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Original Article

Green-Synthesized Zinc Oxide Nanoparticles from Indigenous Medicinal Plants for Multidrug-Resistant Bacterial Infections

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ABSTRACT

Background: When we get infections from bacteria that're resistant to many medicines it is really hard to treat them with the usual medicines. This is why we need to find ways to treat these infections. We think that tiny particles of zinc oxide that are made in a way could help to fight bacteria and heal wounds. Objective: We wanted to see how well a special cream made with these zinc oxide particles worked compared to another medicine called mupirocin. We used this cream on people with skin infections that were resistant to medicines. We also wanted to see if the size of the particles made a difference in how well they fought the bacteria. Methods: We did a study in Karachi, Pakistan from May 2025 to January 2026. We had 84 people in the study. We divided them into two groups. One group used the cream with zinc oxide particles and the other group used mupirocin. They used these medicines twice a day for four weeks. We looked at how many bacteria were on the skin how big the wound. If the wound was completely healed. Results: After four weeks we found that the people who used the cream with zinc oxide particles had bacteria on their skin. The wounds of the people who used the cream with zinc oxide particles were also more likely to be healed. We found that the smaller the zinc oxide particles were, the better they were at killing the bacteria. Our study showed that the cream made with zinc oxide particles worked better than mupirocin at fighting bacteria and healing wounds. This is a sign and we think that we should do more studies, with more people to see if this cream really works. Keywords: Azadirachta indica; Antimicrobial Resistance; Multidrug-Resistant Bacteria; Mupirocin; Nanoparticles; Wound Healing; Zinc Oxide

EDITORIAL INFORMATION

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INTRODUCTION

Antimicrobial resistance has become a problem for health all around the world. Infections that are resistant to medicine cause a lot of sickness, deaths longer stays in the hospital. More money spent on healthcare. The fact that old medicines are not working well as they used to is especially bad for certain bacteria that are hard to treat. These include methicillin- Staphylococcus aureus (MRSA) resistant Enterobacterales and multidrug-resistant Pseudomonas aeruginosa. At the time new medicines to fight bacteria are not being made fast enough to keep up with the spread of these resistant germs. This makes it more important to find ways to treat infections that work differently from the usual antibiotics (1,2).

Nanomaterials have started to get attention as possible medicines to fight germs. Their physical and chemical properties let them work with organisms in many ways. One of these materials is zinc oxide nanoparticles (ZnO-NPs). These have shown they can kill types of bacteria and have other useful properties that make them worth looking into for medical uses (3,4). Possible ways they work include making oxygen molecules breaking the outer layers of bacteria changing how the cell walls let things in and interacting

with parts inside the bacteria. They also release zinc ions that can stop processes in the cell (3–6). This kind of working in than one way is very important for germs that are hard to kill because it is not the same, as the single targets that most regular medicines use.

Conventional methods for making nanoparticles using chemicals and physics often need energy, chemicals like organic solvents or other chemicals that help reduce and keep things stable. These can make it harder to use the nanoparticles in biology on. Green methods offer another way where plant stuff helps make the nanoparticles keep them stable and change their surface (7). Medicinal plants have plant chemicals like polyphenols, flavonoids, terpenoids and other active parts that might act as reducing agents or cover agents when making nanoparticles. Azadirachta indica has been looked at specifically for making ZnO-NPs in a way and checking their antibacterial effects (8). In general using plants to make ZnO has been studied as a way that's better for the environment and might be useful for medical and antibacterial purposes (9,10).

Even though experiments have shown results there are still important questions about how well the biogenic ZnO-NPs work against bacteria that are hard to kill. There are also questions about how they compare to ways of treating infections and how the properties of the nanoparticles affect their ability to kill bacteria. Studies that compare the two have shown that biogenic ZnO might have an antibacterial effect against bacteria found in hospitals (11). The size of the particles might also matter because smaller ZnO-NPs have surface area compared to their size, which could help them interact more with bacteria release ions and get into the bacteria or their protective layers (12,13).. There is still not enough real patient data to fully understand these effects in people, with hard-to-treat skin infections.

The present randomized controlled trial therefore evaluated a topical green-synthesized ZnO nanoparticle formulation derived from A. indica leaf extract in adults with culture-confirmed multidrug-resistant cutaneous or soft-tissue infections. The primary objective was to compare the reduction in quantitative microbial load achieved with 0.5% w/w green-synthesized ZnO nanoparticle ointment versus 2% mupirocin ointment over four weeks. Secondary objectives were to compare wound-healing outcomes between the groups and to examine the relationship between nanoparticle size and antibacterial activity.

