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EVALUATION OF ANTIBACTERIAL OF ZINC OXIDE NANOPARTICLES, ALOE VERA GEL AGAINST MRSA SKIN INJURY

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Key words: MRSA, Aloe Vera gel, ZnO NPs.

ABSTRACT

Microbial resistance to antibiotics increase the risk of infection, so new approach was investigated such as new plant extracts and nano technology, so this study designed to compare the effects of Zinc oxide nanoparticles and Aloe Vera extraction in treatment of experimental skin infection with MRSA. *In vitro*, both were effective against MRSA in well diffusions assay, while in vivo, both were showed antibacterial effects and enhance tissue healing compared with MRSA infective group with priority to Aloe Vera extraction.

INTRODUCTION

The most complex and harmful physical injuriesto clinically evaluate and manage.In addition topain and distress, a large burned area will leave the patient with visible physical scars and invisible psychological sequelae(1).Infectious diseases, whether they are intracellular or extracellular, have always been a global problem to public health, causing millions of deaths each year ,Bacterial resistance to antibiotics has become a global problem nowadays, (2)nanomaterials, such as metal oxide nanoparticles, have appeared to be promising candidates during the last few years. As a result, the science of nanotechnology has significantly advanced due to its wide application (3) Nanoparticles (NPs) are used in many commercial products and new applications in biomedicine, yet their fate, potential toxicity, and mechanisms of translocation in biological cells, (4)

To reduce pain and accelerate the healing process, many natural substances have been traditionally used and more recently have been scientifically studied, such as Aloe (5) Aloe vera has been used in a host of curative purposes including treatment of skin disorders and healing of wounds. The colorless gel that comes from the leaf parenchyma has been used to treat burns because, besides being a potent moisturizing agent, it helps in the healing process of skin lesions and alleviates pain (6)

Methicillin-resistant Staphylococcus aureus (MRSA) is the most pathogen bacterial causing important infections from skin and soft tissue infections such as pneumonia, endocarditis, bacteremia and sepsis (7, 8). It is nonpoisonous; it can accelerate wound healing, reduce blood cholesterol levels, stimulate the immune response and can be biologically decomposed. It has a stronger antimicrobial property compared to chitin in avoiding fungi because it has an active group that will bind to microbes, so it can inhibit microbial growth. (9, 10)

MATERIAL AND METHODS

Thirty swaps were collected from medical implants from (AlYarmouk Teaching Hospital/Baghdad/Iraq), cultured on mannitol salt agar and incubated at 37Cº for 24 hours. The colonie's morphology, Gram stain and Biochemical characteristics ,For inoculum standard, there were homogenized MRSA cells in solution saline (NaCl 0.85 %) and the suspending was diluted to 0.5× 108 CFU·mL−1 using a O.D (11)

Antibiotic Sensitivity Test:

Standard homogenized *S.aureus* was prepared in normal saline and the suspending was diluted to 0.5× 10^8 CFU ml compared with McFarland tubes Antibiotic sensitivity test for *S.aureus* was done by Kirby-Bauer disk diffusion method against Amoxicillin , Cloxocillin , Ampicillin , Cefetrexon and tetracycline .The zone of inhibition were measured (mm) and compared with pretive chart a documented standard, the zone of inhibition (in mm) Clinical and Laboratory Standards Institute (12).

DNA extraction

Genomic DNA was extracted from the detected bacterial isolates according to the protocol of Wizard Genomic DNA Purification Kit, Promega. Quantus Florometer was used to detect the concentration of extracted DNA

Primers Selection

The set of primers 27F (AGAGTTTGATCTTGGCTCAG) and 1492R(TACGGTTACCTTGTTACGACTT) was used for amplification of 16s rRNA for identification of bacteria at gene level (13)

Preparation of Zinc Oxide Nanoparticles (ZnO NPs):

The used preparation procedure was described by (14) but with the insert of modifications. Zinc acetate dehydrate and Sodium hydroxide were purchased. ZnO NPs were synthesized by precipitation method using zinc acetate dehydrate (as a source of zinc) and sodium hydroxide used as precursors and deionized water that used to dilution. 0.1 mole of zinc acetate dihydrate $Zn(CH_3CO_2)_2$.

