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Evaluation of mechanical properties and antibacterial activity of wound dressing fabricated with gum acacia stabilized copper nanoparticles loaded levofloxacin drug nano conjugate

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ABSTRACT

With the advent of nanotechnology, new area has developed in the realm of textile finishing. These textiles can be widely used for hygienic clothing, wound healing, and medical applications in hospitals and other places where bacteria present a hazard. Enhancement of textile materials by nanotechnology is expected to become a trillion dollar industry in the next decade with tremendous technological, economic and ecologic benefits. In the present study, the effect of natural polymer gum acacia coated copper nanoparticles loaded levofloxacin drug nano conjugate fabricated wound dressing on the mechanical properties and antibacterial activity against pyogenic bacteria has been studied. Nano drug conjugate was prepared by one step reduction of metal precursor –polymer-antibiotic suspension with sodium borohydride. Synthesized drug conjugate was characterized by UV Visible spectroscopy, fourier transform infrared (FTIR) spectroscopy and electron microscopy. Nano drug conjugate coating was carried out by pad dry cure method. Fabricated dressing materials were examined in the range of basic physical–mechanical parameters followed by its functional properties. Antibacterial activity was studied against pyogenic bacteria by solid plate assay. Effect of nano drug conjugate on the mechanical properties clearly revealed distinct changes in mechanical and anti bacterial properties Nano coating revealed increased air permeability and no effect on the bursting strength. Anti bacterial activity showed that both the tested bacterial strains were effectively inhibited by the nano drug conjugate coated wound dressing treatment. Further study will helpful to fabricate polymer coated drug conjugate on wound dressing to fight against pyogenic bacterial infection.

Key words: Gum Acacia, Copper Nanoparticles, Drug Nano Conjugate, Wound Dressing, Mechanical Properties

INTRODUCTION

Nanotechnology is concerned with materials whose structures exhibit significantly novel and improved physical, chemical, and biological properties, phenomena, and functionality due to their nanoscaled size [1]. Metal nanoparticles have received attention in recent years because of their potential applications in the various fields of science and technology [2]. One application of Nanotechnology in medicine currently being developed involves employing Nano particles to deliver drugs, heat, light or other substance to specific types of cells. Particles are engineered so that they are attracted to disease cells, which allow direct treatment of those cells. This technique reduces the damage to healthy cells in the body and allows for earlier detection of disease [3,4].

With the advent of nanotechnology, new area has developed in the realm of textile finishing. These textiles can be widely used for hygienic clothing, wound healing, and medical applications in hospitals and other places where bacteria present a hazard [5]. Enhancement of textile materials by nanotechnology is expected to become a trillion dollar industry in the next decade with tremendous technological, economic and ecologic benefits [6,7]

Copper nanoparticles one of the major metallic nanoparticles have recently increased attention in various fields because of their low cost unlike silver and gold nanoparticles and novel optical, catalytic, mechanical, electrical, magnetic, heat conduction and anti microbial properties, which are different from those of bulk metals [8]. This leads to potential application for electronic systems, conductive inks, optical devices and nonlinear optical materials, such as optical switches or photochromic glasses and anti microbials [9,10]. Stabilization of metallic nanoparticles mainly silver, gold and copper nanoparticles is necessary for their stability, functionality, and biocompatibility. Various natural and synthetic polymers have been used as promising stabilization agents of metallic nanoparticles for nanoscale drug carrier systems, especially in oral administration of poorly absorbed therapeutic drugs [11]. Incorporation of the drug into a particulate carrier can protect the active substance against degradation in vivo and in vitro, improve therapeutic effect, prolong biological activity, control drug release rate, and decrease administration frequency. At present, biodegradable polymers like starch, dextran, pullulan, chitosan, and alginate are being used to encapsulate nano formulated therapeutic agents and the formulated therapeutics show better efficacy towards the target [12]. In this study, gum acacia has been selected for the stabilization of copper nanoparticles incorporated levofloxacin nano drug conjugate. There are some natural polymers such as chitosan and gum acacia have been reported as a polymer-based protective agent to stabilize the metal nanoparticles. Gum Acacia is a natural polymer derived from acacia trees. Due to its well steric stabilization effect as adsorbing on the surfaces of colloids and its numerous functional groups such as carboxylate and amine groups, it has been widely used as emulsifiers and capping agents [13-15]. Because of the biocompatibility, biodegradability, nontoxicity and adsorption properties, they were used as a stabilizing agent to prepare various metallic nanoparticles. These polymer protected nanoparticles can be easily integrated into systems relevant for pharmaceutical, biomedical, and biosensor applications [16,17]. In the present study, effect of gum acacia coated copper nanoparticles loaded levofloxacin drug nano conjugate on the mechanical properties and anti bacterial activity against human skin infection causing bacteria has been carried out.

