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Evaluation of Antibacterial and In vivo Wound healing activity of Carbon Dot Nanoparticles

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ABSTRACT: Treating post-surgical wound is one of the major challenges in the field of medical Science due to the several disadvantages posed by the antibiotics. The antimicrobial and Wound healing activity of metal based nanoparticles were well known, but the effects of carbon dot nanoparticles (C-Dots) are less explored. In the present study a wet chemical method for the synthesis of C-Dots from sucrose, glucose and fructose was developed and they were characterized by UV-Visible, Fluorescent spectroscopic techniques and the pharmaceutical applications such as antibacterial and the wound healing activities were evaluated. The study revealed that the C-Dots synthesized from Sucrose (SCD) and Glucose (GCD) showed potent antibacterial activity against both positive and negative bacterial strains at 12.5µl/ml (0.1 ± 0.003) and Fructose (FCD) at 50 µl/ml (0.1 ± 0.01) compared to the vehicle Control (0.61 ± 0.06). The wound healing of SCD (367.8 ± 15.2) was observed better than the Control (280.8 ± 10.72) and FCD (326.8 ± 9.41). GCD (166.8 ± 10.83) skin tissues indicated best healing as compared to FCD (135.8 ± 8.29), SCD (157.3 ± 16.97) and Control (135.8 ± 8.29). The results suggest that C-Dots applied topically possess wound healing activity and have potential applications as a bacteriostatic agent.

Keywords: C-Dots, Microorganisms, antibacterial activity, wound healing activity, Toxicity, Collagenation, Epithelialization, Haemostasis

1. Introduction

Healing of wounds usually takes place in a direction away from its normal course and under- healing, over-healing or no healing of wounds is common.

The emergence of nanotechnology has provided a new therapeutic modality in nanoparticles for use in reduction of wound inflammation, antimicrobials, field of diagnostics [1]. Nanotechnology is gaining tremendous impetus in the present due to its capability of modulating metals and non metals to their nanosize which drastically changes its physical, chemical and optical properties. Antibiotics also known as antibacterials are the drugs used to treat infections. It so happens that some antibiotic cause hypersensitivity and allergic reactions in people. Most anti-microbial drugs, antibiotics included, cause toxic side effects [2]. And these side effects can sometimes prove to be more difficult to manage than the ailment they are meant to cure. Their Long-term health hazards are not known. Another major drawback is the development of resistance to antibiotics by bacteria [3]. Bacterial resistance is the reduced ability of an antibiotic to stop the bacteria causing disease in the host human. Especially, the rise in multidrug resistance among bacterial pathogens has threatened the effective prevention and treatment of bacterial infections, the realm of traditional antibiotics/antimicrobial agents is difficult to meet today's society's expectations. This has motivated a global search for alternative strategies, such as nanotechnology, photo-activated antimicrobial technology [1].

Nano materials in recent years have emerged as a solution that acts as a good substitute for antibiotics for the treatment of wounds. NPs have been shown to possess unusual physical, chemical and biological properties [4]. Nanoparticles (NPs) are defined as particles having one or more dimensions in the order of 100 nm or less. Many researchers found application that Silver nano particles are toxic to bacteria and are currently used in everything from medical devices to sport socks and washing machines to detect microbial growth. Even though Ag NPs-containing dressings are declared to be safe for patients and non-cytotoxic [5], recent studies have shown possible toxic effects on human fibroblasts and keratinocytes [6]. All these disadvantages necessitate the development of new alternatives that can overcome the setbacks of conventional antibiotics and other Nanoparticles.

The Non Metallic Carbon dots nanoparticles synthesized by chemical method and characterized are non toxic and their enormous application in bioimaging can act as an excellent substitute for antibiotics as they possess antibacterial properties and can accelerate skin wound healing.

Most of the antibiotic resistance mechanisms are irrelevant for nanoparticles (NPs) because the mode of action of NPs is direct contact with the bacterial cell wall, without the need to penetrate the cell; this raises the hope that NPs would be less prone to promoting resistance in bacteria than antibiotics.[7] Therefore, attention has been focused on new and exciting NP-based materials for various therapeutic purposes and different pharmacological evaluations [2]. C-dots are relatively nontoxic and easy to synthesize compared to conventional quantum dots which are based on inorganic semiconductors that contain heavy metals, such as Au, Ag, Cd, In, Sn etc. It is well-known that the heavy metals are toxic hence their application is limited. Management of under healing of wounds is a complicated and expensive program and research on drugs that increase wound healing is a developing area in modern biomedical sciences.[8] Several drugs obtained from natural sources are known to increase the healing of different types of wounds.[9,10] Though some of these drugs have been screened scientifically for evaluation of their wound healing activity in different pharmacological models and patients, the potential of many of the traditionally used natural agents remains unexplored. In few cases, active chemical constituents were identified [11-13]

In the present investigation, non-metallic C-Dot NPs were synthesized by wet chemical method from three different compounds namely glucose, sucrose and fructose. The synthesized nanomaterials were characterized with UV-Visible and fluorescence spectroscopic techniques. The antimicrobial activity of the synthesized nanoparticles was tested using the standard micro dilution method, which determines the minimum inhibitory concentration (MIC) leading to the inhibition of bacterial growth. The antibacterial activity of C-dots from sucrose, glucose and fructose was Screened by 96 well Microtitre plate broth dilution method against four bacterial strains - Escherichia coli (ATCC 25922) (gram negative), Klebsiella pneumonia (ATCC 13883) (gram negative), Staphylococcus aureus (ATCC 29213) (gram positive), Staphylococcus epidermidis (ATCC 35984) (gram positive) respectively. The clinical isolates were identified following the standard method. The three wound healing models (incision, excision and dead space wound) were evaluated invivo from the synthesized C-Dots by topical application on the Male albino wistar rats to study the percent of wound contraction, breaking strength and dry tissue weight under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA)

2. Materials and methods

2.1. Micro organisms

Escherichia coli (ATCC 25922) (gram negative), *Klebsiella pneumonia* (ATCC 13883) (gram negative), *Staphylococcus aureus* (ATCC 29213) (gram positive), *Staphylococcus epidermidis* (ATCC 35984) (gram positive) strain

2.2. Chemicals used

Sucrose, Glucose, Fructose, Orthophosphoric acid, Cation adjusted Muller Hinton Broth [CAMHB], Ciprofloxacin(Reference Drug), Acidified water, DMSO, Ketamine injection was procured from Neon laboratories. (Mumbai, India) and xylazine was from Indian Immunological Ltd. (Guntur, India).

2.3. Experimental animals

Male albino Wistar rats weighing between 180-220 g were used. The animals were caged individually after wounding for treatment till completion of wound healing. In each group of different models six animals were used. The experimental protocol was approved by Institutional Animal Ethics Committee and animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA)

2.4. Synthesis of C-Dots

C-dot nanoparticles (C-Dt NPs) were synthesized by acidic oxidation of sucrose, glucose and fructose in separate glass beaker with H_3PO_4at 60^oC till colour changes to the formation of yellow C-dots. In this method 0.698g of sucrose, 0.1396g of glucose and fructose were dissolved in 150ml distilled water in three different glass beakers, then 0.106ml of phosphoric acid was added to each beaker and kept on the heating mantle at 60^oC till the colour changed to pale yellow. The synthesized nanoparticle solutions were then transferred to dialysis bag and subjected for 24 hours of dialysis treatment.

