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Research Article

In vitro and *In vivo* assessment of lawsone microsphere loaded chitosan scaffolds

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Abstract
Objective: To prepare, assess the lawsone loaded chitosan scaffolds for antibacterial and wound healing activity.
Methods: The work was focused is to develop a topical formulation preferably, natural biodegradable scaffolds of lawsone drug for treating skin wounds and bacterial infections. Here, lawsone loaded chitosan microspheres were impregnated into chitosan scaffolds. The lawsone microspheres prepared by emulsification and cross linking method. Incorporation of lawsone loaded chitosan microspheres into chitosan scaffolds by emulsification and freeze drying technique. The lawsone a microspheres characterized by FT-IR, SEM, evaluated through entrapment efficiency, percentage yield, in vitro drug release studies. Lawsone scaffolds was assessed for wound healing ability using albino rats and anti bacterial activity by agar disc diffusion method.
Results: the FT-IR spectra showing almost similar peaks within the same wave length range indicating the there is no possible interaction between the drug and polymer. The SEM of Lawsone microspheres and prepared scaffolds showing oval shape, slightly rough, porous surface, slightly aggregated and interconnected porous structure of the scaffolds was slightly reddish brown in colour. The lawsone loaded chitosan scaffolds had exhibited antibacterial activity against different bacrial strains and wound healing potential in albino rats.
Conclusion: The schematic process of lawsone loaded chitosan microspheres imparts quality in the in the topical formulation and obtained results were judicious.
Keywords: Lawsone, Bioavailability, Scaffolds, Microspheres, Chitosan

1. Introduction

In recent time there is an increase in global utilization of herbal medicine in the treatment of various diseases affecting human. The highly safety profile and low cost of herbal medicines have been reported as the major factors responsible for the increased upsurge in herbal medication. Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects.[1]

However, some limitations of herbal extracts/ plant actives like instability in highly acidic pH, liver metabolism has led to drug levels below therapeutic concentration in the blood resulting in less or no therapeutic effect. Incorporation of novel drug delivery technology to herbal active compounds to minimizes the drug degradation or pre systemic metabolism and serious side effects and improves the ease of administration in the pediatric and geriatric patients.

In phyto-formulation research, developing novel herbal dosage forms (polymeric nanoparticles and microspheres, nanocapsules, liposomes, solid lipid nanoparticles, phytosomes and nanoemulsion) have a number of advantages for herbal drugs, including enhancement of solubility and bioavailability, protection from toxicity, enhancement of stability, enhancement of pharmacological activity, sustained delivery, improving tissue macrophages distribution, protection from physical and chemical degradation. Thus, the nano sized novel drug delivery systems of herbal drugs have a potential future for enhancing the activity and overcoming problems associated with herbal medicines. [2]

In the present work we have designed to formulate a topical formulation for lawsone which is well-known drug as an antibacterial and wound healing agent. Lawsone chiefly obtained from the leafs of *Lawsonia innermis* which has been used in traditional medicine for over 9,000 years as curative agent against a variety of ailments and significantly reported to have anti microbial, wound healing activity, anti-inflammatory, analgesic, hypoglycemic, hepatoprotective, immunostimulant, antidermatophytic, antioxidant, and anticancer properties. It is now considered as a valuable source of unique natural product for development of medicines and also for the development of industrial products [3, 4].

The principal chemical compounds present in whole plant are lawsone and 2-hydroxy-1, 4 napthoquinone which are enormously liable for the specific therapeutic actions. Chemically Lawsone is 2-hydroxy1,4-napthoquinone phenol compound[5]. As the data of thorough literature, the crude leaf extracts and pure lawsone drug doesn't show maximum therapeutic potency as an antibacterial and wound healing agent, because of its less skin absorption levels [6]. Thus, in order to get the better therapeutic efficacy and bioavailability, topical formulation preferably lawsone loaded chitosan microspheres were impregnated into chitosan scaffolds and evaluated for various parameters. Scaffolds are one of the most promising and popular types of wound dressings. Scaffolds of natural origin are preferred over synthetic scaffolds, among them, chitosan based scaffolds were selected.

So the present research work is focused to improve bioavailability and therapeutic potency by applying the principles of novel drug delivery system (NDDS) for lawsone. NDDS is an advance drug delivery system which improves drug potency, stability, site specific targeting with a controlled drug release/ sustained release with high therapeutic index, in turn with greater bioavailability.

2. Materials and methods

2.1. Compatibility studies for drug and polymer

Infra red spectra of lawsone and chitosan were measured by FTIR spectrometer Analytical model- 2202, using KBr pellets from 400 to 4000cm^{-1} with a scanning speed of 2 mm/s 40.

Preparation of KBr disc: Initially a pinch of Potassium Bromide was taken into a granite made motor and pestle and a pinch of pure drug was added, mixed well and this is placed in the disc holder and made into a fine film by the aid of Hydraulic press and this disc was placed in the sample holder and is finally analyzed by the IR instrument. Similarly separate disc was prepared for physical mixture of lawsone and chitosan which was analyzed by using IR instrument.

2.2. Thermal Degradation Test by Melting Point Apparatus

The melting point of the lawsone was determined by taking a small amount of drug in a capillary tube, placed in the melting point apparatus, and the apparatus is turned on and the reading was noted.

2.3. Solubility Studies

Solubility of lawsone was determined by using various solvents like water, ethanol, methanol, acetone and chloroform.