MATERIALS AND METHODS

Chemicals and reagents

All of the chemicals were analytical grade and used as purchased without further purification. Copper (II) sulphate pentahydrate salt, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, of 98% purity (Merck), was dissolved in high purity water. Sodium borohydride (NaBH_4 -Reagent Plus 99%, Sigma-Aldrich) was used as the main reducing agent, Gum acacia (high purity) used as stabilization agents was obtained from SRL chemicals, India Levofloxacin for nano drug conjugate preparation and culture media used for the anti bacterial activity was obtained from Hi-Media, India.

Preparation of gum acacia stabilized copper nanoparticles incorporated levofloxacin drug nano conjugate (GA-CuNp-LF)

Nano drug conjugate was prepared by one step reduction of metal precursor –polymer-antibiotic suspension with sodium borohydride [18]. In this method, aqueous solution of metal precursor copper sulphate penda hydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) was made by dissolving 0.5 g of salt in 50 ml of deionized water followed by drop wise addition of 0.1 M freshly prepared sodium borohydride. Reaction mixture was kept under stirring at room temperature followed by the addition of 5 ml of a solution containing gum acacia (5.0 mg mL⁻¹) and 1 ml of levofloxacin (0.05mg/ml). After stirring for 3 hours at room temperature, the color turned to brownish black. Finally, the solution was centrifuged at 5,000 rpm at ambient temperature, the collected pellet was washed, lyophilized and used for further studies.

Characterization

Primary confirmation of polymer coated nano drug conjugate was carried out by UV-visible spectroscopy to determine surface plasmon absorption maxima using a Shimadzu-1800 spectrophotometer. Fourier transform infrared spectroscopy (FT-IR) for the functional groups. FT-IR was carried out with KBr palletized dried sample in the range of 4000–500 cm⁻¹ using Bruker Optic GmbH Tensor 27. Particle morphology (Shape and size) and elemental composition was studied by field emission scanning electron microscopy equipped with energy dispersive X-ray analysis (FESEM–EDAX) was performed by SUPRA 55-CARL ZEISS, Germany.

Anti bacterial activity

Bacterial strains

Antibacterial activity of nano drug conjugate was tested against two major skin infection causing bacterial strains *Staphylococcus aureus* and *Pseudomonas aeruginosa* were obtained from Microbial Type Culture Collection, Chandigarh, India. Respective bacterial strain was maintained on trypticase soy agar (TSA) slant at 4°C.

Inoculum preparation

A loopful of respective bacterial culture was inoculated from the TSA slant into tryptic soy broth, incubated overnight on a rotary shaker (200 rpm) at 35°C. The inoculums were prepared by diluting the overnight cultures with 0.9% sterile saline to a 0.5 McFarland units standard.

Determination of minimum inhibition concentration (MIC) and minimum lethal concentration

To determine minimum inhibition concentration (MIC) and minimum lethal concentration of the nano drug conjugate, micro dilution colorimetric assay using chromogenic reagent 3-(4, 5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was carried out. [19] Known quantity of bacterial culture thus prepared (0.9 ml) was added into each well of the 96well microplate followed by the addition of 0.1 ml of nano drug conjugate aliquot prepared from the original stock solution. The microtitre plates were incubated in the dark at 28°C for 24 hours. 10 μ L of MTT (5 mg/mL in 0.2 mol/L, pH 7.2, phosphate buffer saline PBS) was added into each well and the plates were incubated another 4 hours. The Minimum inhibitory concentration (MIC) value was defined as the lowest sample concentration that inhibited visible growth of the test bacterium, as indicated by MTT straining.

