimadzu 1601) at 276 nm. Sodium hydroxide (M) was used as blank. The amount of metronidazole present in gelatin nanoparticles was determined by digestion with hydrochloric acid. Briefly, 100 mg of nanoparticles was dispersed in 100 ml of 1M hydrochloric acid in a 100 ml standard flask and kept overnight for 12 h. It was then filtered, diluted and metronidazole hydrochloride content was determined at 320 nm. Hydrochloric acid (1M) was used as blank [21].

Test organisms

The bacterial spp. used for the test were Bacillus subtilis (MTCC 441), Staphylococcus aureus (MTCC 3940), Micrococcus luteus (MTCC 106), Enterobacter aerogenes (MTCC 111), Salmonella typhi (MTCC-734), and Pseudomonas aeruginosa (MTCC 841). All the stock cultures were obtained from Institute of Research and Technology, India. Microbial growth was determined by measuring the diameter of the zone of inhibition. Ciprofloxacin (Himedia, Mumbai, India) is a reference drug used as a control for test organisms [22].

Determination of Minimum inhibitory concentration (MIC)

Antibacterial activities were measured using a dilution technique [23]. Minimum inhibitory concentration (MIC) of GNPs was determined using the microtiter broth-dilution method. The bacterial strains were suspended in sterile physiological Tris buffer (pH 7.4, 0.05 M), homogenized and adjusted to an optical density of 0.05 at 530 nm (equivalent to 1 X 106 CFU/ml). This suspension was used as the inoculums for the test in the agar plates. Bacterial suspensions (100µl) were inoculated using a micropipette. The minimal inhibitory concentration (MIC) was defined as the minimal concentration of the gelatin nanaoparticles which completely inhibited the visible growth (turbidity) of the bacteria in tubes.

Studies on wound healing efficacy

Animals

Healthy Wistar albino rats of either sex weighing 150-250 gm were selected for the study. The animals were individually housed in spacious, clean, polypropylene cages containing paddy husk bedding and fed with a standard pellet diet, marketed by Brooke Bond, Lipton India Limited, Bangalore and water ad libitum in animal house facility and maintained under standard experimental conditions throughout the experiment. The experiments were conducted in accordance with the Institutional Animal Ethical Committee (IAEC), (Ethics Clearance No. 1333/C/10/CPCSEA).

Acute Toxicity Activity

Healthy Wistar albino rats of either sex weighing 150-250 gm were selected for the acute toxicity study. The acute oral toxicity study was carried out by the stair-case method [24]. The animals were fed with increasing doses of 1, 2, 4 and 8 gm/Kg body weight.

Preparation of gelatin nanoparticles impregnated ointment

Gelatin nanaoparticles (5 mg) impregnated with doxocetal was suspended in 0.4 ml saline. The gelatin nanoparticles suspension was uniformly injected into several portions of the inner collagen sponge. The doxocetal doses were 5 mg/scaffold. The doses used in this study were based on some studies [25]. The freeze-dried content was mixed with blank placebo in concentration of 5% (w/w).

Excision wound model

Excision was inflicted on the rats under light anaesthesia [26]. The dorsal fur of the animals was shaved with an electric clipper. Full skin thickness was excised from the marked area to inflict a wound measuring about 500 mm, using a toothed forceps and pointed scissors.

Normal experimental animal model

The rats were divided into three groups of six each.

Group 1: Test group with wound and treated placebo with ointment base.

Group 2: Test group with wound and treated with 5% (w/w) gelatin nanoparticles loaded doxocetal ointment.

Group 3: Test group with wound and treated with standard drug 5% (w/w) povidone iodine ointment [27].

All the formulations were applied twice a day after cleaning with surgical cotton, till the complete epithelialization starting from the day the wound was created. The wound healing process was evaluated by wound contraction percentage and wound closure time.

Evaluation of parameters

Epithelialization period

It was monitored by noting the number of days required for the scar to fall off from the wound surface without leaving a raw wound behind [28].

Measurement of wound contraction

Excluding the day of wounding, the excision wound margin was traced to follow the progressive changes in the wound area plan metrically. The wound surface area was measured by placing a transparent paper over the wound and tracing it out. The same procedure was employed every three days until healing was complete [29, 30]. The traced area of the wound was then evaluated in terms of surface area on a graph sheet [31]. The wound contraction was calculated in terms of percentage in the reduction of the wound area [32], using the following formula:

Percentage of wound contraction = [(Initial wound area - specific wound area) /Initial wound area] x 100.

Statistical analysis

The effect of cross-linking time and the amount of cross-linking agent on the lactic acid release from gelatin nanoparticles were analyzed separately using Repeated Measures Analysis of Variance. When significant differences between the formulations were observed, multiple comparisons by the Duncan test were applied.

