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Silver Nanoparticles as a Novel Therapeutic Approach for Bovine Mastitis: Efficacy and Comparative Analysis an Antimicrobial Perspective

Bilal Ahmed Khan[1,2], M Tariq Javed[1], Imtiaz Chemma[2], Sajid Ali Qaisrani[3], Muhammad Arif Bugti[4], Muhammad Bilal[5], Muhammad Mudassar[6], Obaid Muhammad Abdullah[7], Maryam Hanif[8], Nisar Ahmed Ujjan[9]

[1]Department of Pathology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Punjab, Pakistan.
[2]Rural Poultry Development, Livestock and Dairy Development Department, Government of Balochistan, Spini Road, Quetta, Balochistan, Pakistan.
[3]Department of Veterinary Surgery, Faculty of Veterinary and Animal Sciences, Lasbela University of Agriculture, Water and Marine Sciences- Uthal, Balochistan, Pakistan
[4]Institute of Microbiology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Punjab, Pakistan.
[5]Disease Investigation Laboratory, Livestock and Dairy Development Department, Government of Balochistan, Spini Road, Quetta, Balochistan, Pakistan.
[6]Department of Chemistry, Faculty of Sciences, Superior University Lahore, Punjab, Pakistan.
[7]Department of Veterinary Surgery, University of Animal Sciences, Lahore, Punjab, Pakistan.
[8]Department of Parasitology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Punjab, Pakistan.
[9]Faculty of Veterinary and Animal Sciences, Lasbela University of Agriculture, Water and Marine Sciences- Uthal, Balochistan, Pakistan.

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Correspondence to: Nisar Ahmed Ujjan, Faculty of Veterinary and Animal Sciences, Lasbela University of Agriculture, Water and Marine Sciences- Uthal, Balochistan, Pakistan.
Email: dr.nisarujjan2009@gmail.com

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ABSTRACT

Mastitis is one of the most prevalent and economically important diseases that affects dairy cattle globally. The disease leads to reduced milk production, poor animal health, and increased culling rates. Bacteria are the main cause of mastitis in dairy industry, where extensive antibiotic use has contributed to the emergence of antimicrobial resistance (AMR). To address this significant challenge, antimicrobial nanoparticles have emerged as promising alternative therapy. This study evaluates the efficacy of silver nanoparticles (AgNPs) in the treatment of bovine mastitis. A total of 40 lactating cattle diagnosed with mastitis were randomly divided into two groups (n=20). One of the groups received the treatment of intramammary administration of silver nanoparticles (100 µg) combined with gentamicin (60 mg) for five consecutive days, while the control group remained untreated. The Udder milk samples were collected before and after treatment to assess somatic cell count (SCC), bacterial load, pH, specific gravity, electrical conductivity, and malondialdehyde (MDA) concentration. The Results demonstrated a significant reduction in SCC and bacterial load in the nanoparticle-treated group compared to the controlled one. Additionally, improved milk quality parameters and reduced oxidative stress markers were observed. These findings indicate that silver nanoparticles exhibit potent antimicrobial properties and could serve as an effective therapeutic alternative for managing mastitis while mitigating the risk of AMR. Further studies are warranted to explore their long-term efficacy and safety in dairy herds.

INTRODUCTION

The increase of antimicrobial resistance (AMR) is a big challenge to global health and agriculture particularly in the dairy industry, where bovine mastitis remains a pervasive, economical devastating disease. Bovine mastitis is an inflammation of the mammary gland in dairy animals like cows, sheep, and goats, caused by either infectious or noninfectious factors. It can result from physical damage or microbial infection. The condition leads to significant economic losses due to reduced milk production and affects animal health, milk quality, and public health. The primary cause of clinical mastitis in bovines is biofilm-producing bacteria, such as *Staphylococcus aureus*, which contribute to the severity of the disease (Mohammed *et al.*, 2020; Buzdar *et al.*, 2025).