2.4. Procedure for preparation of lawsone microspheres:

Lawsone loaded chitosan microspheres were prepared by emulsification and chemical cross linking method by following steps are involved in the preparation [7]:

Preparation of drug solution: accurately weighed quantity of lawsone was taken in a beaker, and drug was dissolved by adding few drops of isopropyl alcohol.

Preparation of polymer solution: accurately weighed quantity of chitosan polymer was dissolved in 3% glacial acetic acid solution and stirs it for 1 hr. Then added to previously drug solution.

Preparation of organic phase: to prepare an organic phase, 2000ml of light liquid paraffin oil was stirred in the magnetic stirrer at 2000rpm and added tween 80 (2ml) surfactant.

Preparation of microspheres**:** the organic phase of oil solution along with surfactant was kept under magnetic stirrer at 2000rpm. To this mixture, drug and polymer solution was added drop wise and it was stirred for 1 hr ad then to this solution 25% of glutaraldehyde solution added and stirring was continued for 1 hr and saturated toluene was added. The produced microspheres washed with acetone foe three times and filter the microspheres dried in hot air oven for 2 hrs at 50^0C.

2.4.1. Particle size Determination lawsone microspheres

The particle size and morphology of the prepared microspheres was done by using the Scanning Electron Microscopy (SEM) at an accelerating voltage of 10kV. 9.3mm x 100SE. And the particle size determination of the microspheres was done by using Particle Size Analyser also called as Dynamic Light Scattering which elucidates the mean size of the microparticles within the sample which is indicated by the peak obtained at a desired range of size in microns [8]. Here the little quantity of sample was dissolved in the water and placed in the sample container and the particle size was analyzed at an angle of detection of 90^0.

2.4.2. Entrapment efficiency & Percentage yield

The drug entrapped in the lawsone loaded chitosan microspheres was determined by calculating the difference between the total and the free drug concentrations in the microsphere suspension and the supernatant respectively. The amount of free drug present in the supernatant (w) was assayed by UV-spectrophotometer (Shimadzu UV-1800) at 420 nm. The amount of drug in supernatant was then subtracted from the total amount of drug added (W). In effect, (W-w) will give the amount of drug entrapped. The percentage drug entrapment was calculated using the equation (1) [9]. The percentage yield of the prepared microspheres was calculated by dividing the practical yield (product weight) of microspheres divided by the total amount of all non-volatile components which were used for the preparation of the microspheres.(i.e., drug + excipients) and is calculated according to the equation (3) [10].

Percentage drug entrapment = (W – w) x *100* ------ (1)

Percentage (%) Yield = (Actual weight of product / Total weight of excipients and drug)×100 -----(2)

2.4.3. In-vitro **Drug Release Study**

In-vitro release profile of lawsone from the microspheres was examined in phosphate buffer solution (pH6.8) using USP (XXI) six stage dissolution rate test apparatus (Electro lab TDT-08L). Microspheres equivalent to 100 mg of drug packed in filter paper and was suspended in dissolution medium at 50 rpm and 37 ± 0.5°C. An aliquot of 1 ml was withdrawn periodically at intervals of one hour and same volume of fresh medium was replaced. The samples were filtered through what man filter paper and analyzed spectrophotometrically at 420 nm for amount of drug released.

2.5. Preparation of microspheres impregnated Chitosan scaffolds

Chitosan solution in the concentration of 2% (w/v) was prepared by dissolving chitosan flakes in 0.2 M acetic acid during overnight by stirring. Chitosan solutions were poured into plastic moulds; they were frozen at 20^0C for 24 hr and then, transferred into freeze-drier to obtain highly interconnected porous scaffolds. For stabilization, lyophilized scaffolds were rehydrated in 96% (v/v) ethanol over- night and then in 70% (v/v) ethanol for 1 hr. Incorporation of lawsone loaded chitosan microspheres into chitosan scaffolds was performed by emulsification and freeze drying technique. In this method, 100mg of lawsone loaded chitosan microspheres were homogenized with 50ml of 2% chitosan solution and then poured into moulds. Then, moulds were frozen at 20^0C for 24 hr and then freeze-dried at -80^0C for 2 days [11].

2.6. Characterization of prepared scaffolds

2.6.1. Physical characterization

The prepared scaffolds were examined for physical characterization like colour of the scaffolds before and after loading microspheres, shape, size, etc.,

2.6.2. Morphology and pore size distribution

The morphology and pore size distribution of chitosan was determined by using the Scanning Electron Microscopy (SEM) at an accelerating voltage of 15.0kV.

2.6.3. Swelling studies

Degrees of swelling of the chitosan scaffolds were measured at 25^0C, 32^0C, 37^0 C. Scaffolds of diameter 5.02cm^{2}were dispersed in 25ml of distilled water and incubated for 5hr at each of the above mentioned temperatures. At each temperature a Nylon filter membrane was moistened with distilled water and weighed using a weighing bottle which had an airtight cap to prevent evaporation of water during the weighing process. The sample was then filtered using the same filter membrane at the designated temperature. The filter membrane was weighed using the same weighing bottle. The degree of swelling was calculated using the fallowing equation

Degree of Swelling = Ww-Wd / Wdx 100 --------- (3)

2.6.4. Homogeneity of lawsone microspheres in scaffolds

When randomly cut, four equal sized (2.02cm2) contours of microspheres impregnated chitosan scaffolds were subjected for lawsone content, they showed a good homogenous drug distribution pattern with slightly negligible difference as shown in the Table 1.

