

Antibacterial activity, quality and stability study of creams with new potential silver(I) complexes and *in vivo* case report

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ABSTRACT

The aim of this study was to evaluate the antibacterial activity, quality and stability of creams (at 1 % concentration) prepared with synthesized silver(I) complexes: $[\text{Ag}(\text{Nam})_2]\text{NO}_3 \cdot \text{H}_2\text{O}$ (AgNam), $[\text{Ag}_2(\text{HGly})_2]_n(\text{NO}_3)_{2n}$ (AgGly) (Nam – nicotinamide, Gly – glycine) and silver(I) sulfadiazine (AgSD), which is commercially available. Antibacterial activity was evaluated by agar well diffusion method and in *in vivo* case. The pure silver(I) complexes as well as all three tested creams loaded with AgGly, AgSD and AgNam showed antibacterial potential. Moreover, the creams loaded with AgGly and AgNam showed higher antibacterial effects against *S. aureus* and *B. subtilis* than the cream loaded with AgSD. In terms of appearance, all cream samples were opaque and odourless, and no phase separation was observed. Creams were soluble in water (o/w emulsions) and they had a pseudoplastic behaviour. The pH of the creams was in the range of 4.87–5.75. No visible changes were observed in the case of commercially used AgSD cream during one month testing period at conditions -16 ± 1 °C; 6 ± 1 °C and 56 % relative humidity; 20 ± 1 °C and 58 % relative humidity and 40 ± 1 °C and 75 % relative humidity. However, creams containing AgGly and AgNam changed their colour depending on the tested conditions.

Keywords: silver(I) antimicrobial complexes, creams, evaluation, antibacterial activity, pilot *in vivo* case report

It is well known that silver(I) sulfadiazine (AgSD) is used in various skin injuries and especially in the treatment of burns (1, 2). The main reason for the use of 1 % AgSD cream is the antimicrobial efficiency of this agent and the ability to prevent infections. However,

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several side effects and adverse reactions such as renal toxicity, leukopenia and drug resistance have been reported after applying AgSD. Due to these facts, AgSD cream should not be used on extensive wounds and for long periods (3–5).

In order to develop new, more effective silver(I) drugs with low toxicity and low dosing concentration, we have been preparing and testing new silver(I) compounds (6). Our results indicate that both complexes $[\text{Ag}(\text{Nam})_2]\text{NO}_3 \cdot \text{H}_2\text{O}$ (AgNam), $[\text{Ag}_2(\text{HGly})_{2n}](\text{NO}_3)_{2n}$ (AgGly) (Nam – nicotinamide, Gly – glycine) are potential antimicrobial drugs. The most common dosage forms for commercial antimicrobials based on silver(I) compounds are either colloidal silver nanoparticles (7), a solution with API AgNO_3 (8, 9) or cream with AgSD (10, 11). Just the AgSD-containing cream is used for topical application. Advantageous aspects of distribution through the skin are, for example, the possibility of dermal (local) or transdermal (systemic) delivery, ease of application, prevention of gastrointestinal problems and avoidance of hepatic first-pass metabolism of the drug. Moreover, dermal delivery of the drug used for the treatment of skin diseases minimises the systemic effects of the drugs (12–14).

Due to the fact that our substances are silver(I) complexes (similarly to AgSD), we chose a cream dosage form for our study. In addition, the cream has better applicability than ointment, is expected to be absorbed faster, and its application is also more suitable for acute dermatoses (15, 16).

The aim of this paper was to prepare 1 % creams with newly synthesized silver(I) complexes: AgGly, AgNam and commercially available silver(I) sulfadiazine and compare its properties (physical appearance, pH, viscosity, determine the cream type and study their antibacterial activity and stability affected by temperature and humidity). The most stable creams were applied for the treatment of a decubitus ulcer on the sheep's chest.

EXPERIMENTAL

Antibacterial agents used in this study were $[\text{Ag}_2(\text{HGly})_{2n}](\text{NO}_3)_{2n}$ (AgGly), $[\text{Ag}(\text{Nam})_2]\text{NO}_3 \cdot \text{H}_2\text{O}$ (AgNam) and silver(I) sulfadiazine (AgSD). The complexes AgGly and AgNam were prepared according to the previous procedures (6) and their syntheses, identification and antimicrobial testing results are given in the Supplementary material. Silver(I) sulfadiazine (AgSD) was purchased from Sigma-Aldrich Chemie (Germany).