$2H_2O$was taken and dissolved in 100 ml of deionized water with stirring using a magnetic bar stirrer for the purpose of completely dissolving zinc acetate dehydrate, forming transparent solution. After making sure that the zinc acetate dehydrate are completely dissolved, added sodium hydroxide NaOH gradually with stirring at different quantities for the purpose of changing the pH value of the material to be prepared (using PH meter to measure the pH required), here is a white solution formed. Left it on the magnetic stirrer for 30 minutes at 75 °C, then removed the solution from stirrer. The solution washed and filtered five times with deionized water with a process called Washing and Filtering, the white precipitate is formed. and then dried in the electrical furnace at 100 ° C, the white precipitate separated into a part at calcined at 500 ° C for 3 hours, and part of without calcined. The resulting material was grinding by a mortar to obtain final product (ZnO nano in powder shape), as shown in fig (1).

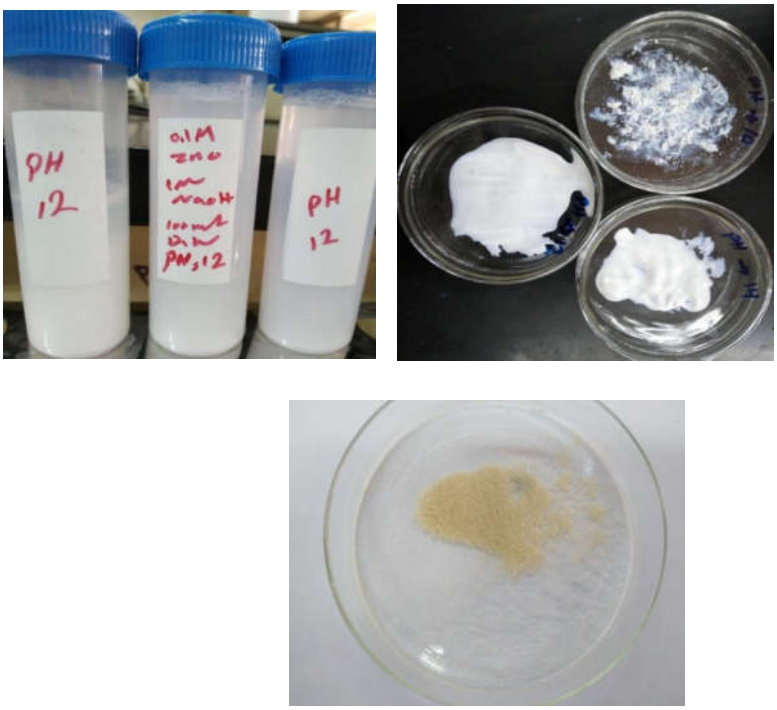


Figure. 1: ZnO nanoparticles

Characterization techniques:

Chitosan/Zinc NPs synthesized were characterized by:

- UV-vis Spectroscopy (Shimadzu, UV-1601PC).
- X-Ray Diffraction (XRD) (Shimadzu, XRD-6000).
- Transmittion Electron Microscopy (TEM) (Philips, CM10).
- Atomic force microscope (AFM) (Angstrom Advanced Inc. , AA2000, Contact mode).

Preparation of A. vera Gel:

The plant of (leaves) *A. vera* was harvested from a (Iraq-Baghdad). Than washed with sterile distilled water to remove dirt and their thick epidermidis was then dissected longitudinally into pieces. The colorless parenchymatous tissue was collected in a sterile container. One hundred grams of the gel was mixed in one liter of 2% dimethyl sulfoxide and kept at 4◦ C. showed fig (2)

Figure(2) Aloe gel

Laboratory animals

Thirty albino male Swiss white mice (17-25) g at 10 weeks age, obtained from Al-Nahrian University. The animals were maintained at a temperature of 25 °C, and had excess free to food pellets and water throughout the experimental work.

