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Original Research Article

Preparation Pharmaceutical Formulation of Sulphathiazole Nanoparticles in a Spray form for Treatment Fungal and Acterial Skin Infections

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Abstract: The Objective of this research to convert sulfamethoxazole to nano-sulfamethoxazole by Sol-gel method and prepare a pharmaceutical formulation of sulfamethaxazole, prepared at a concentration of 0.25% and in the form of a spray to treat fungal and bacterial infections resulting from wounds of skin, its good for heath. The nano particles characterized by powder X-ray Diffraction (PXRD), atomic force microscope (AFM), Scanning electron microscopy (SEM). The results showed successful that nano sphere sulfamethoxazole and it size between (37-55) nm, all these tests showed different type of nanostructures such as nanotubes and nanoparticles, other biological test MIC & MBC show superior biological activity. The stability of the prepared formula was studied for 6 months under temperature conditions (30, 40) degrees Celsius and relative humidity (70, 75) controlled, which showed the stability and stability of the composition. An experiment was also conducted on laboratory animals (mice) by making a wound in them and treating with the prepared formula with a comparison with the normal non-nanostructure.

Keywords: Nanosulphathiazole, Health, AFM, XRD, SEM, TEM, Bacteriological Test, Spray, Wounds, Bacterial Activity.

INTRODUCTION

Nanomaterials have received wide attention in the world due to the unique properties of nanoparticles, which do not exceed one hundred nanometers [1]. The term nanotechnology has made a huge leap in all branches of science, including medical, petrochemical, biological, and others [3, 2]. Nanoparticles are defined as an atomic or molecular grouping ranging in number from a few atoms (or molecules) to a million atoms, with a spherical shape, with a radius less than 100 nm. A nanoparticle with a radius of one nanometer will have twenty-five atoms, most of which are on the surface of the particle. For example, copper nanoparticles with a size of less than 50 nanometers are considered to have high hardness and are not malleable or draggable.

Another property of nanoparticles is the possibility of hanging them inside a liquid or solution without floating or submerging because the interaction between the surface of the particle and the liquid is strong enough to overcome on the density difference between them. Nanocomposites resulting from the addition of nanoparticles to other ordinary materials are known to manufacture new materials, resulting in a significant improvement in the properties of those materials, and now known nanocomposites are nanocomposites [4].

Sulfamethoxazole is an antibiotic derived from sulfonamide (antibacterial), the chemical composition is 4-Amino-N- (5-methylisoxazol-3-yl) benzene sulfonamide. It is slightly soluble in water, freely in acetone, sparingly in 96% ethanol and dissolves in dilute solutions of sodium hydroxide and in dilute acids [5]. It inhibits the activity and reproduction of bacterial cells by inhibiting the manufacture of certain enzymes needed by bacteria in their growth and activity and works on a wide range of gram-negative bacteria, and some gram-positive bacteria. Such as E. coli, Klebsiella, Enterobacter sp., (Morganella morganii), (Proteus mirabilis), Proteus vulgaris and others Sulfamethoxazole works synergistically with Trimethoprimto increase the effect of sulfamethoxazole on various bacteria [6].

The aim of this research is to convert sulfamethoxazole into nano-sulfamethoxazole using the sol-gel method and to formulate it into a pharmaceutical preparation. The formulation, prepared at a concentration of 0.25% in the form of a spray, is intended for the treatment of fungal and bacterial infections resulting from skin wounds, offering health benefits.

MATERIAL AND METHODS

Preparation of Sulfamethaxazole Nanoparticles

The nanoparticles were prepared using the method of gel solutions (Sol gel), which is the simplest method by reducing the size of the particles using materials such as acetic acid, distilled water and ethanol (as a catalyst) at a temperature below 80 ° C using the Probe Sonicator as a catalyst and a hydrolysis agent that converts the material in the final stage. With the increasing temperature of the solution to a white cloud with a concentration of 1% of sulfamethaxazole as shown in Figure (1).

Bioefficacy Examination

Sulfamethaxazole is an antibacterial on several types of Gram-positive and negative bacteria and four types of them were used in the examination: *E coli, Staph.aureus, Candida albicans Pseudo. aeruginosa* by adopting the method of propagation by acre using drilling, as shown in Table 1 and by taking a quantity of bacteria growing in solid medium for 24 hours and diluting it with sterile distilled water, which is equivalent to 108×1.5 Cell/ml, the pits were filled with concentrations and the dishes were incubated aerobically at a temperature of (37ºC) for 24 hours, inhibition areas were observed around the pits [8].