Fabrication of GA-CuNp-LF nano drug conjugate

Fabrication of GA-CuNp-LF nano drug conjugate on the wound dressing material was done by pad-dry-cure method [20]. Sterile wound dressing obtained from the health care centre was cut to the size of 10 \times 10 mm, immersed in minimal inhibitory concentration (MIC) of nano drug conjugate suspension. After curing, sodium lauryl sulphate mediated washing was carried out to remove residual unbound nano conjugate followed by successive washing with sterile distilled water to remove detergent solution. Washed fabric was air-dried and dried pieces were used for further studies. Surface topography of fabricated wound dressing was studied using scanning electron microscopy.

Effect of nano coating on mechanical properties

GA-CuNp-LF fabrication on the mechanical properties of dressing material was studied by the standard methods [21]. Pre treatment of the fabricated material was done for the conditioning at 65 % relative humidity 27°C. The tensile strength was carried out in automated materials testing system. The bursting strength was tested on the bursting strength tester by applying the multidirectional load. Simultaneously, air permeability was tested in air permeability tester (Kato Tech. Co. Ltd., Japan. Initially, the air resistance (Pa.s/m) is measured and its inverse gave the air permeability (m/Pa.s). For friction measurements, The results were expressed as coefficient of friction (μ), which is the ratio of frictional resistance and the normal load.

Agar diffusion assay

Anti bacterial activity of the coated fabric was done using agar diffusion assay. Bacterial strains and inocula preparation was carried out as described earlier. 0.1 ml of the respective bacterial inocula (10⁶ CFU/ml) thus prepared was spread on the sterile Muller Hinton agar (Hi media, Mumbai, India) using sterile cotton swabs and the seeded plates were incubated at 37°C for 24 hours. After the incubation period, the plates were observed for zone of inhibition.

RESULT AND DISCUSSION**Preparation of gum acacia stabilized copper nanoparticles incorporated levofloxacin drug nano conjugate (GA-CuNp-LF)**

Prepared nano drug conjugate stabilized with gum acacia synthesized by one step reduction method was confirmed by changes in colour of the reaction mixture, UV-vis spectroscopy, FTIR and SEM analysis. The reaction mixture offered a change in colour from dark blue to dark brownish black after complete reduction and stabilization of CuSO₄·5H₂O. UV-vis spectroscopy is the first technique which is used in characterization of free and coated metallic nanoparticles because of surface plasmon resonance (SPR) phenomenon. Therefore, metallic nanoparticles display characteristic optical absorption spectra in the UV-vis region, In the present study, the UV-vis spectrum of the chitosan stabilized at different wavelengths ranging from 200 to 800 nm showed the strong absorption peak centering at 575 nm. This characteristic absorption peak at around 575 nm is due to the surface-plasmon band of Cu colloids, which is consistent with that of copper nanoparticles synthesized by other method. Wavelength of the plasmon band stabilizes at 575nm (Figure 1).

Characterization of the synthesized nano drug conjugate was also carried out by FTIR. FTIR analysis helps to detect the functional groups, structure of a compound and purity of the sample in a given environment in terms of frequencies of radiation present in the nanoparticles. The profiles of FTIR spectroscopy of the gum acacia stabilized nano drug conjugate was depicted in figure 2 which reveals the absorption peak at specific wave number indicating NH₂ and OH grouping, C-H stretching vibration of CH₂ symmetry, C=O stretching of amide bonds. FTIR was also confirmed the stabilization of polymer by showing characteristic changes in the vibrational peaks of the

functional group. FTIR spectra of the polar groups O H of polysaccharide have the good ability of coordination reaction with metal ions [22]. When O-H groups and copper ions form coordination bonds, the interactions among the resultant Cu particles and oxygen atoms of O-H groups become stronger with increasing amount of Cu. This can lead to corresponding changes both in the positions and in the strengths of IR spectra of nano drug conjugate. The scanning electron microscopy study reveals gum acacia stabilized levofloxacin-copper nano drug conjugate as spherical particles with the size range of 80-90 nm (Figure 3). Such size distribution analysis of antibiotic nanoparticle conjugates confirms that the particles are well dispersed