2.5. Characterization of C-Dots

Characterization studies such as UV spectrophotmeter (Elico BL165), Fluorescence spectrophotometer (Spectrofluorometer F-2700 Hitachi) were used to analyze the structure and the composition, surface morphology and size and shape respectively of synthesized carbon dots using chemical method.

2.6. Antibacterial activity of C-Dots

Micro dilution method was used for determination of MICs as per the National Committee for Clinical laboratory Standards and Clinical and Laboratory Standard Institute. The MIC assay will be performed in a 96 well microtitre plate. One day before the initiation of experiment the *Escherichia coli* (ATCC 25922) (gram negative), *Klebsiella pneumonia* (ATCC 13883) (gram negative), *Staphylococcus aureus* (ATCC 29213) (gram positive), *Staphylococcus epidermidis* (ATCC 35984) (gram positive) strain was picked from isolated colonies, inoculated into sterile CAMHB broth tubes and incubated overnight at 37ºC to exponential-phase. Post incubation, the cultures will be identified by Gram staining and optical density will be adjusted to that of a 0.5 McFarland Standard (Approximately equivalent to a bacterial suspension 1.0×10^8 CFU/ml), and diluted to $\sim 1.0 \times 10^7$ CFU/ml.

SCD, GCD, FCD was used as test compounds, Ciprofloxacin as the reference compound and saline as vehicle control. The test compounds and control were in µl/ml and ciprofloxacin in µg/ml. The ciprofloxacin was used only as reference standard and was not statistically compared with test compounds. The test compounds were compared with the vehicle control.

150 microliters of CMHB broth containing various concentrations of test compounds working stock dilutions will be placed into wells. Each well will be inoculated with 50 µl of bacterial culture ($\sim 1.0 \times 10^7$ CFU/ml), $\sim 1.0 \times 10^5$ CFU/well, along vehicle control. The different volumes of test samples, ciprofloxacin and controls were added to each assay well in the microtitre plate along with varying volumes of CAMHB and 50 µl of bacterial suspension to make a final assay volume of 200 microlitres in each well. The plates will be incubated at 37ºC for 24 hrs. The plates were incubated at 37ºC for 24 hrs. Post incubation, plates were visually examined for turbidity and the optical densities at 630 nm will be estimated using a Spectrophotometer. The MIC will be defined as the lowest concentration of the test compound that prevents bacterial growth (lack of turbidity or the OD at 630 nm relative to no growth control [14].

The assay was done in duplicates and the obtained results (test compounds *versus* control) were statistically analyzed using ANNOVA software.

2.7. Dose selection for Wound Healing Studies

For the Selection of dose and treatment period, Acute dermal toxicity study was carried out. The doses for topical administration was used based on the acute dermal toxicity study as per OECD guidelines and 1/4of the safe dose was used for Topical application.

2.7a. Acute Dermal Toxicity

Dose range Finding: A range finding study using 1 male and 1 female rat at dose 2000 uL/animal for each test item was carried out in order to establish the dose levels for the main study. One day before the treatment, around 10% dorsal skin area of the each rat was clipped free of hair, without any abrasion. The appropriate amount (2ml) of the test items SCD was applied uniformly over the clipped area of each rat. After the application, the test item was held in contact with the skin for a period of 24-hours, using a porous gauze dressing and bandaged with non-irritating adhesive tape. After the exposure period, the residual test item was wiped gently from the skin using cotton soaked in water.

Limit test: Based on the results from the range finding experiment, limit test was chosen. In the limit test, 5 male and 5 female rats for each test item were exposed to dose 2000 ul/animal. One day before the treatment, around 10% dorsal skin area of each rat was clipped free of hair, without any abrasion. The appropriate amount (2ml) of the test item. SCD was applied uniformly over the clipped area of each rat. After the application, the test item was held in contact with the skin for a period of 24-hours, using a porous gauze dressing and bandaged with non-irritating adhesive tape. After the exposure period, the residual test item was wiped gently from the skin using cotton soaked in water. Animals were observed for 14 days for any abnormality or death.

2.8. Wound Healing Studies

2.8a. Excision wound

The animals were anesthetized using cocktail of ketamine + xylazine (60mg/kg+10mg/kg, ip). An impression was made on the dorsal thoracic region 1 cm away from vertebral column and 5 cm away from ear on the anaesthetized rat. The particular skin area was shaved one day prior to the experiment. The skin of impressed area was excised to the full thickness to obtain a wound area of about $2cm^2$. Haemostasis was achieved by blotting the wound with cotton swab soaked in normal saline. The animals were then grouped and formulation was applied to cover the entire wounded area as follows: Group I: (control), Group II: SCD (0.5ml) formulation, Group III: GCD (0.5ml) and Group IV: FCD (0.5ml) formulation. Animals were treated for 28 days post wounding. Following wound creation, animals were housed individually in cages with species specific enrichment. Post excision, ketoprofen was administered to reduce the pain and stress.

Wound area was measured by tracing the wound on a millimetre scale graph paper on predetermined days i.e., 0, 7, 14, 21and 28 days post-wounding. The tracing was then transferred to 1 mm^2graph sheet, from which the wound surface area was estimated**.** The surface area was then employed to calculate the percentage wound contraction by using the following equation.

$$\%\text{Wound Contraction} = \frac{\text{Initial (day 0) Wound Size} - \text{Specific Day Wound Size}}{\text{Initial Wound Size}} x100$$

The period of epithelisation was calculated as the number of days required after wound creation for the Escher to fall off leaving no raw wound behind.

After 28 days animals were euthanized using CO2, and the wounded skin samples were collected from wound site after euthanizing rats and preserved in neutral buffered formalin. The samples were processed by standardized routine paraffin embedding technique.[15] Prepared blocks were sectioned to the 3 to 5µ using rotatory microtome and mounted on clean glass slide. The prepared slides stained by hematoxylineosin stain. All tissue samples were observed under light microscope. [16]

2.8b. Incision wound

A longitudinal paravertebral incision of 6 cm long was made through the skin and cutaneous tissue on the back with the help of a sharp scalpel [17]. After complete haemostasis, the wounds were closed by means of interrupted sutures placed at approximately 1 cm apart. Animals were treated daily with SCD, GCD and FCD, as mentioned above under excision wound model from 0th day to 14th post-wounding day.[18]

The wound breaking strength was estimated on 15th day by continuous, constant water flow technique. [19]

2.8c. Dead space wound model

This type of wound was created by implanting subcutaneously a 2.5×0.5 cm polypropylene tube in the lumber region in anesthetized rats [20]. Animals were treated with SCD, GCD and FCD topically from 0th day to 9th post wounding day. On the 10th post wounding day, the animals were sacrificed and the granulation tissue harvested on the implanted tube was carefully dissected out along with the tube [21]. The tubular granulation tissue was cut lengthwise to obtain a sheet of granulation tissue. The pieces of granulation tissue were collected, dried at 60° C for 24 hr to get a constant weight and weighed [20, 22].