Minimum Inhibition Concentration (MIC) and Minimum Bacteriostatic Concentration (MBC)

The method adopted the use of a group of double concentrations (mg/ml) after mixing each concentration of the material with the dissolved medium, the dishes were left to harden and then inoculated with bacteria by diffusion method from active cultures planted on the solid Müller medium and the lowest inhibitory concentration was determined from the absence of a halo around the saturated tablet, dishes containing the medium without sulfa were used as control, the concentration that inhibited the apparent growth of bacteria in the dishes was recorded and the lowest inhibitory concentration was selected, and the lowest lethal concentration was selected by replanting tubes that did not They show weak growth or growth by withdrawing 1.0 ml of stuck and spreading it on the surface of the dish and incubating at (37ºC) for 24 hours, after which the dishes that did not show growth were recorded [9].

Characterization of Iron Nanoparticles: Generally, the synthesized nanoparticles were characterized using several instruments such as:

Scanning Electron Microscopy

The scanning electron microscopy (SEM) analysis is utilized to visualize the dimensions, structure, form, and underlying arrangement of aggregated nanoparticles (NPs) by using high-energy electrons to generate a variety of signals on the outermost surface regions of the NPs. The electron-test association indications provided information regarding the surface characteristics, composition, and structure of the materials used in the photographs (Dubey *et al*., 2010).

X-ray Diffraction

X-ray diffraction (XRD) is a characterization technique that offers insights into the crystallographic structure and physical properties of materials. When a single-colored beam of light hits a material, it interacts with the atoms and causes the X-rays to scatter from the atoms in the target material (Ali *et al*., 2020). Hence, XRD analysis yields data on the crystalline arrangement, phase characteristics, lattice parameters, and grain size of a material. The latter parameter is determined by applying the Scherrer equation, which utilizes the widening of the most prominent peak observed in an XRD measurement for a particular sample (Ortiz *et al*., 2004).

Atomic Force Microscope (AFM)

The surface properties, dimensions, and size of the produced FeO nanoparticles were examined using the Angstrom Advanced AA2000 AFM scan, an instrument headquartered in the United States. The AFM pattern was obtained from the contact pattern seen under standard meteorological conditions. A small portion of the sample solution was placed onto a glass slide measuring 1 × 1 cm and allowed to dry before testing (Binnig *et al*., 1986).

Preparation of Formulation of Sulfamethaxazole Nanoscale 0.25%

The composition was prepared through the introduction of carrier, complementary and inert materials that have no effect on the active substance, in addition to being a preservative, which gives the composition a high stability of the active substance without changing the nanomaterial. The shape of the nanomaterial of the active material is distributed between the solutions with the crystalline shape of the nanomaterial because the nanomaterial is unstable in the nanoform transformed into different nanoforms (tubular, spherical) as shown in the image (C-2), and the preparation of the composition is as follows:

Heating the nanomixture at a temperature of 60 degrees Celsius to make the mixture homogeneous and the concentration equal (1%), then A volume of 25 ml, equivalent to 0.25 grams, is withdrawn- Supplement the volume to 100 ml with propylene glycol so that the concentration prepared as a formula is 0.25%.

Automated Analysis

The chemical examination was conducted using HPLC technology according to controlled conditions and according to the following.

Composition: - Each 100 ml has
0.25 gm Sulphathiazole Nano -

Devices Used: - HPLC, Balance, ultra sonic.
Glassware: - Beaker, Volumetric flask, pipit.

– 0.1% Formic acid (85%)

Method of analysis:
Column: 18
Wavelength: 254 nm
Flow rate: 1ml/min
Mobile phase: Acetonitrile - 0.1 % Formic acid. (40% - 60%)
R. Time: Sulpha. 5.1 min

Stander Prepares

– Sulpha 0.01 gm dilutes to 20 ml with Mobile phase.
– Tests prepare: 4 ml dilute to 25 ml with Mobile phase.

Nanomaterial concentration

Area	RT	active ingredient	No.
23205996	5.1 min	sulphathiazolest	1
Area	RT	active ingredient	No.

$$Assay = \frac{Test}{St.} X\ 100$$

$$Assay = \frac{23710605}{23205996} X\ 100 = 102.1\ \%$$

Examination of the Biological Efficacy of the Prepared Composition

The biological examination of the prepared composition was conducted on bacteria and fungi and according to the results obtained, which showed its high effectiveness towards them.

Sterility Examination

The sterility test of the biologically prepared composition was conducted according to the following steps

– Preparation of the medium (Mueller Hinton agar)

Weighing 9.5g of agar and then dissolved with 250ml of distilled water after dissolving it well, the medium is sterilized with autoclave at a temperature of 121ºC and a pressure of 1.5 bar leave the medium to cool and pour into dishes (petri dish).