The following creams excipients were used: propylene glycol, liquid paraffin and tween 80 (Dr. Kulich Pharma, Czech Republic), cetylstearyl alcohol (Molar Chemicals KFT, Hungary), glycerol monostearate (Alfa Aesar, USA), tween 60 (Merck, Germany) and deionised water.

Preparation of creams

We prepared three samples according to silver(I) sulfadiazine cream manufacturing directions stated in the literature (17) and preparation is described below. All cream samples were prepared at 1 % (1 g/100 g) concentration of active complex (AgGly, AgNam or AgSD). Excipients such as cetylstearyl alcohol (5 g), glycerol monostearate (8 g), liquid paraffin (8 g), tween 60 (2 g), tween 80 (3 g) were mixed in a mortar placed in a laboratory water bath, heated up to 60 ± 1 °C. After the dissolution of the mixture, hot water (58 g, 60 ± 1 °C) was

added for homogenization. 1 g of API (AgSD) was first dissolved in propylene glycol (15 g) and then added to the mixture of excipients and homogenized. The same preparation procedure was repeated with AgGly and AgNam. Each sample contained the same amount of excipients and active complexes.

Evaluation of the creams

Physical appearance and pH determination. – The semisolid formulations were evaluated visually for their appearance, colour, smell and phase separation immediately after preparation (18).

The pH of the cream formulations was determined using a digital pH meter (Hanna HI 2211). 1 g of the sample was dissolved in 25 mL of deionized water. The pH values were measured at the temperature of 25 ± 1 °C. The pH was determined on the day of the preparation, after 2 weeks and after a month of preparation. Creams were stored in the refrigerator (at 9 ± 1 °C) and the pH meter was calibrated with standard buffer solutions (pH 4 and 7) before each measurement (19).

Creams type evaluation. – Polar solvent – water (approx. 1 mL) was added to a small amount (approx. 0.5 g) of each cream sample in a Petri dish. The same procedure was repeated using chloroform as a non-polar solvent. The experiment was carried out at laboratory temperature.

Accelerated stability study. – One sample of each cream type was evaluated visually for the change in appearance under different environmental conditions during four time periods (the day of the preparation, 24 hours after preparation, after a week and after a month of preparation), at four different temperatures and three different values of relative humidity (RH). The samples were stored in a freezer at -16 ± 1 °C, in a refrigerator at 6 ± 1 °C and 56 % relative humidity, in a laboratory at 20 ± 1 °C and 58 % relative humidity and in an incubator at a temperature of 40 ± 1 °C and 75 % relative humidity.

Viscosity measurement. – The viscosity of the cream samples (20 g) was measured at 25 ± 1 °C using a digital rotational viscometer (Fungilab ViscoLead ONE). The spindle R5 was used. The spindle was rotated at different RPM values (12–100 RPM). The viscosity was measured immediately on the day of the cream preparation.

Then viscosity of the cream samples (60 g) was measured at different temperature conditions (7 ± 1 , 20 ± 1 and 37 ± 1 °C). The spindle (R5) was rotated at 1 RPM, 4 RPM and 6 RPM. Values of RPM were selected based on suitability for each cream condition affected by temperature. Viscosity was measured immediately on the day of the preparation and after 1 week of storage in the refrigerator (at 9 °C).

In vitro antibacterial activity testing

The antibacterial properties of the active complexes (AgGly, AgNam and AgSD) and creams loaded with silver(I) complexes were evaluated by the agar well diffusion method by a slightly modified process compared to (20). Firstly, AgGly/AgNam/AgSD were dissolved in distilled water (10 mg mL^{-1}), with final concentration 1 % of silver(I) complexes. Because of the low solubility of AgSD ($0.34 \text{ mg}/100 \text{ mL}$ at 25 °C) (21), the prepared suspension was used for further antibacterial study in this case. Secondly, 10 mg of creams con-

taining active complexes AgGly/AgNam/AgSD at their 1 % concentration were used for the testing.