2.9. Data Compilation and Statistical Analysis

The micro dilution assay for determination of MIC was performed in duplicates and the Data is presented as the mean values with ±SD as Error bars. * indicate statistically significant differences ($p<0.05$) test Compounds compared to control. For the wound healing studies GraphPad Prism 5.0 was used for statistical analysis. Column statistics were estimated and a two tailed t-test was used to check the significance of percent wound contraction of the treatments relative to control. A p level < 0.05 was considered to be significant.

3. Results

3.1. Synthesis of carbon dots

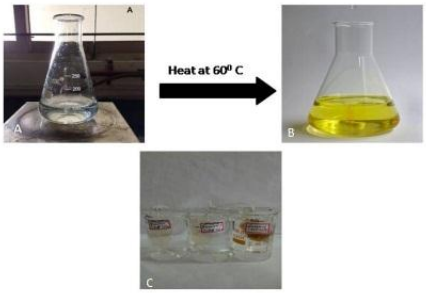


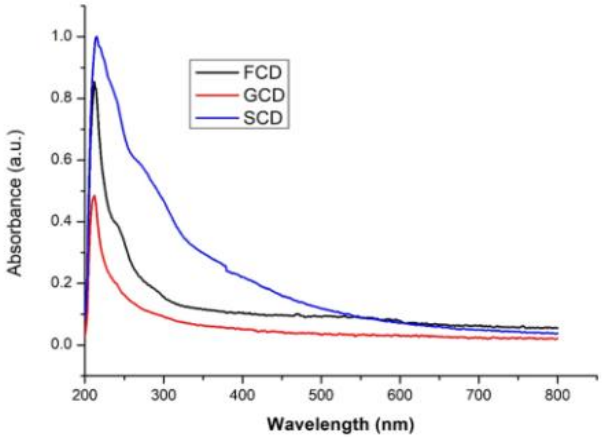
Figure 1: Synthesis of C dot nanoparticles – A) Sucrose/Glucose//Fructose solution (Before) B) synthesized C dot nanoparticles (After). C) Dialysis of synthesized nanoparticles

C dot nanoparticles synthesis adopting acidic oxidation process at 60 c was primarily confirmed by colour change of the reaction mixture from colourless to yellow clearly indicating the formation of C dot nanoparticles (Fig. 1).

The characteristic yellow colour due to the slow release of C dots on heating which provides a convenient signature of their formation. The synthesized nanoparticle solution were then filled into the dialysis bag and kept for dialysis treatment in water for 24 hours with timely changing of water in the beaker.

3.2. Characterization of Carbon dots

When UV-Visible light is passed through a sample, the transmittance of light by the sample is measured. From the transmittance (T), the absorbance



can be calculated as A=-log (T). The absorbance spectrum is obtained which shows the absorbance of a sample at different wavelengths.

Figure 2: UV-Visible spectra of synthesized C-dots

The absorbance of the C-dots at UV and Visible spectrum is shown in (Fig. 2) Single significant peak in UV region was observed. This might be some absorption shoulders both the n–π* transition of the C = O and a π–π* transition of the conjugated C = C. The absorbance peak value is recorded and used for the fluorescence spectrophotometer.

An emission peak at around 500 nm was observed. It should be mentioned here that the EG solution itself is non-emissive in the visible region, confirming the bright fluorescence to be originating from the synthesized C-dots.

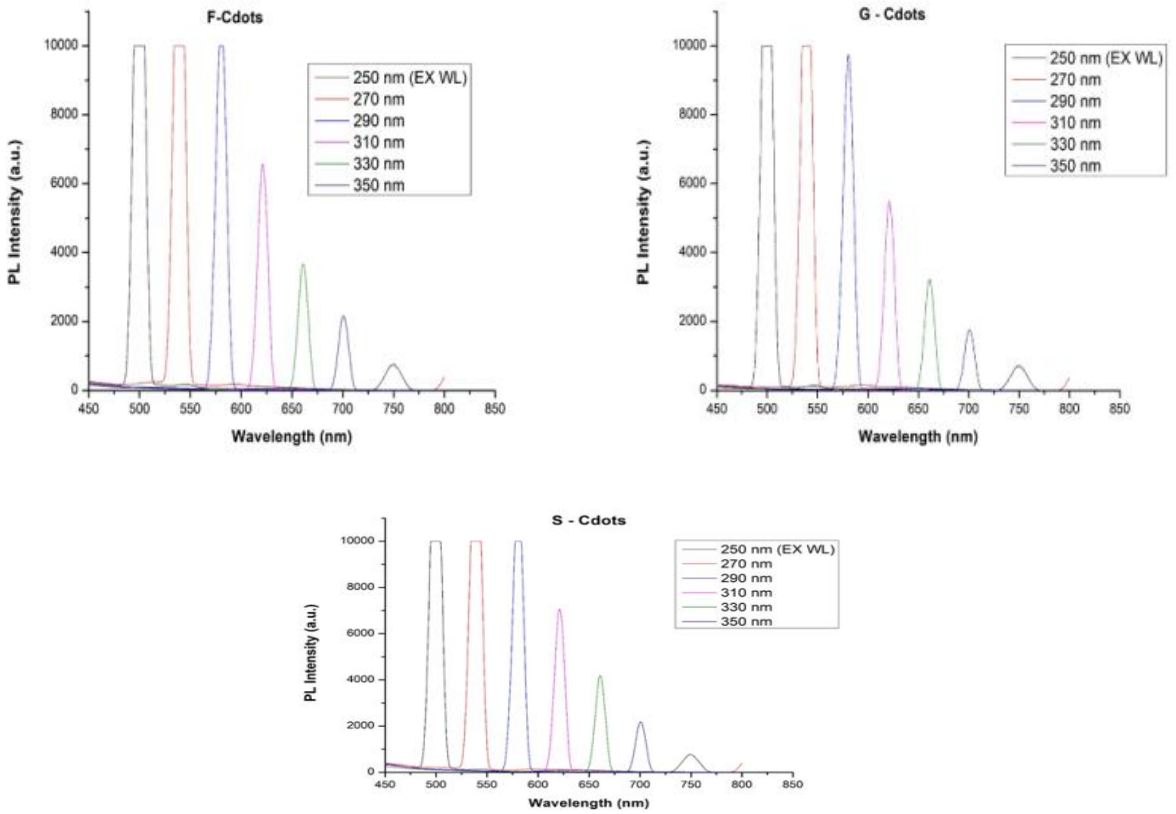


Figure 3. Fluorescent spectra of c-dot s excited at excitation edge of 250-350 nm.

The emission spectra recorded at different excitation wavelengths (250 to 350 nm) showed the fluorescence emission has a red shift as the excitation wavelength is increasing (Fig.3).Interestingly, with increase in the excitation wavelength from 250 nm to 350 nm, the emission from C-dots gradually shifted to higher wavelengths accompanied with decreased fluorescence intensity. This bathochromic shift of emission from C-dots has also been reported previously [14]. The strong fluorescence exhibited by the C-dots is thought to be responsible for the quantum confinement of the passivity surface energy traps [11].