Take 0.01g equivalent of the active substance and dissolve in 10 ml of distilled water after sterilization with (Balautocliff) and then take B lup and brush on the agricultural dish and placed in the incubator for 24 h at a temperature of 37 ºC also put another dish of the medium (Blanc), and read and results on the second day.

The presence of contamination through the culture media is figured out in case of any growth of bacteria or fungi if there is evidence ofcontamination andthe absence of evidence of the absence of contamination. Sterility test proves that there is no contamination of the composition.

Examination of the Substance by Creating a Wound in Laboratory Mice

The effectiveness of the sulfa nanospray was examined and compared to the standard material in the treatment of open wound in laboratory animals (white mice), the duration of the effectiveness examination lasted 3 weeks, starting with the stage of preparing laboratory animals and making the wound and then leaving the open wound for a period of time until the redness of the area is noticed as an indication of acute inflammation and then the wound was treated The preparation until the state of healing and the return of the skin of the mouse to the normal situation with attention to a number of observations and put the mice in cages with the provision of appropriate conditions and ensure that there is no injury or symptom prevents the examination process, shaved the dorsal area of the mice and the wound was caused by a surgical scalpel treatment of mice with the preparation separately daily and recording observations that occur during the treatment process while checking the behavioral changes shown by the animal during it. Toxicity test Preparation of the concentration of the standard substance Weighed 0.125gm of standard sulfamethoxazole and diluted 100ml of distilled water, weighed 00.25gm of standard 100ml distilled water, weighed 0.5gm of standard sulfamethoxazole and diluted 100ml distilled water and given to white mice orally for two weeks and saw clinical signs daily.

Preparation of the concentration of nanomaterials by taking 12.5ml of nanosolution and adding 100ml of distilled water, 25ml of nano solution add 100ml distilled water, 50ml of nano solution add 100ml distilled water and the mice are given orally for two weeks and see clinical signs daily.

Laboratory Animals

In this experiment, thirty-five white mice were used, each group consists of (5) mice, with weights ranging between 25-30 gm, and each group is placed in a single cage with the provision of proper conditions. All groups are dosed with standard sulfamethaxazole and nanoscale in one dose per day for two weeks in different concentrations and by 0.1ml / 10gm of body weight for mice and the groups were divided according to the following Mambin:
The First Group: consists of five white mice, the control group was given only distilled water.
The Second Group: divided into three small groups as shown:

A group of five white rats, dosed with standard sulfa at a concentration of 0.125gm/100ml distilled water and given orally once for two weeks.

A group of five white rats, dosed with the standard sulfa at a concentration of 0.25gm/100ml of distilled water and given orally once for two weeks.

A group of five white rats, dosed with the standard sulfa at a concentration of 0.5gm/100ml distilled water and given orally once for two weeks.

The Third Group was divided into Three Small Groups as Shown:

A group of five white mice dosed with sulfa nanoparticles at a concentration of 12.5ml/100ml distilled water, equivalent to the standard material, and given orally once for two weeks.

A group of five white mice, dosed with sulfa nanomaterial at a concentration of 25ml/100ml of distilled water, equivalent to the standard material, and given orally once for two weeks.

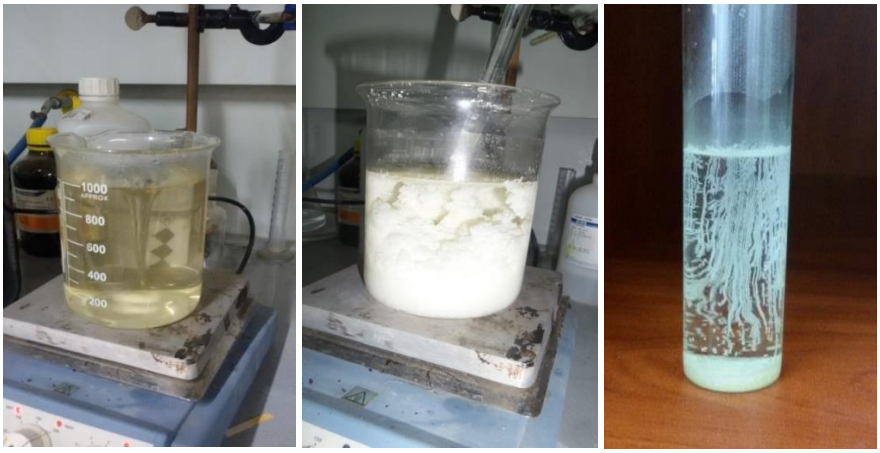
A group of five white mice, dosed with sulfa nanomaterial at a concentration of 50/100 ml, equivalent to the standard material, distilled water and given orally once for two weeks.

