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ANTIMICROBIAL AND HISTOLOGICAL EFFECTS OF NANO-NEOMYCIN SOLUTION AGAINST DIFFERENT MICROBIAL POPULATION

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Abstract

In the present study, pomegranate nanoparticles solution was synthesized by Sol gel method. Which show hexagonal morphology with size average (50±10) nm scanned by (SEM) and (TEM). The synergestic solution of (Neomycin and Nano-Pomegranate extract) scanned by Atomic Force Microscopy (AFM) analysis provided the information about the distribution of particles in new solution. Beside the phenolic compounds, flavonoids and tannin played crucial role in the significant antibacterial activity and the synergestic solution observed in F1 and F2 at range (9.5-12.5) mm against Staphylococcus aurous, E.
coli between (5-11.5) mm, Bacillus sp. (3.9-5.5); Proteus vulgaris (4.1-5.1) mm; Salmonella typhimurium at ranges (5.8-6.5) mm, klebsiella pneumonia (3.8-4.8) mm and listeria monocytogenes (3.9-5.6) mm. the Synergistic effect of the solution increased the efficacy of their work compared to used them individually, this was shown through increasing the inhibition zone. These results were demonstrated through histopathological examination to organs examined, that identical to normal when given different concentrations of these solutions, and proof of their non-toxicity. Overall, the results elucidated a rapid, cost-effective, environmentally friendly and convenient method for synthesis, which could be used as a potential antimicrobial agent against multidrug-resistant bacteria.

Keywords: *Nano Extract, Antibacterial Effect, Nano Technical Test.*

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Introduction

In the last years, antibiotics resistance increased rapidly due to excessive use of different antibiotic with high environmental pollution as a serious of public health (1, 2). *S. aureus* has a big role in the epidemiology and pathogenesis of different maladies (3, 4). as a result, the emergence of resistant *S. aureus* strains, especially methicillin-resistant *Staphylococcus aureus* (MRSA) at rate 94.3% ; which observed may differences depend on geographic area, sources of clinical specimens, and the collection site of isolates (5,6). The frequent use of antibiotics in the treatment of diseases has several adverse impacts, including the introduction of antibiotic residues into the human food chain and the spread of antibiotic-resistant bacteria strains resulting in poor rate of diseases healing (7, 8). higher and abuse of antibiotics not only effect on targeted therapy but also prevent rapid recovery of diseases and promote growth, moreover, using animal feces containing bacteria or antibiotic as natural fertilizers which increase the spread of bacterial population (9). MRSA in food animals has the potential to cause animal illnesses, also a zoonotic concern in both animals and humans through direct contact, pollution of environment and products of animal, and so on (7). The presence of tetracycline in poultry foods was closely related to outbreaks of resistant staphylococci in the environment. Furthermore, Enterobacteriaceae group especially *E. coli* showed highest rates of antibiotic-resistant represented by ampicillin (98%), amoxicillin (80%) and gentamicin (5%) respectively (10,11). Numerous dietary additives and Antimicrobial substances have been related to health concerns. Therefore, the usage of natural, alternative substances is a significant topic (12). Bacteria resistant to antibiotics remedies is increasing therefor, nanotechnology offers a chance to overcome this issue (13). Metal NPs, also known as nanobiotics, have been proposed as potential antibacterial agents. Possibility of using nanoparticles against continuous microorganism's resistance and multidrug-resistant extremely interested (14, 15). The metals used for these NPs are almost exclusively heavy metals, like gold, zinc or Nano extract, and is based on low price, efficient, non-poisonous and ecofriendly method for green synthesis. Mechanisms of nanoparticle depend mainly on their type, shape, and size. Alternative, natural plant products in Nano form alone or conjugated with antibiotic are effective against a vast number of microorganisms (14, 16, 17). NPs have the ability to penetrate the bacterial cell wall and damage their cellular membrane or binding with receptors on cell surfaces thus, increases their effectiveness to inhibiting bacterial resistance (18). The aim of this study was to outline the inhibitory effect of Nano pomegranate extract made by sol gel method against different bacteria and discuss new strategy for overcoming multidrug resistance (MDR), through conjugating Nano extract with antibiotic and scanning the particles of new solution by electron microscopy (SEM and TEM) secondly.

Materials and Methods

Preparation of Nano pomegranate extract:

100 g of pomegranate peels powder dissolved with 100 ml of ethanol alcohol stirred using Ultrasonic Probe Sonicator type (USA) with 10 ml of concentrated acetic acid is added and 100 ml of distilled water in 80 degrees Celsius for 4 hours for 5 days until the color of the solution changes as evidence of hydrolysis and the beginning of the Nano phase (19).