Mouse wound infection Wound distance:

In order to produce skin injury, the mice were anesthetized with and (IP)injection of both of xylazine (5 mg/kg) and ketamine (75 mg/kg). Then the hair on the flank .The cleaned by soap and sterile D.W before shaved area than drying skin wound was induced using lancet sterile in which line of superficial

Experimental design

Mice were divided into five major groups (each group 5 mice) as the follows:

- Group1:(n=6) negative control group
- Group2: (n=6) mice were subjected to superficial skin wound without any treatment (wound only) and considered as positive control group.
- Group 3: (n=6) mice were subjected to superficial skin wound and infected with MRSA (0.1 ml of 0.5×10^8 CFU ml)
- Group 4 (n=6) mice were wounded and infected with MRSA then treated with Zinc Oxide NPs
- Group 5:(n=6) mice were wounded and infected with MRSA then treated with *A. Vera* Gel.

RESULT AND DISCUSSION

Isolation and Identification *S. aureus*:

Fifty specimens were collected over a period of a month between August and December 2019.While the isolates on Blood agar showed color yellow-gray colonies are (4-3) mm in diameter on the zones of β-hemolysis.This description is mentioned by (Figure 3)

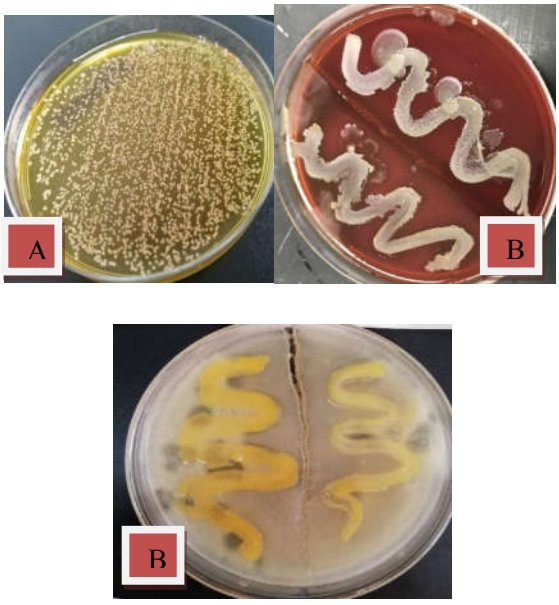


Figure (3): *S.aureus*: (A) On mannitol salt agar (B) Blood agar

S.aureus C) Milk agar at 37°C for 24 hrs. Figure (4) show various levels susceptibilities to different antibiotics among isolates that were observed by Disk diffusion method. The isolates of S. aureus (n=22) showed different susceptibility towards 5 antimicrobial agents used, there were about 14(%) resist to Azithromycin, 12(%) resist to cefoxitin, 6(%) resist to Gentamicin and 16(%) resist to Trimethoprim . There was less resistance to Levofloxacin 4(%) than other antibiotics, all the results of AST .isolates was multi-resistance for antibiotics with a high level against, Gentamicin, Azithromycin, Cefoxitin , Trimethoprim and Levofloxacin result was similar to that acquired by (15)

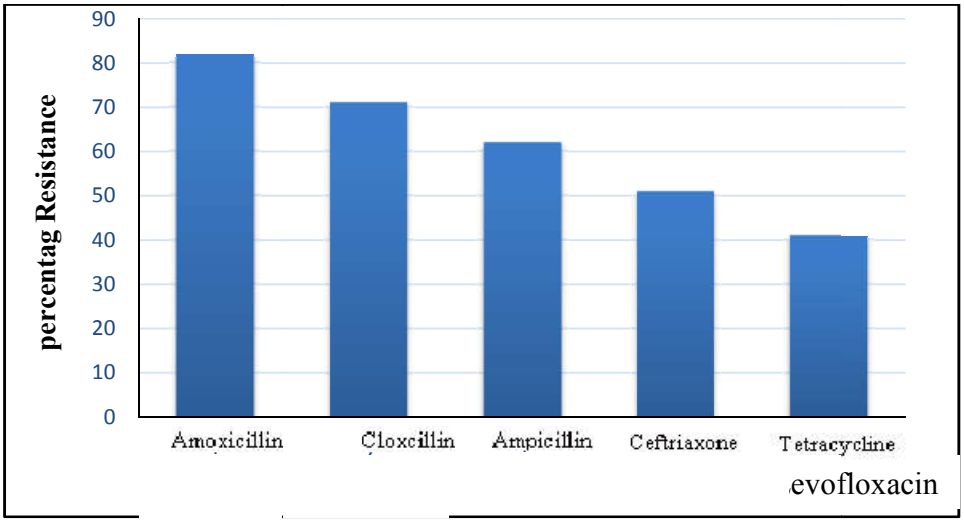


Figure (4): Antibiotic susceptibility test of *S.aureus*

Confirmation of *S. aureus,a* using Vitek 2 System organism with probability (98-99) %