MATERIALS AND METHODS

Study Design and Setting

A study was done at one hospital in Karachi, Pakistan. The study happened between May 2025 and January 2026. The study had people who were put into two groups. Each person got their treatment for four weeks. The study looked at how the treatment worked.

Participants and Eligibility Criteria

People who were 18 to 65 years old and had infections that were confirmed with a lab test were checked to see if they could join the study. The infections had to be on the skin or in the tissue. The infection had to be caused by bacteria that did not respond to medicines. This included MRSA or bacteria that were resistant to medicines. People who had infections in the blood very bad kidney problems were allergic to zinc or were taking medicines that weaken the immune system were not allowed to take part.

A total of 112 people were checked. Out of these 84 people met the rules to join the study. They were then put into groups.

Randomization, Allocation Concealment and Blinding

People were put into two groups in numbers. One group got the treatment. The other group got a medicine called mupirocin. The way people were put into groups was done through a computer. The computer made sure the groups were balanced. The people who picked the groups were not told who got which treatment. They used numbered envelopes that were not see-through. These envelopes were made by a pharmacist who was not part of the study.

The two treatments looked different. So it was not possible to keep the people who gave the treatments blind.. The people who checked the results the lab workers and the people who looked at the data did not know which group each person was in.

Intervention and Comparator

People, in the treatment group got a special ointment made with zinc oxide nanoparticles. These nanoparticles were made using a leaf extract from a plant called A. Indica. The ointment was put on the cleaned wound twice every day for four weeks.

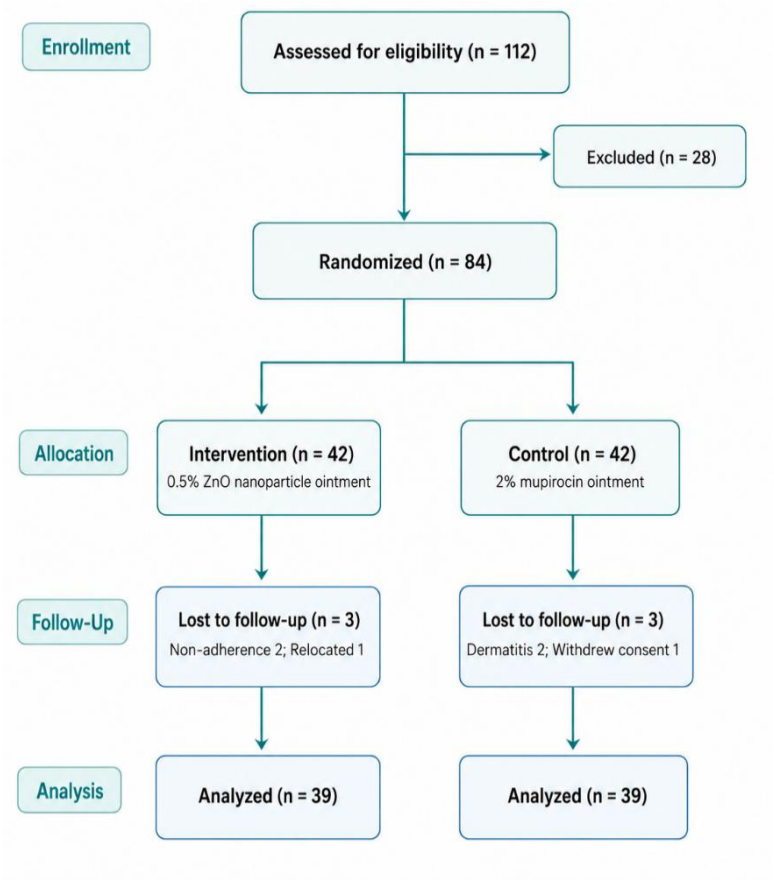

Figure 1 CONSORT Flowchart

Participants in the control group got 2% mupirocin ointment put on the cleaned wound two times every day for the four-week time of treatment. People in both groups had a check-up by a doctor every week. The way well people followed the treatment was watched during these meetings and by looking at how they used the wound dressings.

Outcome Assessment

Outcomes were checked when the study started, at week 2 and at week 4. The main result was how much the number of microbes changed during treatment. The amount of germs was checked using the number of colonies found in wound samples, which included tissue samples and swab samples.