Bovine mastitis can be caused by a number of different etiological agents including bacteria or fungus (Sonmez and Erbas, 2017). The presence of yeasts in breastfeeding animals' milk is significant in terms of zoonotic transmission and may have an impact on the safety and quality of milk and milk products (Sartori *et al.*, 2014). *Streptococcus dysgalactiae, Streptococcus uberis, Coagulase-negative staphylococci (CNS), Staphylococcus*

aureus, and *Escherichia coli* are the most frequent bacterial infections that cause mastitis. Since the development of broad-spectrum antibiotics, immunosuppressive corticosteroids, and anticancer medications, their prevalence has significantly increased (Hasanin *et al.*, 2022). Resistance to numerous bacteria has developed as a result of prolonged antibiotic use (Schmidt *et al.*, 2020). Since at least a century ago, the idea of treating infected mastitis using intramammary infusions of antiseptic treatments has been around (Barkema *et al.*, 2006).

There are one or more of the common antibiotics used to treat infections are ineffective or lead to develop resistance against more than 70% of bacterial diseases (Odonkor and Addo, 2011). The development of novel, efficient antibacterial agents appear to be of at most significance. Metals including silver (Ag), copper (Cu), gold (Au), titanium (Ti), and zinc (Zn), each of which has distinct properties, potencies, and spectrums of action, have been known and employed for their antibacterial capabilities since antiquity (Dizaj *et al.*, 2014).

Recent nanotechnology has opened up several scientific and technological sectors to new possibilities. Numerous researchers are becoming increasingly interested in pharmaceutical nanotechnology due to its benefits. After more than 20 years of research, novel dosage has improved therapeutic effects and physicochemical features have been created by using nanoparticles in drug delivery systems. Many different kinds of nanoparticles and their derivatives have drawn a lot of interest because of their antibacterial properties. Some of the metal nanoparticles that have been found as having antibacterial capabilities include Ag, titanium dioxide (TiO2), silicon (Si), copper oxide (CuO), zinc oxide (ZnO), gold (Au), calcium oxide (CaO), and magnesium oxide (MgO) (Dizaj *et al.*, 2014).

At concentrations as low as 6.25 g/mL, CuNPs exhibit little toxicity on fibroblast cell lines and inhibit the growth of Staph aureus, one of the principal mastitis-causing bacteria. CuNPs at a dosage of 6.25 g/mL were more effective than IM gentamicin therapy for treating S aureus. Due to the compound's safety, efficacy, and affordability, CuNPs present a possible choice for developing a novel therapy regimen against bovine mastitis. The usage of antibiotics in people and animals can be considerably diminished by doing research on the use of NPs as antimicrobials against a wide range of pathogens with further study in this direction (Taifa et al., 2022).

The Zn-Al LDH/GA nanocomposite was regarded as one of the best substitutes for sanitizers and/or post-milking teat dip. At 2500, 5000, and 1250 g/mL, it was deadly to Gram-positive and -negative bacteria, respectively. The synthesised Zn-Al LDH, Zn-Al LDH/GA nanocomposite, and the loading on GA through Zn-Al LDH were all characterised. Gallic acid was also added to Zn-Al LDH to aid increase the growth inhibition of the mastitis-causing bacteria and therefore reduce their capacity to produce biofilms (Mohammed *et al.*, 2020).

In this study explores the efficiency of silver nanoparticles as unique treatment approach for bovine mastitis, comparing their performance with conventional treatment. The research aims to provide insights into potential of AgNPs as an effective, cheap and more suitable alternative to antibiotics. Moreover, the results of this study could pave the way for development of innovative strategies to compete diseases such as mastitis, mitigate AMR and increase the productive performance and health of dairy herds.

MATERIALS AND METHODS

Synthesis of nanoparticles

Nanoparticles of silver oxide were synthesized by eco-friendly method reported by Pattanayak and Nayak, (2013) (Pattanayak and Nayak, 2014). One molar solution of silver nitrate was prepared by using deionized water. To prepare one molar solution of 169.87 grams of silver nitrate was added to 1 liter of deionized water and completely dissolved by using hot plate magnetic stirrer. NaOH added drop by drop with the help of burette into one molar solution of copper salt under continuous stirring at room temperature for stabilization. Then crushing was done and particles well be crushed into fine particles. After crushing the particles was placed in muffle furnace at 700°C for 7 hours for drying.

Procedure for Silver Nanoparticles

The preparation of the silver nanoparticles is done by include a chemical reduction process. With 1% Tri-sodium solution, a 0.001 M solution of silver nitrate was created. In order to prepare, 50 ml of 0.001 M AgNO 3 were heated to boiling. Drop by drop, 5 ml of 1% Tri-sodium solution were added. Through a magnetic stirrer, the process will get constant heated. Additionally, vigorous mixing was used until the colour turns yellow. The mixture was taken off the heat and stirred until it has reached room temperature. The formed particles were filtered and drying in hot air oven at 70°C for overnight. Then crushing was done and particles well be crushed into fine particles. After crushing the particles was placed in muffle furnace at 700°C for 7 hours for drying. Finally, the color of nanoparticles was changed to black from yellow.

