Journal of Pharmaceutical Research International

Volume 35, Issue 4, Page 32-43, 2023; Article no.JPRI.97595
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Determination of the Anti-inflammatory and Anti-microbial Activity of a Cosmetic Product Silver Stop® Cream

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Authors' contributions

This work was carried out in collaboration among all authors. Authors ZPD, TPP and IMG did the conceptualization, data validation and literature searches. Authors FH and TPP did the formal analysis. Authors II and TT did the data validation. Authors TT, TPP and II did the study investigation. Authors ZPD, IG, TPP and II wrote the original draft of the manuscript. Author TPP and II wrote, reviewed and edited the manuscript. Authors TPP, II and TT did the data visualization. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2023/v35i47321

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/97595

Original Research Article

Received: 08/01/2023
Accepted: 13/03/2023
Published: 17/03/2023

ABSTRACT

The anti-inflammatory and antimicrobial effects of a cream containing extracts of African geranium (*Pelargonium sidoides* DC.), black elderberry (*Sambucus nigra* L.) and St. John's wort (*Hypericum perforatum* L.) in colloidal nanosilver (AgNPs) at a concentration of 30 ppm, denoted as SILVER

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STOP® cream (SS® cream) was examined *In vitro*. The research was performed with *Staphylococcus aureus* (ATCC-6538 and two clinical strains). Suspension tests for determination the time of antimicrobial action of SS® cream were used. SS® cream showed significant antimicrobial activity. In suspension with a density of 10^4 cells.mL^{-1}*S. aureus* died after 60 minutes of exposure to SS® cream. In suspension with concentration of 10^6 cells.mL^{-1}, after 2 h only single cells remained viable. The anti-inflammatory effect was evaluated using human epithelial cell line HT-29, incubated with a type strain of *S. aureus.* After treatment with SS® cream, results have shown a statistically significant reduction of the levels of the pro-inflammatory cytokinesIL-6, IL-8, IP-10 and IL-1β.

Keywords: Colloidal nanosilver AgNPs; Staphylococcus aureus; Pelargonium sidoides; Hypericum perforatum; Sambucus nigra; antimicrobial activity; anti-inflammatory activity.

1. INTRODUCTION

Staphylococcus aureus is a Gram-positive bacterium known as a major pathogen causing various bacterial skin infections [1,2]. It is able to cause not only skin and soft tissue infections and inflammations, but also bacteraemia, endocarditis, intravascular infections, pneumonia, osteomyelitis, septic arthritis, pyomyositis, mastitis, endophthalmitis, parotitis, urogenital infections, central nervous system Infections, etc. [3]. S. aureus spread worldwide and is characterized by rapidly increasing antibiotic resistance [2]. *S. aureus* is a wide spread bacterium, and infections caused by it play an extremely important role in a number of diseases. Among them are such as atopic dermatitis, psoriasis, allergic asthma, pulmonary cystic fibrosis, food poisonings, multiple sclerosis, sarcoid, osteomyelitis and others. Virulent strains of *S. aureus* are able to secrete various exotoxins and enzymes, thereby inducing inflammatory reactions and activating inflammatory cells, such as keratinocytes, helper T cells, innate lymphoid cells, macrophages, dendritic cells, mast cells, neutrophils, eosinophils and basophils. Activated inflammatory cells express various cytokines and induce an inflammatory response. *S. aureus* can also induce host cell death through apoptosis, pyroptosis, necroptosis, autophagy, etc. Knowledge of the pathogenic mechanisms of *S. aureus* provides a basis for the targeted treatment of the diseases caused by this species. In the therapy of infections caused by methicillin-susceptible strains of *S. aureus*, success can be achieved with ß-lactam antibiotics such as cephalosporins, oxacillin, or nafcillin. However, antimicrobial drugs from other classes are used against methicillin-resistant strains. The glycopeptide antibiotics vancomycin and teicoplanin are most often relied upon. Other classes of antimicrobials are now available to treat staph infections. However, all of them have adverse effects, and to achieve lasting success, the use of additional therapies is also required [3]. Most of the infections caused by *S. aureus* are associated with the development of inflammatory processes. Inflammation is considered an evolutionary developed process with a dual role: a pathological condition or host defense against the pathogens [4-7]. It is well known that prolonged, inflammation can result in the development of rheumatoid arthritis, inflammatory bowel disease, asthma, and pancreatitis [8] as well as predisposition to cancer [7]. “An increase in the levels of cytokines, cytokine receptors, adhesion molecules, immuno-regulatory factors, and several other mediators is the key mechanism of inflammation” [5]. As chronic inflammation could lead to irreversible consequences, many studies are focused on the development of anti-inflammatory products. Unfortunately, many market-available medicines are associated with adverse side effects. The application of the modern research methods has led to advances in our understanding of the mechanism of skin immune responses to the important skin pathogen *S. aureus*. Liu et al. [9] reported “new insights related to the innate and the adaptive immune mechanisms in host defense and inflammatory reactions in response to skin infections caused by *S. aureus*. Antimicrobial peptides, antigen recognition receptors, and inflammasome activation play a role in innate immunity to the infections, while T cells and their effector cytokines play a key role in the adaptive immunity against skin infections caused by *S. Aureus”*. Certain mechanisms have been identified by which this bacterium contributes to abnormal skin inflammation, such as in exacerbations of atopic dermatitis [9]. All these data point to a need for new therapeutic approaches and agents to combat skin infections and skin inflammations, caused by *S. aureus*.