Anti bacterial activity

In recent years, great developments have been made in the field of pharmacology using nanotechnology principles. The rapid expansion of nanotechnology promises to have great benefits for society that increase the residence time of drugs on the target area and subsequently enhance the bioavailability of drugs without affecting normal tissues [23]. The present study shows effective inhibition of the tested bacterial strains by the GA stabilized CuNp-LF nano drug conjugate. Micro dilution colorimetric assay using chromogenic reagent 3-(4, 5-dimethyl thiazol-2-yl)-2-5-dephenyl tetrazolium bromide (MTT) was carried out to determine MIC of the tested bacterial strains. MIC values of nano drug conjugate against all the tested bacterial strains were lesser than free antibiotic. It can be seen that nano drug conjugate showed high antibacterial efficacy (Figure 4,5). Higher efficacy of antibacterial activity was mainly associated with increased capacity of entry of drug from polymer coated copper nano conjugate and causes specific interaction with the cellular system of the bacteria

Nano coating and its effect on mechanical properties and antibacterial activity

Synthesized nano drug conjugate was coated on the wound dressing by pad dry cure method. Characterization of the coated material was carried out by SEM Surface topography of the wound dressing studied by SEM showed uniform dispersion of the nano conjugate particles finely embedded on the fibre surface with the size range of 70-85nm (Figure 6). Influence of nano drug conjugate coating on the mechanical properties was studied by standard methods. From the figure 7 and 8, it is clear that tensile strength of the fabric in warp-direction was found to be reduced. But distinct changes was not recorded in fabric weft direction and the percentage of strain was reduced in both warp and weft directions Significant changes (P=5%) was recorded in air permeability (Figure 9). Higher rate of air permeability was observed in nano drug conjugate coated wound dressing. Air permeability plays a major role in the function of textile materials used to provide an indication of the breathability of coated fabrics. Uniform distribution and nano size of nano conjugate improved air permeability and hence its breathability. But, nano coating did not cause any change in bursting strength and coefficient of friction which are the most commonly studied parameters used to determine degrees of smoothness or roughness (Figure 10-12). Antibacterial activity of nano drug conjugate coated wound dressing was studied by agar diffusion assay. 21.0 and 24.5mm of zone of inhibition was recorded against *Staphylococcus aureus* (Figure 13 a) and *Pseudomonas aeruginosa* (Figure 13b). Because of amino and hydroxyl group of the polymers, they have unique ability to form complexes with various chemical groups which facilitates binding of the nano drug conjugate to the fibre surface. This interesting property would lead to fabrication of various dressing materials used for UV protection and medicament This results confirms that GA stabilized nano drug conjugate fabricated wound dressing shows effective inhibition against the pathogenic bacteria which would suggests the possible utilization of fabricated wound dressing to prevent fatal infection.