RESULTS

In the present work the solubility studies of docetaxel were performed in common solvents. A specific amount of drug was dissolved in specific amount of different solvents at room temperature and observed only by the visible inspection. The result suggested that, it is sparsely soluble in water, acetone, isopropanol, methylene chloride and it exhibit good solubility in methanol, ethanol and dimethyl formamide. It also exhibit insolubility in ether. Results are expressed in (**Table 1).**

Table 1 Solubility studies of Docetaxel in various solvents

S.No	Solvent	Solubility
1	Water	Sparingly soluble
2	Methanol	Good solubility
3	95% Ethanol	Good solubility
4	Isopropanol	Sparingly soluble
5	Methylene chloride	Sparingly soluble
6	Acetone	Sparingly soluble
7	Ether	Practically insoluble
8	Dimethyl formamide	Good solubility

The sample was scanned in the range of 200-400 nm using Shimadzu 1700 UV/visible spectrophotometer to determine the λ max. The absorption maxima of Docetaxel were found at 230 nm. The spectra were shown in **(Fig. 1).**

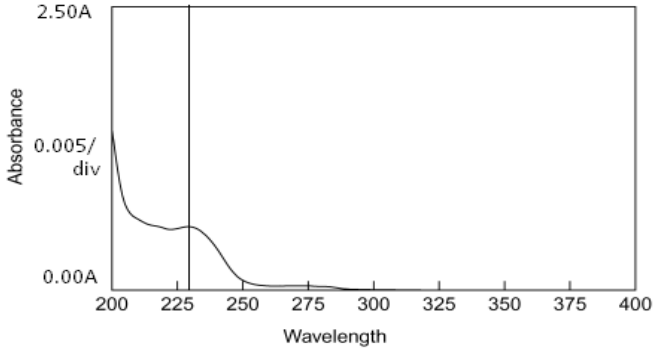

Fig. 1 UV spectra of Docetaxel

Amount of gelatin for the preparation was optimized by preparing the nanoparticles at different amount viz. 50, 100 and 200 mg keeping other variables constant as described in the general procedure of preparing gelatin nanoparticles. The effects of amount of gelatin on the particle size, shape, size distribution and drug entrapment efficiency are reported in **Table 2.**

Table 2: Effect of amount of gelatin on particle size and drug entrapment efficiency of gelatin nanoparticles

Formulation code	Amount of gelatin (mg)	Amount of glutaraldehyde (µL)	Stirring rate (rpm)	Temperature (° C)	Size (µm) ±SD	DEE±SD (%)
G1	50	200	600	40	5.71±0.13	70.01±1.2
G2	100	200	600	40	11.75±0.11	71.3±2.1
G3	200	200	600	40	26.84±0.11	83.2±1.9

Glutaraldehyde concentration for the preparation was optimized by preparing the nanoparticles at different concentration viz. 50, 100, 200 and 300 µL keeping other variables constant as described in the general procedure of preparing gelatin nanoparticles. The effects of glutaraldehyde concentration on the particle size, shape, size distribution and drug entrapment efficiency are reported in **Table 3.**

Table 3 Effect of amount of glutaraldehyde on particle size and drug entrapment efficiency of gelatin nanoparticles

Formulation code	Amount of gelatin (mg)	Amount of glutaraldehyde (µL)	Stirring rate (rpm)	Temperature (°C)	Size (µm) ±SD	DEE (%) ±SD
GA1	100	50	600	40	24.52± 1.03	73.1±2.1
GA2	100	100	600	40	17.34±1.40	76.9±2.3
GA3	100	200	600	40	9.52±0.39	80.11±2.8
GA4	100	300	600	40	6.16±0.32	83.7±2.2

Particle size and size distribution of gelatin nanoparticles were determined using laser light diffractometry equipment (Mastersizer X, Malvern Instrument, UK). The average particle size was expressed as the volume mean diameter in micrometers. The results are given in Table 4 and 5.

Table 4 Effect of stirring rate on particle size and drug entrapment efficiency of gelatin nanoparticles

Formulation code	Amount of gelatin (mg)	Amount of glutaraldehyde (µL)	Stirring rate (rpm)	Temperature (°C)	Size (µm) ±SD	DEE ±SD (%)
S1	100	200	200	40	32.6± 0.21	79.10±2.1
S2	100	200	400	40	21.43±0.11	81.0±2.4
S3	100	200	600	40	11.3±0.14	82.03±2.8
S4	100	200	800	40	9.30±0.32	83.50±3.4

Table 5 Effect of temperature on particle size and drug entrapment efficiency of gelatin nanoparticles

Formulation code	Amount of gelatin (mg)	Amount of glutaraldehyde (µL)	Stirring rate (rpm)	Temperature (°C)	Size (µm) ±SD	DEE ±SD (%)

T1	100	200	600	40	8.35±0.22	80.10±2.3
T2	100	200	600	50	18.16±0.98	76.03±1.9
T3	100	200	600	60	22.42±0.67	70.19±2.2

The surface morphology of nanoparticles was observed scanning electron microscopy (SEM). The samples for SEM were prepared by lightly sprinkling the nanoparticles powder on a double adhesive tape which stuck to an aluminum stub. The stubs were then coated with gold to a thickness of about 300^0A using a sputter coater. These samples were than randomly scanned and photomicrographs were taken which are shown in Fig. 2 a and b.