"Different plants possess antimicrobial and anti-inflammatory properties. In the context of the increasing resistance of microorganisms to antibiotics, the use of plant extracts is more examined nowadays. A very good results are reported for African geranium (*Pelargonium sidoides* DC.), black elderberry (*Sambucus nigra* L.), St. John's Wort (*Hypericum perforatum* L.) etc". [10-12]. Aqueous extracts of these and other plants also show a very good antiviral effect, demonstrated *in vitro* by [13] against Herpes simplex viruses, HSV-1 and HSV-2. Other research demonstrated *in vitro* antibacterial activity of extracts and isolated components of *P. sidoides* against different bacteria including *S. aureus*, as well as immunostimulatory properties [14]. This herb has shown a beneficial effect in the treatment of respiratory tract infections as well as other infections. Experimental results of *in vitro* studies have indicated that the bioactive constituents of *P. sidoides* may not have a direct antimicrobial effect. Their action is probably expressed in the prevention of microbial binding to host cell receptors, inhibition of key enzymes and the production of antimicrobial effector molecules such as nitric oxide and interferons from the host cells [15].

The *in vitro* results with methanol-acetone extracts from *H. perforatum* L. show high activity of *H. perforatum* against *S. aureus* and other microorgnisms [16]. The flowers and fruits of the elderberry tree (*S. nigra* L) also demonstrate well expressed activity *in vitro* against *S. aureus*, as well as anti-inflammatory action [17,18]. "The extracts from *S. nigra* exhibit various health-beneficial effects not only *in vitro*, but and *in vivo*, including antioxidant, anti-inflammatory, anti-cancer, anti-influenza, antimicrobial, anti-diabetic, cardiovascular protective and neuro protective activities" [19]. Elderberry has a high potential for reducing cellular oxidative stress, as well as for preventing inflammatory processes. Ferreira et al. [20] demonstrated "anti-inflammatory activity of black elderberry, establishing a dose-dependent inhibition of nitric oxide release by lipopolysaccharide-stimulated RAW 264.7 cells pre-exposed to elderberry extracts. The authors also reported antioxidant protection of cells after exposure to elderberry extract (50 µg/mL) by preventing up to 90% of tert-butyl hydroperoxide (t-BOOH)-induced toxicity. Furthermore, elderberry extracts prevent cellular glutathione depletion, inhibit the production of reactive oxygen species (ROS), abnormal morphological changes and DNA fragmentation, in response to t-BOOH oxidative damage". A dose-dependent anti-inflammatory activity of elderberry fruit extracts has been observed [20]. It is due to the synergistic effect between the phenolic and polar compounds contained in it, such as sugars, etc. Elderberry extracts also show powerful antioxidant protection against cytotoxic factors, contributing to the reduction of intracellular ROS. These extracts have strong anti-inflammatory and antioxidant potential, which may be due to the phytochemical profile (eg, anthocyanin, phenolic acid) of elderberry. These two bioactivities are crucial to reduce or prevent some disorders related to inflammatory status and ROS production [20]. In experiments with rats, *H. perforatum* has been shown to have anti-inflammatory properties and significantly reduce all inflammatory parameters. In addition, extracts from this plant have a strong wound healing effect. It is most likely mainly due to the increase in the activation of fibroblast cells, which have a role in wound repair by closing the damaged area, as well as by the stimulation of collagen production by this type of cells [21]. The data presented above indicate that these plants show a potential to be an effective and cheaper substitute for conventional drugs because they are readily available and the extracts can be easily prepared by decoction or infusion.

Today, silver nanoparticles (AgNPs) are used worldwide in a wide range of products such as medical devices and consumables, water filters, detergents, cosmetics and more, due to their antimicrobial properties [22]. AgNPs are widely used in the treatment of injuries, burns and microbial infections [23]. In our previous studies of the *in vitro* antimicrobial activity of AgNPs, we found a good effect of colloidal nano-silver at concentrations of 20 ppm, 24 ppm and 30 ppm against different microbial species including *S. aureus*. Colloidal nano-silver with a concentration of 30 ppm showed the highest antimicrobial activity [24,25].