The tested Gram-negative bacteria (*Escherichia coli* CCM 3988, *E. coli*; *Salmonella typhimurium* CCM7205, *S. typhimurium*; *Klebsiella aerogenes* CCM 7797T, *K. aerogenes*) and Gram-positive bacteria (*Staphylococcus aureus* CCM 4223, *S. aureus*; *Enterococcus faecalis* CCM 4224, *E. faecalis*; *Bacillus subtilis* CCM1999, *B. subtilis*) were obtained from the Czech collection of microorganisms (CCM).

Frozen glycerol stock cultures were maintained at $-20\text{ }^{\circ}\text{C}$. Before experimental use, cultures were transferred to LB (Lysogeny broth, Luria-Bertani) medium (Sigma-Aldrich, USA) and incubated for 1 day aerobically at $37\text{ }^{\circ}\text{C}$ with agitation except for *B. subtilis*, which was grown aerobically at $30\text{ }^{\circ}\text{C}$. Cultures were then subcultured in LB medium and incubated for 16–20 h aerobically at $37\text{ }^{\circ}\text{C}$ / $30\text{ }^{\circ}\text{C}$ and used as the source of inoculum for each experiment.

Plate count agar (HIMEDIA, India) medium was cooled to $45\text{ }^{\circ}\text{C}$ after autoclaving, and inoculated with liquid overnight bacterial culture to a cell density of $5 \times 10^5\text{ cfu mL}^{-1}$. 20 mL of this inoculated agar was poured into a Petri dish (ϕ 90 mm). Once the agar was solidified, five mm diameter wells were punched in the agar and filled with 50 μL of samples. Gentamicin sulfate (Biosera, France) at the concentration of $50\text{ }\mu\text{g mL}^{-1}$ was used as a positive control. The dilution medium for the positive controls was sterile distilled water. The plates were incubated for 24 h at $37\text{ }^{\circ}\text{C}$. Afterwards, the plates were photographed and the inhibition zones were measured by the ImageJ software. The calculation of the antibacterial activity was reported by Baláž *et al.* (22).

Statistics

Statistical analyses were performed with the software Minitab 18. Significant differences were determined by one-way ANOVA and Tukey's pairwise comparison of the mean. Values are given in average mean \pm standard deviation (SD). Significant differences between the means of corresponding data (pH, viscosity and ATB) of positive control (cream base) and complexes (creams with complexes) are marked with: * for $p < 0.05$; ** for $p < 0.01$; *** for $p < 0.001$, n stands for the number of replications. Except for the p -values, we included 95 % confidence intervals for differences in means for particular groups (Tukey's pairwise comparison). Values in each group followed by the same letter are not significantly different.

A case of *in vivo* application

The sheep was a 4-year-old Charolais ram that had been admitted to the Clinic of Ruminants of the University of Veterinary Medicine and Pharmacy in Košice for treatment of a decubitus ulcer on its chest. No data on the historical background of the lesion were available. Neither systemic nor local treatment was done on the farm. The ram was in good body condition (90 kg) and was fed hay and commercial ration throughout the treatment period. Clinical examination did not reveal any abnormality from healthy general status (no fever, tachypnea, and tachycardia). The skin lesion was 20 cm long and 18 cm wide covering almost the whole xiphoid region. The skin was hyperaemic, swollen, with increased sensitivity, undermined by a flat capsule containing pus and a small number of necrotic membranes. The capsule communicated with the skin surface with the fore small fistulas.

Bacteriological culture from the pus and skin swabs was performed. The necrotic tissue was removed and the whole lesion was rinsed with an iodine solution (0.1 %). The local treatment with the same solution was performed daily for the next two weeks. No systemic reaction of the sheep was recorded during the treatment. Two-week local treatment with 0.1 % iodine solution improved partially the skin condition, but despite repeated treatment, the decubitus ulcer persisted with a continued pus formation and hyperaemic, and oedema skin reaction.

A cream containing AgSD, a commercially used drug for the prevention and treatment of bacterial infections, was used for the treatment for three days. After completion of the application of the cream, the lesion was monitored for the next three days, and as there was no significant improvement, subsequently the cream containing AgNam was applied to the skin, again for three days.

The animal underwent repeated haematological and complex biochemical examinations (protein, energy, hepatic and mineral profiles). The haematological parameters were determined on an automatic haematological analyser ABC-vet (Horiba ABX, France) and biochemical parameters on an automated biochemical analyser Alizé (Lisabio, France) using commercial diagnostic kits (Randox, UK). Serum concentrations of total immunoglobulins were measured by zink-sulphate test according to McEwan *et al.* (23).