3.3. Antibacterial activity of C- Dots

All the four organisms were found susceptible to the C-dots from sucrose, Glucose and fructose. The

MIC concentration of SCD ,GCD , FCD against *E.coli and K.pneumoniae*, the carbon Dots from Sucrose and Glucose showed the antibacterial activity (MIC) at a concentration 12.5µl (0.1±0.003) where as the carbon dots from fructose showed MIC at a higher concentration of 50µl (0.1 ± 0.01) lesser than the control(0.61 ± 0.06). (Fig. 4 &5, Table 1).

As similar to the two gram positive clinical strains (S.aureus and S.epidermidis), the carbon Dots from Sucrose and Glucose showed the antibacterial activity (MIC) at a concentration 12.5µl (OD -0.1 ± 0.001) but the carbon dots from fructose showed antibacterial activity (MIC) at a higher concentration of 50µl (OD-0.1 ± 0.01) lesser than the control (0.58 ± 0.05, 0.46 ± 0.01). (Fig 6 &7, Table 2).

The result of this investigation showed that the (SCD) and (GCD) showed good antibacterial activity against both gram negative Escherichia coli and Klebsiella pneumonia and gram positive Staphylococcus aureus and Staphylococcus epidermidis at a lesser concentration when (FCD) (Fig. 8). All the three C-dots have shown potent antibacterial activity when compared to the control

3.4. Wound Healing activity of C- Dots

3.4a. Excision wound

Effect on excision and incision wound—Test compound SCD, GCD and FCD produced a significant decrease ($P<0.05$) in percent wound contraction on day 14 onwards, when compared to control. (Image 1, Table 3, Fig. 9).

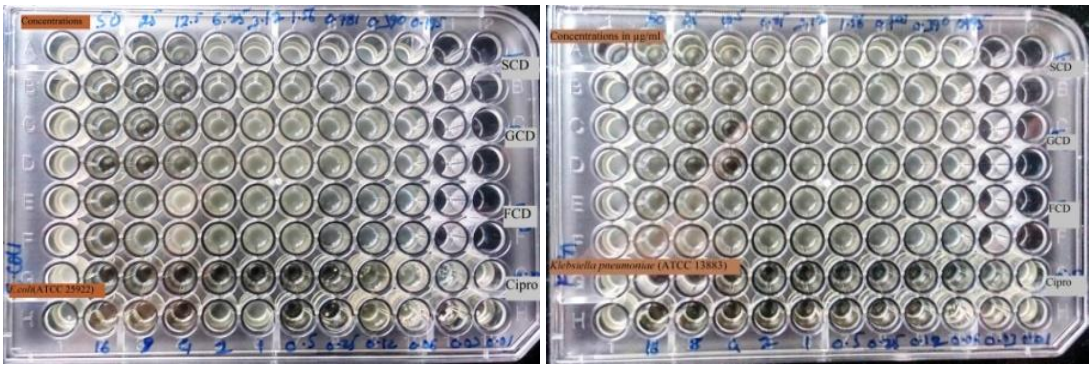


Figure. 4. MIC of test compounds (SCD, GCD and FCD), Control and Ciprofloxacin on *E.coli* and *K.pneumoniae*

Table 1. MIC of SCD, GCD and FCD versus Control on *E.coli* and *K.pneumoniae*

OD at 630nm ant different concentration (Mean ± SD)								
Test compound Concentration	**Vehicle Control**		**SCD**		**GCD**		**FCD**	
	E.coli	*K.pneumoniae*	*E.coli*	*K.pneumoniae*	*E.coli*	*K.pneumoniae*	*E.coli*	*K.pneumoniae*
50 µl/ml	0.61 ± 0.06	0.48 ± 0.01	0.1 ± 0.01*	0.09 ± 0.003*	0.09 ± 0.002*	0.08 ± 0.01*	0.1 ± 0.01*	0.1 ± 0.01*
25 µl/ml	0.61 ± 0.06	0.48 ± 0.01	0.1 ± 0.004*	0.09 ± 0.003*	0.1 ± 0.003*	0.11 ± 0.01*	0.22 ± 0.02*	0.37 ± 0.01*
12.5 µl/ml	0.61 ± 0.06	0.48 ± 0.01	0.1 ± 0.003*	0.09 ± 0.02*	0.1 ± 0.003*	0.1 ± 0.01	0.41 ± 0.01*	0.39 ± 0.01*
6.25 µl/ml	0.61 ± 0.06	0.48 ± 0.01	0.34 ± 0.01*	0.36 ± 0.03*	0.41 ± 0.05*	0.31 ± 0.03*	0.4 ± 0.02*	0.4 ± 0.01*
3.125 µl/ml	0.61 ± 0.06	0.48 ± 0.01	0.42 ± 0.01*	0.45 ± 0.02	0.45 ± 0.03*	0.4 ± 0.03	0.39 ± 0.09*	0.41 ± 0.02
1.562 µl/ml	0.61 ± 0.06	0.48 ± 0.01	0.42 ± 0.02*	0.45 ± 0.03	0.46 ± 0.03*	0.41 ± 0.01	0.4 ± 0.01*	0.41 ± 0.01
0.781 µl/ml	0.61 ± 0.06	0.48 ± 0.01	0.44 ± 0.05*	0.46 ± 0.05	0.4 ± 0.001*	0.39 ± 0.02	0.36 ± 0.01*	0.39 ± 0.01
0.39 µl/ml	0.61 ± 0.06	0.48 ± 0.01	0.43 ± 0.06*	0.46 ± 0.05	0.4 ± 0.01*	0.38 ± 0.01	0.37 ± 0*	0.38 ± 0.01
0.19 µl/ml	0.61 ± 0.06	0.48 ± 0.01	0.47 ± 0.05*	0.5 ± 0.06	0.42 ± 0.01*	0.43 ± 0.02	0.39 ± 0.003*	0.42 ± 0.02

SCD and GCD showed antibacterial activity at a concentration of 12.5µl/ml at a lesser concentration compared to FCD at 50 µl/ml. All the three test compounds showed good activity when compared to vehicle control.

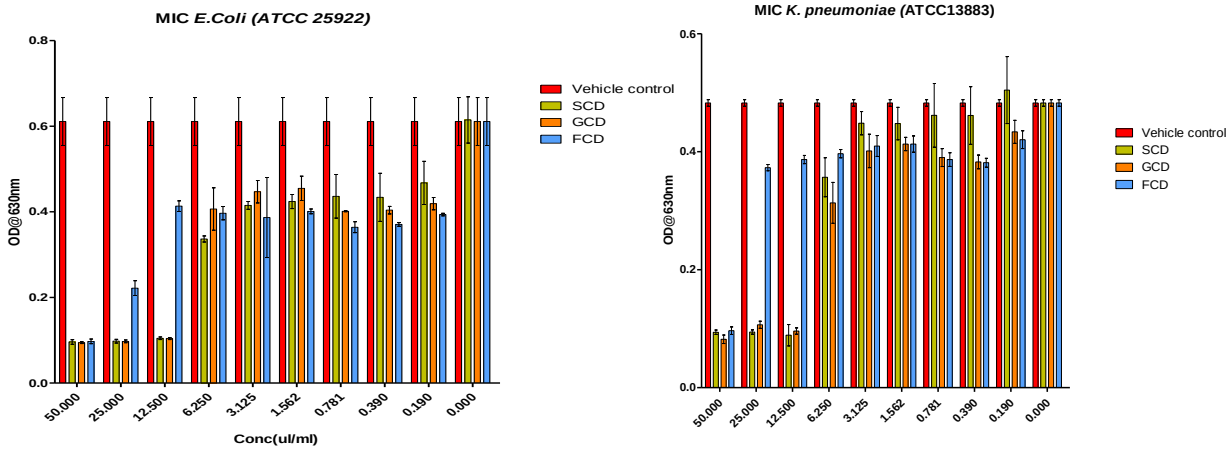


Figure 5: Comparative results of test compounds (Concentration verses OD) of gram negative *E.coli* , *K.pneumoniae* and control

