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Wound healing potential of naturally synthesized silver nano-particles

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ABSTRACT

Nanoparticles have been in raising demand in many fields of science and biotechnology and extends its applications in biomedical and pharmaceutical areas. Out of all the metals silver leads the way in this technology in view of its practical applications. They are used to preserve, diagnose many diseases and mainly used in external applications on the body emphasizing their antimicrobial and healing abilities. On the other hand, the synthesis of nano particles has been a very serious concern due to its huge cost of production, toxicity and dosing issues. Bio synthesis or green synthesis using herbals and microbes to synthesize nano particles showed a path to overcome the above hurdles and make the synthesis of silver nanoparticles more commercial and can be adopted on large scale. In this research silver nano particles were synthesized using purified extracts of leaves of Lannea coromandelica and were confirmed using UV spectroscopy. The produced nanoparticles exhibited surface plasmon resonance at 420nm in UV spectroscopy. They were investigated for their wound healing activity in excision wound model in wistar albino rats for a 16 day study. These nano particles were found to be very successful and showed wound healing property. Thus produced nano particles showed similar activity competent enough with that of the standard, Betanidine. These silver nano particles can be used efficiently in therapies that target infections and wounds that are provoked and delayed healing due to infections.

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INTRODUCTION

Silver nano particles have now a days gaining value in a number of arenas of biomedicine and biotechnology. In view of their practical applications and pros over other technologies, these are used as tools of diagnosis and medicine. Nano particles are synthesized in various techniques each of them has their own pros and cons. Out of all the herbal or green synthesis of nano particles had been an emerging now a days in nanotechnology where safety and health is concerned. The above-mentioned types of synthesis are considered as nature friendly, efficient and less toxic approaches to humans and ecosystem as well.

Out of all the metals, Silver is considered as sacred and one of the precious with respect to the medical applications parallel to gold and copper in human history since 500 AD. From the past days it is used

as water, wine and milk storage containers on the belief that it kills micro-organisms [1] . Even though there was no established scientific evidence to prove the same, it had been widely used in household and in health care also. The mechanism of action and the toxicity of silver was not established till late 90's but the usage is not restricted as its roots are carried in from ages. The higher efficacy of silver to fight against infections and thus helpful in treating the open wounds and surgical wounds. Most of the marketed formulation such as ointments and gels also incorporate silver in them. Even though they use silver as wound healing agent, the toxicity behind its synthesis and their derivatives could not overcome the side effects and cost burden need to synthesize them. Considering these disadvantages nano silver synthesis has been flexed towards the investigations of cheaper and economical ways to produce silver nano particles so as to use them in pharmaceutical industry [2].

Numerous investigations had been made to prove the effect of herbals to synthesis of silver nano particles. There had been plants that are used for the purpose they are Neem, Soap nuts, geranium, Citrus, Tea, Coffee, Tannic acid and many other microorganisms too. In this research the silver anno particles were synthesized biologically using the aqueous extract of leaves of Lannea coromandelica. They are synthesized using the reduction property of the extract due to the presence of rich poly phenols. They can be used to reduce the silver into silver nano particles. Thus, produced silver nano particles are investigated for their wound healing properties [3].

MATERIALS AND METHODS

Extraction of Leaves of Lannea

Leaves of Lannea coromandelica were collected from the campus of Sree Venkateswara University, Chittore and duly authentified and the herbarium sample is preserved in the college library. The leaves were shade dried and finely ground to powder. 50gm of this powder was extracted with 250 ml of methanol using a Soxhlet apparatus till exhausting of drug. The obtained extract was filtered using a whattman filter paper to get a clear filtrate and was used for further experiments.

EXPERIMENTAL ANIMALS

Adult wister albino rats ranging between 180-250gm of both sex were kept in normal conditions in the lab in PPE cages at room temperature and fed with normal pellet feed and water ad libitum. The rats were separated into 3 groups of rats in each groups.