RESULTS AND DISCUSSION

Evaluation parameters of the creams

Physical appearance and pH determination. – All cream samples immediately after preparation were opaque and odourless, and no phase separation was observed. The colour of cream with API AgSD (1 %) and AgNam (1 %) was white, but 1 % cream with AgGly was light grey on the day of the preparation.

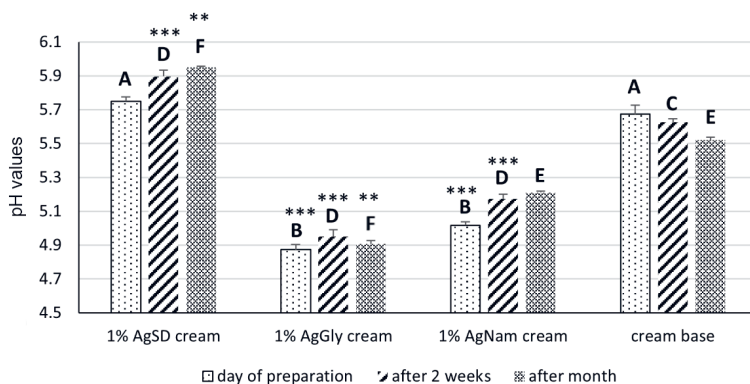


Fig. 1. pH values of the creams loaded with AgGly, AgNam, and AgSD at 1 % concentration on the day of the preparation, after 2 weeks and after 1 month ($n = 3$, results shown as mean \pm SD). Differences between the means of cream base and creams with complexes at a particular time point (day of preparation, after 2 weeks, after 1 month) were considered significant when $p < 0.05$. The same letter above the bars means that the results do not differ significantly at $p < 0.05$ by Tukey's pairwise comparisons. Significant differences are marked with: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

All cream samples had pH values in the acidic range. The pH values slightly fluctuated during the testing period. A decrease in pH was observed after the addition of API AgNam and AgGly to the cream base (cream without active substance). A cream containing silver(I) sulfadiazine had a higher pH value compared to the cream base. After 1 month, the pH of the cream base decreased, however, the pH of the creams containing AgSD, AgGly and AgNam increased. The physiological pH of the *stratum corneum* varies in the range from 4.1 to 5.8 (24). Most topical formulations have a pH between 3 and 8 (25). Comparing pH values in dependence of creams composition, the most statistically significant differences were observed in the case of creams with AgGly.

Creams type evaluation

The cream sample with API AgSD (1 %) was soluble in water and insoluble in chloroform the same as the 1 % cream loaded with AgGly and AgNam. It confirms that creams are o/w emulsions, as it was suggested by composition (ratio of excipients).

Accelerated stability study

In general, under ambient conditions (in the laboratory at 25 ± 1 °C and 60 % relative humidity) the stability of the cream is assumed and no visible changes in the appearance should be observed (25). Indeed, no visible changes were observed immediately after the preparation of the samples. The results of the accelerated stability study are summarized

Table I. Colour of creams loaded with AgGly, AgNam, and AgSD at 1 % concentration stored in different environmental conditions

Storage conditions		Colour		
		AgSD	AgGly	AgNam
Day of the preparation		white	light grey	white
After 24 h	40 °C, 75 % RH	white	light brown	beige
	20 °C, 58 % RH	white	beige	light beige
	6 °C, 56 % RH	white	light grey	white
	-16 °C	white	light grey	white
After 1 week	40 °C, 75 % RH	white	dark brown	brown
	20 °C, 58 % RH	white	brown	beige
	6 °C, 56 % RH	white	light beige	white
	-16 °C	white	light grey	white
After 1 month	40 °C, 75 % RH	white	dark brown	brown
	20 °C, 58 % RH	white	dark brown	light brown
	6 °C, 56 % RH	white	brown	beige
	-16 °C	white	light grey	white

RH – relative humidity, temperature ± 1 °C

in Table II. The changes were more or less visible depending on the storage of the creams at the different temperatures (40 ± 1 , 20 ± 1 , 6 ± 1 and -16 ± 1 °C) and RH (75, 58 and 56 %).