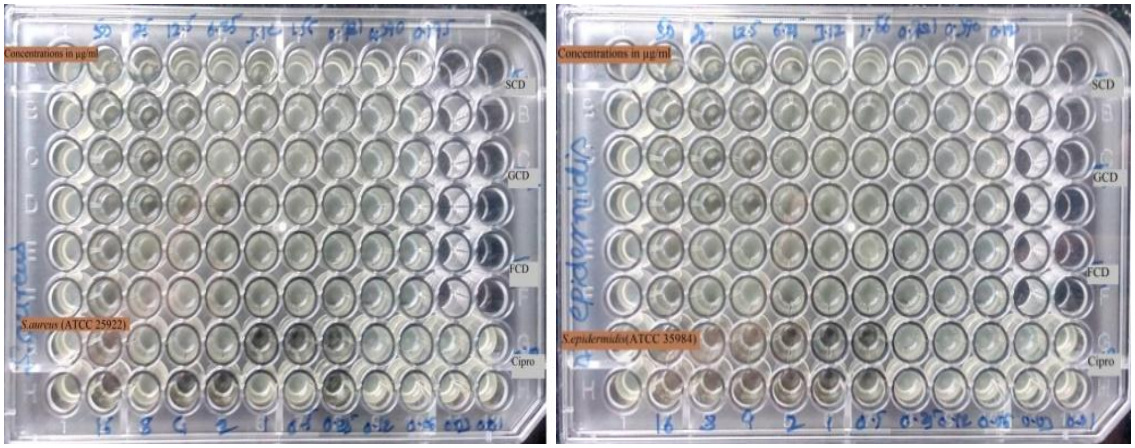


Figure 6. MIC of test compounds (SCD, GCD and FCD), Control and Ciprofloxacin on *S.aureus* and *S.epidermidis*

Table 2. MIC of SCD, GCD and FCD versus control on *S.aureus* and *S.epidermidis*

OD at 630nm ant different concentration (Mean ± SD)								
Test compound Concentration	**Vehicle Control**		**SCD**		**GCD**		**FCD**	
	S.aureus	*S.epidermidis*	*S.aureus*	*S.epidermidis*	*S.aureus*	*S.epidermidis*	*S.aureus*	*S.epidermidis*
50 µl/ml	0.58 ± 0.05	0.46 ± 0.01	0.08 ± 0.01*	0.1 ± 0.003*	0.09 ± 0.005	0.1 ± 0.003*	0.1 ± 0.01*	0.1 ± 0.01*
25 µl/ml	0.58 ± 0.05	0.46 ± 0.01	0.09 ± 0.004*	0.1 ± 0.003*	0.1 ± 0.002	0.1 ± 0.004*	0.24 ± 0.02*	0.33 ± 0.03*
12.5 µl/ml	0.58 ± 0.05	0.46 ± 0.01	0.1 ± 0.001*	0.09 ± 0.005*	0.1 ± 0.002	0.1 ± 0.003*	0.3 ± 0.04*	0.35 ± 0.04*
6.25 µl/ml	0.58 ± 0.05	0.46 ± 0.01	0.32 ± 0.02*	0.32 ± 0.03*	0.26 ± 0.01	0.3 ± 0.02*	0.33 ± 0.05*	0.37 ± 0.02*
3.125 µl/ml	0.58 ± 0.05	0.46 ± 0.01	0.33 ± 0.02*	0.38 ± 0.05*	0.35 ± 0.01*	0.31 ± 0.02*	0.36 ± 0.04*	0.38 ± 0.03*
1.562 µl/ml	0.58 ± 0.05	0.46 ± 0.01	0.39 ± 0.08*	0.39 ± 0.08*	0.35 ± 0.003*	0.34 ± 0.01*	0.37 ± 0.05*	0.37 ± 0.03*
0.781 µl/ml	0.58 ± 0.05	0.46 ± 0.01	0.41 ± 0.04*	0.42 ± 0.03	0.38 ± 0.003*	0.37 ± 0.01	0.42 ± 0.05*	0.4 ± 0.01
0.39 µl/ml	0.58 ± 0.05	0.46 ± 0.01	0.41 ± 0.03*	0.43 ± 0.04	0.38 ± 0.02*	0.38 ± 0.01	0.41 ± 0.05*	0.39 ± 0.02
0.19 µl/ml	0.58 ± 0.05	0.46 ± 0.02	0.45 ± 0.04	0.47 ± 0.05	0.4 ± 0.02	0.41 ± 0.01	0.44 ± 0.05	0.41 ± 0.01

SCD and GCD showed antibacterial activity at a concentration of 12.5µl/ml at a lesser concentration compared to FCD at 50µl/ml. All the three test compounds showed good activity when compared to control.

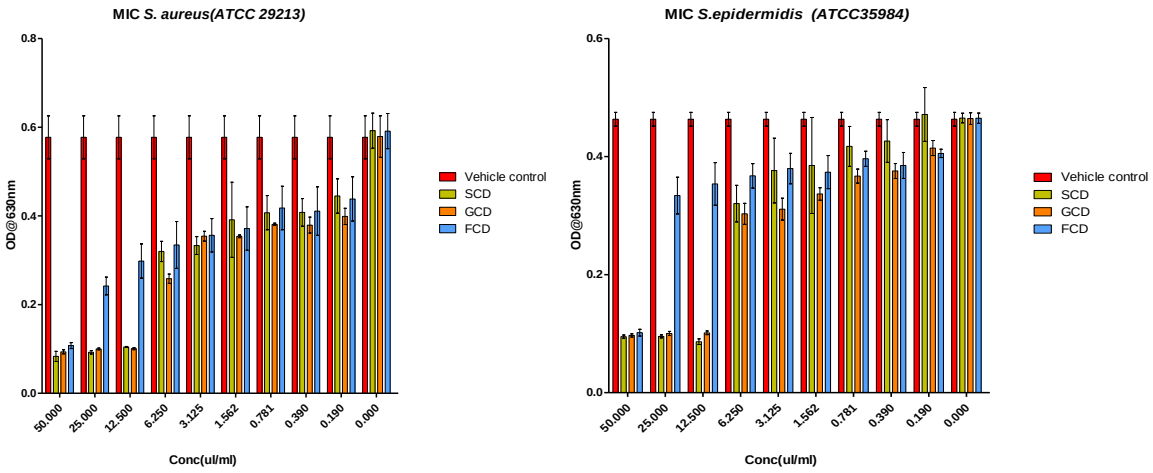


Figure 7: Comparative results of test compounds (Concentration verses OD) of gram positive *S.aureus* , *S. epidermidis* and control.