2.6.5. Release studies of scaffolds

In-vitro release studies were carried out with lawsone microspheres loaded chitosan scaffolds in 2 ml eppendorf tube with PBS, at 37^0C with 15 rpm agitation. In the studies, chitosan scaffolds of 10 mm diameter and 1 mm thickness were used. At the specific time intervals, complete solution was removed and filtered with 0.45 mm filter. Lawsone concentration was determined using UV spectrophotometer by measuring absorption at 420 nm. Buffer solution in the Eppendorf tubes was replenished after each measurement. All experiments were performed in triplicates [11].

2.7. *In-vitro* Antibacterial activity

The antimicrobial activity of microspheres impregnated chitosan scaffold was done by agar disc diffusion/ filter paper disc method. Nutrient broths with bacterial inoculums were incubated at16h at 37^0C. Bacterial broth for each strain was diluted in 1ml normal saline (0.89% NaCl). Bacterial inoculums 0.1ml inoculated on solid Nutrient agar in petriplates. The bacterial inoculums were spreaded by glass spreader until totally absorbed in agar layer for the development of uniform bacterial growth [12,13]. The culture plates seeded with test organisms were allowed to solidify and the scaffold was placed and gently pressed on to the surface of the agar medium. The plates were then incubated at 37°C for 48 hrs. The zones of inhibition were measured and recorded. The test organisms selected for this study are *Bacillus subtilis (Gram positive), E. coli (Gram negative),* because these are the commonly used bacterial strains that make the wound healing process slow.

2.8. Wound healing activity of lawsone microspheres loaded chitosan scaffolds

2.8.1. Selection of animals

Wistar rats of either sex weighing 150–200 g were used in the study, after obtaining the approval of the Institute's Animal Ethics Committee (SPSP: 1016/PO/E/S/CPC SEA/2016/009). Animals were fed on a standard pellet diet and water *ad libitum* and maintained at 24–28°C temperature and relative humidity (30% - 70%). Animals marked as fasted were deprived of food for 16 hours, but had free access to water.

2.8.2. Grouping of animals

Two wound models were used for the study, excision wound model and incision wound model, comprise three animals in each group. The groups were:

Group 1: Excision wound model Control which is not treated with the Lawsone scaffold (n=3)

Group 2: Incision wound model Control which is not treated with the Lawsone scaffold (n=3)

Group 3: Animals were used for Excision wound model (n=3)

Group 4: Animals were used for Incision wound model (n =3)

Group 5: Animals treated with Betadine ointment (n=3)

2.8.3. Excision wound model

The wound site was prepared following the excision wound model. Two groups of three animals each were used. The rats were anesthetized prior to and during infliction of the experimental wounds. The surgical interventions were carried out under sterile conditions using diethyl ether. Wound of 300 sq. mm on dorsal lumbar region was made. Animals were closely observed for any infection and those which showed signs of infection were

separated and excluded from the study and replaced. The scaffolds containing lawsone of 100mg which was loaded in chitosan microspheres were applied on the wound area and the animals were observed for wound closure at 0^{th}, 5^{th}, $10^{th}$and 15^{th}days and for period of epithelialization[14].

% Wound contraction=final area/initial areaX100

2.8.4. Incision wound model

In incision wound model, 2 cm long paravertebral incision were made through the full thickness of the skin on either side of the vertebral column of the rats, after all the animals of each group were anesthetized under light ether anesthesia. No local or systemic antimicrobials were used throughout the experiment. All groups were treated same as in excision model, the both edges kept together and stitched with Black silk surgical thread and a curved needle was used for stitching. The continuous threads on both wound edges were tightened for good closure of the wound. After stitching, wound was left undressed, and then chitosan loaded lawsone scaffolds were applied topically on the wound. Animals were observed.

2.8.5. Statistical analysis

The data are expressed as the mean ±S.E.M. The difference among means has been analyzed by one-way ANOVA. A value of $p < 0.001$ was considered as statistically significant.

3. Results

3.1. Compatibility studies FTIR study:

The FTIR spectra of drug, polymer and their physical mixture are shown in Fig 1, 2 & 3. From the FTIR spectra of drug, polymer and their physical mixture shows almost similar peaks within the same wavelength range indicating the presence of similar functional groups in both the chemical structures thus suggesting that there is no possible interaction between the drug and polymer during the process.

3.1.2. Thermal degradation by melting point apparatus:

The melting point of the drug measured by using Melting Point Apparatus was found to be 195^0C.

3.1.3. Solubility studies

Lawsone was freely soluble in methanol, 0.1N HCl, Glacial acetic acid, insoluble in water.

3.1.4. Particle size determination and characterization of lawsone microspheres

The mean particle size of microspheres prepared in this study was in the range of 66.7µm. Fig 9 shows typical particle size distribution. The prepared microspheres were free flowing, slightly yellow colored powder. The colour of the microspheres changed from yellow to yellowish orange when cross-linking time and concentration of glutyraldehyde-saturated toluene increased. This phenomenon may be caused by increasing the degree of chitosan cross-linking. Fig 4 elucidates the SEM photograph of the chitosan microspheres treated with glutyraldehyde. As may be seen, spherical to oval microspheres with slightly rough and porous surface were formed. The SEM picture indicates that the microspheres were slightly aggregated and the appearance of the microspheres surface and their mean particle size were only slightly affected by the experimental cross-linking conditions (time and amount of the cross-linking agent).