Moreover, degradation processes were observed on a time scale from one day to one month, and the colour changes were monitored immediately after cream samples preparation, after 24 hours, after 1 week, and after 1 month of creams storage.

No visible changes were observed in the case of commercially used AgSD cream during the whole period of testing, even at the temperature of 40 ± 1 °C and 75 % RH. However, the colour and overall appearance of the creams containing AgGly and AgNam have been changed in dependence on the environmental conditions. The cream base (without silver(I) compounds), was stable and did not change colour either after preparation or after a week. While AgNam cream slightly changed its colour, AgGly cream changed more significantly. After 24 hours and 1 week of storage, AgNam cream remains stable (white colour) at 6 ± 1 °C and 56 % RH (in the refrigerator) and at -16 ± 1 °C (in the freezer). Oppositely, the colour is slightly changed to light beige at 20 ± 1 °C; 58 % RH and to beige at 40 ± 1 °C; 75 % RH after 24 hours. After 1 week of storage, the beige colour at 20 ± 1 °C; 58 % RH was changed to brown colour at 40 ± 1 °C; 75 % RH.

In the case of 1 % AgGly cream, for positive temperatures with the appropriate relative humidity values, gradual darkening of the sample occurred (from light grey to beige to light brown after 24 hours of storage and from light beige to brown and dark brown after 1 week of storage).

While 1 % AgGly cream changes colour also at 6 ± 1 °C and a little bit at -16 ± 1 °C after 1 month of storage, 1 % AgNam cream remains almost the same. Therefore, AgNam cream was used for the next *in vivo* examination.

Viscosity measurement

The viscosity of the creams decreases with increasing RPM values. All studied samples had pseudoplastic behaviour. A similar observation was described by Mei X. Chen in the case of formulation of the gels and creams with zinc(II) and copper(II) sulphate (26).

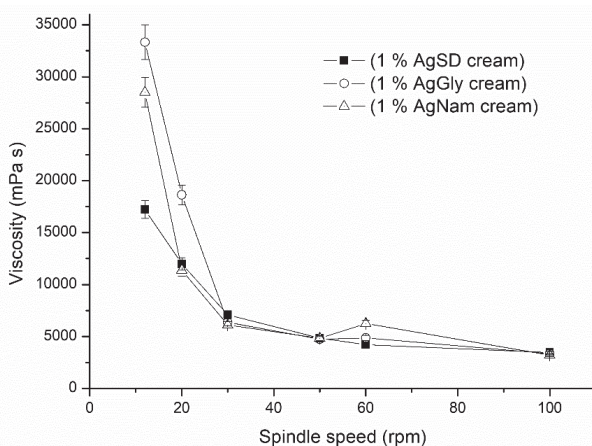


Fig. 2. Viscosity measurement of tested creams at different RMP ($n = 3$, results shown as mean \pm SD).

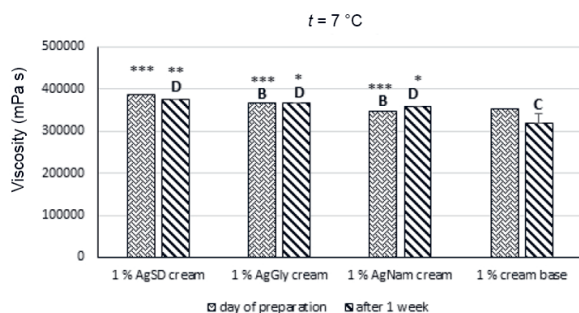


Fig. 3. Viscosity of the creams measured on the day of the preparation and after 1 week of storage at 7 ± 1 °C ($n = 3$, results shown as mean \pm SD). Differences between the means of cream base and creams with complexes were considered significant when $p < 0.05$. Significant differences are marked with: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

As can be seen in Figures 3 and 4, the viscosity decreases with increasing temperature, ranging approximately from 3 to 4×10^5 mPa s at 7 ± 1 °C (Fig. 3), from 8×10^4 to 1×10^5 at 20 ± 1 °C and from 5 to 6.2×10^4 mPa s at 37 ± 1 °C; resp. similar behaviour is expected and has been observed in the case of sunscreen creams (27). The viscosity decrease is attributed to the break of intermolecular interactions in the interfacial region, which provides the loss of colloidal stability. In addition, the viscosity does not change significantly over time, except for the cream base where a more significant decrease was observed at 7 ± 1 °C after a week compared to creams containing effective complexes. Moreover, the viscosity of creams with AgSD, AgGly and AgNam complexes at 7 and 20 ± 1 °C slightly increased compared to the base, which indicates an increase in the colloidal stability of creams by adding complexes. Comparing viscosity values in dependence of creams composition, the most statistically significant differences were observed in the case of cream with AgSD at 7 ± 1 °C.