Other results included how much the wound area changed, which was measured using a tool. The health of the wound was checked using a score that went from 0 to 12. The study also noted if the wound was completely closed by week 4.

A separate look at the data checked how the size of ZnO nanoparticles was connected to how they killed bacteria. The nanoparticles in the study were said to be between 15 and 50 nm. The size of the nanoparticles was linked to how much bacteria was reduced.

Sample Size

The number of people needed was based on a guess that the effect would be 0.65, an 80% chance of finding an effect and a 5% chance of a false result. After thinking about 15% of people possibly leaving the study the total number planned was 84 people, which meant 42, in each treatment group.

Statistical Analysis

We looked at the variables by using the average and how much they varied from that average. For the variables we looked at how often they happened and what percentage that was. We checked if the continuous variables were normally distributed by using the Shapiro-Wilk test. We compared the outcomes between groups by using a special kind of test called an independent-samples t test. We also looked at how things changed within a group over time by using a paired-samples t test.

We wanted to see how things changed over time so we looked at the results from the beginning after two weeks and after four weeks. We used a kind of analysis to see if there were any differences in how the groups changed over time. We also looked at how the size of the nanoparticles affected the reduction in load by using a Pearson correlation analysis.

We used a computer program called SPSS version 28.0 to do all the analysis. We considered something to be statistically significant if the p value was, than 0.05. The results we talk about below are based on the 78 participants who had data after four weeks. We always say how many people were included in each analysis.

RESULTS

Participant Flow and Follow-up

Between May 2025 and January 2026 the doctors looked at 112 patients to see who could be in the study. They found that 84 patients were suitable and could be part of the study. These 84 patients were then divided into two groups. One group got the synthesized ZnO nanoparticle treatment and the other group got the mupirocin treatment. Each group had 42 patients.

Six patients did not finish the four weeks of treatment. In the synthesized ZnO nanoparticle group two patients did not take the treatment twice a day like they were supposed to so they were not part of the study anymore. One patient moved away during the study. In the mupirocin group two patients got dermatitis. Had to change their treatment. One patient decided they did not want to be part of the study

So 78 patients finished the four weeks of treatment. In each group 39 patients. Gave data for the last week of treatment.

Baseline Characteristics

The two groups of patients were very similar. They were similar in age whether they were men or women how big their wounds were at the start how many bad germs were in their wounds at the start and what kind of germs they had. The patients in the synthesized ZnO nanoparticle group were 42.1 years old on average and the patients in the mupirocin group were 41.1 years old on average.

In the synthesized ZnO nanoparticle group, 26 out of 42 patients had a type of germ called MRSA. This is 61.9% of the patients in this group. In the mupirocin group 24 out of 42 patients had MRSA. This is 57.1% of the patients in this group. The green-synthesized ZnO nanoparticle group also had 16 patients with a type of germ called multidrug- P. Aeruginosa. This is 38.1% of the patients in this group. The mupirocin group had 18 patients with this type of germ. This is 42.9% of the patients, in this group.

Table 1. Baseline demographic and clinical characteristics of randomized participants (N = 84)

Characteristic	Total (N = 84)	Intervention (n = 42)	Control (n = 42)
Age, years, mean ± SD	41.6 ± 11.8	42.1 ± 12.1	41.1 ± 11.6
Male, n (%)	48 (57.1)	25 (59.5)	23 (54.8)
Female, n (%)	36 (42.9)	17 (40.5)	19 (45.2)
Baseline wound surface area, mean ± SD	14.8 ± 4.2	14.6 ± 4.4	15.0 ± 4.1
Baseline microbial load, mean ± SD	7.42 ± 0.61	7.45 ± 0.63	7.39 ± 0.60
MRSA, n (%)	50 (59.5)	26 (61.9)	24 (57.1)

Characteristic	Total (N = 84)	Intervention (n = 42)	Control (n = 42)
MDR P. aeruginosa, n (%)	34 (40.5)	16 (38.1)	18 (42.9)

SD, standard deviation; MRSA, methicillin-resistant Staphylococcus aureus; MDR, multidrug-resistant.

Baseline hypothesis-testing p values have not been retained because baseline characteristics in a randomized trial are more appropriately presented descriptively.