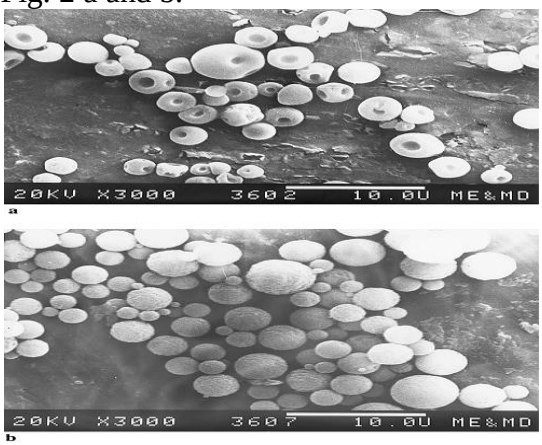

Fig. 2 SEM photomicrograph of (a) Plain nanoparticles (b) Docetaxel loaded gelatin Nanoparticles

In the current study, we have for the first time researched on the effect of the docetaxel drug loaded gelatin nanoparticles for antibacterial effect. The present study is to develop and characterize docetaxel drug loaded gelatin nanoparticle-loaded for antibacterial effect. Six Bacterial pathogens *Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus, Enterobacter aerogenes, Salmonella typhi, Pseudomonas aeroginosa* are used for experimental study (Table: 6).

Table 6: Antibacterial activity of docetaxel drug loaded gelatin nanoparticles

S.No	Bacterial name	Zone of inhibition in mm		
		Docetaxel drug loaded gelatin nanoparticles	Marketed drug	Reference Drug
1.	Bacillus subtilis	13±0.34	9±0.61	14±0.42[a]
2.	Staphylococcus aureus	13±0.85	8±0.42	16±0.49[b]
3.	Micrococcus Luteus	13±0.91	8±0.92	15±0.48[c]
4.	Enterobacter aerogenes	20±0.85	12±0.43	13±0.39[d]
5.	Salmonella typhi	17±0.91	9±0.06	16±0.72[a]
6.	Pseudomonas aeroginosa	23±0.71	16±0.38	17±0.54[d]

Keys: Reference drugs: a-Streptomycin, b-Gentamycin, c-Ampicillin, d-Erythromycin

The study concludes that the minimum inhibitory concentration (MIC) results which revealed the details of mean MICs of docetaxel drug loaded gelatin nanoparticles are 25µg in six bacterial strains. The concentration of docetaxel drug loaded gelatin nanoparticles that completely inhibited bacterial growth. The docetaxel drug loaded gelatin nanoparticles showed there was no significant effect on antibacterial. Maximum zone of inhibition was found due to the presence of *Pseudomonas aeruginosa, Micococcus luteus, Enterobacter aerogenes*.

The rats of either sex were fed with increasing doses (1, 2, 4 and 8 gm/Kg body weight) of docetaxel-loaded gelatin nanoparticles for 14 days. The doses went up to 8 gm/Kg body weight but there were no signs of toxicity and mortality. The animals were physically active and consumed food and water as normal and also did not exhibit any abnormal behavior. However in the current study, a dosage of 5 gm/Kg body weight was utilized for the preparation of the docetaxel-loaded gelatin nanoparticles -impregnated ointment. Studies on docetaxel-loaded gelatin nanoparticles-impregnated ointment for its wound healing property on excised wound showed significant results in terms of epithelialization period and wound contraction and was close to the standard (Povidone iodine ointment) used. (Table: 7; Figure 3).

Table 7: Wound healing activity of Docetaxel-loaded gelatin nanoparticles in experimental rats (excision wound model).

Groups	Post woundling days Wound area (mm^2) and percentage of wound contraction				Period of epithelialisation
	0- day	6th day	12th day	18th day	
Simple ointment	243±1.12	226.26 ± 2.1	166.62± 1.79	65.77 ± 1.4	23

Docetaxel-loaded gelatin nanoparticles in pregnated ointment base (5% w/w)	246± 0.93	203.71 ± 1.81*	121±1.72	45.26±1.9*	16
Povidone Iodine ointment 5% (w/w)	247± 1.05	212.63± 1.73	126.26± 2.1	40.64± 2.1*	14

Values are mean of six individual observations in each group Mean±SEM. 'P' denotes statistical significance * P<0.05

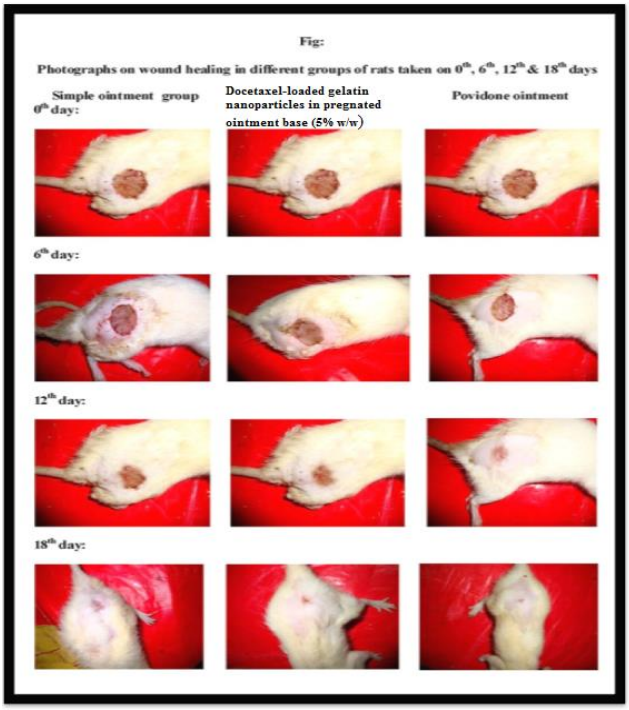

Figure 3: Photographs on wound healing in different groups taken 0th, 6th, 12th, 18th days

