Green synthesized silver nanoparticles for cream formulation: its anti-inflammatory and healing activities

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Abstract

Among several forms of metallic nanoparticles used in biomedical applications, AgNPs are the most fascinating and widely used. Therefore, this study was carried out to evaluate the anti-inflammatory activity of *Ehretia cymosa*-AgNPs cream. Different extracts of the plant (methanol, ME; n-hexane, NE; ethylacetate, EE) were used to synthesize AgNPs, which were characterized by UV and FTIR spectroscopy. The AgNPs were used to prepare creams, and the physical properties of the creams were evaluated. Anti-inflammatory activities of the creams were evaluated by carrageenan induced rat paw edema method on albino rats. The biosynthesized AgNPs had UV absorption spectra in the range 400-450 nm. The creams had pH of 4.6-5.6 similar to the pH of the skin. Cream formulation NO (no extract) had no significant anti-inflammatory activity within 6 h of treatment while formulations NE, ME and EE had a significant anti-inflammatory activity within 6 h of treatment. The efficacy of the cream formulation of the extracts after 6 h of treatment was in the order EE > ME > NE. There was a significant anti-inflammatory activity with the AgNPs cream formulations. Formulations MS, NS and ES had 87.1, 90 and 100% anti-inflammatory activity, respectively in 4 h of treatment. The ES, MS and NS cream formulations had 100% healing in 4, 5 and 6 h, respectively, while only formulation EE had 100% healing in 6 h. Other cream formulations containing the extract could not achieve 100% healing in 6 h. The study established that *Ehretia cymosa*-synthesized AgNPs cream formulations had a faster healing ability compared with the positive control and *Ehretia cymosa* cream formulations.

Keywords: Silver nanoparticles, *Ehretia cymosa*, cream formulation, anti-inflammatory activity
1. Introduction

Creams are semi-solid emulsions of oil and water. They are topical preparations which could be medicated or non-medicated. Medicated creams contain active pharmaceutical ingredients (API) most often used as antimicrobial agent, anti-inflammatory agent, anti-acne agent among others, while non-medicated creams do not contain API. They are often used because of their emollient and moisturizing properties. Silver Nanoparticles (SNPs) have gained acceptability and applicability in many sectors like medical, pharmaceutical, chemical, health care, consumer, construction, food, and textile [1]. Nanoparticles are small particles in size range of 1-100 nm which confers an advantage over micro-sized particles in terms of rate of activity [2, 3].

There are several forms of metallic nanoparticles used in biomedical applications; silver nanoparticles (AgNPs) are the most fascinating and most widely used due to their remarkable activities such as antimicrobial, anti-inflammatory and anti-coagulant [4,5,6]. Several studies on the biomedical applications of biosynthesized silver nanoparticles are available in the literature, each with different focus [7, 8]. Also, many researchers have reported the anti-inflammatory potentials of biosynthesized silver nanoparticles [9-12]. Inflammation is the body's way of protecting itself from infection, illness, or injury. Symptoms include pain, swelling, redness, immobility and heat.

*Ehretia cymosa* is a shrub, which belongs to the family Boraginaceae. It is found abundantly in western, central and eastern Africa. It is commonly called Shekutu in Luhya, Kenya and Jàokè in Yoruba, Nigeria. It is used in traditional folklore medicine as a mild laxative, treatment of muscle stiffness, toothache, inflammation and healing of fractured bone [13]. The stem is used as a chewing-stick for maintaining tooth and gum hygiene. This study was carried out to evaluate the anti-inflammatory activity of a cream formulation incorporated with silver nanoparticles biosynthesized with different extracts of *Ehretia cymosa*.

2 Materials and Methods

2.1 Leaves Collection and Extraction

The leaves of *Ehretia cymosa* plants were collected and identified at the Forest Research Institute of Nigeria (FRIN), Ibadan with voucher No. 112440. The leaves were dried and milled into powder with a blender. The powdered leaves of *Ehretia cymosa* was extracted with three different solvents (N-hexane, ethylacetate, and methanol). The filtrates were concentrated to dryness with a rotary evaporator.
2.2 Synthesis of silver nanoparticles

About 0.1g of the powdered extract was dissolved in 100 ml of distilled water at 60 °C for 1 h; it was allowed to cool and then filtered with Whatman No. 1 paper. The filtrate was centrifuged at 4000 rpm for 15 min. Thereafter, 1 ml of the centrifuged filtrate was transferred into 40 ml of 1 mM aqueous silver nitrate solution and was observed for colour change.

2.3 Characterization of *Ehretia cymosa*-synthesized silver nanoparticles

The synthesis of *Ehretia cymosa*-silver nanoparticles were monitored by measuring the absorption spectra in the wavelength range of 300-700 nm using T90+ UV/Vis spectrometer and the spectrum was recorded and the maximum absorption wavelength was determined. The silver nanoparticles synthesized were also characterized by Transform infrared (FTIR) spectroscopy which was carried out with PerkinElmer spectrum spectrometer version 10.03.02 to detect the biomolecules responsible for the biosynthesis of the silver nanoparticle.

2.4 Cream preparation

The creams were prepared according to Table 1 with each cream formulation containing 2 g of either the extract from the different solvents or the AgNPs synthesized from the extract of these solvents. Two (2) gram of the extract or the AgNPs was mixed with 0.1 g of chlorocresol and the required quantity of distilled water with the aid of heat to about 70 °C. Nine (9) gram of emulsifying wax, 15 g of white soft paraffin and 6 g of liquid paraffin were melted together at 70 °C. The chlorocresol and extract solution was added to it at same temperature (70 °C) and stirred until it was cold. The pH of the formulations was determined with a digital pH meter. Each gramme (1g) of the formulation was dissolved in 100 ml of distilled water in a beaker and the pH reading was recorded in triplicate [14].