3.1.5. Entrapment efficiency, drug content & percentage yield

The average percent drug entrapment efficiency was found to be 71.2±2.92– 85.2±2.01%. The drug content of the microspheres was found to be 75.2-82.9 %. The Percentage yield of the prepared microspheres formulations was found to be 83.8-92.03 %.

3.1.6. *In-vitro* Drug Release Study

The percentage cumulative amount of the drug released from the microspheres was found to be 94.5 % in 8 hr.

3.2. Physical Characterization scaffolds

The scaffolds were red in colour with good porous nature & uniform diameter and shown in fig 6.

3.2.1. Morphology and Pore size distribution

It is known that the microstructure such as pore size and its distribution, porosity as well as pore shape has prominent influence on cell intrusion, proliferation and function in tissue engineering. The cross section morphology of chitosan scaffold is shown in fig 5. The interconnected porous structure of the scaffolds was influenced by the presence or absence of cross linking agent.

3.2.2. Porosity/ Water uptake/ Swelling studies

The ability of a scaffold to preserve water is an important aspect to evaluate its property for skin tissue engineering. The water binding ability of the chitosan scaffold could be attributed to both of their hydrophilicity and the maintenance of their structure. The swelling property of the scaffolds is mainly influenced by the presence or absence of the cross linking agent. The scaffolds treated with cross-linking agent exhibits low swelling index than the uncross-linked one. However the cross linked scaffolds did not show obvious difference regardless of the glutyraldehyde concentration. Similarly the swelling property and the cross-linking property is also influenced by the duration and temperature of the freeze-drying conditions, and the refreezing-drying procedure will cause the reduction of the porosity, hence the volume for water storage, leading to the decrease of the swelling capacity. However the scaffolds with the fallowing swelling index are high enough for skin tissue engineering. Further from table 1, it is noted that the swelling property increases with the increase in the temperatures.

3.2.3. Homogeneity of lawsone microspheres in chitosan scaffold

When randomly cut, four equal sized (2.02cm2) contours of microspheres impregnated scaffolds were subjected for lawsone analysis, they showed a good homogenous drug distribution pattern with slightly negligible difference.

3.2.4. Drug release studies from scaffolds:

The release of the drug from the scaffold was slightly delayed when compared to the microspheres alone. The distribution of the microspheres within the scaffold is uniform and achieved good homogenity. The release profile of microspheres from various scaffolds is shown in the fig 7. From the graph it was noted that the release of the drug from the microspheres loaded scaffold was immediate at acidic pH and the release at other pH was prolonged. The release from the microspheres probably takes place first into the pores present in scaffold and this prolongs the release of the molecules from the fibrous scaffold. Also, chitosan is a natural protein; it is also known as rapid biodegradable material. This study demonstrated that biodegradation rate of chitosan microspheres decreased when they incorporated into the scaffolds. The cumulative drug release values from lawsone loaded chitosan scaffolds were given in table 2.

3.3. *In-vitro* Antibacterial activity of the chitosan scaffold

The antimicrobial activity on different bacterial strains such as *E. coli, Bacilus subtiliss* was done for the chitosan scaffold impregnated with lawsone microspheres showed a high resistance zone formation (i.e., Zone of Inhibition) of about 12-14mm, where this zone formation is due to the release of the drug from the scaffold following diffusion phenomena. The Antimicrobial activity is done only to confirm whether the drug is released from scaffold and to check the resistance to the common bacterial species. Here the antibacterial activity is done for two different bacterial species belonging to categories i.e., Gram *+ve*, Gram *–ve* species and the chitosan scaffold exhibited very good antibacterial activity and finds its way in wound healing applications.

3.4. Effect on excision and incision wound

The results of excision wound model are show in table 3. The lawsone loaded chitosan scaffolds exhibited significant wound healing activity as compared to control in excision wound model shown in fig 8 and 9. The Percentage closure of original wound area was calculated at different time intervals. The measurement on 5^{th} and 10^{th} day showed that the percentage closure of the original excision wound area was found to be 14.8 and 02.86 (control group), 5.033 and 1.48 (lawsone scaffolds). The tested extracts significantly ($p < 0.0001$) promoted wound closure compared to control. On 15^{th} day the extent of percentage wound closure was 02.35 (control group), 0.43 (lawsone scaffolds). It was observed that the wound contracting ability of the scaffold treated groups showed significant wound healing from the 5^{th} day onwards. The wound closure time was lesser; as well as the percentage of wound contraction was more with the scaffold treated group. The epithelisation of wound with scaffold treated groups was found to be earlier as compared to control. In the scaffold treated group rats, wounds were completely healed in 15 days where as in the control animals it took more 20 days.