The epithelialization period for the wound treated with the docetaxel-loaded gelatin nanoparticles - impregnated ointment (5% w/w) was 16 days, when compared to wound treated with povidone iodine ointment (5% w/w) which was 14 days and the simple ointment, 23 days. The percentage of wound contraction was measured and calculated on 6th, 9th, 12th and 18th day. On the 18th day, the animals treated with the docetaxel-loaded gelatin nanoparticles - impregnated ointment (5% w/w), showed a wound area and wound contraction when compared to the animals treated with povidone iodine ointment (5% w/w), which showed a wound area and a wound contraction , whereas the wound treated with simple ointment showed an area and a contraction.

DISCUSSION

Solubility, crystallinity, and the crystal properties of an active pharmaceutical ingredient (API) play a critical role in the value chain of pharmaceutical development, manufacturing, and formulation [33]. Solubility studies were necessary to check for the pharmacopoeial specifications. Because these properties are all solvent dependent, solvent screening is of fundamental and foremost importance to the pharmaceutical industry [34]. Similar studies elsewhere reported that,

Loxoprofen is soluble in water, methanol and freely soluble in ethanol, practically soluble in diethyl ether, acetone and chloroform [35]. Gelatin nanoparticles have the potential to be an efficient, viable, safe and cost-effective system for administration of Docetaxel on account of their biodegradability, biocompatibility, suitability for oral applications and low immunogenicity [36]. Docetaxel-loaded nanoparticles were characterized to evaluate the effect of the different amount of gelatin on mean particle size, size distribution [37]. The particle size of the gelatin nanoparticles varied from 4.61±0.13 µm to 16.84±0.11 µm with varying amount of gelatin from 50 mg to 200 mg. The average particle size of nanoparticles increased with increasing amount of polymer solution, which got dispersed into larger droplets. The drug entrapment efficiency varied from 75.01±1.2% to 81.2±1.9%. The highest entrapment efficiency was found with G2 and the size of nanoparticles was also sufficiently low, therefore this formulation was selected as optimum. The pore size ranging from 10 µm to 400 µm could benefit the preservation for tissue volume, provide the temporary mechanical function and also deliver the biofactors [38]. The particle size and structure would increase the bioactivity into maximum range [39, 40]. A study reported that the average diameters of the hydrated gelatin methacrylate nanoparticles were 4.9±3.6, 5.5±5.2 and 5.0±6.9 respectively [41]. Similar studies elsewhere reported that the encapsulation of Oxybenzone into gelatin nanoparticles with increasing drug and polymer ratio from 1:6 to 1:2 caused the particle size shift from around 12.46 µm to 14.67 µm [42]. This particle size variation paved a way for the maximum percentage of drug loading efficiency [43]. In this study, the particle size of gelatin nanoparticles decreased from 22.49±1.03 µm to 5.16±0.32 µm with increasing amount of glutaraldehyde from 50 µL to 300 µL. The drug entrapment efficiency varied from 71.1±2.1% to 79.7±2.2%.

The particle size of gelatin nanoparticles decreased from 24.6± 0.21 µm to 6.30±0.32 µm with increasing stirring rate from 200 rpm to 800