Figure 1. UV visible absorption spectra of GA-CuNp-LF drug nano conjugate

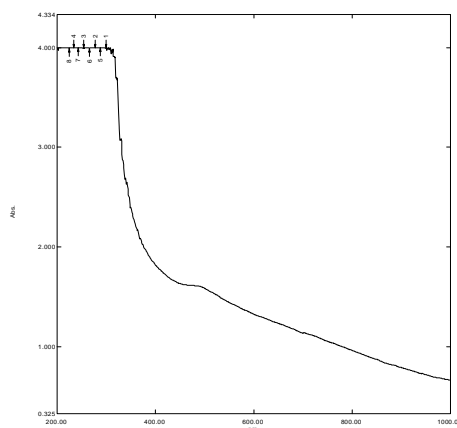


Figure 2. FTIR spectra of the GA-CuNp=LF

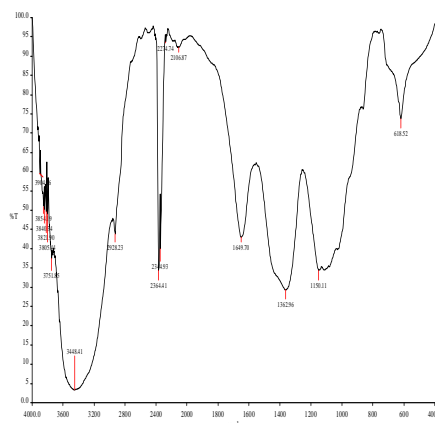


Figure 3. Scanning electron microscopy (SEM) of GA-CuNp-LF drug nano conjugate

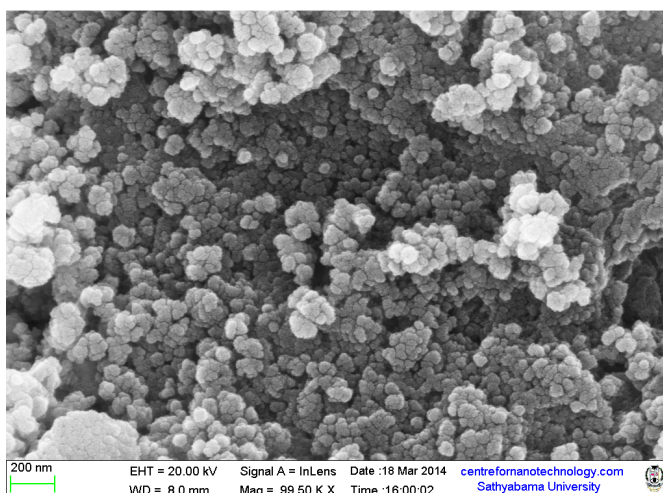


Figure 4. MIC and MLC (mg) of GA-CuNp-LF nano drug conjugate against *Staphylococcus aureus*

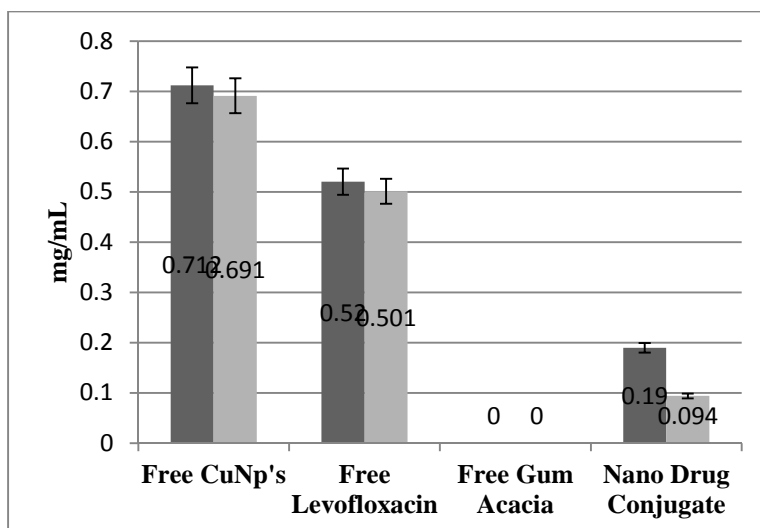


Figure 5. MIC and MLC (mg) of GA-CuNp-LF nano drug conjugate against *Pseudomonas aeruginosa*

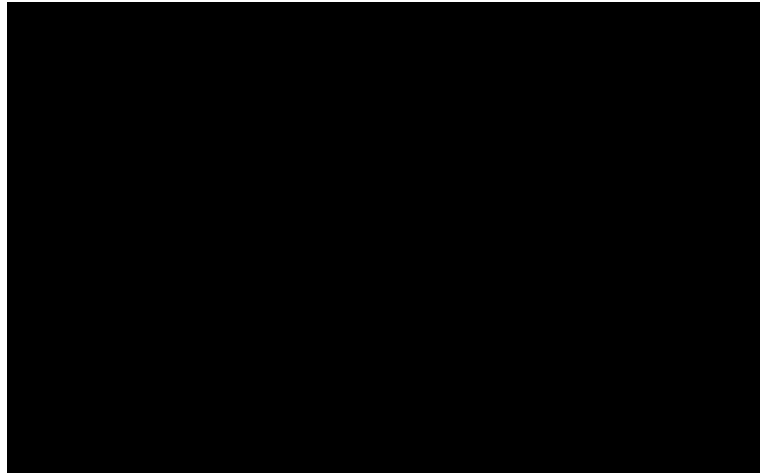


Figure 6. Scanning electron microscopy (SEM) image of GA-CuNp-LF nano drug conjugate fabricated wound dressing