Green Synthesis of silver nanoparticles

50ml of 1mM AgNO3 was added to 1ml, 5ml, 10ml of lannea extract separately in different beakers and volume is made up to 200ml using distilled water. The mixture is centrifuged at 18000 rpm for 30 min to separate any granular material. The supernatant liquid was collected and heated at 50ºC to 60ºC [4] . the colour change in the resultant solutions were noted and then they are named as SNP 1, SNP 5, SNP 10 respectively.

Evaluation

The formation of silver nano particles was evaluated by subjecting the reaction mixture to UV-Vis spectrum (UV-Vis Spectrophotometer UV 2450 (SHIMADZU) by diluting the mixture with distilled water. This estimation was done at 30 min, 1, 2 and 3 hrs intervals of heating [5].

Wound healing potential

Rats were given anesthesia before creation of wound as per Morton (Morton and Malone, 1972). The hair on the back skin of the rats was removed by wiping with an available depilatory with the help of a cotton swab. A round wound of diameter 400 mm^2 was made on the animals by cutting the dorsal skin. The total wound is opened and the %closure is noted down on 1^{st},4^{th},7^{th},10^{th},13^{th} and 16^{th} days after wounding the rats [6]. The area of the wound is measured in sqmm on a graph sheet and was taken as initial wound area.

I: Control group with simple ointment base;

II: Standard group received topical application of Betanidine (5% w/w) ointment;

III: Received simple ointment containing silver nanoparticles (5%v/w), topically.

The ointment, standard drug and silver nanoparticles were applied on wound on daily basis everyday up to 16^{th} day. The wound area of each animal was noted on the 1^{st},4^{th},7^{th},10^{th},13^{th} and 16^{th} days in sqmm by using graph sheet. They were observed thoroughly for epithelialization and contraction of wound. The total epithelialization time (ET) was noted after observing the fall of scab showing no raw wound area. The % protection was calculated on the 16^{th} day by using the below formula [7].

$$\%\ protection \quad = 100 - (Final\ X\ 100)/Initial$$

Statistical Analysis

The results are presented as meanSEM and analysed in One-way ANOVA followed by Dunnett's test. The values of p<0.01 were considered significance.

Figure 2.

Table 1: Woundhealing activity of silver nanoparticles

Group	Wound area (sqmm)						% protection	ET
	1stDay	4thDay	7thDay	10thDay	13thDay	16thDay		
Control (base)	400±0.44	380.5±0.76	373.6±0.42	309.3±0.33	221.1±0.55	157.33±0.3	66.38	24
Betadine (5% w/w)	400±0.365	367±0.35*	279.6±0.4*	232.6±0.9*	93.6±0.33*	7.16±0.3**	98.450	16
SNP-10 +base	400±0.307	350.1±0.3*a	250.6±0.3*a	201.5±0.42*	151.3±0.1*	13.16±0.2**	97.18	17

Results were represented as mean±SEM, n=6; *P<0.01 significant compared to control;**more significant; ^{a} significant compared to std