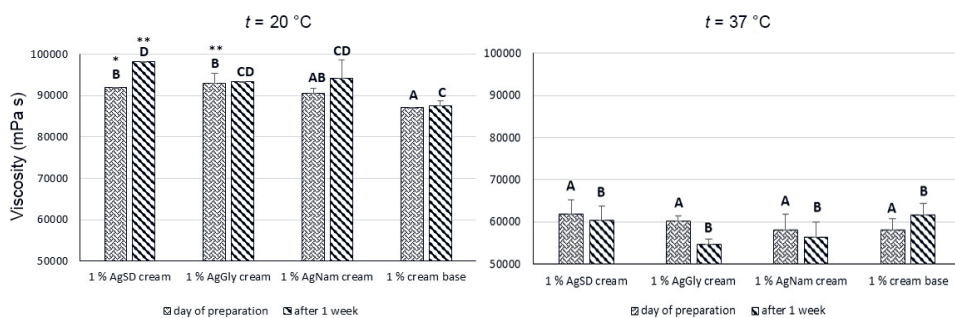


Fig. 4. Viscosity of the creams measured on the day of the preparation and after 1 week of storage at 20 ± 1 and 37 ± 1 °C ($n = 3$, results shown as mean \pm SD). Differences between the means of cream base and creams with complexes were considered significant when $p < 0.05$. The same letter above the bars means that the results do not differ significantly at $p < 0.05$ by Tukey's pairwise comparisons. Significant differences are marked with: * $p < 0.05$, ** $p < 0.01$.

In vitro antibacterial activity testing

For comparison, the antibacterial activity of prepared creams with bioactive substances as well as their pure bioactive ingredients were tested against six strains of bacteria and three Gram-negative (*E. coli*, *S. typhimurium*, *K. aerogenes*) and three Gram-positive (*S. aureus*, *E. faecalis*, *B. subtilis*) bacterial strains were used (Fig. 5). As a standard, gentamicin in the concentration of $10 \mu\text{g mL}^{-1}$ was used.

All three tested silver(I) complexes AgGly, AgNam and AgSD showed antibacterial potential. AgGly showed the strongest inhibition effect among the tested substances and its relative inhibition zone was in the range of 50–104 %. The most inhibited was *E. faecalis* and the lowest inhibition by AgGly showed *B. subtilis*. Except for *E. coli*, the AgGly complex was more active as a commercial AgSD complex against all bacteria. AgSD and AgNam showed similar inhibition ratios against bacterial strains, the range of AgSD was: 21–103 % RIZD and the range of AgNam was: 38–78 % RIZD. AgSD and AgNam inhibited the most *E. coli* and on the other hand the lowest inhibition both complexes showed against *E. faecalis*. However, the AgNam complex was more effective against all selected Gram-negative bacteria compared to AgSD. The new tested compounds showed promising activity against Gram-positive as well as Gram-negative bacteria. Silver(I) complex AgGly showed significantly strongest inhibition than the other two tested complexes against four bacterial strains: *K. aerogenes*, *S. aureus*, *E. faecalis* and *B. subtilis* (Fig. 5).

All three tested creams loaded with AgGly, AgSD and AgNam (at 1 % concentration) showed also antibacterial potential (Fig. 6). The highest antibacterial activity showed suspension of the cream with AgSD, and the range of relative inhibition zone was: 18–68 %.