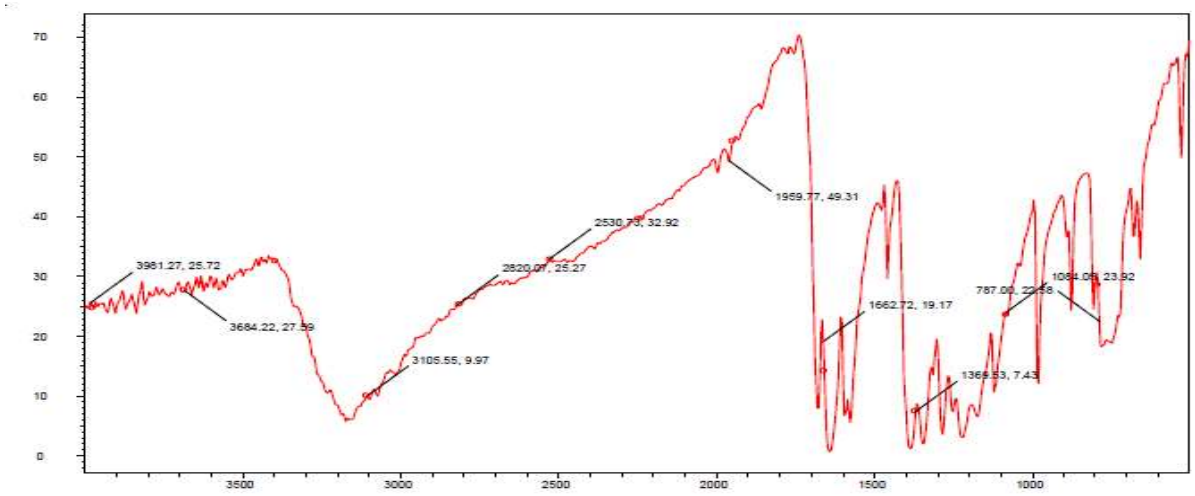

Figure 1: FTIR spectrum of lawsone

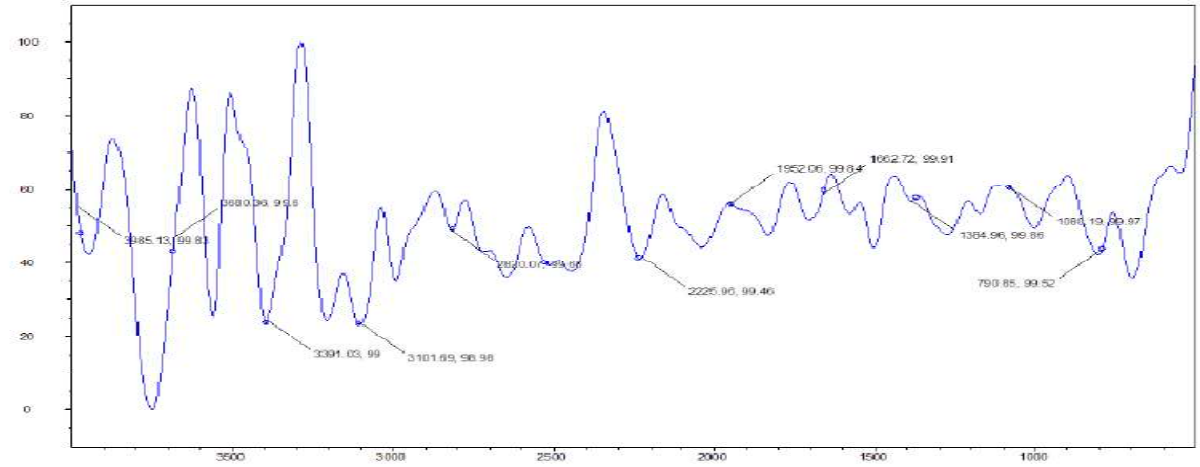

Figure 2: FTIR spectrum of chitosan

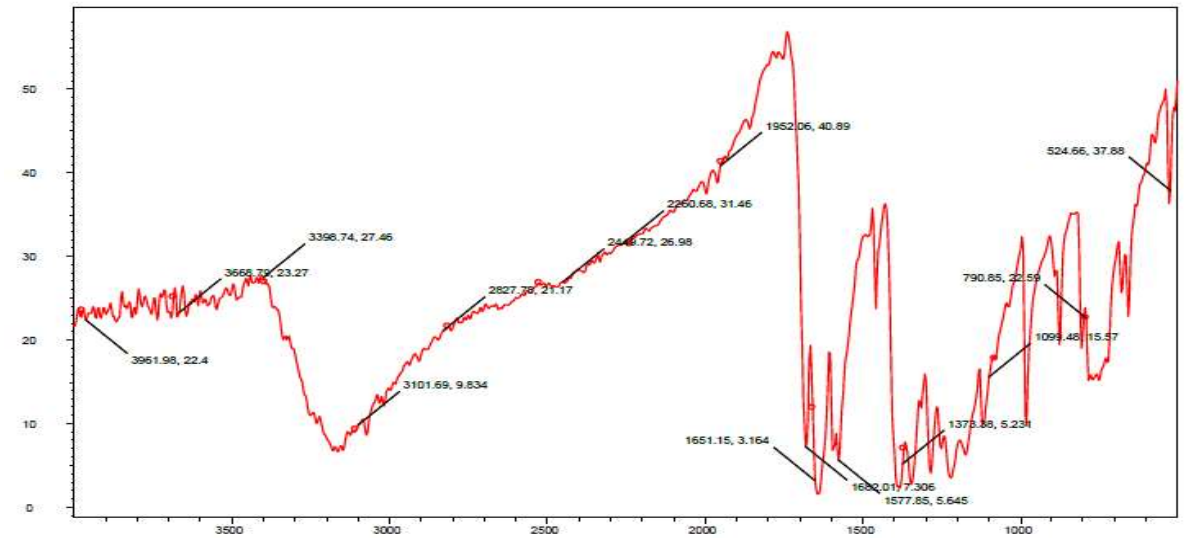

Figure 3: FTIR spectrum of physical mixture of lawsone and chitosan

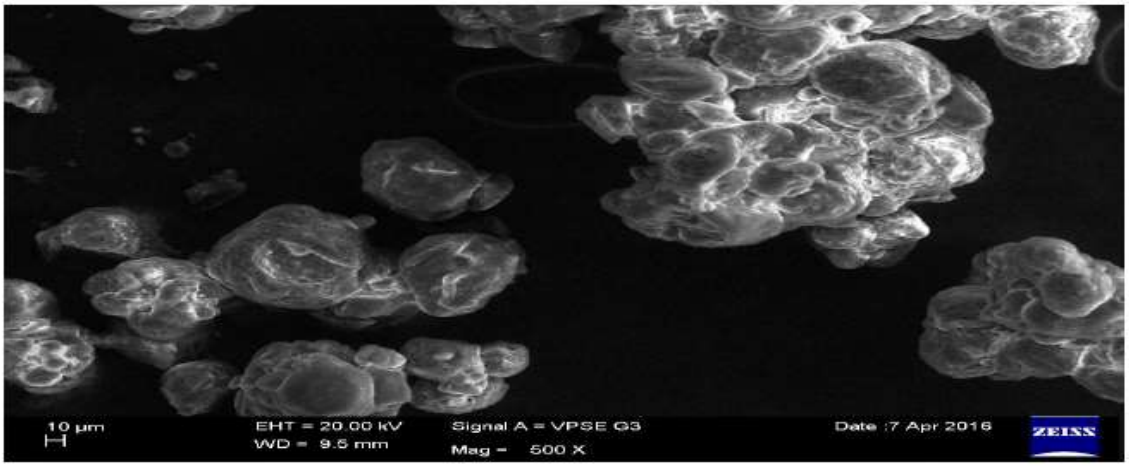

Figure 4: SEM images of lawsone microspheres