Characterization of Nanoparticles

The characterization of nanoparticles' size, shape, and structural details was carried out

Zeta Sizing

Prepared nanoparticles were delivered to National Textile University's high-tech lab in Faisalabad for zeta sizing.

Scanning Electron Microscopy (SEM)

Samples were sent to Hi-tech lab of University of Agriculture Faisalabad for SEM analysis. During analysis the AgNPs, the sample were ready by putting small drop of Nps suspension on the clean silicon wafer/carbon coated copper grid, dried properly in air for proper adhesion. This dried samples were further coated with thin layer of platinum for increasing the conductivity and prevent charge effects. The samples were then mounted on aluminum stub by help of conductive carbon tape and introduced into SEM chamber under vacuum conditions. The image was obtained under proper accelerating voltage and magnification to get high resolution surface morphology and structural details of Ag NPs.

Collection of Milk Samples before Treatment

Before milk sample collection, a physical examination of udder was done and clinical signs and case history was also conducted. Aseptically, mammary secretions samples were collected. The guidelines followed for collection of milk samples aseptically from every quarter was National Mastitis Council. The vigorous scrubbing of each teat was performed with the help of 70% ethyl alcohol soaked with cotton gauze. The few streams of milk from fore quarter were discarded and then SFMT paddle was used for the collection of milk samples for detection of subclinical mastitis. The collection of samples was done within the sterilized test tubes. The transportation of samples was performed by the samples on crushed ice within the thermos after which transported to University of Agriculture, Faisalabad. Department of Clinical Medicine and Surgery, Mastitis Research within two hours of collection examination started.

Polymerase Chain Reaction (PCR)

The PCR technique is based on the enzymatic replication of DNA, where a short fragment of DNA is amplified using primer-mediated enzymes. DNA polymerase synthesizes new DNA strands complementary to the template DNA, adding nucleotides to the pre-existing 3'-OH group. For DNA extraction, the boiling method was used, a widely employed technique for bacterial DNA isolation (Dashti et al., 2009). In this method, bacterial colonies were added to 200 µL of lysis buffer in an Eppendorf tube and boiled for 10 minutes to lyse the cells. The tube was then immediately placed in an ice box for 5–10 minutes to facilitate cell debris precipitation. Following this, the sample was centrifuged at 12,500 rpm for 5 minutes at room temperature. The supernatant, containing the extracted DNA, was carefully transferred to a separate tube and stored at -20°C for later use. The primer sequence used for bacterial identification (Table 1).

Table 1

Primer Sequence for PCR

Pathogens	Forward primers	Reverse primers	Annealing temperature	Product size (bp)	Reference
S. aureus	Sau 327 GGA CGA CAT TAG ACG AAT CA	Sau 1645 CGG GCA CCT ATT TTC TAT CT	64 ^{0}C	1318	(Riffon *et al.*, 2001)
E. coli	Eco 223 ATC AAC CGA GAT TCC CCC AGT	Eco 455 TCA CTA TCG GTC AGT CAG GAG	64 ^{0}C	232	

Selection of Animals

Animals were subjected to surf field mastitis test (SFMT) at the Livestock Research Farm University of Agriculture Faisalabad. Animals with clinical and subclinical mastitis were selected based on SFMT.

Treatment Protocol

Animals suffering from subclinical mastitis were divided into two treatment groups each with twenty animals. Group A was given a silver nanoparticles (AgNps) while, Group B was given antibiotic gentamicin (60 mg) for a duration of 5 days (Table 2).

Table 2

Experimental design

Animals Groups	No of Animals	Treatment	Dose Rate	Route of Treatment	Duration of Treatment
A	20	Silver Nanoparticles (AgNps)	AgNps (100 µg)	Intramammary	5 Days
B	20	Antibiotics	Gentamicin (60 mg)	Intramammary	5 Days

Parameters performed

Surf Field Mastitis Test (SFM)

3% surf field solution by combining 6 teaspoons of surf with half a liter of water, stirring, filtering and heating the mixture. Examine the mixture for preparation or gel formation after swirling it for 30 seconds with an equal volume of 3% solution added to milk (in case of mastitis) as method describe by (Muhammad *et al.*, 2010).