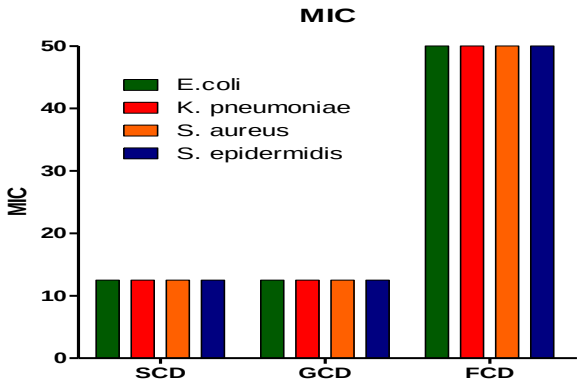


Figure 8: C-Dots of Sucrose and Glucose shows better MIC compared to C-Dots of Fructose on all Bacterium.

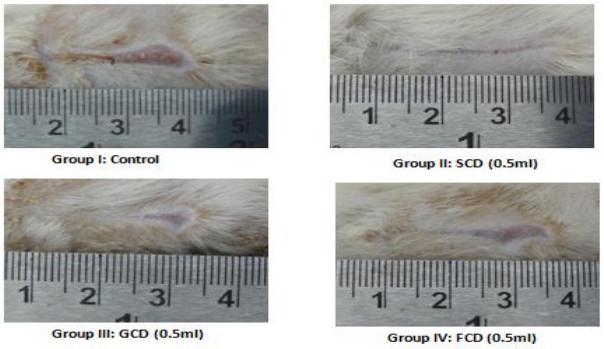


Image. 1 Excision wound contraction on day 28

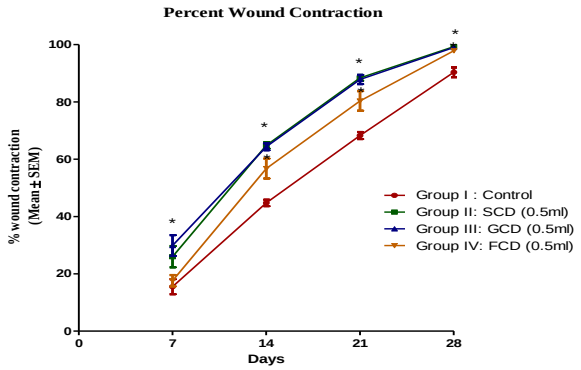


Figure 9. Percent Wound contraction

Table 3. Percent Wound contraction

	Mean ± SEM			
	Group I: (control)	Group II: SCD (0.5ml) formulation	Group III: GCD (0.5ml) formulation	Group IV: FCD (0.5ml) formulation
Day 7	15.52 ± 2.63	25.99 ± 3.7	29.87 ± 3.64	17.61 ± 1.98
Day 14	44.77 ± 1.13	64.84 ± 1.09*	64.36 ± 1.21*	56.74 ± 3.47*
Day 21	68.26 ± 1.23	88.39 ± 1.02*	87.83 ± 1.6*	80.35 ± 3.39*
Day 28	90.36 ± 1.72	99.31 ± 0.45*	99.02 ± 0.33*	97.85 ± 0.29*

*p<0.05 compared to Control

3.4b. Incision and dead space wound

The breaking strength of 15 days old incision wound was significantly increased by treatment of SCD, GCD and FCD.

Effect on dead space wound—the dry tissue weight was significantly increased (P<0.05) by the treatment of SCD, GCD and FCD when compared to control (Table 4, Fig 10)

In histopathology observations (Image 2) Group 1(control) skin sections show least collagen fibbers deposition in extra cellular matrix along with least neovascularisation. The wound healing in group 2(SCD) observed better than the Group 1 and 4(FCD). The histopathological observations are well developed epithelium, better collagen and elastic fibre deposition in extra cellular matrix, evidence neovascularisation and haemorrhage. Group 3 skin tissues indicate best healing in as compared to Group 4, 2 and 1. The group samples indicate almost complete epithelisation, enough collagen deposition in extra cellular matrix and few number of fibroblast and inflammatory cells are visible. Group 4 skin samples show better tendancy towards healing. Histosections show new epithelisation and adequate collagen deposition and elastic fibbers in extra cellular matrix. Few inflammatory cells are noticeable and neovascularisation is absent. The wound healing in Group 2 is better than Group 1

Table 4: Breaking Strength and Dry tissue weight

Group	Mean ± SEM	
	Breaking strength(g)	Granulation tissue weight (mg)
Group I: (control)	280.8 ± 10.72	90.83 ± 2.9
Group II: SCD (0.5ml) formulation	367.8 ± 15.2*	157.3 ± 16.97*
Group III: GCD (0.5ml) formulation	368.8 ± 14.22*	166.8 ± 10.83*
Group IV: FCD (0.5ml) formulation	326.8 ± 9.41*	135.8 ± 8.29*
*p<0.001 compared to Control		