The present work aimed to evaluate the potential of two cosmetic products to reduce the levels of several inflammation-related cytokines - IL-1β, IL-6, IL-8, and IP-10. The anti-inflammatory effect of the drug against a human epithelial cell line, which is in a state of inflammation caused by *Staphylococcus aureus*, was studied. The second goal of the study was to perform studies to evaluate the antimicrobial activity of SILVER STOP® cream containing extracts of African geranium (*P. sidoides*), black elderberry (*S.*

nigra) and St. John's Wort (*H. perforatum*) in colloidal nanosilver (AgNPs) at a concentration of 30 ppm against *S. aureus.*

2. MATERIALS AND METHODS

2.1 Cosmetic Product

The anti-inflammatory and antimicrobial effect of SILVER STOP® cream (SS® cream), prepared by the patent of Baiti [26], containing extracts from African geranium (*Pelargonium sidoides* DC.), black elderberry (*Sambucus nigra* L.) and St. John's Wort (*Hypericum perforatum* L.) [27] in colloidal nano-silver (AgNPs) [28] et at a concentration of 30 ppm (from EVODROP AG, Switzerland) was tested.

The combination of the same plant extracts in AgNPs at a concentration of 30 ppm, denoted as SilverStop solution, was also used in the anti-inflammatory tests.

The epithelial human cell line HT-29 was obtained from American Type Culture Collection (ATCC), and cultured to a monolayer in DMEM (Dulbecco's modified Eagle's medium, Gibco, UK), supplemented with 10 % fetal bovine serum, at 37°C and 5% CO_2. In about 90% of the cells monolayer, cells were passaged by incubation with 0.25% trypsin and 10 mM EDTA solution for 10 minutes at 37°C.

To determine the anti-inflammatory effect, eukaryotic cells were cultured in 24-well plates at a concentration of 2 ×10^5 cells.mL^{-1}. The medium was changed every 2 days for a total of 14 days, supporting not only a monolayer formation (3-4 days) but also the maturation of cellular receptors. The resulting monolayer was washed twice with Phosphate buffered saline (PBS) buffer.

DMEM (1 mL) containing different preparations was added to each well and incubated for 20 hours at 37°C. The preparations were as follows:

- control DMEM;
- DMEM with *S. aureus* 1x10^5 CFU/ml;
- DMEM with 10% SilverStop;
- DMEM with 10% SilverStop and *S. aureus* 1x10^5 CFU.mL^{-1};
- DMEM with 10% Cream "SilverStop";
- DMEM with 10% Cream "SilverStop" and *S. aureus* 1x10^5 CFU.mL^{-1}.

After incubation, the cell-free supernatant was used to determine the cytokine expression. IL-8, IP-10, IL-1β, and IL-6 were assessed using ELISA (enzyme-linked immunosorbent assays) according to the manufacturing instruction (Diaclone, USA).

2.2 Microorganisms

Pure cultures of *S. aureus* subsp. *aureus* ATCC – 6538 and of two clinical strains were used.

2.3 Nutrient media

Mueller Hinton agar and Mueller Hinton broth (BUL BIO NCIPD - Sofia), Columbia blood agar and the selective medium Chapman agar (Biolab Zrt. H-1141, Budapest Ov. Utra 43) were used. *The cultivation* of the microorganisms was carried out at 35-37°C for 18-24 under aerobic conditions.

2.4 The Antibacterial Effect

The antibacterial effect was studied by *determination of the time of antimicrobial action* of SS® cream. A suspension of the tested microbial strains with a concentration of 10^5 cells.mL^{-1} in an amount of 0.1 mL was added to 0.9 mL of SS® cream, as well as to 0.9 mL of sterile water as a control of the microbial growth, where the final concentrations became 10^4 cells.mL^{-1}. Also, a suspension of each of the tested microbial strains with a concentration of 10^7 cells.mL^{-1} in an amount of 0.1 mL was added to 0.9 mL of SS® cream, as well as to 0.9 mL of sterile water as a control of the microbial growth, where the final concentrations became 10^6 cells.mL^{-1}. After homogenization for 1 min on a Vortex apparatus (Heidolph - Labimex, Bulgaria) and different time intervals for exposure of the microorganisms to SS® cream tested (5 min, 10 min, 20 min, 40 min,60 min and 120 min) cultures were made from each of the samples on Mueller-Hinton agar, which were incubated at 37°C for 24-48 h under aerobic conditions. After cultivation, the number of colonies developed (Colony forming units - CFU) was determined.

2.5 Statistical Analysis

Data were analysed statistically according to the method of Student–Fisher with *t*-test using Microsoft® Office Professional Plus Excel 2013. The average values and their standard deviations were calculated. Student's t-test analysis for independent samples was applied to determine the statistical dependence and reliability of the results. The Student's *t*-test was counted for 3 results in each group. Significance of the differences was defined at significance level $p < 0.05$.

