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Development of biosynthesized silver nanoparticles based formulation for treating wounds during nursing care in hospitals

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ABSTRACT

Silver nanoparticles (AgNPs) have been emerged as significant wound healing agents because of their improved mechanical properties. However, the green synthesized silver nanoparticleshave reported significant wound healing action in Albino rats which was validated by the measurement of wound closure rate. Silver nanoparticles were efficiently synthesized using *Euphorbia milii* leaf extract. The UV–visible spectra recorded the effect of the reaction time on AgNPs synthesis and was indentified that the peak became shaper with an increase in time, which corresponds to increase in the number of nanoparticles formed from the reduction of silver ions present in the aqueous solution. X-ray diffraction technique and corresponding XRD patterns confirmed the biphasic nature of the biosynthesized silver nanoparticles. However, low magnification TEM images presented monodispersed AgNPs with their size ranging from 20 to 30 nm while SAED diffraction pattern disclosed their crystalline nature. Furthermore, the wound healing activity of AgNPs was examined through the excision wound model by measuring the rate of wound closure and Group II (treated with 10% Ointment base with biosynthesized AgNPs) revealed significant wound healing activity over Control group and Group I (treated with Standard Nitrofurazone ointment) in Albino rats.

1. Introduction

Wound healing, significantly intends tore pair in the shortest time with nominal side effects [1]. In the recent times, wound infection is treated as one of the major cause of mortality followed by surgery [2]. Wound infections that are caused by pathogenic bacteria are becoming serious problems for the patients. Therefore, instant monitoring of these infections along with preventive measures and therapeutic care is mandatory [3]. In addition, destructive effect of certain microorganisms on wound healing has been extensively studies and has been considered that they are the reason behind delayed wound healing. *Pseudomonas aeruginosa* is observed to be one of the most common pathogen instigating these infections [4]. Antimicrobial treatment which has the ability to regulate colonization along with proliferation of the pathogens is considered to be the most important aspect of the wound care [5]. Consequently, antimicrobial agents like iodine compounds, silver compounds, chlorhexidine, and acetic acid are more frequently employed in the prevention and treatment of wound infections [6,7]. An ideal treatment should be able to protect the wounds against microbial interactions [8]. Metals like Silver, gold, and zincare of immense consideration over a period of time, owing to their bacteriostatic and bactericidal properties as well as due to their wide spectrum of activity [9].

Nanoparticles are particulate dispersions or solid particles with their size ranging from 1 to 100 nm [10]. The modification of microparticles to nanoparticles (< 100 nm in diameter) corresponds to an increase in the surface area along with changes in other properties. Contact surface of metals is significant to impart antibacterial activity; The larger the surface area of the nanoparticles, the greater is the extent which allows their interactions with organic and inorganic molecules [11]. In the recent time, nanomedicine is an advancing field with rapid expansion due to the exploration of new nanomaterials in a wide range of medical equipment and technology. Silver nanoparticles (AgNPs) are well renowned to exhibit exceptional physicochemical properties along with momentous antibacterial properties, consequently making them more advantageous for the progression of unconventional products against multidrug resistant microorganisms [12] as well as against some Gram-negative bacteria like *Pseudomonas aeruginosa* [13]. However, AgNPs have grabbed immense attention due to their non-toxicity towards human body even at low concentrations together with their broad spectrum of antibacterial properties [14]. Recently, the research of nanoparticles is reaching high progress, resulting in enormous increase

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of their medicinal applications and also extended to other fields of non-infectious wound healing [15].

In this work, we showed the use of new bio synthesized AgNP based ointments for wound healing process. The polyphenols of *Euphorbia milii* plant extract are responsible for reduction and stabilization of fabricated AgNPs. The fabrication of nano-silver based ointment using bio synthesized silver nanoparticles would be an efficient technique towards wound healing and unlocks a novel path for paramedical studies.

2. Experiments

2.1. Materials

Silver nitrate ($AgNO_3$) was used as a silver precursor which was supplied by Sigma-Aldrich, Shanghai. Petroleum jelly, solvents and other chemicals were also purchased from Sigma-Aldrich, Shanghai. All the solutions were made by means of deionized water.

2.2. Extract Preparation

1 g of dried *Euphorbia milii* leaves were crushed to make into powder. Thus obtained powder was dispersed in 100 mL distilled water by gentle stirring and heated at 100 °C for about 30 min. The extract was then filtered through mesh, followed by Millipore filter (0.2 μm), and kept at −20 °C before apply.