a) Chitosan plain scaffold b) Microsphere loaded scaffold

Figure 5: Morphology of scaffolds

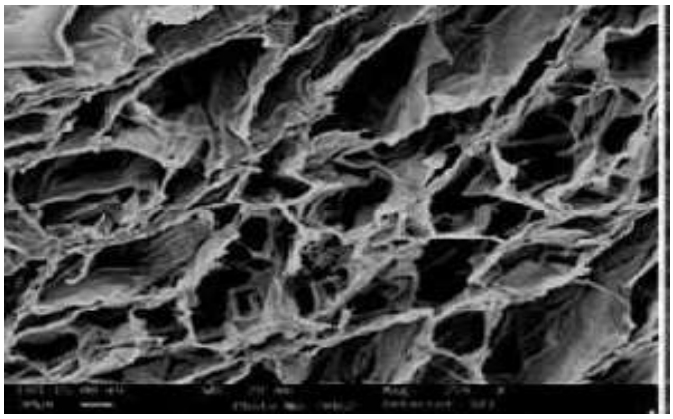

Figure 6: SEM image of chitosan scaffold

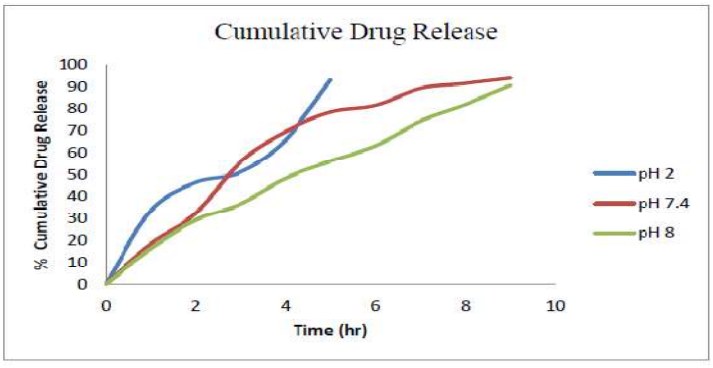

Figure 7: drug release of scaffolds at different pH's

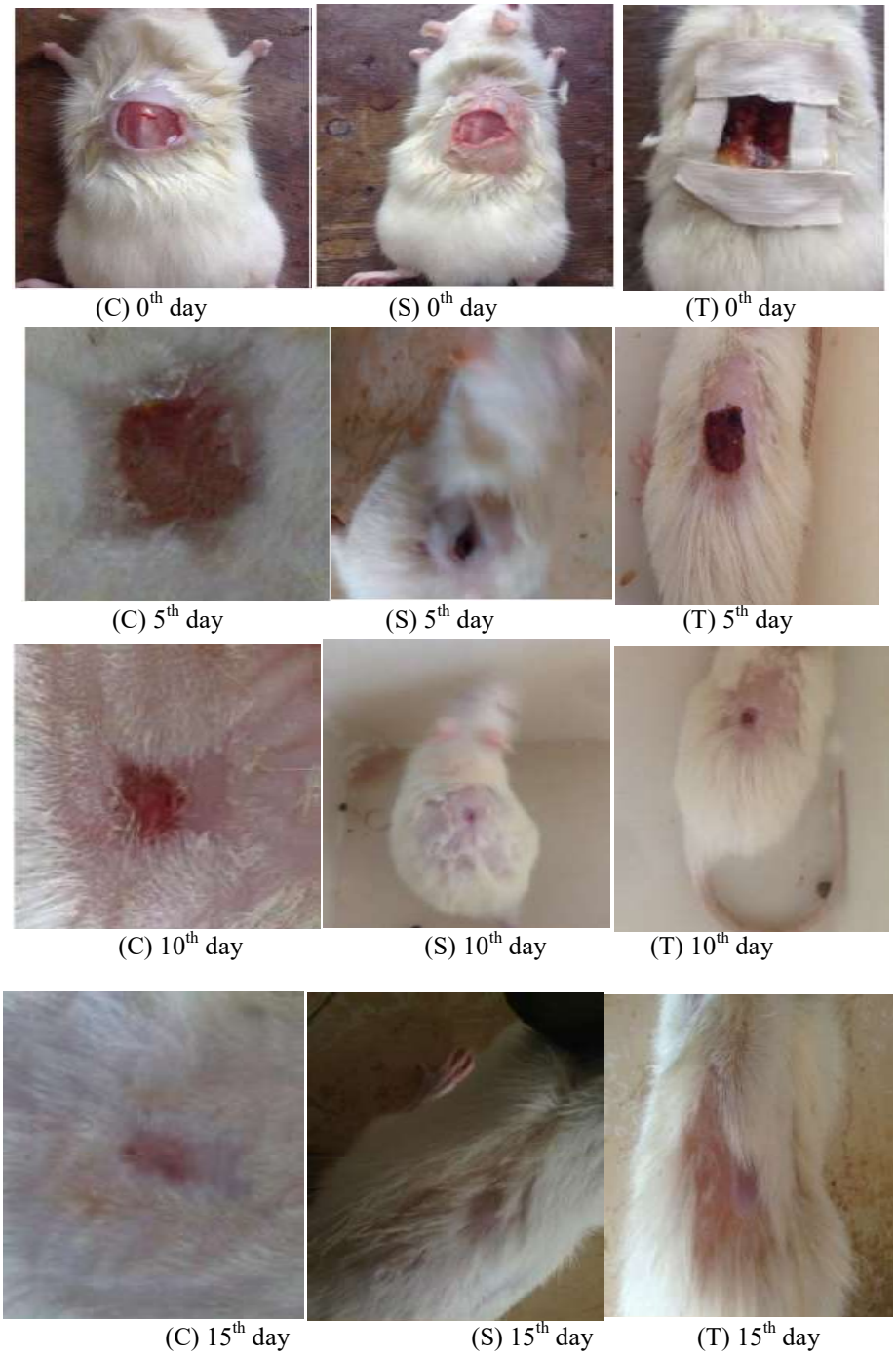