2.5 Evaluation of anti-inflammatory activity of the cream formulations

Carrageenan-induced rat paw edema method according to established protocol [15] was adopted in evaluating the anti-inflammatory activity of the creams. Seven groups of six albino rats were used. Edema was induced by injecting Carrageenan into the sub-planar tissue of the right hind paw in all animal groups. The linear paw circumference was measured before and after induction and also at an hourly interval after the application of the cream formulations.
Table 1. *Ehretia cymosa* extract and AgNPs cream formulations

| Ingredients (g)         | NO  | NE   | EE   | ME   | NS   | ES   | MS   | Diclo |
|-------------------------|-----|------|------|------|------|------|------|-------|
| Extract/AgNPs           | -   | 2.0  | 2.0  | 2.0  | 2.0  | 2.0  | 2.0  | 2.0   |
| Emulsifying wax         | 9.0 | 9.0  | 9.0  | 9.0  | 9.0  | 9.0  | 9.0  | 9.0   |
| White soft paraffin     | 15.0| 15.0 | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 | 15.0  |
| Liquid paraffin         | 6.0 | 6.0  | 6.0  | 6.0  | 6.0  | 6.0  | 6.0  | 6.0   |
| Chlorocresol            | 0.1 | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  | 0.1   |
| Distilled water, to     | 100.0| 100.0| 100.0| 100.0| 100.0| 100.0| 100.0| 100.0 |

NO, No extract; NE, N-hexane extract; EE, ethylacetate extract; ME, methanol extract; NS, N-hexane extract-AgNPs; ES, Ethylacetate extract-AgNPs; MS, Methanol extract-AgNPs; Diclo, Diclofenac cream

3. Results and Discussion

Peak absorption wavelength for all the three *Ehretia cymosa* extract-mediated silver nanoparticles were observed in the range of 400-450 nm. This confirms that the color change that occurred was due to the conversion of silver nitrate to silver nanoparticles with the extract serving as the a reducing agent. Similar range of absorption wavelength of silver nanoparticle was observed in other studies [7, 16, 17].

Transform infrared (FTIR) spectroscopy was carried out to identify and confirm the biomolecules responsible for the synthesis, reduction, capping and stabilization of the silver nanoparticle. As revealed in Figure 1, the FTIR spectrum shows absorption peaks at 3356.37, 2091.85, 1629.96, 1311.71, and 1038.94 cm\(^{-1}\) which is an indication that a capping agent responsible for bio-reduction is present.

The normal human physiological skin pH is in the acidic range [18]. As presented in Table 2, the pH of all the cream formulations was within acidic range (4.6-5.6). This is an indication that the formulations may not cause skin irritation.
Table 2. The pH of cream formulations

| Formulation | pH      |
|-------------|---------|
| NO          | 5.87±0.02 |
| NE          | 5.25±0.10 |
| ME          | 5.10±0.07 |
| EE          | 4.80±0.11 |
| NS          | 5.20±0.02 |
| MS          | 5.30±0.02 |
| ES          | 4.95±0.01 |
| Diclo       | 5.50±0.06 |

Figure 1. FTIR spectrum of the synthesized AgNPs

The anti-inflammatory activity of the creams is presented in Figures 2, 3 and 4. Formulation NO (cream without any active ingredient) did not have any significant anti-inflammatory activity
within 6 h of treatment while the N-hexane extract (NE), methanol extract (ME) and ethyl acetate extract (EE) had significant anti-inflammatory activities within 6 h of treatment (Figure 2). The NE, ME and EE creams had 94.5, 96.7 and 100% anti-inflammatory activities, respectively in 6 h. The efficacy of the cream formulations from the extracts after 6 h of treatment was in the order EE > ME > NE.

There was a significant anti-inflammatory activity with the AgNPs cream formulations (Figure 3). Methanol extract silver nanoparticle (MS), N-hexane extract silver nanoparticles (NS) and ethyl acetate extract silver nanoparticle (ES) cream formulations had 87.1, 90 and 100% anti-inflammatory activities, respectively in 4 h of treatment. The ranked order of the anti-inflammatory activity was ES > NS > MS.

The ES, MS and NS cream formulations had 100% healing in 4, 5 and 6 h, respectively while only formulation EE had 100% healing at 6 h (Figure 4), while NE, ME (cream formulations containing the extract) and formulation containing diclofenac, the standard drug could not achieve 100% healing in 6 h. Silver has been used traditionally as a therapy for wound healing especially burns [19]. The formulation of silver sulfadazine cream was based on the knowledge of the traditional use of silver [20]. The wound healing potentials of silver nanoparticle have been reported severally in literature [21-24].

![Figure 2. Percentage (%) anti-inflammatory activity of *Ehretia cymosa* cream formulations](image-url)
Figure 3. Percentage (%) anti-inflammatory activity of *Ehretia cymosa*-AgNPs cream formulations

Figure 4. Percentage (%) healing with time on application of the cream formulations
4 Conclusion

Metallic nanoparticles especially silver nanoparticles have widely gained acceptability and applicability in biomedicals. In this study, the anti-inflammatory activity of a cream formulation incorporated with silver nanoparticles biosynthesized with *Ehretia cymosa* leaf extract was evaluated. The study established that *Ehretia cymosa*-synthesized silver nanoparticles cream formulations had a better anti-inflammatory properties and a faster healing ability compared to the positive control (diclofenac) and *Ehretia cymosa* cream formulations.

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