rpm. Results revealed that the particle size of nanoparticles was controlled by stirring rate. These results show that a high stirring speed produced smaller nanoparticles due to the smaller emulsion droplets produced by a higher stirring speed, which provided more energy to disperse the oil phase in water [44]. Results also suggested that there was a stirring rate limit for a particular polymer concentration. Higher stirring rate did not result in further reduction in mean diameter significantly. The stirring rate of 800 rpm was found to be optimum for gelatin nanoparticles, as the drug entrapment efficiency was highest i.e. 80.03±2.8% at this stirring rate. The stirring speed also affected the size of the formation of nanoparticles. A recent study revealed that upon increasing the stirring speed from 300 rpm to 1200 rpm, there is a gradual decrease in the particle size from 16.0 to 10.2 μm. The of particle size of gelatin nanoparticles increased from 6.45±0.22 μm to 20.42±0.67 μm when increasing temperature from 40 to 60°C and entrapment efficiency decreased from 76.10±2.3% to 67.19±2.2% . The particle size distribution of drug may influence properties of the pharmaceutical interest such as the flow properties, packing densities and compressibility segregation characteristics [45]. The size of the cell aggregates coated with gelatin nanoparticles were 50.1±18.3 and 194.3±33.0mm. The gelatin nanoparticles size was controlled by adjusting the concentration of gelatin in the aqueous phase. An increase in size of the gelatin was achieved by adjusting the concentration in aqueous droplets in emulsion and thus the size of the final gelatin nanoparticles varies [46]. Surfactants also control the size of nanoparticles by reducing the size and the aggregation tendency of the gelatin droplets during emulsification process [47]. SEM was used to investigate the morphology as well as particle size of nanoparticles. Nanoparticles displayed a spherical shape with a smooth surface and no aggregation was observed. No difference was observed in the morphological properties of nanoparticles due to presence of the drug. Normally, all of the synthesized nanoparticles showed a highly porous structure, where there is a number of nanofibrous gelatin/silica bioglass composite walls, several tens of nanometers in size, were created through the nanoparticles [48]. This structure was achieved by the unique phase separation of the gelatin/silica hybrid mixtures during TIPS at -70^{0}C [49]. The porous structure is strongly affected by the additional variable not considered here, such as nonsolvent/solvent ratio, polymer concentration and temperature [50]. The hydrated gelatin nanoparticles have smooth surfaces and have roughly spherical in shape was observed in earlier studies [51]. The erythromycin loaded gelatin nanoparticles also showed very smooth and uniform surface during SEM microphotographs. The morphology of the *Sphingomoans sp* HXN-200 loaded gelatin nanoparticles observed that the outer surface of the nanoparticles was smooth and non-porous [52]. The cell aggregates loaded into gelatin have a smooth surface and spherical in shape. Gelatin nanoparticles loaded with lactic acid have the morphology of very smooth and uniform surface with no physical pores on the surface of the nanoparticles [53]. A wound is a breach in the normal tissue continuum resulting in a variety of cellular and molecular sequences. Wound may be accidental or as a result of planned surgical interventions in the tissue of the body. The term "wound" is generally applied to more superficial form of tissue damage where as "injury" is used for damages of depart structures. Wound has a variety of effects on the tissue including mechanical separation of functional structure such as blood vessels (bleeding) deformation occurs due to tissue tension, biological effects which results immediately leading to inflammatory responses and secondary effects which occurs at a later stage such as infections [54]. Wound healing is a complex process of restoring cellular structures and tissue layers in damaged tissue together to its normal state and commencing in the fibroblastic stage where the area of the wound undergoes shrinkage [55]. It comprises of different phases such as contraction, granulation, epithelization and collagenation [56]. Wound contraction is a process that occurs throughout the healing process, commencing in the fibroblastic stage where the area of the wound undergoes shrinkage [55]. The wound repair and healing process involves steps that include inflammation and haemostasis around the site of injury. The proliferative phase, characterized by epithelialization, angiogenesis and collagen deposition and the final remodeling phase, characterized by wound contraction resulting in apparently a smaller amount of scar tissue, repair of the connective tissue and epithelium that leads to a healed wound [56]. The progression from an injured site to a healed wound is potentially slowed down or arrested by a number of different events and conditions; the most significant being colonization of the wound bed by micro-organisms [57]. This would ultimately lead to the

production of a variety of toxins and proteases by the micro-organisms in the wound bed resulting in a prolonged inflammatory response. Though the host's inflammatory response is vital and effective to eliminate the microbial pathogens, over time it could also lead to the damage of the surrounding tissues [58]. The use of anti-microbial prophylaxis is vital in reducing the wound's microbial load. Once a wound becomes infected, healing is delayed [59]. Increased bacterial burden on the surface and in the wounded tissue increases the metabolic requirements of the wound and of the host's response to that heavy bacterial load. A bacterium produces endotoxins, exotoxins, proteases and creates local tissue injury [60]. The presence of a bacterial burden in a wound stimulates a pro-inflammatory environment. The presence of bacteria also induces the migration of monocytes, macrophages and leukocytes, all of which are in co-ordination initially but later produce a response that is exaggerated and deleterious, leading to delayed or failure to healing [61]. This is referred to as a phenomenon known a 'Bioburden', which is defined as the metabolic load imposed by bacteria in the wound bed. Bacteria compete with normal cells for available oxygen and nutrients. Apart from this, bacteria and bacterial products such as endo-toxins and metallo-proteinases can cause disturbances in the wound healing phases [61]. The impregnated leucocytes in the wound bed kill phagocytic bacteria by mechanisms that involve an oxidative and the consumption of significant amounts molecular oxygen, thereby depraving the oxygen required for basic wound metabolisms. In addition to the white blood cells inflammatory response needed to kill bacteria increases the release of damaging oxygen free radicals. Thus, from a patho-physiologic point, treating an infection reduces the wound's bacterial burden which in turn effects the dynamics of oxygen delivery and its utilization within the wound [62]. Study of wound healing effect of docetaxel-loaded gelatin nanoparticles was carried out using excision wound model in order to establish the antiseptic activity of the drug [63]. In the present study, sincere effort has been attempted to establish the scientific validity of the wound healing effect of the docetaxel-loaded gelatin nanoparticles. Antioxidants have been reported to play a significant role in improving the wound healing process and protecting the tissues from oxidative damage [64]. Showed that treated healed wound group contained a large amount of fibroblast proliferation, collagen synthesis and neo-vascularisation, which resulted in increased wound tensile strength and accelerated wound healing. This is in coherence with the current study which revealed that dressing the wound with docetaxel-loaded gelatin nanoparticles 5 % (w/w) significantly enhanced the process of wound healing.