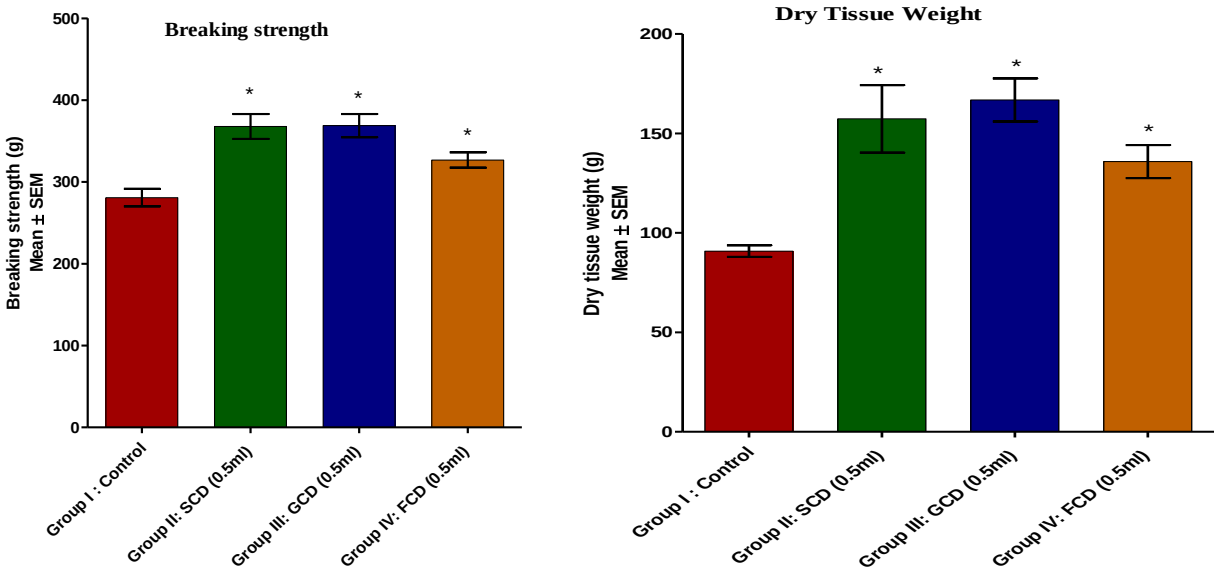
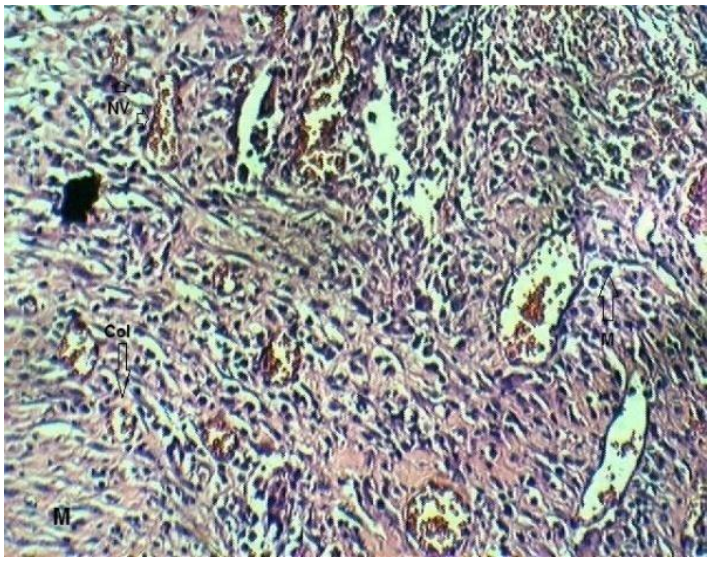
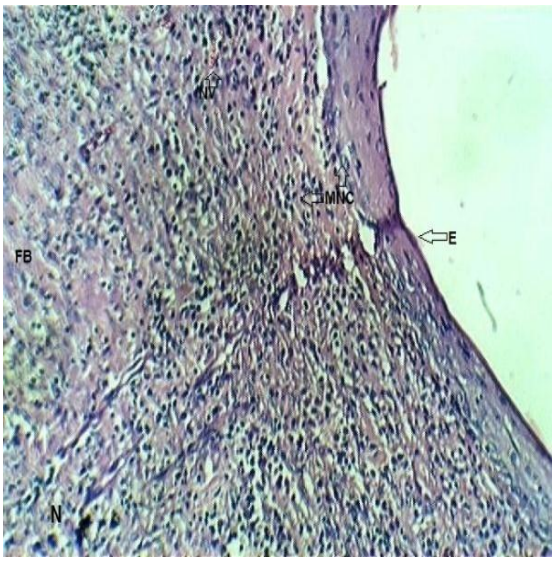


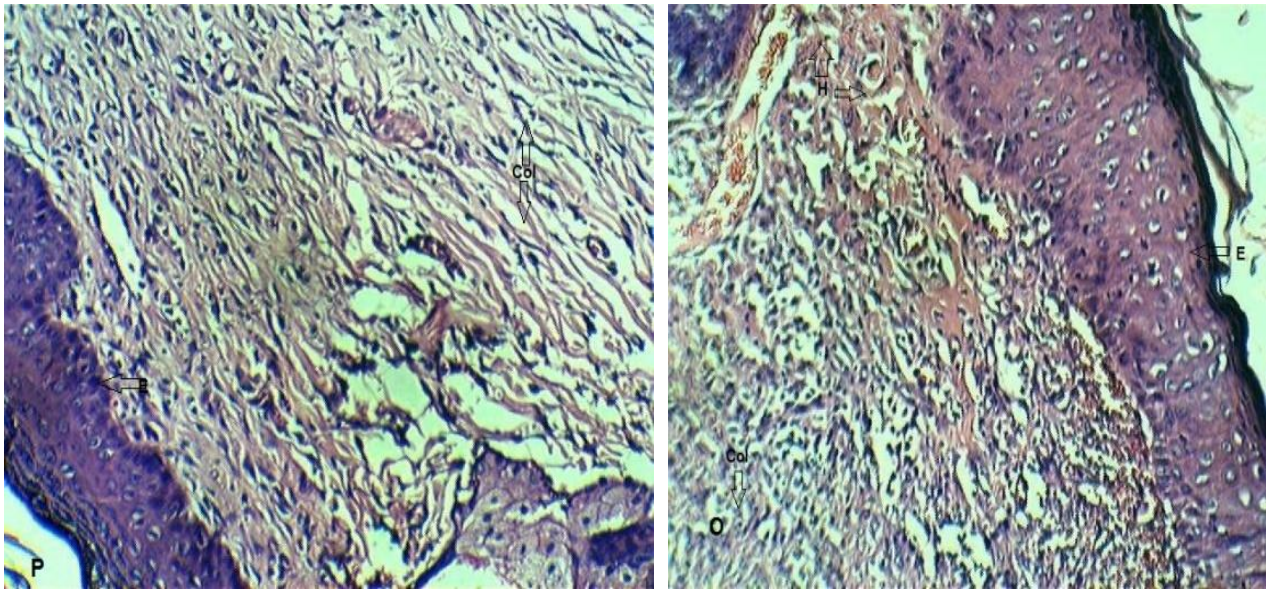
Figure 10. Breaking Strength and Dry tissue weight



Group 1 skin shows least collagen fibbers (Col) along with neovascularisation (NV). (H & E, 40X)



Group 2 skin shows new epithelisation (E) and collagen deposition (FB) with few fibroblasts. (H & E, 40X)



Group 3 skin almost complete epithelisation (E), collagen deposition (Col) and few numbers of fibroblast and inflammatory cells are visible. (H & E, 40X)

Group 4 skin shows more better epithelisation (E), better collagen and elastic fibre deposition (Col),along with neovascularisation (NV) and haemorrhage (H). (H & E, 40X)

Image 2. Histopathological observation of skin samples

4. Discussion

The C-Dots synthesized from all the three sources (Sucrose, Glucose and Fructose) showed good antibacterial activity on all the four bacterial strains. Due to the non toxic nature of C-dots when compared antibiotics which often pose potential toxicity and resistance to bacteria especially in situations when high dosage is required the nanosized nonmetallic carbon dots can act as an good substitute to the antibiotics . The SCD and GCD have shown potent antibacterial activity with against both gram positive and negative strains at a far lower concentration than that of FCD which signifies that the presence of number of carbon dots are more in the C-Dots synthesized from sucrose and glucose than fructose. This may be due to the structural differences present between the sources sucrose, glucose and fructose. The action of these nanoparticles may be by inhibiting the synthesis of peptidoglycan layer and the C-dots generating “holes” in the bacterial cell walls thereby increasing the permeability of cell wall resulting in the leakage of the cell contents and eventually death [23,24]. SCD, GCD and FCD have shown potent wound healing activity in Excision, Incision and dead space wound models. Collagenation, wound contraction and epithelization are crucial phases of wound healing. [25] The phases of inflammation, macrophasia, fibroplasia and collagenation are intimately interlinked. Thus an intervention into any one of these phases by drugs could eventually either promote or depress one, other or all phases of healing [26]. Growth hormone is known to promote the healing process by enhancing epithelial cell proliferation and cell collagen formation. Collagen is the family of protein, which provide structural support and it is the main component of tissue such as fibrous tissue and cartilage. The collagen synthesis is stimulated by various growth factors [27]. Growth hormone is also known to promote the proliferation of fibroblasts and fibroblast proliferation form the granulation tissue. In the dead space wound model, C-Dots treatment increased granuloma tissue weight. The exact mechanism by C-Dots, increased the granuloma tissue weight of granulation tissue is still not known.