3. RESULTS

Our results indicate that *S. aureus* increases the concentration of the pro-inflammatory cytokines compared to untreated cell line samples – in DMEM media. Around 3-fold increase was measured for IL-8 (Fig. 1) and IP-10 (Fig. 2). Around 2-fold higher levels were observed for IL-1β (Fig. 3). The smallest increase – around 1.5-fold was obtained for IL-6 (Fig. 4). This result demonstrates that in our experiments IL-8 and IP-10 are the most sensitive markers for *S. aureus*-induced inflammation.

Next, data revealed no overexpression of IL-8 after the treatment with both cosmetic products (Fig. 1). No over-expression was measured after the application of the products on cell lines in a state of inflammation caused by *S. aureus*. The measured levels were similar.

The next marker we studied was IP-10. Data revealed that better protection expressed as inhibition of the over-expression of IP-10 might be observed after the application of SilverStop in comparison with the cream (Fig. 2).

The same was observed for the other studied marker – IL-1β– 60-fold lower (Fig. 3). SS® cream reduced the induction of IL-1β around 1.5-fold.

Considering IL-6, the most pronounced reduction was obtained after the application of SS® cream– around 9-fold (Fig. 4). The IL-6 reduction after the application of Silver Stop was calculated to be around 6-fold lower.

The results from the studies by the suspension method showed that SS® cream exhibited significant activity (Fig. 5) against *S. aureus* when in suspension with a density of 10^4 cells.mL^{-1}. After 5 minutes of exposure to SS® cream, the amount of *S. aureus* decreased by about 44% compared to the untreated control, and after 40 minutes only single cells remained viable in some samples. No growth of *S. aureus* was observed after 60 minutes of exposure to SS® cream.

When in suspension with a density of 10^6cells.mL^{-1} (Fig. 6), under the action of SS® cream the quantity of the studied *S. aureus* strains was reduced with about 18% within 10 min. After 60 minutes, the amount of *S. aureus* decreased by about 75% compared to the untreated control, and after 2 h only single cells remained viable in some samples. No growth of *S. aureus* was observed after 24 h of exposure to SS® cream.

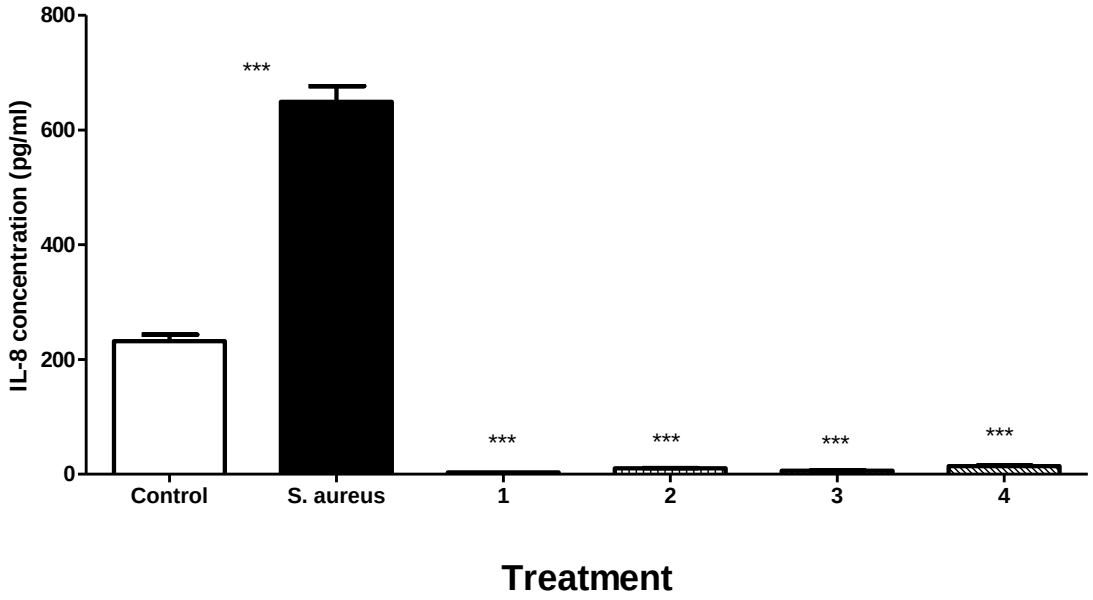

Fig. 1. Concentration of IL-8 (pg/ml) after various treatments: 1 – SilverStop; 2 – SilverStop + *S. aureus*; 3 –SS® cream; 4 - SS® cream + *S. aureus*. Data are from at least three independent experiments, $p < 0.05$