Preparation of synergism solution:

2.5 gm. of Neomycin sulfate dissolved in 50 ml of Nano pomegranate extract with complete volume to 100 ml by distilled water separately and mixing in Ultrasonic machine for 15 min. with 15 C° to yield new solution.

Scanning of Nanoparticles Pomegranate Solution by TEM, SEM

The determined the character of particles of solution analysis by electron microscope with a magnification power up to X2000.

Scanning by probe microscope (SPM or AFM)

Study the surface of Nano scales of new solution obtained from synergism by microscope probe and collect data obtained as 2 or 3 dimensional grid to generate an image of whole surface.

Inhibitory activity

The antibacterial assays done using agar diffusion method with Muller Hinton Agar against different bacterial strains. The antibacterial activity was determined by measuring the zone of inhibition around each well (4).

Experimental Design

The study was achieved using (20) Albino mice (weighted 22- 25g) at 10 weeks age, housed in polycarbonate cages an ambient temperature of 27±2°C with 12h-light and 12h-dark cycle. Animals were obtained from animal house of Alrazi-center and given food and water ad libitum. The experiments on animals were performed based on the guide lines of the institutional animal ethics committee. In this study, 20 mice were randomly divided into 2 groups (ten in each group). The experimental group designed to treat orally with Nano-Neomycin for 1month by gastric lavage. The control group treated with Distilled water.

Histopathology

After the treatment days, the histological analysis was performed by examining the morphological changes induced by Nano-drug, over the liver, kidney, testis and heart. These organs were collected and fixed with 10% formalin, embedded in paraffin, and cut into 5-µm thick sections. The fixed sections were stained for analysis using hematoxylin and eosin (H and E) staining. The sections were examined under binocular microscope (Olympus CH-2,

Tokyo, Japan), and photomicrographs of the fixed organs were obtained.

Results

The shapes of the Nano particles of pomegranate solution were investigated using scanning electron microscopy (SEM). The morphology of NPs is mostly spherical/ agglomerated in shape, and typical SEM images are depicted in Fig. 1 and average size of pomegranate NPs is ~64.5 nm. Transmission electron microscopy images provided exact.

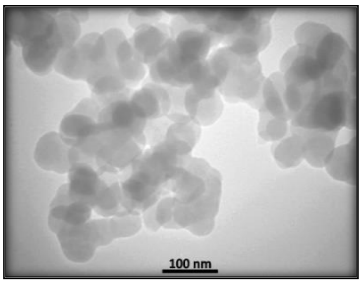

Figure (1) Image of Nano pomegranate extracts solution scanned by tonal electronic microscope (TEM)

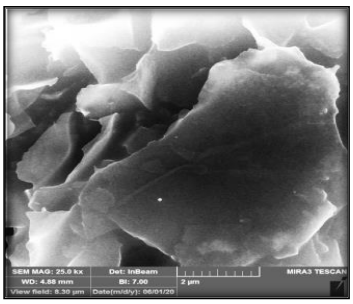

Figure (2) Image of Nano pomegranate extracts solution scanned by electron microscope (SEM)

Morphology and size of the synthesized NPs at different magnification as in Fig. 2 which seen hexagonal Quartzite shapes were observed with an average diameter ranging from 50 to 75 nm. Atomic force microscopy (AFM) commonly used to determine of the size of particles of new solution (Nano pomegranate extract & Neomycin) was 403.6 nm with threshold 2576 nm in 300 number of new particles, coverage area 47.14% and density 750000 particles/mm3 beside the 3D view of the sample surface over a 2 x 2 mm scan show uniform height distribution as in Fig. 3 (20).