REFERENCES

1. Ichinohe N, Kuboki Y, Tabata Y. Bone regeneration using titanium nonwoven fabrics combined with fgf-2 release from gelatin hydrogel nanoparticles in rabbit skull defects. Tissue Eng Part A 2008; 14:1663-71.
2. Choy YB, Cheng F, Choi H, Kim KK. Monodisperse gelatin nanoparticles as a drug delivery vehicle: release profile and effect of cross linking density. Macromol Biosci 2008; 8:758-65.
3. Cheng F, Choy YB, Choi H, Kim KK. Modeling of small-molecule release from crosslinked hydrogel nanoparticles: effect of cross linking and enzymatic degradation of hydrogel matrix. Int J Pharm 2011; 403:90–5.
4. Tanaka A, T. Nagate, and H. Matsuda, "Acceleration of wound healing by gelatin film dressings with epidermal growth factor. Journal of Veterinary Medical Science, vol. 67, no. 9, pp. 909–913,2005.
5. Chong EJ, T. T. Phan, I. J. Lim et al., "Evaluation of electrospun PCL/gelatin nanofibrous scaffold for wound healing and layered dermal reconstitution," Acta Biomaterialia, vol. 3, no. 3, pp. 321– 330, 2007.
6. Amegbor K, Metowogo K, Eklu-Gadegbeku K, Agbonon A, Aklikokou KA, Napo-Koura G, Gbeassor M. Preliminary evaluation of the wound healing effect of Vitex doniana sweet (Verbenaceae) in mice. Afr J Tradit Complement Altern Med. 2012;9(4):584-90.
7. Agyare C, Dwobeng AS, Agyepong N, Boakye YD, Mensah KB, Ayande PG, Adarkwa-Yiadom M. Antimicrobial, Antioxidant, and Wound Healing Properties of Kigelia africana (Lam.) Beneth. and Strophanthus hispidus DC. Adv Pharmacol Sci. 2013; 2013:692613.
8. Houghton PJ, P. J. Hylands, A. Y. Mensah, A. Hensel, and A. M. Deters, "In

vitro tests and ethnopharmacological investigations: wound healing as an example," Journal of Ethnopharmacology, vol. 100, no. 1-2, pp. 100–107, 2005.
9. Sharma P, Jha AB, Dubey RS, Pessarakli M. Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. Journal of Botany. 2012; 2012: 1-26.
10. Martin A. The use of antioxidants in healing," Dermatological Surgery, vol. 22, no. 2, pp. 156–160, 1996.
11. Ahmed TA, Aljaeid BM. Preparation, characterization, and potential application of chitosan, chitosan derivatives, and chitosan metal nanoparticles in pharmaceutical drug delivery. Drug Des Devel Ther. 2016; 10: 483–507.
12. Kim KK, Pack DW. Microspheres for drug delivery. In: Ferrari M, Lee AP, Lee J, editors. BioMEMS and Biomedical Nanotechnology: Biological and Biomedical Nanotechnology. Vol. 1. New York: Springer; 2006. p. 540.
13. Shanmugasundaram N, Sundaraseelan J, Uma S, Selvaraj D, Babu M. Design and delivery of silver sulfadiazine from alginate microspheres-impregnated collagen scaffold. J Biomed Mater Res B Appl Biomater. 2006; 77:378-88.
14. Jackson JE, Kopecki Z, Cowin AJ. Nanotechnological Advances in Cutaneous Medicine. Journal of Nanomaterials. 2013; 2013: 1-8.
15. Schmidtchen A, Holst E, Tapper H, Björck L. Elastase producing Pseudomonas aeruginosa degrade plasma proteins and extracellular products of human skin and fibroblasts, and inhibit fibroblast growth. Microb Pathog 2003;34:47-55.
16. Roopa G, Bhat SR.Wound- healing activity of a prepared long acting gel loaded with ciprofloxaciliin HCL microspheres in albino rats. Pharmacologyonline. 2010; 2: 1010-1016.
17. Magharla DD, Nandhakumar S, Vankayalu DS, Suresh C. Preparation of poly (epsilon-caprolactone) microspheres containing etoposide by solvent evaporation method. Asian J of Pharm Sci. 2010; 5(3):
18. Mortko CJ, Sheth AR, Variankaval N, Li L, Farrer BT. Risk assessment and physicochemical characterization of a metastable dehydrate API phase for intravenous formulation development, J Pharm Sci. 2010;99(12):4973-81.
19. Sahitya G, Krishnamoorthy B, Muthukumaran M. Importance of preformulation studies in designing formulations for sustained release dosage forms. Int J Pharm Tech. 2013; 4(4): 2311-31.
20. Grant DJW, Higuchi T. Solubility Behavior of Organic Compounds, John Wiley.1990.
21. Karthikeyan K, Durgadevi R, Saravanan K, Shivsankar K, Usha S, Saravanan M. Formulation of Bioadhesive Carbomer Gel Incorporating Drug-Loaded Gelatin Microspheres for Periodontal Therapy. Tropical Journal of Pharmaceutical Research. 2012; 11 (3): 335-34.
22. Pandey MK, Singh GN, Sharma RK, Sneh S. Antibacterial activity of Eclipta alba (L.) Hassk. Journal of Applied Pharmaceutical Science. 2011;01 (07): 104-107.
23. Alves SH, Cury A. Estudo comparative entre as technicas de diluic ao em caldo para Candida. Revista de Patologia Tropical. 1992; 34: 259-262.
24. Jalalpure SS, Patil MB, Prakash NS, Hemalatha K, Manvi FV. Hepatoprotective activity of fruits of *Piper longum* L. Indian Journal of Pharmaceutical Sciences. 2003; 65, 363-366.
25. Saravanan M, Bhaskar K, Maharajan G, Pillai KS. Ultrasonically controlled release and targeted delivery of diclofenac sodium via gelatin magnetic microspheres. Int J Pharm 2004; 283:71-82.
26. Morton JJP and Malone MH. Evaluation of vulnerary activity by an open wound procedure in rats. Arch. Int. Pharmacodyn. Ther., 1972; 196: 117-126.
27. Kalyon R, Shivakumar H, Sarkar S. Wound Healing Potential of Leaf Extracts of *Ficus religiosa* on Wistar albino strain rats. *Int J Phar Tech Res*. 2009; 1(3): 506-508.
28. Rashed AN, Afifi FU, Disi AM. Simple evaluation of the wound healing activity of a crude extract of Portulaca oleracea L. growing in Jordan. J Ethnopharma. 2003; 88:131-136.
29. Manjunatha BK, Vidya SM, Krishna V, Mankani KL. Wound healing activity of