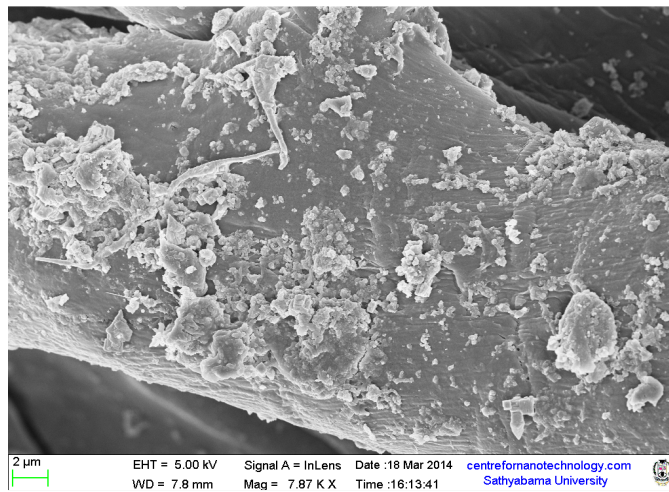


Figure 7. Effect of GA-CuNp-LF nano drug conjugate fabrication on the average breaking load (Kg) of the wound dressing

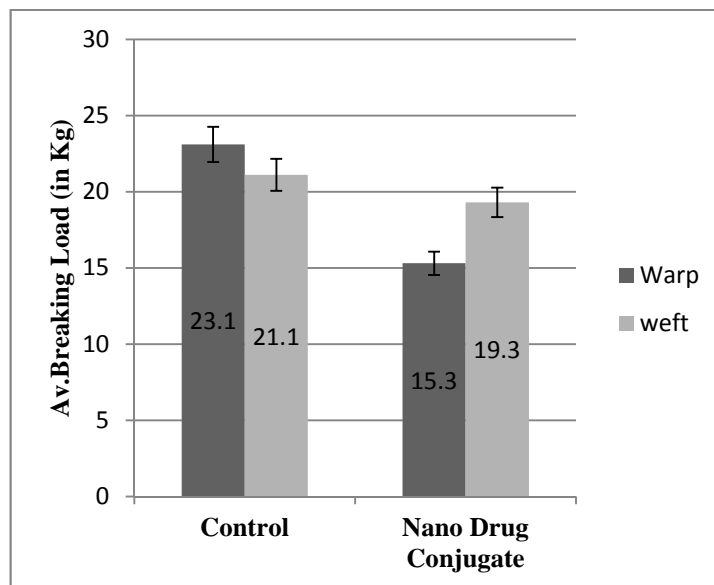


Figure 8. Effect of GA-CuNp-LF nano drug conjugate fabrication on the strain (%) of the wound dressing