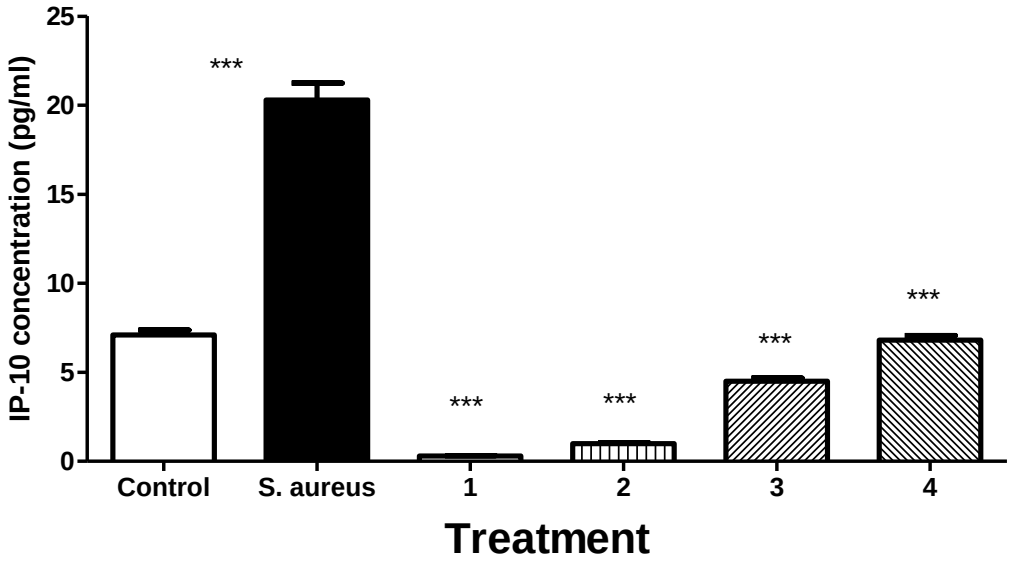

Fig. 2. Concentration of IP-10 (pg/ml) after various treatments: 1 – SilverStop; 2 – SilverStop + *S. aureus*; 3 –SS® cream; 4 - SS® cream + *S. aureus*. Data are from at least three independent experiments, p < 0.05

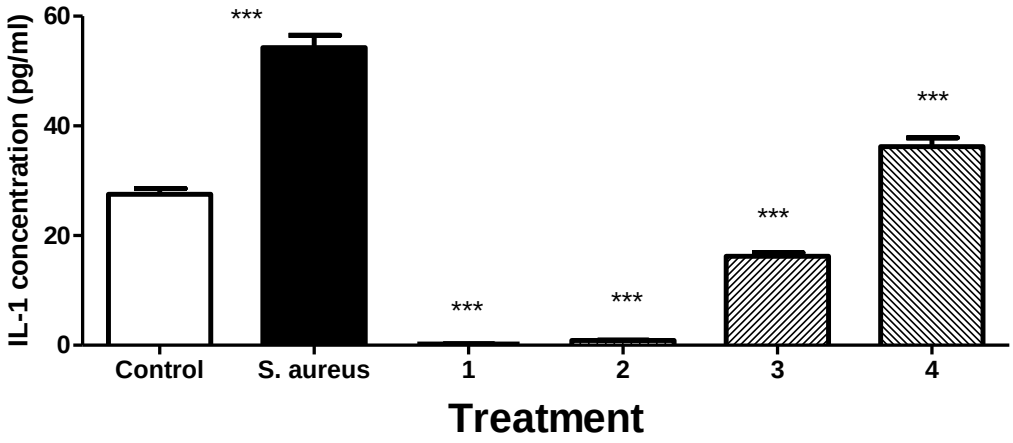

Fig. 3. Concentration of IL-1β (pg/ml) after various treatments: 1 – Silver Stop; 2 – Silver Stop + *S. aureus*; 3 –SS® cream; 4 - SS® cream + *S. aureus*. Data are from at least three independent experiments, p < 0.05

4. DISCUSSION

The results of the present research demonstrate that both preparations (Silver Stop and SS® cream) decrease the concentration of the four pro-inflammatory cytokines in the two cases – control samples (DMEM) and cell lines in a state of inflammation caused by *S. aureus*.

Based on the reported data, it could be concluded that the ability of Silver Stop to decrease the concentration of IL-8, IP-10, and IL-1β is greater compared to the effect of SS® cream. In contrast, the effect of SS® cream was found to be more pronounced on IL-6 compared to SilverStop.