5. Conclusion

The study reveals that Carbon dots possess antibacterial properties and substantiate their use for the treatment of wound as the SCD, GCD and FCD have shown potent wound healing activity in Excision, Incision and dead space wound models, C-Dots applied topically promoted the breaking strength, wound contraction. The C-Dots synthesized from sucrose (SCD) and glucose (GCD) possesses good antibacterial activity when compared to carbon dots synthesized from fructose (FCD).

The C-Dots are inorganic, eco friendly, non toxic in nature and can be inexpensively synthesized on large scale using a single step pathway by a wet chemical method and these C-Dot nanoparticles can act as excellent substitute to the antibiotics which pose potential toxicity and resistance to bacteria on a long term use. They open the possibility of development and formulation of a new generation of bactericidal materials.

References

[1]. X. Xiuli Dong, M.A. Awak, N. Tomlinson, Y. Tang, Y-P. Sun, L. Yang, Antibacterial effects of carbon dots in combination with other antimicrobial reagents. *PLoS ONE,* 12(2017) 1-16.

[2]. L. Wang, C. Hu, L. Shao, The antimicrobial activity of nanoparticles: present situation and prospects for the future, *International Jouranl of Nanomedicine.* 12(2017): 1227–1249.

[3]. J. Tian, K. Y. Wong Kenneth, Chi-Ming Ho, Chun-Nam Lok, Wing-Yiu Yu, Chi-Ming Che, C. Jen-Fu, K. H. Tam Paul, Topical Delivery of Silver Nanoparticles Promotes Wound Healing, *Chemmedchem*,12(2017) 1227–1249.

[4]. M. Pulido Moran, J. Moreno Fernandez, C. Ramirez Tortosa, M. Ramirez-Tortosa, Curcumin and Health. *Molecules*, 21(2016)264-286.

[5]. S. Schreml, R. Szeimies, L. Prant, M. Landthaler, P. Babilas, Wound healing in 21st century, *Journal of the American Academy of Dermatology*, 63(2010) 866–881.

[6]. L. Zhang, T. Webster, J. Nanotechnology and nanomaterials: Promises for improved tissue regeneration, *Nanotoday*. (2009), 4, 66-80.

[7]. P.V. Asha Rani, M. Prakash Hande, Suresh Valiyaveettil, Anti-proliferative activity of silver nanoparticles, *BMC Molecular and Cell Biology*. 10 (2009) 1-14.

[8]. S. Enoch, D.J. Leaper, Basic science of wound healing, *Surgery,* 26 (2008) 31–37.

[9]. K. Maiti, K. Mukherjee, A. Gantait, B. P. Saha, P.K. Mukherjee, Curcumin- phospholipid complex: Preparation, therapeutic evaluation and pharmacokinetic study in rats, *International Journal of Pharmaceutics,* 330(2007)155-163.

[10]. J. Li, J. Chen, R. Kirsner, Pathophysiology of acute wound healing, *Clinics in Dermatology,* 25(2007) 9-18.

[11]. T. Yoshikawa, Y. Tsutsumi, S. Nakagawa, Development of nanomedicine using intracellular DDS. *Nihon Rinsho,* 64 (2006) 247-252.

[12]. D.F. Emerich, C.G. Thanos, Nanotechnology and medicine, *Expert Opinion on Biological Therapy,* 3(2003) 655-663.

[13]. T. K. Biswas, B. Mukherjee, Plant medicines of Indian origin for wound healing activity: *A review, The International Journal of Lower Extremity Wounds,* 2(2003) 25 -39.

[14]. C. N. Baker, S. A. Stocker, D. H. Culver, C. Thornsberry, Comparison of the E Test to agar dilution, broth microdilution, and agar diffusion susceptibility testing techniques by using a special challenge set of bacteria, *Journal of Clinical Microbiology,* 29(1991) 533–538.

[15]. J.V. Kamath, C. Rana, R. Chowdhury, Pro-healing effect of Cinnamomum zeylanicum bark, *Phytotherapy Research,* 17(2003) 970-972.

[16]. J. S. Reddy, P. R. Rao, M. S. Reddy, Wound healing effects of Heliotropium indicum, Plumbago zeylanicum and

Acalypha indica in rats, *Journal of Ethnopharmacology,* 79(2002) 249-251.

[17]. K. H. Lee, Studies on the mechanism of action of salicylates II, effect of vitamin A on wound healing retardation action of aspirin, *Journal of Pharmacological Sciences*, 57(1968)1238-1240.

[18]. H. P. Ehrich, T. K. Hunk, Effect of cortisone and anabolic steroids on tensile strength of healing wound, *Annals of Surgery,* 170(1969) 203 - 206.

[19]. S. N. Somayaji, A. P. Jacob, K. L. Bairy, Effect of tolmetin and its copper complex on wound healing, *Indian J Exp Biol,* 33(1995) 201- 204.

[20]. P. N. Padmaja, K. L. Bairy, D. R. Kulkarni, Pro healing effect of betel nut and its polyphenols, *Fitoterapia,* 65 (1994) 298-300.

[21]. S. R. Corton, V. Kumar, T. Collins, Robbins pathologic basis of diseases, Harcourt Limited, New Delhi, India, 2003.

[22]. T. C. Williams, L. A. Frohman, Potential therapeutic indication for growth hormone releasing hormone in the condition other than growth retardation, *Pharmacotherapy*, 6 (1986), 311-318.

[23]. K. Vibha, Y. Rakesh Kumar, T. Aseem Prakash, C. Anil, V. Promila, G. Prashant, S. Vijay Kumar, Comparative evaluation of antibacterial effect of nanoparticles and lasers against Endodontic Microbiota: An in vitro study, *Journal of Clinical and Experimental Dentistry,* 10(2018) e1155–e1160.

[24]. W.R. Rolim, M. T. Pelegrino, Brunade Araújo Lima, L. S. Ferraz, F. N. Costa, J. S. Bernardes, T. Rodigues, M. Brocchi, A. B. Seabra, Green tea extract mediated biogenic synthesis of silver nanoparticles: Characterization, cytotoxicity evaluation and antibacterial activity. *Applied Surface Science*. 463 (2019) 66-67.

[25]. A. Vijayakumar, M. Jeyaraj, M. Selvakumar, E. Abirami, Pharmacological activity of silver nanoparticles. *Research journal of life sciences, bioinformatics , pharmaceutical and chemical sciences*. (2019).

[26]. R. Manikandan, R. Anjali, M. Beulaja, N. M. Prabhu, A. Koodalingam, G. Saiprasad, P. Chitra, M. Arumugam, Synthesis, characterization, anti-proliferative and wound healing activities of silver nanoparticles synthesized from *Caulerpa scalpelliformis. Process biochemistry, 79*(2019) 135-141.

[27]. B. Turakhia, S. Chikkala, S. Shah, Novelty of Bioengineered Iron Nanoparticles in Nanocoated Surgical Cotton: A Green Chemistry, *Advances in Pharmacological Sciences.* (2019), 1- 10.

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Competing Interests:

The authors declare that they have no competing interests.

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