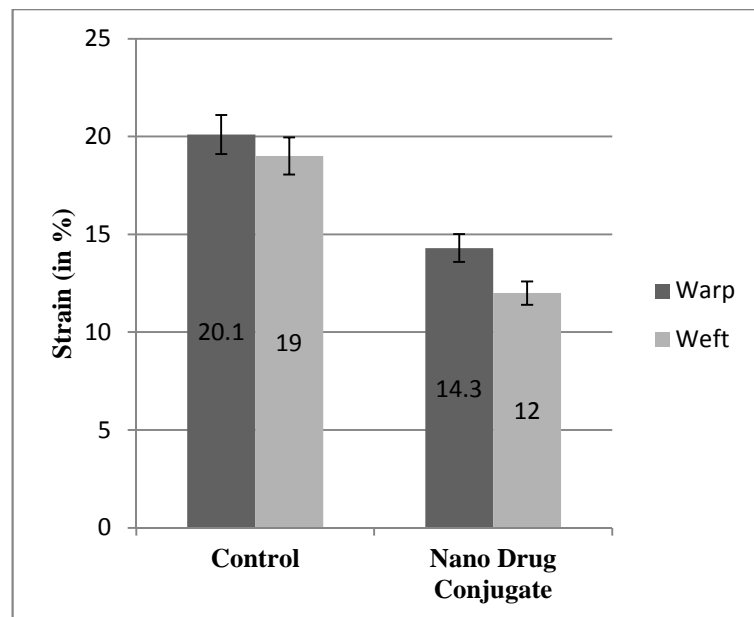


Figure 9. Effect of GA-CuNp-LF nano drug conjugate fabrication on the air permeability ($\text{m}^2/\text{kPa}\cdot\text{S}$) of the wound dressing

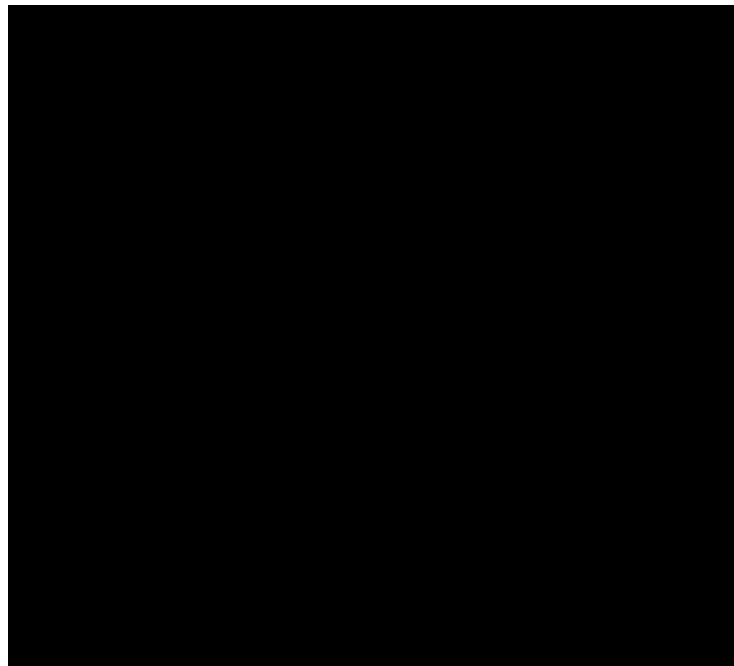


Figure 10. Effect of GA-CuNp-LF nano drug conjugate fabrication on the Burst Strength (kg/cm^2) of the wound dressing

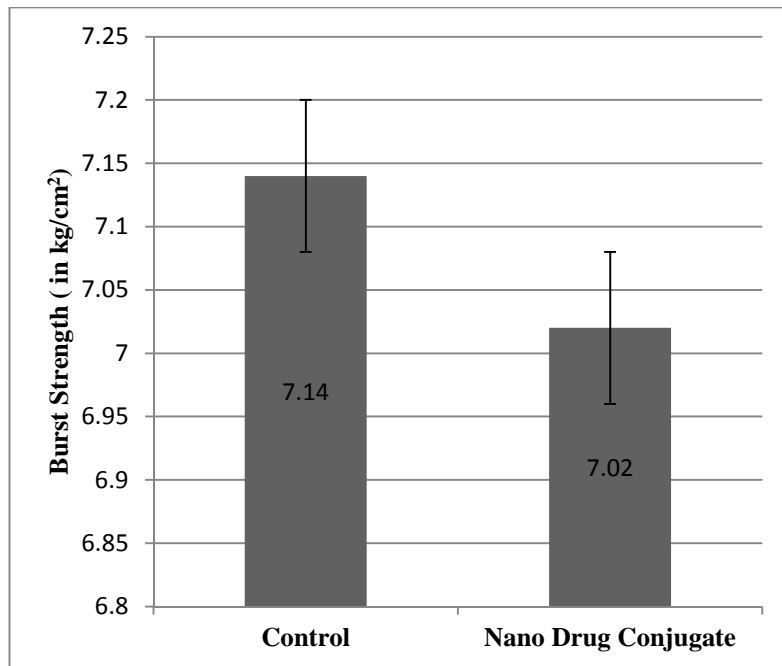


Figure 11. Effect of GA-CuNp-LF nano drug conjugate fabrication on the coefficient of friction (fabric to fabric) of the wound dressing