It is well-known that the IL-1 familyis involved in acute and chronic inflammation [29] and thus plays a significant role in the regulation of inflammation processes [30]. The IL-1 gene family is characterized by three constituents - IL-

1α, IL-1β, and IL-1 receptor antagonist (IL-1RA) [31]. IL-1βis released after proteolytic cleavage and is most probably involved in the inflammatory process [29] as its overexpression and following inflammatory cascade may result in the development of autoimmune diseases [32]. Based on the pathophysiological relevance of IL-1β, many studies have been focused on the development of drugs and products for targeting this interleukin [33]. By now several drugs have been approved such as anakinra, rilonacept, and canakinumab [33]. Our work provides a basis for further examinations of the potential of SilverStop to alter the IL-1β overexpression.

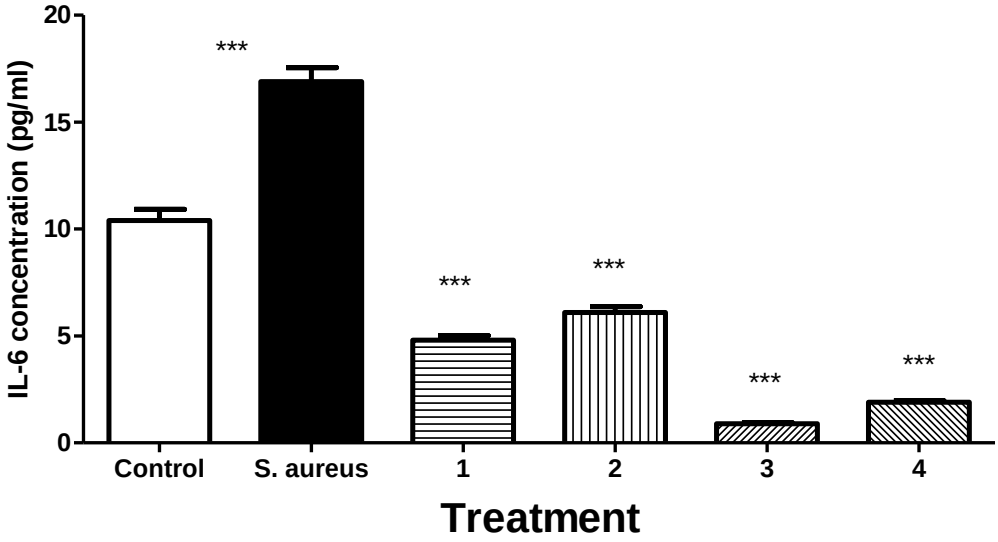

Fig. 4. Concentration of IL-6 (pg/ml) after various treatments: 1 – SilverStop; 2 – SilverStop + *S. aureus*; 3 –SS® cream; 4 - SS® cream + *S. aureus*. Data are from at least three independent experiments, p < 0.05

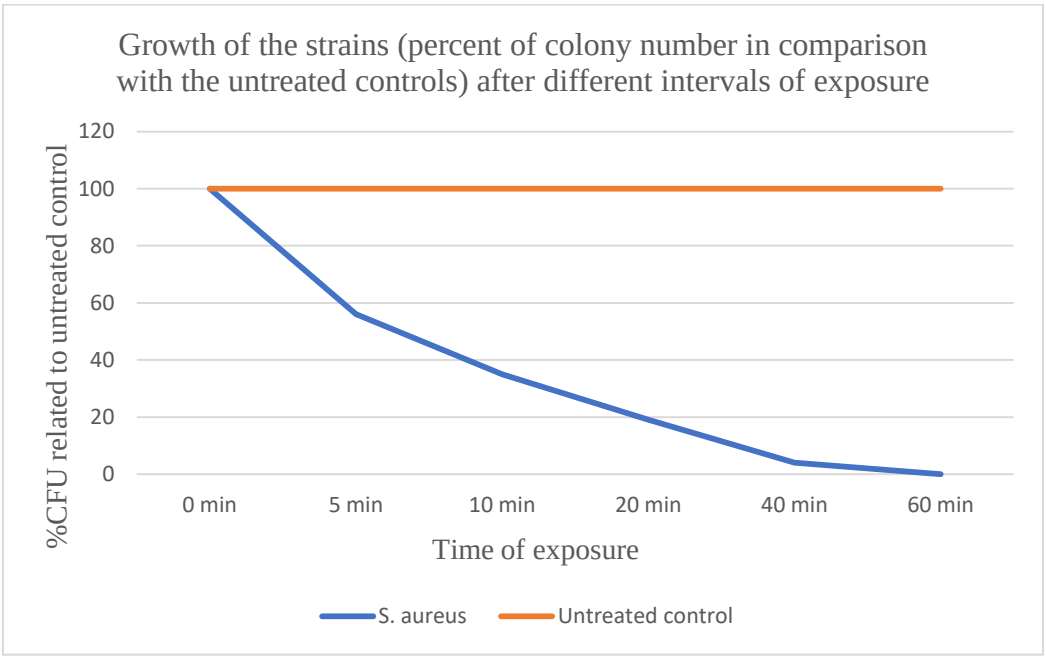

Fig. 5. Antimicrobial effect of SS® cream on *Staphylococcus aureus* in suspensions with a density 10^4 cells.mL^{-1}. Data are from three independent experiments