Somatic Cell Count

Using platinum loop, 0.01 ml of milk will be transport to the designated location. Gently spreading the milk with the loop itself to cover the entire 1cm2 area on the glass slide. The slide will allow drying, and after the smear is completely dry, it is ready to stain. 10000 cells per ml are considered normal if no of cell are >200000, it is indication for bacterial infection as method described by (Berry and Broughan, 2007).

Bacterial Strain in Milk

Presence of bacterial strains in milk will be tested by inoculating the milk sample on blood agar and McConkey agar medium by method described by (Schmidt *et al.*, 2012).

Ph of Milk

Ph of milk will be determined by using Ph meter. Normal Ph of milk is 6.7 by method described by (Choudhary *et al.*, 2019).

Electrical Conductivity

Electrical conductivity meter will be calibrated using a 0.1M KCI solution. Each fresh buffalo milk sample had its electrochemical content (EC) tested individually, with the electrochemical sensor being cleaned with distilled water between measurement as method described by (Norberg, 2012).

Specific Gravity

Milk sample will be collected in any jar. Lactometer will be placed in milk sample without touching side of jar. Note down the lactometer reading as method described by (Nonga *et al.*, 2015).

Antioxidant Biomarkers

For the detection of antioxidant markers such as malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), in milk samples. The milk collected form respective animals and was centrifuged at 4000 rpm for 10 minutes to separate the fat layer. Based on a reaction with Thio barbituric acid, a photometer approach was used to measure the level of

lipid per oximeter (TBA). It forms a crimson product when it interacts with malondialdehyde produced by peroxidation processes in an acidic solution as method described by (Zigo *et al.*, 2019). SOD activity was assed based on ability to inhibit reduction of nitro blue tetrazolium, have absorbance recorded at 560 nm. The activity of CAT was measured by analyzing the decomposition of hydrogen peroxide at 240 nm. Similarly, the level of GPx, LDH, ALP, and AST were measured using commercially available enzyme assay kits, according to the manufacturer's instructions.

Statistical Analyses

Statistical analysis was done by one-way and two-way ANOVA and Tukey's multi-comparison test using GraphPad Prism software 8.1.

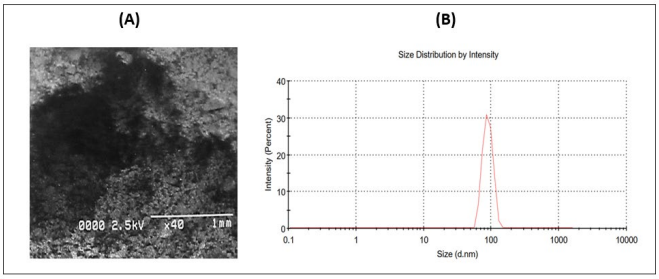
RESULTS

Characterization of Nanoparticles

Results of the Zeta sizer test revealed that the average size of silver nanoparticles was 97.5± 23.76 nm. The Shape of nanoparticles was assessed through Scanning Electron Microscopy and results revealed that the shape of nanoparticles of silver oxide was found to be varied from ellipse to round and was observed through 100x magnification (Figure B).

Figure 1

Characterization of Cu and Ag Nps (A). Scanning Electron Microscopic analysis of Cu and Ag nanoparticles (B).



Distribution of Zeta Sizer

Conformation of Mastitis from Milk Sample and Prevalence

Milk samples collected from the animals were subjected to Surf Field Mastitis Test (SFMT). The milk sample and 3% surf solution were taken in equal amounts, mixed for half a minute, and waited for gel formation. 90 animals were tested for mastitis, out of which 20 were found positive, as indicated by the Surf Field Mastitis test. Milk samples from these animals positive for mastitis were preserved for bacterial isolation for further process. During this the prevalence of mastitis was observed 22.2 % (total animals 90 samples, positive 20 samples).

Bacterial Isolation and Identification

Milk samples were inoculated on the different agar plates and then incubated overnight for bacterial growth and colonies were observed.