Primary Microbiological Outcome

Among the 78 participants with available four-week measurements, microbial load decreased substantially in both groups but the reduction was greater in the ZnO nanoparticle group. At week 4, mean microbial load was 2.14 ± 0.48 in the intervention group compared with 3.85 ± 0.62 in the control group. The between-group mean difference at week 4 was −1.71 (95% CI, −1.96 to −1.46; p < 0.001).

The mean absolute reduction from baseline was 5.31 ± 0.52 in the intervention group and 3.54 ± 0.58 in the control group, corresponding to a between-group difference in reduction of 1.77 (95% CI, 1.52 to 2.02; p < 0.001).

Table 2. Four-week primary microbiological outcome among participants with available outcome data (n = 78)

Outcome	Intervention (n = 39)	Control (n = 39)	Mean difference (95% CI)	p value
Microbial load at week 4, mean ± SD	2.14 ± 0.48	3.85 ± 0.62	−1.71 (−1.96 to −1.46)	<0.001
Absolute reduction from baseline, mean ± SD	5.31 ± 0.52	3.54 ± 0.58	1.77 (1.52 to 2.02)	<0.001

Repeated-measures analysis across baseline, week 2, and week 4 demonstrated a statistically significant time-by-treatment-group interaction (p < 0.001), indicating that the trajectory of microbial-load reduction differed between the treatment groups over the four-week study period.

Wound-Healing Outcomes

At week 4, mean wound surface area was 3.2 ± 1.1 in the intervention group compared with 6.8 ± 1.9 in the control group (p < 0.001). Relative to baseline, mean wound surface area decreased by 11.4 ± 3.8 in the intervention group and by 8.2 ± 3.1 in the control group. Clinical wound assessment scores at week 4 were also lower in the ZnO nanoparticle group, with a mean score of 1.8 ± 0.7 compared with 4.3 ± 1.2 in the control group (p < 0.001). Complete wound closure was documented in 28 of 39 participants (71.8%) in the intervention group and 14 of 39 participants (35.9%) in the control group (p = 0.002).

Table 3. Secondary outcomes at four weeks among participants with available outcome data (n = 78)

Outcome	Intervention (n = 39)	Control (n = 39)	p value
Wound surface area, mean ± SD	3.2 ± 1.1	6.8 ± 1.9	<0.001
Clinical wound assessment score (0–12), mean ± SD	1.8 ± 0.7	4.3 ± 1.2	<0.001
Complete wound closure, n (%)	28 (71.8)	14 (35.9)	0.002

Nanoparticle Size and Antibacterial Response

The reported correlation analysis demonstrated an inverse relationship between nanoparticle size within the investigated 15–50 nm range and reduction in bacterial load (p < 0.001). Smaller nanoparticle preparations, particularly those reported within the 15–25 nm range, were associated with greater bacterial-load reduction than larger preparations within the investigated range.

DISCUSSION

The current study, which was a controlled trial showed that people who used the green-synthesized ZnO nanoparticle ointment had bigger short-term drops in the amount of bacteria and better results when it came to healing wounds compared to those who used topical mupirocin. After four weeks the group that got the ointment had an average level of bacteria that was 1.71 units lower than the group that got the usual treatment. The drop from the starting point was also bigger for the group using the ZnO nanoparticle ointment. The size of the wound the scores for how the wound looked and the number of wounds that fully healed were all better for the ZnO nanoparticle group (11). These results match what other studies have found before which showed that the ZnO nanoparticles made in a way have strong antibacterial effects against bacteria that are important in medical cases.

The way the bacteria reacted to the treatment makes sense from a point of view. This is because ZnO nanoparticles can stop bacteria from living in ways. Unlike medicines that usually work on just one specific target the tiny ZnO particles can affect bacteria by breaking their outer cover causing stress from oxygen letting out zinc and reacting with parts inside the bacteria (3-6). The way the nanoparticles are covered by plants might also change how they work and how stable they are. Other studies using ZnO nanoparticles made from A. Indica have also shown that they can fight bacteria, which supports why this way of making them is worth looking into. Still these explanations about how they work are ideas that need more proof because the study did not check for things, like how much oxygen is created how much the bacterias outer layer is damaged, how the zinc moves or other similar details (8).