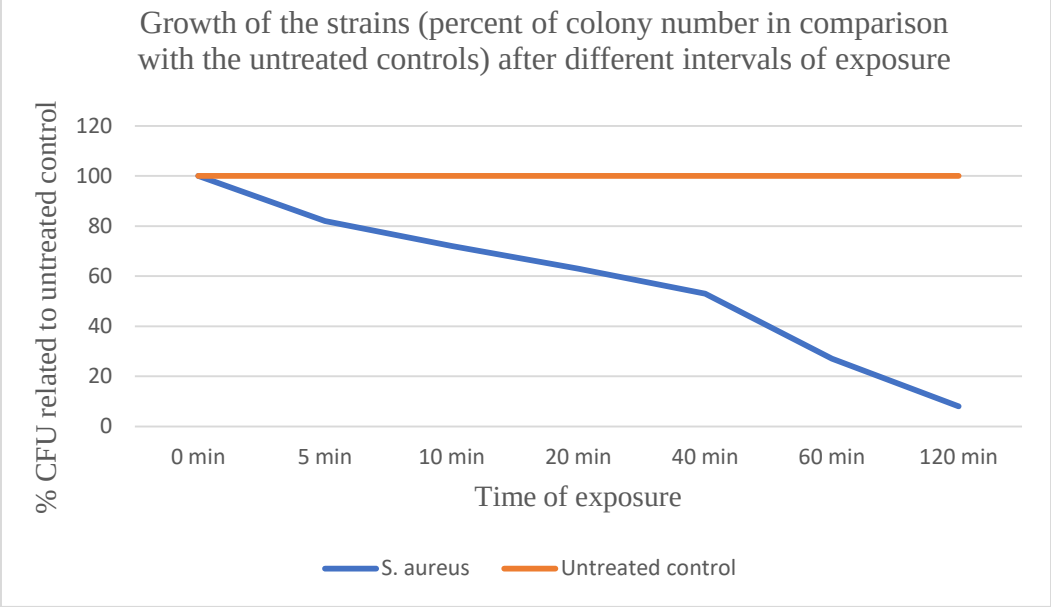

Fig. 6. Antimicrobial effect of SS® cream on *Staphylococcus aureus* in suspensions with a density 10^6 cells.mL^{-1}. Data are from three independent experiments

Further, overexpression and upregulation of IL-8 have been reported to be related to several cancers including breast cancer [34]. Nevertheless, studies have revealed a significant increase in post-menopausal women with osteoporosis and bone loss [35]. Thus, the reduction of this marker may provide significant advances with such health problems. In our study, IL-8 was significantly reduced by Silver Stop.

The results of the present microbiological studies show that *S. aureus* dies in a short time under the influence of the tested cream, especially when it is in a lower concentration. This indicated that early in the development of the infection, topical application of SS® cream would be very effective for fast healing. In an advanced infectious process, when the concentration of staphylococcal cells is high, treatment with the cream would also be very effective, although in a high concentration the bactericidal effect of the cream is slower. This implies its longer application. The anti-inflammatory properties of SS® cream, established in the present studies, also play a large role in the expected high efficiency in combination with its pronounced antibacterial effect.

The results of current studies correspond to those of other authors, who also demonstrate *in vitro* antimicrobial activity of products containing AgNPs. Silver nanoparticles show effective antimicrobial properties due to their large surface area, which provides better contact with microorganisms. Silver ions penetrate the cells, leading to aggregation of damaged DNA and thus affecting protein synthesis [36]. Our results are in line with those of Mativandlela [37] and Kolodziej [38], which found antibacterial properties of *P. sidoides* against Gram-positive and Gram-negative bacteria, as well as improvement of immune functions at different levels in the body [37,39]. Jekabsone et al. [40] have investigated the antibacterial, anti-inflammatory and cytoprotective capacity of *P. sidoides* DC root extract, as well as a proanthocyanidin fraction from the plant's root. They found antimicrobial activity of the tested agents on both *S. aureus* and other Gram-positive microorganisms, as well as on Gram-negative bacteria such as *Escherichia coli*, etc. A higher activity against *S. aureus* had been found compared to that against *E. coli*. Both agents tested prevented LPS-induced fibroblast death. Their application has resulted in reduced LPS-induced release of interleukin-8 and prostaglandin E2 from the fibroblasts and IL-6 from the leukocytes, blocking the expression of IL-1β, iNOS and the surface presentation of CD80 and CD86 in the macrophages treated with LPS + IFNγ, and the expression of IL-1β and COX-2 in LPS-treated leukocytes. The plant extract and tested fraction have been shown to exhibit strong antibacterial, anti-inflammatory and gingival tissue protective properties under