Leucas hirta. Indian J Pharma Sci. 2006; 68(3):380-384.
30. Srinivas BR, Kumar RKR, Naidu Vam, Madhu Sudhana K, Agwane SB, Ramakrishnan S, Diwan PV. Evaluation of antimicrobial, antioxidant and wound healing potentials of H. integrifolia. J Ethnopharm. 2008;115:249-256.
31. Sadaf F, Sallem R, Ahmed M, Ahmad SI, Navaidul-Zafar. Healing potential of cream containing extract of S.indicus on dermal wounds in guinea pigs. J Ethnopharm. 2006; 107:161-163.
32. Werner S, Breeden M, Hübner G, Greenhalgh DG, Longaker MT. Induction of keratinocyte growth factor expression is reduced and delayed during wound healing in the genetically diabetic mouse. J Invest Dermatol. 1994;103(4):469-73.
33. Bordawekar S, Kuvadia Z, Dandekar P, Mukherjee S, Doherty M. Interesting Morphological Behavior of Organic Salt Choline Fenofibrate: Effect of Supersaturation and Polymeric Impurity. Cryst. Growth Des. 2014; 14 (8): 3800–3812.
34. Lee, T.; Kuo, C. S.; Chen, Y. H. Solubility, Polymorphism, Crystallinity, and Crystal Habit of Acetaminophen and Ibuprofen by Initial Solvent Screening. Pharm. Technol. 2006, 30 (10), 72–92.
35. Venkatesan P, V. Sree Janardhanan, R.Manavalan, K.Valliappan. Preformulation parameters characterization to design, development and formulation of loxoprofen loaded nanoparticles International Journal on Pharmaceutical and Biomedical Research (IJPBR) Vol. 2(3), 2011, 107-117.
36. Lohcharoenkal W, Wang L, Chen YC, Rojanasakul Y. Protein Nanoparticles as Drug Delivery Carriers for Cancer Therapy. Bio Med Research International. 2014; 2014: 1-12.
37. Wei Y, Gao L, Wang L, Shi L, Wei E, Zhou B, Zhou L, Ge B. Polydopamine and peptide decorated doxorubicin-loaded mesoporous silica nanoparticles as a targeted drug delivery system for bladder cancer therapy. Drug Deliv. 2017; 24(1):681-691.
38. Hollister SJ, Porous scaffold design for tissue engineering. Nature Mater. 2005; 4: 518-524.
39. Yao ZQ, Ivanisenko Y, Diemant T, Caron A, Chuvilin A, Jiang JZ, Valiev RZ, Qi M, Fecht HJ. Synthesis and properties of hydroxyapatite-containing porous titania coating on ultrafine-grained titanium by micro-arc oxidation. Acta Biomater. 2010; 6(7):2816-25.
40. Fan X, B. Feng, Y. Di, X. Lu, K. Duan, J. Wang, J. Weng, Applied Surface.2012.
41. Nguyen AH, McKinney J, Miller T, Bongiorno T, McDevitt TC. Gelatin methacrylate nanoparticles for controlled growth factor release. Acta Biomater. 2015; 13:101-10.
42. Champion JA, Walker A, Mitragotri S. Role of particle size in phagocytosis of polymeric microspheres. Pharm Res. 2008; 25(8):1815-21.
43. Patel M, Jain SK, Yadav AK, Gogna D, Agrawal GP. Preparation and characterization of oxybenzone-loaded gelatin nanoparticles for enhancement of sunscreening efficacy. Drug Deliv. 2006; 13(5):323-30.
44. Chin SF, Azman A, Pang SC. Size Controlled Synthesis of Starch Nanoparticles by a Microemulsion Method. Journal of Nanomaterials. 2014; 2014: Article ID 763736. P:1-7.
45. Jayanthi B, Madhusudhan S, Mohanta GP, Manna PK. Preformulation Characterisation, Designing And Formulation Of Aceclofenac Loaded Microparticles ", Int. J. Drug Dev. & Res.2012, 4(3): 186-196.
46. Zhang M, Yang B, Liu W. Li S. Influence of hydroxypropyl methylcellulose, methylcellulose, gelatin, poloxamer 407 and poloxamer 188 on the formation and stability of soybean oil-in-water emulsions. Asian Journal of Pharmaceutical Sciences. 2017. In Press.
47. De Clercq K, Schelfhout C, Bracke M, De Wever O, Van Bockstal M, Ceelen W, Remon JP, Vervaet C. Genipin-crosslinked gelatin nanoparticles as a strategy to prevent postsurgical peritoneal adhesions: In vitro and in vivo characterization. Biomaterials. 2016; 96:33-46.
48. Bencherif SA, Braschler TM, Renaud P. Advances in the design of macroporous polymer scaffolds for potential applications in dentistry. J Periodontal Implant Sci. 2013; 43(6):251-61.