A further finding was the reported inverse relationship between nanoparticle size and antibacterial response, with smaller particles within the investigated range associated with greater bacterial-load reduction. Size-dependent antimicrobial effects of ZnO nanoparticles have previously been linked to changes in surface-area-to-volume ratio, contact with bacterial membranes, dissolution characteristics, and interactions with microbial structures (12,13). The present observation is therefore compatible with existing experimental evidence. However, interpretation should remain cautious until the nanoparticle characterization method, batch-level size distribution, number of observations contributing to the correlation, and experimental unit used in the size–activity analysis are fully reported. These details are particularly important because batch-level measurements treated as independent patient-level observations could overstate statistical precision.

The more favorable wound-healing measures observed with the ZnO nanoparticle formulation may reflect a combination of improved local microbial control and biological interactions of nanomaterials with the wound environment. Nanoparticle-based materials have been investigated for infection control and wound-healing applications, while broader nanomedicine research has emphasized their potential to influence local drug delivery and tissue-repair processes (15,16). Plant-derived surface constituents may additionally contribute biological activity, although the present trial did not independently quantify phytochemical composition or separate the biological effects of the plant-derived coating from those of ZnO itself. Consequently, the observed clinical response should be attributed to the tested formulation as a whole rather than to an individual mechanistic component.

Safety is really important when we think about using metal oxide nanoparticles to help heal wounds. We know that ZnO nanoparticles can be bad for cells and cause problems like oxidative stress and cytotoxic responses. This happens when the conditions are just right so we need to think about how much of the nanoparticlesre used what they are like how long they are used for and the context in which they are used. In our study two people in the control group got dermatitis. Had to change their treatment. We do not have a list of bad side effects so we cannot say for sure if one group had more problems than the other (17).

Our study is good because we did things like randomly assign people to groups keep the assignments secret and make sure the people assessing the results did not know who was in which group. We also used a comparator checked the results many times and looked at how well the wounds were healing and the microbes that were present. Using nanoparticles to fight microbes is an idea because they can work in many ways not just one way like regular antibiotics. Our study gives us some idea of how this might work in life which is important because a lot of what we know about this is still in the experimental stage.

The metal oxide nanoparticles, like the ZnO nanoparticles are what we are really interested in. The metal oxide nanoparticles have a lot of potential to help us. We need to do studies that look at the bad side effects, like treatment discontinuations, serious bad side effects and exposure to zinc-containing nanoparticles so we can learn more, about the metal oxide nanoparticles and how they can be used to help people with wounds (18,19).

There are some limitations that affect how we understand the results. First this study was done at one place. It had a small number of people so we do not know if the results would be the same in other places. Second some people who were part of the study did not give us information about how they were doing after four weeks so we only looked at the information from the people who did give us data. We did not have

a plan for what to do with missing information so we cannot be sure that our results are completely accurate. Third the different treatments looked and felt different so the people in the study and the people giving the treatments could tell which one was which and this could have affected the results. Fourth we only looked at what happened after four weeks so we do not know what happens in the term like if the infection comes back or if there are bad effects from the treatment. Fifth we need information about how the tiny particles were made what they are like and how they work so that other people can do the same study.

The study only used one treatment to compare to the new treatment and this is a problem because the study included different types of infections including some that are hard to treat. We do not have information about which infections the comparison treatment works well for so we cannot be sure that it was a fair comparison. Future studies should choose comparison treatments that're fair for all the different types of infections and they should do tests to see which treatments work best for each type of infection. They should also tell us everything they did to take care of the wounds. They should look at the safety of the new treatment and how it is made because we need to know a lot more, than just if it kills germs or not (20).

The results show that more research is needed before these treatments can be used widely. Big studies that involve several places should plan ahead for how they will look at certain groups of people make sure they follow up with everyone or have clear ways to handle missing information. They should also check the nanoparticles carefully use the ways to test for germ resistance keep track of any bad reactions and watch people for a longer time. These kinds of studies could show if the good results seen in the term can be repeated in different places with different kinds of bacteria different types of wounds and different batches of nanoparticles.

CONCLUSION

In this study that compared treatments, people who used a special ointment made from green-synthesized zinc oxide nanoparticles from neem plants had better results. They had bacteria and more healing in the short term than people using 2% mupirocin. A link was found between the size of the nanoparticles and how well they killed bacteria. However the details needed to understand this part of the study are not complete. These results give some support for looking more closely at plant-made zinc oxide nanoparticle treatments. More research is needed before these treatments can be used regularly in clinics. Larger studies with reports, full analysis of all participants complete details, about the materials, proper testing methods checking for safety and longer follow-up are all needed.

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