Isolation on Blood Agar Medium

Milk samples were inoculated on the surface of blood agar plates with the help of a loop. These plates were incubated overnight at 37 ^{0}C and the growth of bacterial colonies was observed. On the surface of blood agar following characteristics were shown by bacterial colonies. *S. aureus* has convex, round and sharp colonies with 1-4 mm in diameter on blood agar medium. They have light to golden yellow color colonies. While the E. coli showed Convex, Circular, Green metallic sheen with 1-3 mm size (Figure 2-a).

Isolation of S. aureus on Mannitol Salt Agar (MSA)

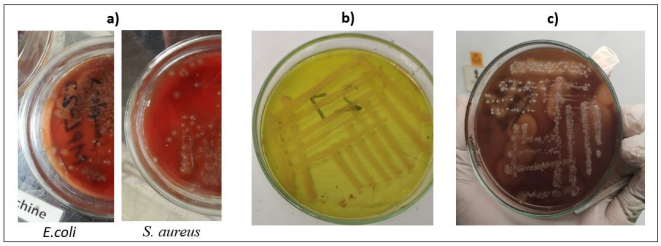
Mannitol salt agar (MSA) is used for the specific culturing of only S. aureus colonies. Suspected colonies of S. aureus were picked from the blood agar medium and inoculated on the surface of Mannitol Salt Agar (MSA). S. aureus has small colonies surrounded by yellow zones. On the surface of MSA, S. aureus could ferment mannitol and convert the color of the medium from red to yellow. The colony showed curvilinear elevation; surface of colony was smooth with 1-4 mm size (Figure 2-b).

Isolation of E. coli on MacConkey Agar Medium

Specific culturing of E. coli colonies was carried out on the surface of the MacConkey agar medium. E. coli could ferment lactose which is specific for E. coli to differentiate it from other bacteria. The E. coli showed morphological characteristics such as round shape, convex elevation, opaque structure and smooth fresh surface with 2-3 mm size (Figure 2-c).

Figure 2

Bacterial isolation on various agar medium a) Bacterial Growth on Blood Agar b) Staph Colonies on MSA c) E. coli colonies on MacConkey Agar

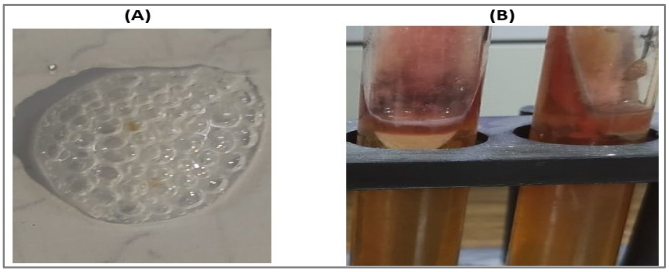


Conformation of E. coli and S. aureus using biochemical tests

Figure 3-A the formation of bubbles immediately after mixing bacterial colonies with hydrogen peroxide on a glass slide indicated a positive result for the Catalase test. *S. aureus* and *E. coli* both were found to be positive for the Catalase test. Similarly Figure 3-B represents a loop full of bacterial colonies was steaked into the slant of the TSI agar medium in the glass tube. The change in color of the slant from red to yellow and the acidic slant confirms the presence of E. coli colonies.

Figure 3

Biochemical tests for conformation of E. coli and S. aureus. (A) represents Catalase test while (B). Triple Sugar Iron tests.

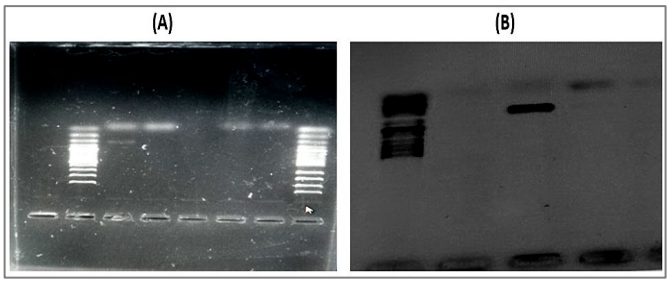


Conformation by PCR

After gel electrophoresis image, the PCR amplification results for Staphylococcus aureus are shown. The presence of clear, distinct bands at the expected molecular weight in the test lanes confirms the presence of S. aureus DNA. The two lanes on the edges contain a DNA ladder (marker), which helps determine the size of the amplified DNA fragments. The uniform bands in multiple lanes indicate successful amplification, suggesting that the samples tested positive for S. aureus (Figure 3 A). while (Figure 3 B) represents the PCR results for *Escherichia coli*. The DNA ladder on the left side serves as a reference for fragment size. A strong, single band in one of the lanes suggests the presence of *E. coli* DNA in that sample, while other lanes either show faint or no bands, indicating negative or weak amplification.