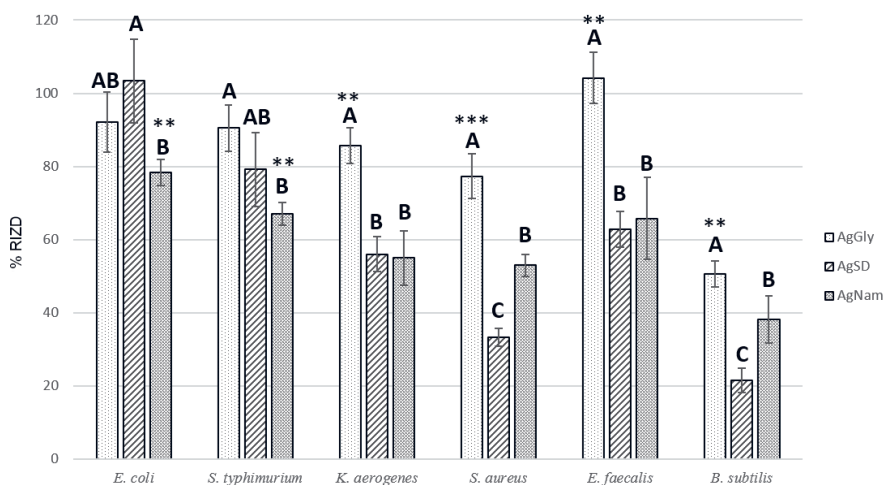


Fig. 5. Antibacterial test activity of bioactive compounds at 1 % concentration. % RIZD means the percentage of relative inhibition zone diameter. Columns with the appropriate marking represent Gram-positive and Gram-negative bacteria. ($n = 3$, results shown as mean \pm SD). The statistics were performed in each bacterial strain. The same letter above the bars means that the results do not differ significantly at $p < 0.05$ by Tukey's pairwise comparisons. Significant differences are marked with * for $p < 0.05$, ** for $p < 0.01$ and *** for $p < 0.001$.

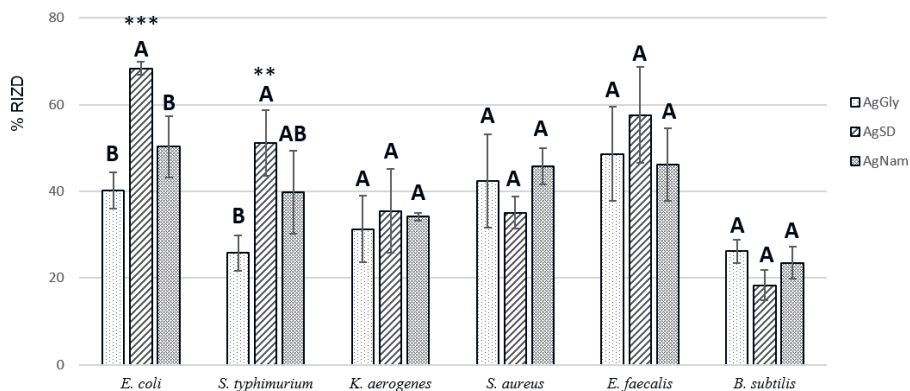


Fig. 6. Antibacterial activity of creams loaded with bioactive compounds AgGly, AgNam and AgSD at 1 % concentration of complexes. % RIZD means the percentage of relative inhibition zone diameter. Columns with the appropriate marking represent Gram-positive and Gram-negative bacteria ($n = 3$, results shown as mean \pm SD). The statistics were performed in each bacterial strain. The same letter above the bars means that the results do not differ significantly at $p < 0.05$ by Tukey's pairwise comparisons. Significant differences are marked with * for $p < 0.05$, ** for $p < 0.01$ and *** for $p < 0.001$.

The most inhibited was *E. coli* and the lowest inhibition the cream showed against *B. subtilis*. The cream including AgNam showed inhibition against bacterial strains in the range of 23–50 %. The strongest inhibition was exhibited against *E. coli*, and the lowest against *B. subtilis*. The cream containing AgGly showed the highest inhibition effect against *E. faecalis* and the lowest against *S. typhimurium*.

While AgGly cream activity decreased compared to AgSD cream, in the case of AgNam cream the higher antibacterial activity against Gram-negative bacteria *S. aureus* and *B. subtilis* was maintained compared to the activity of AgSD cream.

Significant differences were observed in the cream containing silver(I) complex AgSD compared to the other two creams against two bacterial strains *E. coli* and *S. typhimurium* (Fig. 6).