2.3. Preparation of Silver Nanoparticles

20 mL of *Euphorbia milii* aqueous extract was mixed in 80 mL of 1 mM $AgNO_3$ solution and placed in stirring under dark conditions for 30 min, followed by centrifugation at 3000 rpm for about half an hour. Further, the pellet and supernatant were isolated and the pellet was placed for 2 h for drying at 40 °C in microwave oven.

2.4. Wound Healing Assay

2.4.1. Preparation of Ointments

About 0.5 g of the biosynthesized AgNPs samples were added with 4.50 g of petroleum jelly and further stirred uniformly to give 10% AgNP formulated ointments. Thus generated ointments could be used as topical applicators [16].

2.4.2. Treatment and Excision on Animals

Male albino rats weighing about 160–1100 g were used to investigate the wound healing activity of the *Euphorbia milii* mediated AgNPs. Large polypropylene cages, bedded with rice husk were used to house the rats and proper ventilation was maintained in the room under standard conditions with temperature 28–29 °C and 12 h light/dark cycle all through the experimental duration. All the rats were supplied with standard pellet diet and water regularly. All these animals were well adapted to the surroundings one week former to their experimental usage. In this study, all the experimental procedures and protocols were followed according to the standard guidelines, approved by the institutional animal care and committee. The dorsal sides of all the rats were shaved on the day before experiment. Using a sterile surgical blade, 50 mm^2 predetermined area was excised under anaesthetic conditions. All were divided into three groups with each group consisting of three animals.

Control: Control animals.

Group-I: Wound and Standard ointment.

Group-II: Wound and 10% Ointment base with biosynthesized AgNPs.

2.4.3. Percentage of Wound Healing

The ointments were regularly applied over the excised area and covered with dressings in subsequent days. The reductions in wound size were regularly measured and the recorded changes were photographed. The wound area measurement was carried out from the day of excision with an interval of three days till complete epithelisation is observed. The area of the wound healing was calculated by means of meter ruler in both treated and control groups on 4th, 7th, 10th and 13th day. The percentage of wound healing was calculated using the following formula [17].

$$\%\text{of WH} = \frac{WA_0 \times WA_n}{WA_0} \times 100$$

where WH = Wound Healing; $WA_0$is Wound area on day 0; $WA_n$is Wound area on day n; and n = 4, 7, 10 and 13th day.

2.5. Characterization

UV–vis spectroscopy was used as an analytical tool to track AgNPs formation using Jasco UV–vis 950 spectrophotometer. A diluted nano silver colloid was used for UV–vis measurements. The morphology of the obtained nanoparticles was observed using a JEOL-2100F electron transmission electron microscope (TEM). A droplet of AgNPs dispersion was placed on an ultrathin carbon film and dried at room temperature before observation under microscope. X-ray diffraction (XRD) was detected on an X-ray diffractometer (Rigaku, D/max-2500 using a Cu tube). A dried powder of AgNPs was used for XRD measurements. Fourier transform infrared spectroscopic analysis for prepared materials was analyzed to know the surface capping and also reduction of AgNPs by using JASCO FT-IR 4100 instrument in the diffuse transmittance mode at a resolution of 4 cm^{-1}. The AgNPsand plant extract powder samples were mixed with dried KBR powder and grounded well using mortar pestle and made pellet using pellet maker. The obtained pellets were used for FTIR measurements.

3. Result and Discussion

Upon addition of the extract into the flask with aqueous silver nitrate solution, it was observed that the color of the medium changed to yellowish brown within an hour, indicating the formation of AgNPs. The spectra were recorded after at 20, 60, 90, and 120 min. UV–vis spectra was used to record the effect of the reaction time on AgNPs synthesis and was identified that the peak became shaper with an increase in time. The SPR band of AgNPs occurred at 457 nm and even after 1 h of incubation only slight variation was observed (Fig. 1). The observed increase in intensity could be because of the increase in the number of nanoparticles formed from the reduction of silver ions present in the aqueous solution.

X-ray diffraction technique and corresponding XRD patterns were used to investigate the phase of the prepared nanoparticles as shown in

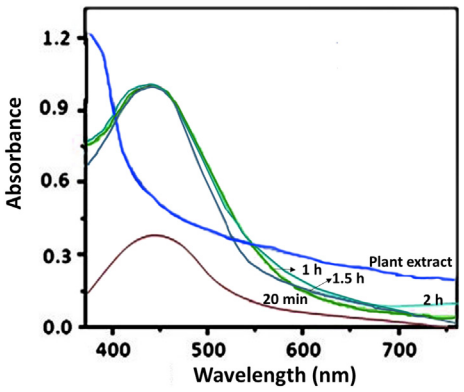

Fig. 1. UV–vis spectra of the biosynthesized AgNPs.