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ScienceDirect

European Journal of Integrative Medicine 7 (2015) 243–250

European Journal of INTEGRATIVE MEDICINE

www.elsevier.com/eujim

Original article

Can green synthesized propolis loaded silver nanoparticulate gel enhance wound healing caused by burns?

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Received 12 December 2014; received in revised form 4 March 2015; accepted 4 March 2015

Abstract

Introduction: Nanotechnology can offer new opportunities in the fight against infection. The aim of current work was to investigate an eco-friendly method for synthesis of silver nanoparticles (AgNP) which have the ability to load lipophilic compounds onto their surface.
Methods: Pharmaceutically acceptable hydrophilic lipid (Gelucire® 50/13) has been used as a reducing agent for in situ reduction of silver nitrate so as to obtain silver nanoparticles. Propolis is used as model molecule for loading onto surface of AgNP owing to its well reported broad range of pharmacological activities including anti-inflammatory, antioxidant and antimicrobial activity. Propolis loaded silver nanoparticles (PLSN) were prepared and characterized for silver content, surface plasmon resonance, particle size, XRD, FTIR, TEM, antibacterial activity and burn wound healing in wistar rats.
Results: Propolis constituents were successfully loaded onto surface of AgNP using the proposed conceptual method. The formation of PLSN having size 24.3 ± 2.5 nm was confirmed using surface plasmon resonance, FTIR, XRD and TEM. The combination of propolis with AgNP significantly reduced minimum inhibitory concentration of AgNP alone when tested against *Staphylococcus aureus*. PLSN gel showed comparable burn wound healing in wistar rats when tested against marketed silver sulfadiazine gel.
Conclusion: The use of Gelucire® as solubilizing agent for lipophillic drugs was effectively utilized for loading lipophillic constituents of propolis onto the AgNP. This potentially provides an effective method for the green synthesis of AgNP which can be used to load lipophillic molecules onto their surface whenever such combination is required.

Keywords: Gelucire 50/13; Silver nanoparticles; Propolis; Burn wound; MIC; Infection

Introduction

Nanoparticles are at the leading edge of the rapidly developing field of nanotechnology. Their unique size dependent properties make them superior in many areas of human activity. Silver nanoparticles (AgNPs) show broad spectrum antibacterial activity against various strains of bacteria including *Salmonella*, *Staphylococcus* and *Pseudomonas* [1–3]. Several mechanisms have been proposed for antibacterial activity of silver nanoparticles. AgNP cause structural changes (formation of 'pits') in the bacterial cell membrane thereby increasing its permeability which in turn leads to loss of cell content and death of cell. Another mechanism is the formation of free radicals by AgNP which causes bacterial cell death. Further, silver ions released by the nanoparticles interact with the thiol groups of many important bacterial enzymes leading to their inactivation is one of the proposed mechanisms. The bacterial cells contain sulfur and phosphorus (e.g. DNA) which are soft bases. Silver, a soft acid has a tendency to react with base which creates problems in the DNA replication of the bacterial cell and subsequently kills the cells. Another AgNP mechanism is the alteration in the phosphotyrosine profile of bacterial peptides which modulate the signal transduction in bacteria leading to the stoppage of growth [4,5]. Moreover, since silver attacks a broad range of targets in the microbes, so it is difficult for them to develop resistance against silver [6]. In spite of having such potential, widespread use of

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http://dx.doi.org/10.1016/j.eujim.2015.03.002
1876-3820/

AgNPs is limited. A number of approaches are available for the synthesis of AgNP including reduction in solutions [7], chemical and photochemical reactions in reverse micelles [8], thermal decomposition of silver compounds [9], and electrochemical method [10]. One of the reasons for its limited use includes use of toxic and flammable chemicals for their synthesis. Thus there is a need to investigate cost effective and eco-friendly method for synthesis of AgNPs. There are well reported methods on green synthesis of AgNPs using different biological sources such as plant extracts [11–14], bacteria [15], fungi [16] and enzymes [17].