The antibacterial effect of creams loaded with metal ion compounds has been tested by Chen (26) and Selvaraj (28). Also in these studies, a promising ATB effect was observed against selected strains of bacteria.

A case of *in vivo* application

Haematological examination performed immediately after admission of the animal to the clinic showed a significant increase in leukocyte counts to 85.2 G/L (physiological standard: 5.1–11.1 G/L), and the concentration of total immunoglobulins was also increased to 49.5 UZST (physiological standard: 26–38 UZST). Both parameters reflect the inflammatory process in the patient's body. After surgical removal of necrotic tissue and treatment with iodine solution, leukocyte counts were reduced to 15.2 G/L. Other tested haematological, as well as biochemical parameters, were not significantly affected, only calcium concentrations were slightly reduced to 1.80–1.89 mmol L⁻¹ (physiological value: 2.25–3.0 mmol L⁻¹) during the patient's entire stay in the clinic. The bacteriological examination of

the pus and skin swabs yielded *Trueperella pyogenes*, *Staphylococcus* spp. and *Streptococcus* spp. The strains of Staphylococcal bacteria are the most frequent cause of skin dermatitis in sheep, affecting mainly udder and lip skin (29).

Firstly, AgSD cream was used for the treatment for three consecutive days. After this time, however, there was no improvement, in addition, the skin around the wound was hyperaemic, dried and cracked after application, so the treatment was stopped.

Whereas accelerated stability tests have shown high sensitivity of AgGly cream to temperature and humidity, a cream containing AgNam was used for the next therapy. After two-day applications, the condition of the wound improved, the hyperaemia and oedema of the skin gradually disappeared, the wound has been closing without drying the skin, and only a very small amount of new pus was formed. The leukocyte counts were 13.8 G/L, and the concentration of total immunoglobulins did not change, it remained increased to 49.5. Although an experiment was performed on one animal and with previous therapy (AgSD cream), this case study indicates that even the AgNam cream can potentially have a therapeutic effect on this type of decubitus ulcer. However, it is clear that more detailed studies on a larger representative sample are required.

Systemic antibacterial therapy is usually not used in the treatment of skin dermatitis given the requirements for milk and meat withdrawal time. Therefore, the local administration of iodine on inflated skin represents the most common treatment procedure on sheep farms. Veterinary surgeons and farmers are mostly applying either solutions or ointments based on povidone-iodine (30). The invisible effect of the first treatment with iodine solution and the second with AgSD was observed probably due to multiple bacterial contaminations of the lesions. Some of these bacteria might possess higher resistance to the used active antibacterial substances. In the study, the susceptibility of the *Streptococcus* spp. and *Trueperella pyogenes* to the tested antibacterial creams was not examined. Moreover, the lesion could be also contaminated with anaerobic bacteria, like *Fusobacterium necrophorum* and *Dichelobacter nodosus*.

CONCLUSIONS

The aim of this study was to prepare 1 % creams with a silver(I) complexes AgNam, AgGly (Nam– nicotinamide, Gly – glycine) and AgSD (commercially available) and evaluate their antibacterial activity, quality and stability. After the preparation, all cream samples were opaque and odourless, and there wasn't any phase separation. The colour of the creams was white (AgSD and AgNam) and light grey (AgGly), they were o/w emulsions with pseudoplastic behaviour and their pH values were in the range of 4.87–5.75. The accelerated stability testing has indicated the effect of external conditions on the stability of AgGly and AgNam creams and it is recommended to store these creams at lower temperatures, humidity and without access to light (in the fridge). Higher antibacterial activity of the creams with AgGly and AgNam was observed against Gram-negative bacteria *S. aureus* and *B. subtilis* in comparison to AgSD cream and *in vivo* case report of the most stable AgNam cream confirms that the utilisation of new silver(I) complex enhanced its antimicrobial effect comparing to AgSD.

Supplementary material is available upon request.

Conflicts of interest. – The authors declare no conflict of interest.

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Authors contributions. – Conceptualization, Z.V.; methodology, S.S., Š.L.M, J.H., L.T., D.M. and P.M; analysis S.S., M.G. D.M. and P.M; investigation, S.S. and Z.V; writing, original draft preparation, Z.V., S.S., M.G., M.R. and P.M; writing, review and editing, Z.V., M.G. and M.R. All authors have read and agreed to the published version of the manuscript.

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