conditions mimicking periodontitis and are suitable for the treatment of the disease [40]. Papies et al. [41] through *in vitro* and clinical studies proved that the root extract of *P. sidoides* DC. exhibits antiviral and immunomodulatory properties, limiting the severity of symptoms and the duration of the disease in infections with several viruses of the upper respiratory tract, as well as with SARS-CoV-2. It was found that the cellular entry stage of SARS-CoV-2 is significantly reduced by pretreatment with this extract administered at doses of 10–100 µg/ml. Using sequential ultrafiltration, the authors have found that *P. sidoides* root extract separated into fractions containing either prodelphinidins with different degrees of oligomerization or small molecular constituents such as benzopyranones and purine derivatives. Prodelphinidins with a low degree of oligomerization and small molecular constituents are most effective in inhibiting the entry of SARS-CoV-2 already at 10 µg/ml and have effects on immune gene regulation. A downregulation of multiple proinflammatory genes (CCL5, IL6, IL1B) was found, which was accompanied by an increase in the levels of the anti-inflammatory TNFAIP3 at 48 h post-infection. At high concentrations (100 µg/ml), moderately oligomerized prodelphinidins have reduced the spread of SARS-CoV-2 most effectively and showed marked immune gene modulation [41].

Antimicrobial effect of *H. perforatum* extract against *S. aureus* was demonstrated by [42,43]. The antibacterial and antiviral effects of *H. perforatum* are due to partial control of the transcription factor NF-κB and the involvement of some serine/threonine kinases of the protein kinase C family. The main constituents responsible for pain relief in *H. perforatum* are hyperforin and hypericin [21]. The antimicrobial effects of *S. nigra* extract are due mainly to its high phenolic content [44,45]. The potential molecular mechanisms of action of *S. nigra* extract are related to the regulation of some key signaling pathways and molecular targets [19]. Olejnik et al. [46] have experimentally tested the anti-inflammatory and antioxidant effects as well as the therapeutic properties of *S. nigra* fruit extract on lipopolysaccharide-activated RAW264.7 macrophages. A reduction in the secretion of IL-6, TNF-α and prostaglandin E2 was demonstrated, also observed with treatment of LPS-stimulated macrophages. These effects are complemented by increased nitric oxide production following elderberry extract administration in response to inflammatory stimuli in RAW264.7 cells. The treatment with the fruit extract of *S. nigra* results in a decrease in the expression of pro-inflammatory genes and a reduction in the increased production of inflammatory mediators, which are crucial for the initiation and progression of inflammatory processes, which are an important risk factor for the development of various diseases [46]. Santin et al. [47] have elucidated experimentally the anti-inflammatory and relaxant effects of a lyophilized aqueous extract obtained from the flowers of *S. nigra* in *in vivo* and *in vitro* studies of the inflammation processes on isolated rat vascular and airway smooth muscle. The authors found that the flower extract of *S. nigra* represents an important tool in the management of acute inflammation. It exerts anti-inflammatory effects by modulating the functions of macrophages and neutrophils, including the production of inflammatory mediators and cell migration, by promoting efferocytosis and effectively affecting acute inflammation. It has also been shown to have a relaxing effect on both vascular and non-vascular smooth muscle tissues [47]. The antimicrobial activity of SS® cream and successful topical application for treatment of some skin diseases were reported in our previous study [48]. Our preliminary *in vitro* studies [9,35,36] have shown that the antimicrobial action of SS® cream is mainly due to the herbal extracts it contains, as well as to the colloidal nanosilver in the final concentration applied. They are synergistic in terms of antibacterial activity.

5. CONCLUSION

The tested antimicrobial product SILVER STOP® cream (SS® cream) containing extracts of *P. sidoides*, black elderberry (*S. nigra*) and *H. perforatum* in colloidal nanosilver exhibited significant antimicrobial activity *in vitro* against *S. aureus*. When in suspension with a density of 10^4 cells.mL^{-1}, *S. aureus* died after less than 1 hour at presence of SS® cream. In suspension of 10^6 cells.mL^{-1}, almost all cells of the tested strains were inactivated after 2 h of exposure to SS® cream. This activity is due to the synergistic antimicrobial and anti-inflammatory action of the plant extracts included in the product, as well as the colloidal nanosilver contained in it. The results of the present study indicate that the product is promising for use as an alternative to conventional therapy of staphylococcal infections.

Our results demonstrate that SS® cream decreases the concentration of the four pro-

inflammatory cytokines in the state of inflammation caused by *S. aureus*.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial Interests OR personal relationships that could have appeared to influence the work reported in this paper.

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