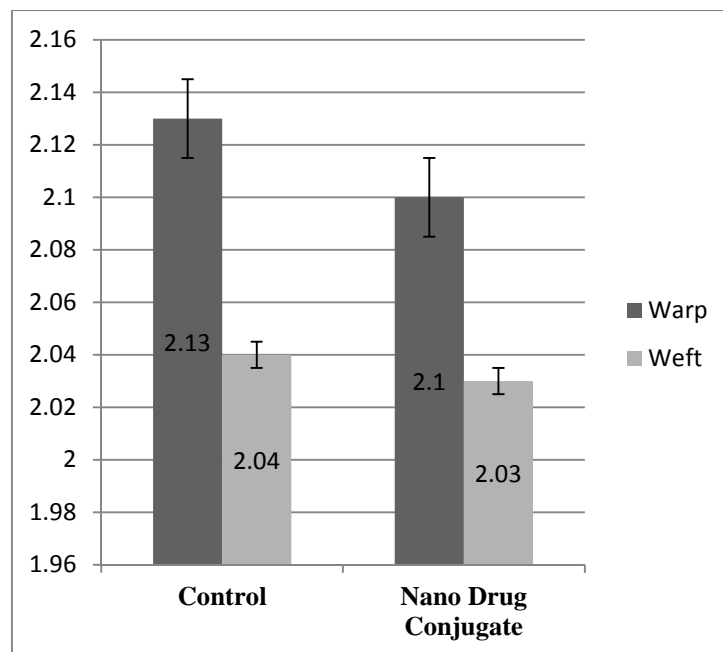


Figure 12. Effect of GA-CuNp-LF nano drug conjugate fabrication on the coefficient of friction (fabric to metal) of the wound dressing

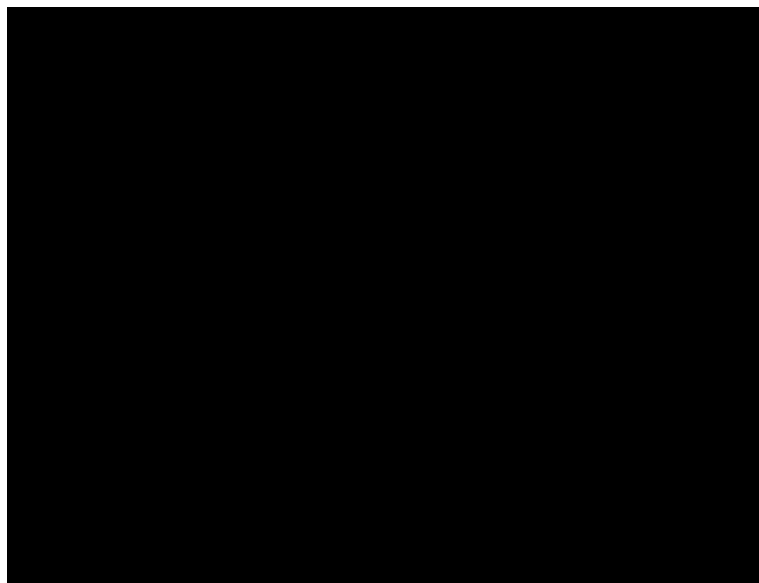


Figure 13. Zone of inhibition (mm) of nano drug conjugate fabricated wound dressing against (a). *Staph. aureus*



(b). *P. aeruginosa*



CONCLUSION

The present study, biocompatible polymer gum acacia stabilized copper nanoparticles –levofloxacin (GA-CuNp-LF) nano drug conjugate synthesized by one step reduction fabricated on wound dressing showed effective inhibition against the tested bacterial strains. Nano drug conjugate coating revealed distinct changes in mechanical properties. Therefore, the possible utilization of GA-CuNp-LF fabricated wound dressing to fight against skin infection causing bacteria known to cause high mortality. Toxicity studies will be studied to confirm the biocompatibility. In future, development of wound dressing will form a new revolution in medicine.

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