Figure 8: Comparison of wound site by excision wound model in control (C), standard (S), and lawsone chitosan scaffolds treated (T) on 0th day, 5th day, 10th day, 15th day intervals

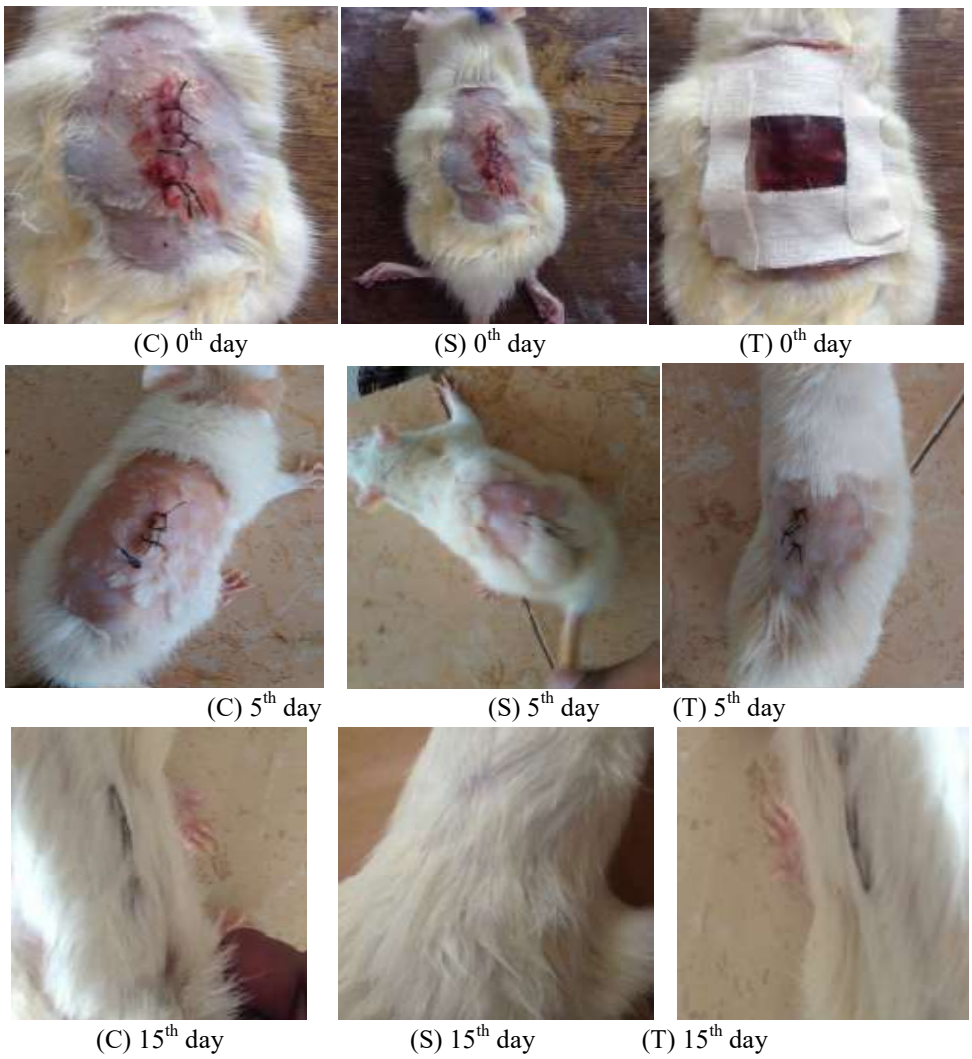

Figure 9: Comparison of wound site by incision wound model in control (C), standard (S), and lawsone chitosan scaffolds treated (T) on 0th day, 5th day, 15th day intervals