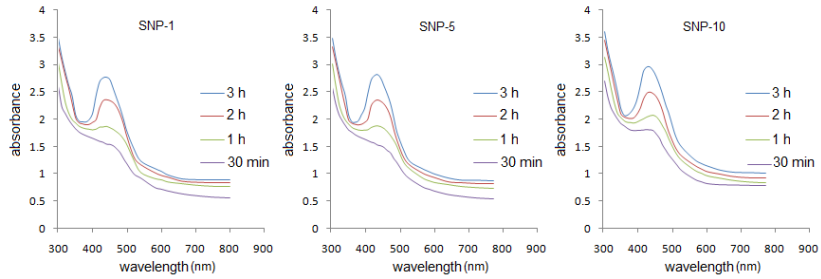

Figure 1: UV spectra of silver nano particles

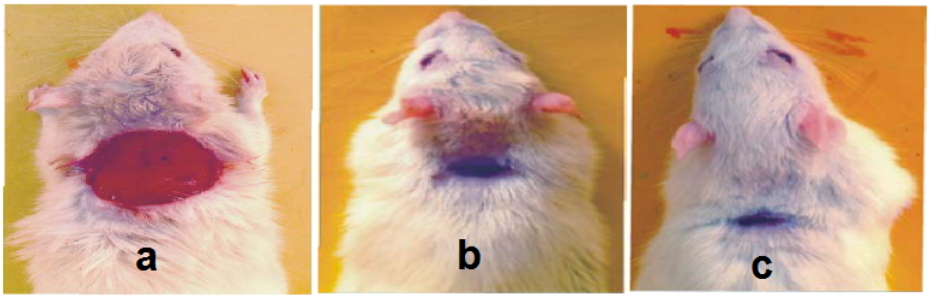

Figure 2: Wound healing of silver nanoparticles on, a. 1^{st} day, b. 8^{th} day and c. 16^{th} day

RESULTS AND DISCUSSION

The method used for synthesis of silver nano particles is valid and the extract of leaves of Lannea coromandelica has helped in successful production of silver nano particles which was evident from the colour change in the silver nitrate solution while heating. The solution was initially pale yellow to orange colour. This turned into dark brown colour which suggests the formation of silver nano particles. The intensity of colour also indicate the complete reduction of silver into silver ions which forms nano particles.

The spectral analysis of the solution under UV exhibited the surface plasmon resonance at 420nm which is indicative of formation of silver nano particles. Nano particles show the surface plasmon vibrations at a higher intensity in UV spectroscopy [8]. The peaks observed at the intervals of 30 min, 1hr, 2hrs, 3hrs respectively and are illustrated in Figure 1. The peak noted at the end of 3^{rd} hour gives the absorption maximum at 420 nm. The differences in heights are due to the formation of a greater number of nano particles. The formation of nano particles is proportional to the heights of peaks. The peaks in the third hour are sharp and crisp which indicates that the formed nano particles are all even sized and are in contrast with the smudged and blunt peaks at 1^{st} and 2^{nd} hours. The peaks at shorter wavelengths is due to the formation of silver nano particles due to the intervention of secondary metabolites which are present in the methanol extract of lannea leaves. The most commonly reported reducing and antioxidant agents in the extract are mainly poly phenols

and flavonoids [1]. These are majorly helpful for the reduction of silver in to silver ions and thus formation of nano particles (Table 1).

The wound healing activity of silver nanoparticles was investigated on the excision wound model in wistar albino rats. As SNP 10 showed a greater antibacterial activity and low particle size, it has been selected as a test sample to study the wound healing activity. The effect of silver nanoparticles, was better than the standard and control rats (i.e., simple ointment base group). Interestingly, wound healing is observed in all the three groups at the end of the study. The healing of wound during the course is shown inFigure 2.

CONCLUSION

Silver is one of those metals which is used as well as household and as medical, diagnosis and therapeutic agent. It is clearly evident from the literature and researches that the silver nano particles are very potent and efficient as an anti-microbial and wound healing agent when compared to other synthetic drugs and herbal extracts alone. The synthesis of silver nano particles had been a very serious concern as it uses synthetic chemicals which are known to cause side effects and toxicity. Considering this, green synthesis or herbal synthesis of silver nano particles had been adopted in the current research to produce nano particles. So, the methanol extract of leaves of Lannea coromandelica had been used to produce silver nano particles since this biomimetic synthesis was proven to have relatively safer and cost effective. Thus produced silver nano particles were tested for their wound healing activity and were proven to show a better activity compared to the standard drug betanidine. The potency and effect of silver nanoparticles was proven yet the toxicity was to be considered for establishing it as a therapeutic agent.

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Conflict of Interest

Authors declare no conflict of interest.

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