Figure 3

Diagram Represents the PCR amplification results for bacterial identification.



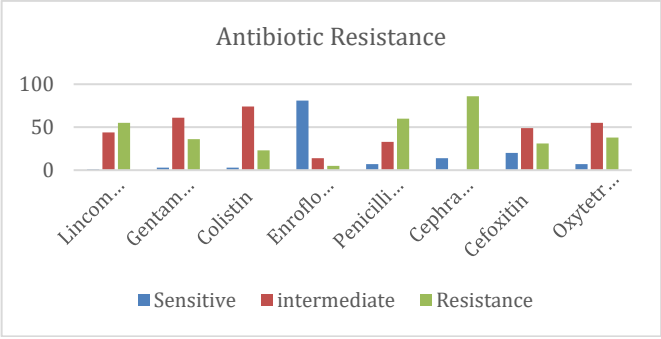
(A) PCR result for *Staphylococcus aureus*, showing distinct bands corresponding to the expected DNA fragment sizes, confirming the presence of *S. aureus* in positive samples. (B) PCR result for *Escherichia coli*, where a strong, specific band indicates the presence of *E. coli* in the respective sample.

Antibiotic Sensitivity Test for S. aureus

All the isolated colonies of S. aureus were checked for the Antibiotic Resistance test using the Kirby-Bauer Disc Diffusion method. The antibiotic sensitivity test for all eight antibiotics was evaluated based on the zone of inhibition around the discs. Results were noted as resistant, sensitive, or intermediate. High resistance was shown by S. aureus isolates against Penicillin (60%), Cephradine (86%) and Lincomycin (55%). Relatively low resistance was shown against Cefoxitin (31%), Gentamycin (36%), Oxytetracycline (38%), Colistin (23%) and Enrofloxacin (5%) (Figure 4).

Figure 4

Graphical representation Antibiotic Sensitivity profile of S. aureus

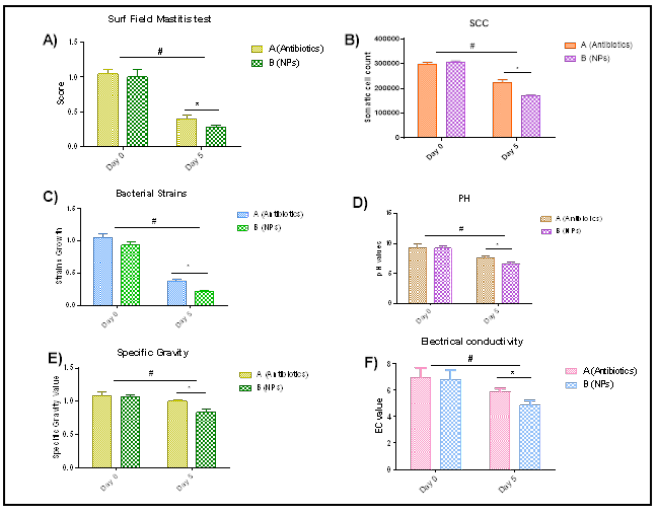


Effect of NPs on Milk Quality Assessment Tests

Milk sample collected form cow were checked for milk quality assessments test and results are presented in Figure 5 representing Surf field mastitis score, Somatic cell count, Bacterial strain in milk, milk pH, Electrical Conductivity, and specific gravity in NPs and antibiotics treated animals. However, in NPs treated milk after 5 days of treatment the Surf field mastitis score (0.27 ± 0.03) significantly ($p<0.05$) decrease compared to antibiotic group (0.40 ± 0.07) (Figure 5-A). Additionally, the Somatic cell count in NPs treated group (167799 ± 7355.3) after 5 days of treatment showed decreasing impact compared with the antibiotic control group (223755 ± 16270) (Figure 5-B). Besides, a decline observed in bacterial strain growth on day 5 in NPs treated group (0.215 ± 0.021) in contrast with antibiotic treated control group (0.375 ± 0.035) (Figure 5-C). Moreover, Ph also observed to decrease on day 5, however, the decline was significantly lower in NPs treated group (6.55 ± 0.48) compared with antibiotic control group (7.58 ± 0.46) (Figure 5-D). Similarly, the Specific Gravity and Electrical Conductivity decline on day 5 in both groups (Figure 5-E, F). The value of Specific Gravity in NPs treated group are (0.83 ± 0.08) lower compared with Antibiotic control group (1.0 ± 0.03). Similarly, value of Electrical Conductivity in NPs treated group are (4.88 ± 0.54) lower compared with Antibiotic control group (5.89 ± 0.38).