Table 1: swelling studies and drug content of chitosan scaffolds

No of counters	Swelling studies			Drug content
	25^0C	32^0C	37^0C	
1	40%	51%	64%	30.6mcg
2	42.43%	40.56%	68.61%	36.4mcg
3	42.01%	56%	67.49%	41.0mcg

Table 2: Cumulative drug release from chitosan scaffold

Time (hr)	Cumulative drug release from chitosan scaffolds		
	pH-2	pH-7.4	pH-8
1	33.4	18.4	16.23
2	46.3	32.3	29.24
3	51.0	55.6	36.54
4	86.6	69.4	48.23
5	93.5	78.3	36.06
6	96.3	81.4	62.93
7		89.33	74.03
8		91.69	81.87
9		94.23	90.78

Table 3: effect of lawsone scaffolds on healing of excision wound model (in mm^2)

S. No	Groups	0^{th} day	5^{th} day	10^{th} day	15^{th} day
1	Control	22.48±0.009	12.30±0.166	08.06±0063	02.35±0.19
2	Standard (Betadine)	29.72±0.0086	14.18±0.014	2.86±0.017	0.23±0.004
3	Test (LLCS)	28.65±0.007	5.033±0.0202	1.48±0.013	0.42±0.004

Values are the ±SEM, one way ANOVA, a***p<0.0001;in parenthesis values showing percentage closure of original excision wound, LLCS-lawsone loaded chitosan scaffolds

4. Discussion

The enormous therapeutic potential of herbal drugs should be explored through some value added drug delivery systems. For oral and topical administration of drug molecules, lipid solubility and molecular size are major limiting factors for crossing the biological membrane and entering the systemic circulation. Several plant extracts and phytomolecules, despite having excellent bioactivity *in vitro* demonstrate less or no *in vivo* actions due to their poor lipid solubility or improper molecular size or both, resulting poor absorption and poor bioavailability. Hence, there is a great potential in the development of novel drug delivery systems for the plant actives and extracts.

The prepared formulation was fulfilled with the results obtained. Many studies indicate that plant products are potential drugs as anti bacterial and wound healing agents and largely preferred. In this work, we showed the applications NDDS to lawsone bio active compound to emphasize its anti bacterial activity and wound healing activity of by natural and biodegradable polymer chitosan loaded scaffolds.

The lawsone loaded chitosan microspheres prepared by emulsification-chemical cross linking method were spherical to oval in morphology, slightly aggregated with porous surface, exhibited a good entrapment efficiency of 71.2±2.92 to 85.2 ±2.01, drug content of 75.2-82.9%, high percentage yield of 83.8-92.03% and showed the drug release for a period of 8hr. Later on the optimized microsphere formulation was impregnated into chitosan solution and fabricated into scaffolds. The plain scaffolds were white in colour where as the microspheres loaded scaffolds were slightly reddish brown in colour, the scaffold exhibited good homogeneity and acceptable swelling index with an intercalated porous network like structure when analyzed by SEM. The release of the drug from the scaffolds was slightly extended (9hr) when compared to microspheres alone.

Microspheres loaded chitosan scaffolds exhibited good in-vitro antibacterial activity with a zone if inhibition of 12-14mm.

Wound healing is characterized by three stages, viz., inflammation, proliferation, and remodeling. The proliferative phase typically demonstrates angiogenesis, collagen deposition,
granulation tissue formation, epithelialization and wound contraction. In angiogenesis, new blood vessels grow from endothelial cells. In fibroplasia and granulation tissue formation, fibroblasts grow and form a new provisional extracellular matrix by excreting collagen and fibronectin. In epithelialization, epithelial cells crawl across the wound bed to cover it. Fibronectin, the major glycoprotein secreted by fibroblasts, has important functions of chemoattraction for macrophages, fibroblasts and endothelial cells, promoting re epithelialization and acting as a transduction agent in wound contraction. Wound contraction occurs by myofibroblasts, which establish a grip on the wound edges, bringing them in apposition. The *in-vivo* results manifest the potent wound healing activity of scaffolds as evident from the wound contraction. Furthermore, the period of epithelialization was shorter in the treated wounds. Topical application of scaffolds is effective in faster wound contraction due to the larger availability at the wound site. In excision and incision wound model, scaffolds showed prominent significance ($p<0.0001$) and faster wound contraction compared with control groups.

The present study demonstrated that, the wound healing property was effective and faster for topically applied lawsone microspheres loaded chitosan scaffolds than the natural wound healing mechanism and conventional antibacterial therapy alone. Finally it is concluded that, the aim of formulation and impregnation of lawsone loaded chitosan microspheres into a natural, biodegradable polymeric scaffolds was achieved and the results obtained were satisfactory.

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