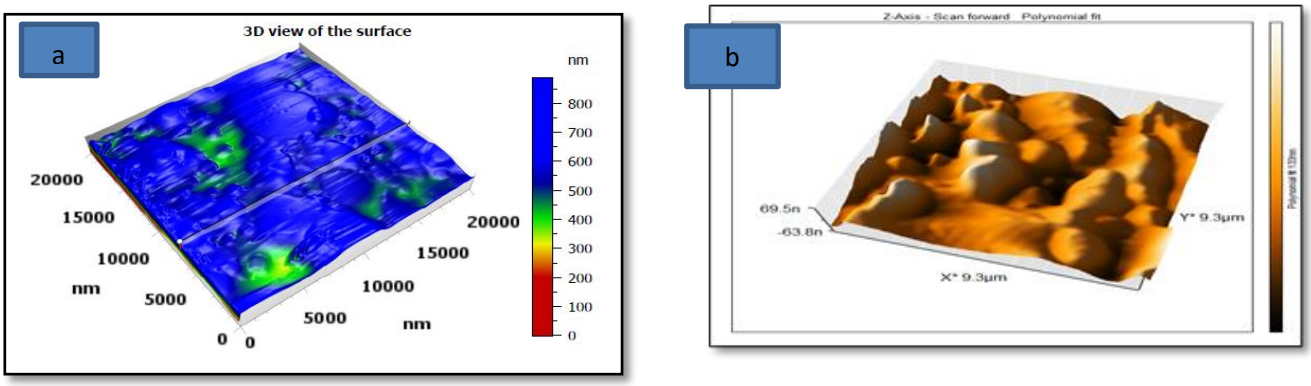

Figure (3) Images (a & b) of Nano- Neomycin solution scanned with AFM & Particle size - Threshold detection.

The present study represented that size of particles has greater efficacy on that inhibition the growth of bacteria and increased solubility with well accumulation on the cell surface of bacteria lead to better antimicrobial activity (21).

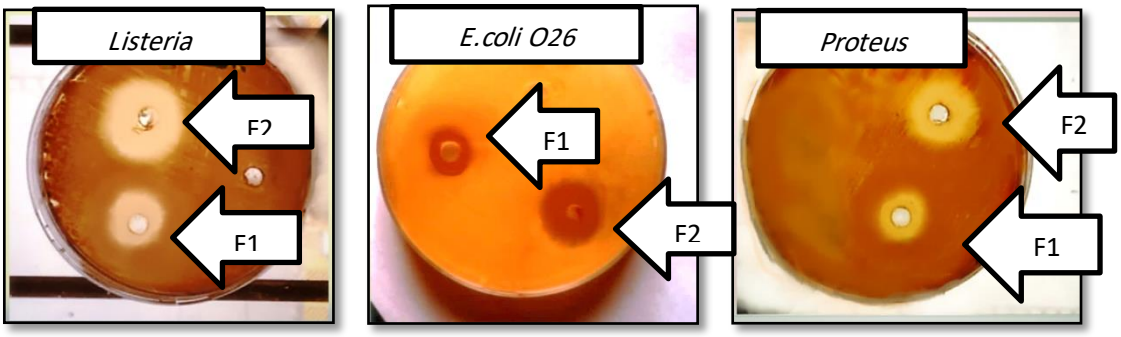

Figure (4) Inhibition zone of Nano pomegranate extracts alone (F1) & Nano pomegranate conjugated with neomycin 1% extract (F2) against different bacterial sp.

Table (1) Inhibition zone of (Nano pomegranate & Neomycin) solution against different bacteria.

No.	Type of bacteria	Inhibition zone of Nano pomegranate extract	Inhibition zone of (new solutionF1) Nano pomegranate & neomycin (1%)	Inhibition zone of (new solutionF2) Nano pomegranate & neomycin (2.5%)
	Staphylococcus aurous	7.5	9.5	12.5***
	E.coli	6.5	8.5	11.5***
	E.coli O_{126}	3. 5	4.0	5. 5
	E.coli $O_{157}H_7$	4. 5	5. 0	6. 5
	Bacillus sp.	3.5	3.9	5.5
	Klebsiella pneumonia	3.4	3.8	4.8
	Listeria monocytogenes	3.6	3.9	5.6
	Salmonella typhimurium	5.3	5.8	6.5
	Proteus vulgaris	3.8	4.1	5.1

The recent study shows inhibition zone of Nano pomegranate extract according to *Staphylococcus aurous* 7.5 mm, *Escherichia coli* 6.5 mm, *E.coli* O_{26} 3.5 mm, *E.coli* $O_{157}H_7$ 4.5 mm, *Bacillus sp.*3.5 mm, *Proteus vulgaris* 3.8 mm, *Salmonella* 5.3 mm, *klebsiella* 3.4 mm and *listeria* 3.6. While the new solution in F1 and F2 range (9.5-12.5) mm on *Staphylococcus aurous, E. coli* between 8.5-11.5 mm, *Bacillus sp.* between 3.9-5.5; *Proteus vulgaris* ranges