Molecular diagnosis of *S.aureus*:The results shwen that multiplex PCR analysis for both strain were confirmed by AST and Vitek 2 system gave positive results for multiplex Polymerase chain reaction (PCR).showed figure(5)

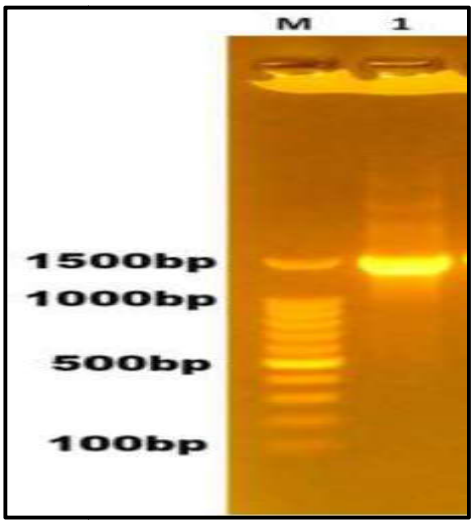
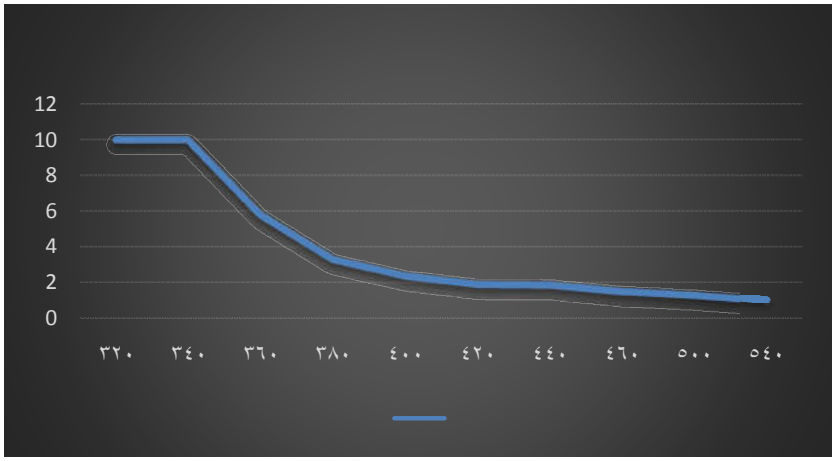


Figure (5) : Agarose gel (1%) electrophoresis (100v/mAmp for 90min) of amplified *16s rRNA* (1500pb) from bacterial DNA stained with ethidium bromide. Lane M. 100 bp DNA ladder, Lane 1. Unknown bacterial isolates

Study of Zinc NPs Nanoparticles characterization:

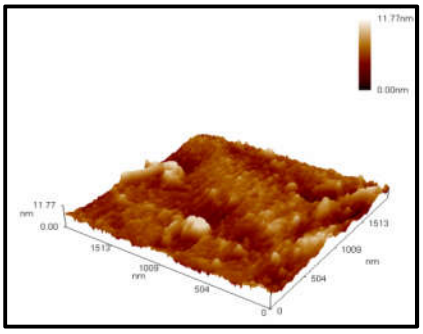
Spectral Properties of the Zn NPs Nanoparticles: Figure (6) revealed a strong surface plasmon placed around 340 nm. This indicates the creation of Zn NPs. It is not likely to detect at 280 nm since it does not comprise any aromatic amino acids, the 215 nm was selection



Figure(6) Absorption spectra of **Zinc NPs** nanoparticles.