49. Lei B, K.H. Shin, D.Y. Noh, I.H. Jo, Y.H. Koh, W.Y. Choi, H.E. Kim, Nanofibrous gelatin-silica hybrid scaffolds mimicking the native extracellular matrix (ECM) using thermally induced phase separation, J. Mater. Chem.2012; 22: 14133-14140.
50. Guillen GR, Y. Pan, M. Li, E.M.V. Hoek, Preparation and characterization of membranes formed by nonsolvent induced phase separation: a review, Ind. Eng. Chem. Res.50 (2011) 3798-3817.
51. Solorio LD, Eran L. Vieregge b, Chirag D. Dhami a, Phuong N. Dang a, Eben Alsberg. Engineered cartilage via self-assembled hMSC sheets with incorporated biodegradable gelatin microspheres releasing transforming growth factor-β1. Journal of Controlled Release 158 (2012) 224–232.
52. Wang L, Kai-Chee Loh a, Yen Wah Tong Immobilization of growing Sphingomonas sp. HXN-200 to gelatin microspheres: Efficient biotransformation of N-Cbz-pyrrolidine and N-Boc-pyrrolidine into hydroxypyrrolidine derivatives. Journal of Biotechnology 182–183 (2014) 74–82.
53. Dinarvand R, Mahmoodi S, Farboud E, Salehi M, Atyabi F. Preparation of gelatin nanoparticles containing lactic acid--effect of cross-linking on drug release. Acta Pharm. 2005; 55(1):57-67.
54. Kim JY, Kawabori M, Yenari MA. Innate inflammatory responses in stroke: mechanisms and potential therapeutic targets. Curr Med Chem. 2014;21(18):2076-97.
55. Chitra S, Patil MB, Ravi K. Wound healing Activity of Hyptis suaveolens (L) Poi (Laminiaeae). Int J Pharm Tech Res. 2009; 1:734-744.
56. Ayyanar M, Ignacimuthu S. Traditional Knowledge of Kani tribals in Kouthalai of Tirunelveli hills, Tamil Nadu, India. Journal of Ethnopharmacology. 2005; 102:246-255.
57. Werner S1, Krieg T, Smola H. Keratinocyte-fibroblast interactions in wound healing. J Invest Dermatol. 2007;127(5):998-1008.
58. Wright JB, Hansen Dl, Burrell RE. The comparative efficacy of two antimicrobial barrier dressing: in vitro examination of two controlled release of silver dressing. Wounds. 1998; 10(6): 179-188.
59. Madsen SM, West H, Danielsen L, Rosdahl VT. Bacterial colonization and healing of venous leg ulcers. APMIS. 1996; 104:895-5.
60. Koziel J, Potempa J. Protease-armed bacteria in the skin. Cell Tissue Res. 2013; 351(2): 325-337.
61. Warriner R, Burrell R. Infection and the chronic wound: a focus on silver. Adv Skin Wound Care. 2005; 18(8):2–12.
62. Sorensen LT. Wound Healing and Infection in Surgery: The Pathophysiological Impact of Smoking, Smoking Cessation, and Nicotine Replacement Therapy. A Systematic Review. Annals of Surgery. 2012; 255(6): 1069-1079.
63. Bhaskar A, Nithya V. Evaluation of the wound-healing activity of Hibiscus rosa sinensis L (Malvaceae) in Wistar albino rats. Indian J Pharmacol. 2012;44(6):694-8.
64. Kahkeshani N, Farahanikia B, Mahdaviani P, Abdolghaffari A, Hassanzadeh G, Abdollahi M, Khanavi M. Antioxidant and burn healing potential of Galium odoratum extracts. Res Pharm Sci. 2013; 8(3):197-203.