4.1-5.1mm; *Salmonella* 5.8-6.5 mm, *klebsiella* 3.8-4.8 mm and *listeria* 3.9-5.6 mm Table (1). The synergism solution composed of Nano-pomegranate with antibiotic like Neomycin sulfate show potentially antibacterial activity (22). Due to highly efficiency, the ability to penetrate the tissues of different organs made as a good drug highly stabile with perfect effect to different type of bacteria. Beside Pomegranate consider rich source of polyphenols, flavonoids and tannin compounds have potent antioxidant properties used to treat diarrhea and dysentery in small animals (1, 2, 8). The combination of the Nano-pomegranate with antibiotic solution gave a high efficacy to inhibition the bacteria compared when both substances were employed separately showed in Fig. (F1 & F2) and (Table 1). The maximum zone of inhibition was obtained against Gram-positive and negative bacteria chiefly in F2 of *E. coli* 12.5 mm and *S. aurous* 11.5 mm. Synergistic effect of Nano-pomegranate and Neomycin displayed huge effectiveness against the pathogens as a combinatorial therapy. The direct interaction of Nano particles to the bacterial cell surface, changes the permeability of cell membrane, and facilitate entry inside the cell accelerate of induce oxidative stress that inhibition of bacterial growth, finally their death (23). Nowadays synergistic study becomes a very important tool to increase the antibacterial activity of current antibiotics through available natural materials against a number of dreadful multi-drug resistant bacteria (24).

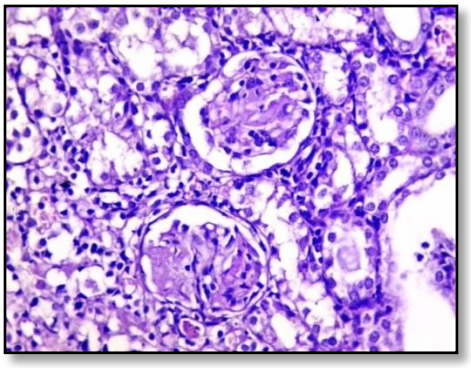

Fig. 5: Histopathological section of kidney of mice mice
doses orally for 1 month with Nano drug showing showing
normal histological structure of renal tubules (x40).

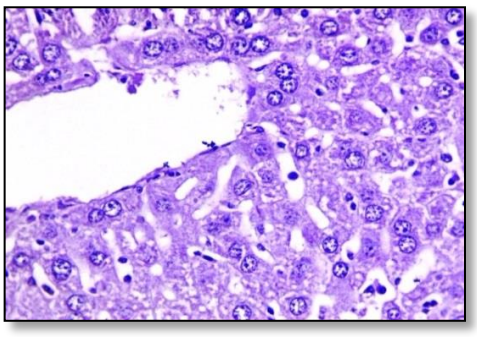

Fig. 6: Histopathological section of liver of
doses orally for 1 month with Nano drug
Normal structure of hepatic cells (x40).

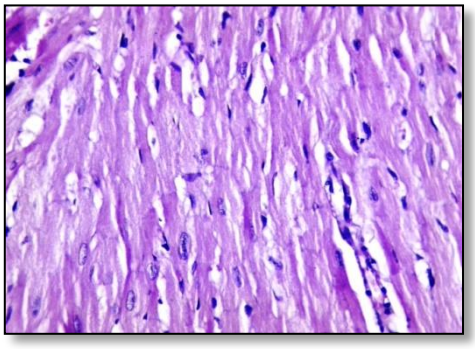

Fig. 7: Histopathological section of heart of mice mice doses orally for 1 month with Nano drug showing showing normal histological structure appearance of cardiac (x40). muscles fibers (x40).

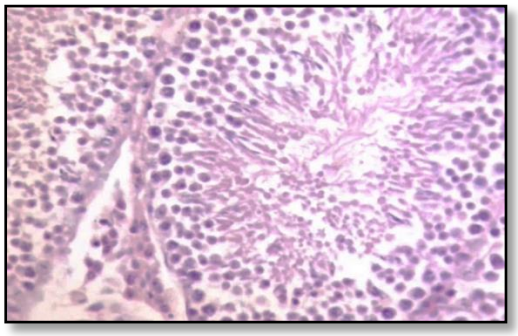

Fig. 8: Histopathological section of testis of doses orally for 1 month with Nano drug normal development and maturation of spermatogoni cells with presence of sperms

Histological results showed that liver tissue is still similar to normal structures, also other sections like kidney, testis and heart disappeared any structural changes. This gives indicators that prepared Nano solution is safety and gave amazing results due to highly antioxidants of pomegranate employed.

Conclusion

In the present study, Nano-pomegranate solution synthesized by using the green approach sol gel method was used for the synthesis process. The synthesized Nano-pomegranate extract solution have shown antibacterial efficacy against different bacteria. While the combination of the Nano-pomegranate with antibiotic solution showed a vast efficacy to inhibition the bacteria compared when both substances were employed separately. Therefore, the synergistic solution may be used as chemotherapeutic or preventive agents against the pathogens, also employed as beneficial formulation of antibacterial products in pharmaceutical industries, in addition for preparing Nano-medical therapy in future.

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