Atomic Force Electron Microscopy (AFM)

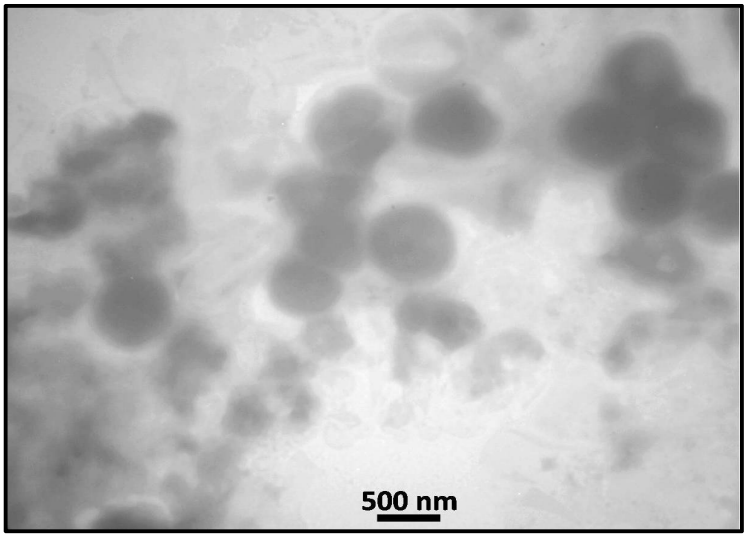
The AFM micrograph acquired for the **Zinc** NPs Figure (7) shows the surface roughness alterations and the surface roughness change [root mean square (Rp)] values were recognized. For the sample the roughness value was ٤٢ nm and the section analysis of the sample's grain size value was 20 nm.



Figure(7): AFM for Chitosan/Zinc NPs nanoparticles

Transmission Electron Microscope (TEM Analysis):

(TEM) imaging, the Zn NPs suspensions according (15).



Figure(8): TEM for ZnO NPs

Antibacterial Activity :

About antibacterial activities of *A. vera* gel showed MDR strains except five of them were inhibited by *A.vera* gel extractat.

Zn NPs and Aloe vera Gel Assay

The well diffusion agar method (WDA) was used to detection *S. aureus* to word Zn NPs at concentration 128 µg\ ml and Aloe vera Gel. The result was recorded below in Table (1) The inhibition zone size reached (11 and) mm recpectively while (16) proved the activity of ZnO NPs with acetic acid on Staph. aureus in mutton meat.

Table(1): Inhibition zone well diffusion agar method.

Inhibition zone (mm)	**ZnNPs at conc(128 µg\ ml)**	**Aloe vera Gel**
	15 ± 1.06	7 ± 0.33
LSD value		
*Each value is the mean of three replicate (mean ± SE) values with difference letter have significant different * (P<0.05) .		

Histopathology: Skin section from negative control group showed normal architecture (Figure 9)

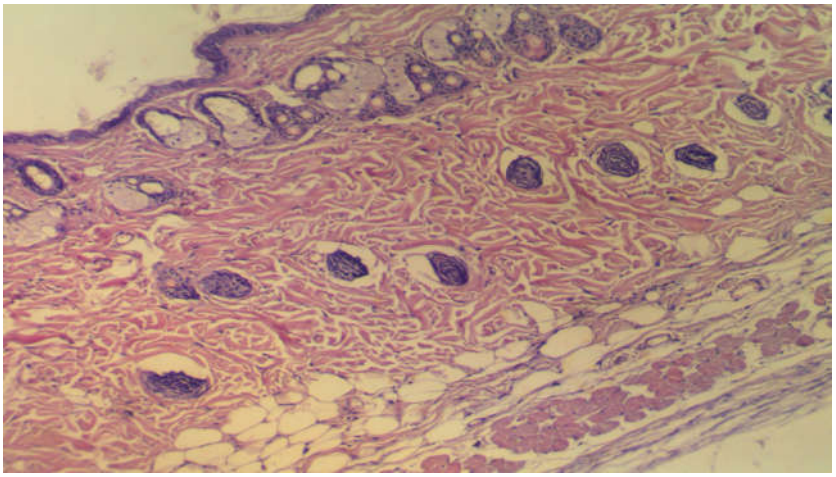


Figure (9): Histological section from negative control group showed normal skin histology (H&E stain; 200×)

In the first group –positive control group (wound only), at 7 day post injury the skin showed incomplete regeneration of the epithelial cells of the epidermis which extended under the necrotic debris, while the dermis showed mild infiltration of MNCs (Figure 10) .