Figure 5

Effect of nanoparticles treatment on various Milk Quality Assessment parameters in Cow with Subclinical mastitis.



A) Represents Surf field mastitis score, B) Represents Somatic cell count, C) Represents Bacterial strain in milk, D) Represents milk pH, E) Represents Electrical Conductivity, and F) Represents specific gravity. Values are represented as mean ± SD. Mean values were significantly different (*$P<0.05$ Antibiotic and NPs; #$P<0.05$ Day 0 and Day 5).

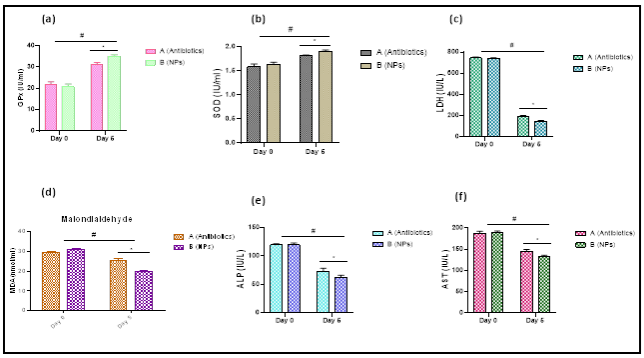
Effect of NPs on milk Antioxidant Biomarkers and Enzymatic markers

The effect of copper and silver nanoparticles on the Milk Antioxidant biomarkers including Glutathione Peroxidase (GPx), Superoxide Dismutase (SOD), and Malondialdehyde (MDA), while Lactate Dehydrogenase (LDH), Alkaline Phosphatase (ALP), and Aspartate Aminotransferase

(AST) are important enzymatic markers were observed in milk on day 1 and 5 in all groups. When compared with the control group, the level of GPx, SOD, increased in NPs treated group on day 5 (Figure 6 a-b). While values of LDH, MDA, ALP and AST decreased on day 5 in both groups, however there was significantly high decline in NPs treated group compared with control (antibiotic) (Figure 6 c-f).

Figure 6

Effect of nanoparticles treatment on cow milk antioxidant and enzymatic biomarkers infected with subclinical mastitis.



(a) Shows GPx (b) Shows SOD (c) indicates LDH (d) indicates MDA (e. represents ALP and (f) represents AST. Values are represented as mean ± SD. Mean values were significantly different (*P<0.05 Antibiotic and NPs; #P<0.05 Day 0 and Day 5).

DISCUSSION

Mastitis is an inflammation of the mammary glands, and its causes can be both infectious and noninfectious. Microbes and physical damage both contribute to inflammation. It has a significant negative impact on the economy of the dairy sector and has a major impact on the milk output of animals, especially cows (Ashraf and Imran, 2020). Several different types of bacteria can cause mastitis. More than 137 bacteria have been linked to the occurrence of mastitis in dairy cattle, and *E. coli, Streptocoous species,* and *Straph aureus* are responsible by 80% of the infections (Abdullah and Alwan, 2017). *E. coli* primarily impact dairy animals from environments with its strain O157: H7 is also harmful to humans as well (Radostits *et al.*, 2006).