Propolis is a resinous substance collected by honeybees from buds and leaves of trees and plants, and consists of a mixture of pollen as well as enzymes secreted by bees. Its composition depends on the geographical location and variety of vegetation surrounding the hives. It has attracted much attention in recent years owing to its various biological properties including antimicrobial, antioxidant, antiulcer, etc. [18,19]. The antimicrobial property of propolis has been widely reported [20–22]. Caffeic acid phenethyl ester (CAPE), a lipophillic constituent has been identified as a main active ingredient of propolis. There are reports suggesting the utility of CAPE in treatment of human patients who have been severely burned [23,24]. Most of the time, a combination of AgNPs with lipophillic molecules is desirable but there is a gap in the current literature presents lacuna in this area. To the best of our knowledge no method is available which may combine AgNPs with lipophillic molecules without use of organic solvents. Further the method investigated for synthesis of AgNPs should also present favorable sites onto the surface of AgNPs for effective loading of lipophillic molecules to achieve desired combination. Literature suggests that the combination of AgNPs with propolis may prove fruitful in the treatment of wounds resulting from a burn. A burn being defined as a type of injury to flesh or skin caused by heat, electricity, chemicals, friction, or radiation [25]. Biogenic synthesis of silver and gold nanoparticles using ethanolic and aqueous extracts of Indian propolis has been reported in the literature [26]. However, the synthesis is carried out at alkaline conditions (pH ~10.6) which make it unsuitable for topical use for wound treatments. Further aqueous extract of propolis is devoid of CAPE due to its inherent water insolubility which is principal constituent of propolis having attributed with wide range of pharmacological activities.

Thus, the objective of the current work was to investigate eco-friendly method for the synthesis of AgNP and further to assess its ability for combination of lipophillic constituents with AgNPs. Gelucire 50/13 (GL) contains stearic acid, tripalmitic acid, esters of glycerol along with mono- and di-fatty acid esters of PEG-1500. It has been widely reported as solubilizing agent for poorly water soluble drugs due to the presence of hydrophilic PEG and glycerol molecules [27]. The structure of GL reveals large number of –OH groups and the literature suggests that hydroxyl groups play an important role in situ reduction of $AgNO_3$ [28]. Hence, GL was screened for in situ reduction of $AgNO_3$.

In the current work, propolis loaded AgNP was prepared and characterized for silver content, surface plasmon resonance, particle size, X-ray diffractometry and transmission electron microscopy. The prepared propolis loaded AgNP was also analyzed against clinically isolated strain of *Staphylococcus aureus*. Additionally, topical gel of propolis loaded AgNP was prepared and assessed for wound healing activity.

Materials and methods

Materials

$AgNO_3$ was purchased from Central Drug House, Mumbai, India. Gelucire 50/13 (GL) and propolis were obtained as generous gift from Gattefosse, France and Nature's Laboratory, UK. All the chemicals were of analytical reagent grade and used without further purification.

Methods

Preparation of silver nanoparticles (AgNP)

Green synthesis of AgNP was carried out by heating (50–55 °C for 5 min) 10^{-3} M of $AgNO_3$ solution containing 0.1% (w/v) of Gelucire 50/13 in deionised water.

Preparation of propolis loaded silver nanoparticles (PLSN)

Propolis constituents were extracted using hydrophilic lipid i.e. GL. Propolis:gelucire extract (PGE, 0.5:1, w/w) was prepared by addition of propolis in molten GL. An aqueous solution (0.15%, w/v) of PGE was prepared containing 10^{-3} M of $AgNO_3$ which was subjected to heating (50–55 °C for 5 min) in order to obtain PLSN.

Preparation of physical mixture of propolis and silver nanoparticles (PMSN)

PMSN was prepared by mixing the already synthesized AgNP with propolis extract (0.05%, w/v).

Characterization

UV-vis spectroscopy

UV-vis spectroscopy (V-530, Jasco, Japan) is the most common method used to confirm the formation of AgNPs, since nano-silver exhibit surface plasmon resonance (SPR) at about 400–500 nm wavelength in UV-vis spectroscopy and this was the basis for our further study [2,4,8]. All colloidal solutions i.e. AgNP, PLSN, PMSN prepared for the study were scanned in the range of 200–800 nm wavelength in UV-vis spectroscopy.

Particle size analysis

The particle size of all the prepared colloidal solutions was measured by BIC 90 Plus Particle Size Analyser (Brookhaven Instruments Corporation, USA).

Determination of silver content

The silver content of AgNP, PLSN and PMSN colloidal solutions was determined by using atomic absorption spectroscopy (AAS) (Varian spectraAA 220 spectrometer).