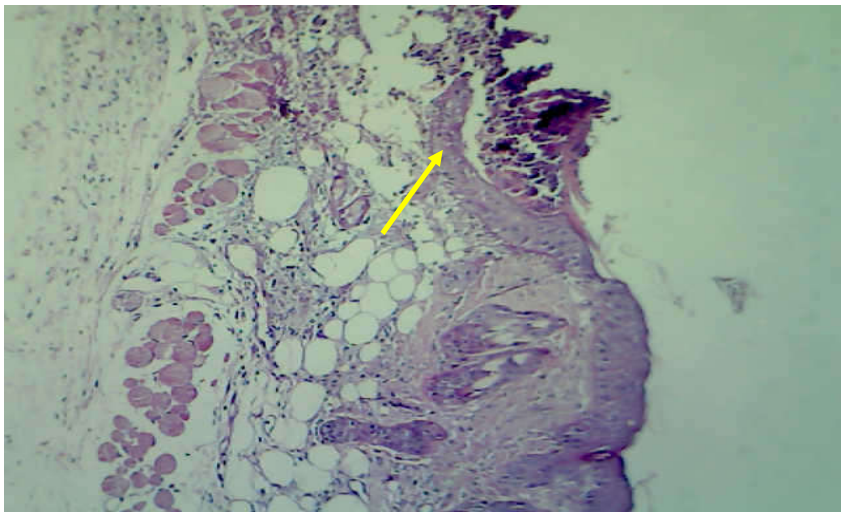


Figure 10: Histopathological section (positive control group), 7 days post injury showed incomplete regeneration of the epidermal epithelia (arrow) under the necrotic tissue and proliferation of MNCs in the dermis (H&E stain; 200×)

The Histopathological changes in the third group (infected group) characterized by present of necrotic tissue in the injured site with neutrophils infiltration extended to the dermal layer (Figure 11).

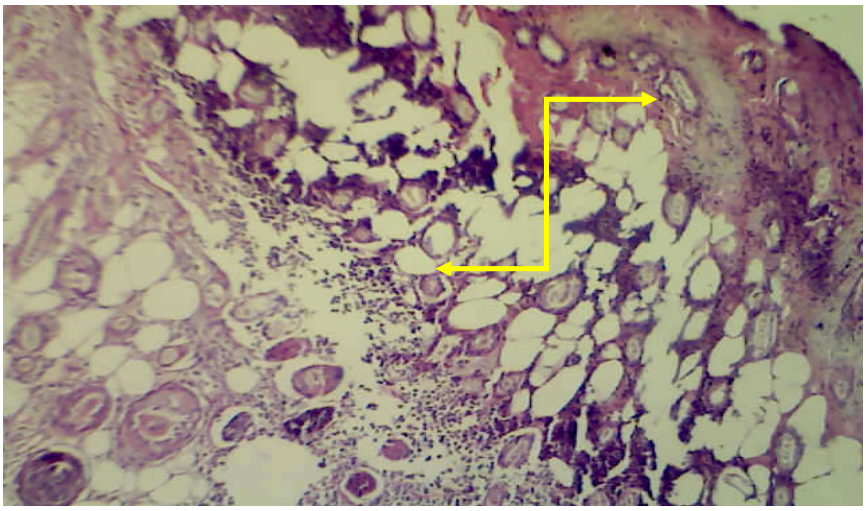


Figure 11: Histopathological section (infected group), 7 days post injury showed necrotic tissue (arrow) in the injured site with neutrophils infiltration extended to the dermal layer (H&E stain; 200×)

The histopathological section in the 4th group (treated with ZnONPs) showed incomplete regeneration of the epithelial layer under the necrotic tissue (Figure 12) and mild infiltration of inflammatory cells mainly neutrophils and MNCs in the dermal layer.