One of the key diseases that negatively affects the dairy industry's economics is mastitis. According to estimates, treating mastitis in American dairy cattle costs between 33 and 38 percent of the country's overall health care expenditures, or close to 1.7 billion US dollars each year (Abd EL-Tawab *et al.*, 2018). As a vaccination has not yet been produced, antibiotic therapy is the main method utilised to address the causes of mastitis. The use of antibiotics to treat mastitis increases the burden on the dairy industry and fuels the crisis of antimicrobial resistance (AMR) (Alekish *et al.*, 2018). Hence, in order to treat animal and human infections related to the development of antibiotic resistance, safer, less invasive methods with efficient bactericidal activity were necessary (Manuja *et al.*, 2021). Copper and silver nanoparticles can endure high temperatures and pressure and have antibacterial capabilities. Also, these are secure in terms of public health. Many different kinds of nanoparticles and their derivatives have drawn a lot of interest because of their antibacterial properties. Some of the metal nanoparticles that have been found as having antibacterial capabilities include Ag, titanium dioxide (TiO2), silicon (Si), copper oxide (CuO), zinc oxide (ZnO), gold (Au), calcium oxide (CaO), and magnesium oxide (MgO) (Zhang *et al.*, 2022).

The goal of the current study was to identify the best alternative antimicrobial resistance (AMR) treatment for cow mastitis in its subclinical form. In a nutshell, silver nanoparticles and Gentamicine were given to 40 dairy cows positive for subclinical mastitis were treated in 2 equal groups, respectively. Although genatmicine was shown to be efficient in controlling mastitis, its efficacy was significantly inferior to that of the group treated with copper and silver. The experiment to test the effectiveness of gentamicine and copper and silver combination nanoparticles against *Staph. aureus* on blood agar revealed that the number of bacteria was reduced at high rate in the copper and silver combination treatment group, compared to in the control group. It demonstrated on *Staph. aureus* with copper and silver nanoparticle were effective, which is a key factor in the development of the subclinical type of mastitis. Gentamicine was shown to be beneficial in the current trial to manage the mastitis, although its effectiveness was much lower than the group treated with nanoparticles.

Various studies have been conducted to investigate the impact of nanoparticles on sub clinical form of mastitis. The findings of current studies were inline the with the research conducted by Kalinska *et al.* (2019) (Kalińska *et al.*, 2019). They stated that nanoparticles are efficient to use as these have good interaction with the biological system, Efficient bioavailability, smaller dose required and good utilization in living system. In their trials, found the effect of AgNPs, CuNPs, and AgCuNPs on pathogen species commonly involved in udder inflammation (e.g., *Staphylococcus aureus* and *Escherichia coli*). The results shown that commercially available NPs of good quality and did not have a toxic effect on mammary gland tissue. AgNPs, CuNPs, and AgCuNPs also influenced or decreased the viability of pathogens.

Similarly, Wernicki *et al.* (2014) (Wernicki *et al.*, 2014) found that the best preparations for preventing the growth of infections were those containing copper and silver nanoparticles, whereas the treatment including gold nanoparticles had a substantially smaller effect. Platinum nanoparticles did not exhibit any biocidal activity towards the tested microorganisms at the applied doses. The antibacterial activity of the silver nanoparticles caused the complete eradication of the viable cells of the microorganisms isolated from mastitis patients at concentrations of 50 and 25 ppm within 30 minutes

Likewise, Kovalenko *et al.* (2020) (Kovalenko *et al.*, 2020) examined the medication mastitnano-analgesic BelGAU's and antibacterial properties. Together with other chemicals, it contains distilled water, arginine, dexpanthenol, copper, and silver nanoparticles. At a concentration of 104 cells/ml, it was discovered that samples 1 and 2 showed stronger antibacterial action

against clinical polyresistant strains of the Staphylococcus genus. There were active compounds containing silver nanoparticles at concentrations of 0.15 and 0.12 mg/mL, respectively. The clinical strain of R. aeruginosa 185 was more resistant to Mastitnano-BelGAU when tested therapeutic samples were compared to the strain of E. coli 197.

In the current investigations, the experiment to test Gentamicine and Silver Nanoparticles against E. coli on Blood Agar revealed that the number of bacteria was reduced way more in the Nanoparticles treated group, as comparision to the Control group treated with gentamicine. Research of the bases of results of parameters tested show that silver and copper nanoparticles were more effective against E. coli, which only slightly contributes to the development of subclinical mastitis.

CONCLUSION

The use of antibiotics induces bacteria to be become resistant to them, which is why the threat of antibiotic resistance is reduced by using metal oxides nanoparticles, a novel technology that has a significant positive effect. It is determined that mastitis is very important in the production of dairy products. It needs to be made available for public use. Chronic mastitis has a detrimental effect on the dairy sector and the socioeconomic status of the owners. The world has to be educated about antibiotic resistance, the effects of antibiotics, and novel nanoparticle treatments for mastitis.

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