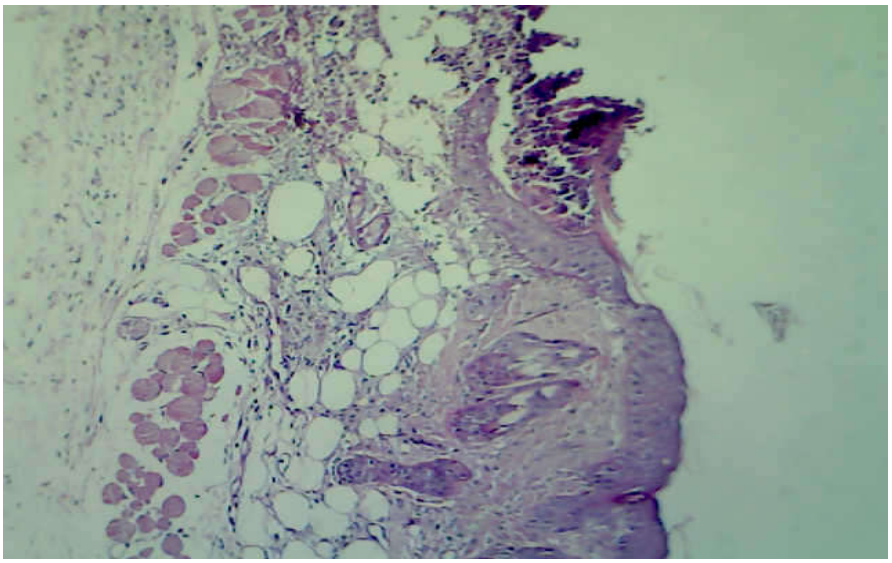


Figure 12: Histopathological section (ZnONPs group), 7 days post injury showed incomplete regeneration of the epithelial layer under the necrotic tissue (H&E stain; 200×)

In the 5th group (treated with Aloe Vera Gel), 7 day post injury the skin showed complete regeneration of the dermal epithelia and infiltration of inflammatory cells mainly neutrophils in the dermis and hypodermis (Figure 13).

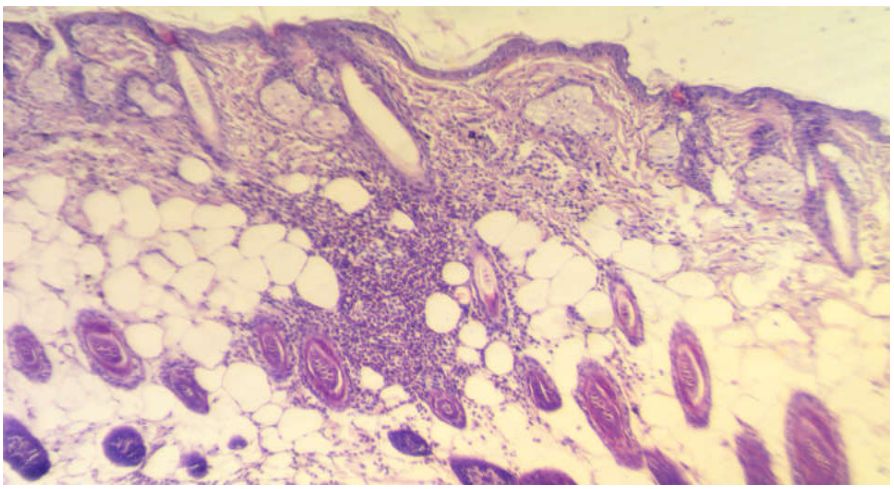


Figure 13: Histopathological section in skin (Aloe Vera group), 7 days post injury showed complete regeneration of the dermal epithelia and infiltration of inflammatory cells mainly neutrophils in the dermis and hypodermis (H&E stain; 200×).

In the third group (infected with MRSA), the severe tissue damage and failure of skin healing can be attributed to the pathogenicity of MRSA which characterized by its resistance and the ability to evade the immune system in addition to their important such as (toxins, enzymes, adhesion proteins, and others) (17) for example that *S. aureus* strain express PVL protein caused necrotizing and the current result may same idea to explain the acute necrosis in the skin, also the infiltration of neutrophils in epidermis and subcutaneous tissue, also an effective immune response against *S. aureus* in and during the first 24 hr, neutrophils recruitment to the site of infection is required for skin wounds of mice inoculated with MRSA developed neutrophilic abscesses (18). The 5th group which treated with nanoparticles showed a good healing and this may contributed to the antibacterial properties of the ZNO since nano-sized ZnO is safe and does not irritate skin (19)

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