Changes in the Weights of White Mice after Dosing

After the end of the examination, clinical signs and side effects resulting from dosing are recorded, as are changes in the weights of mice, which are important signs of acute toxicity screening.

RESULTS AND DISCUSSION

By sol gelsulfamethazole particles, which took the form of a white cloud with low temperature of the solution, as in Figure 1.



A B C

Figure 1: Showing the shape of the sulfamethaxazole solution with temperatures, (A) the transformation of color to transparent crystal with elevated temperature, (B) the appearance of the white cloud with hypothermia (C) the formation of nanocrystals

Characterization of Nanoparticles

Examination of the atomic force microscope AFM

The examination shows the regularity of the grain size of the nanomaterial prepared in the form of monolithic tubes, and to prove this, a test (TEM) was performed and that the average grain size of the material is 60.05 nm shown in Table (1) and Figure (2).

Table 1: Shows the diameter of sulfa nanoparticles according to the solution volumes evaluated with SPM

Avg. Diameter:60.05 nm	<=10% Diameter:40.00 nm
<=50% Diameter:60.00 nm	<=90% Diameter:75.00 nm

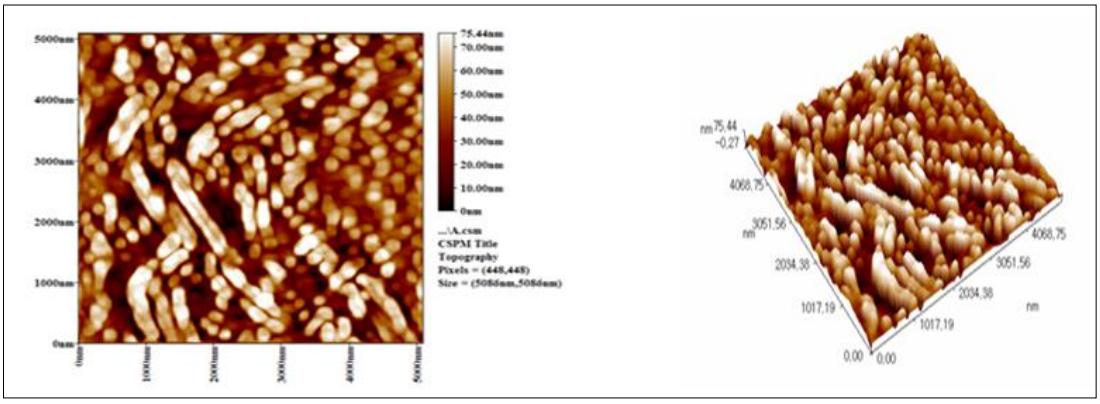


Figure 2: AFM scan shows the shape of sulfamethaxazole nanoparticles and their sizes ranging from up to 75 nm

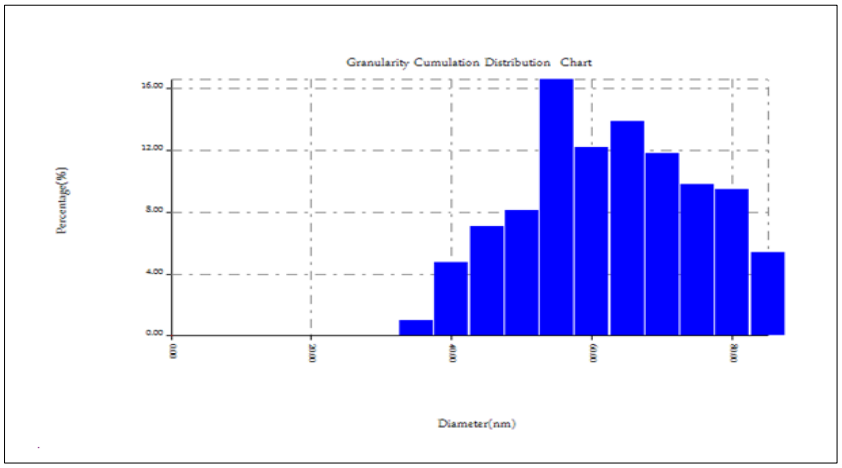


Figure 3: Showing the particle distribution diagram of sulfamethoxazole nanomaterial

X-Ray Diffraction Test

X-ray diffraction patterns of samples examined and obtained using a device

Shimadzu XRD-2700SS, with wavelength, voltage and current, 15406nm (40kV), (30mA) respectively and with a scan step (0.2º) and Figure (5.4) shows the X-ray diffraction patterns of the prepared samples and the crystal size of the nanomaterial (12) nm according to the spark equation.

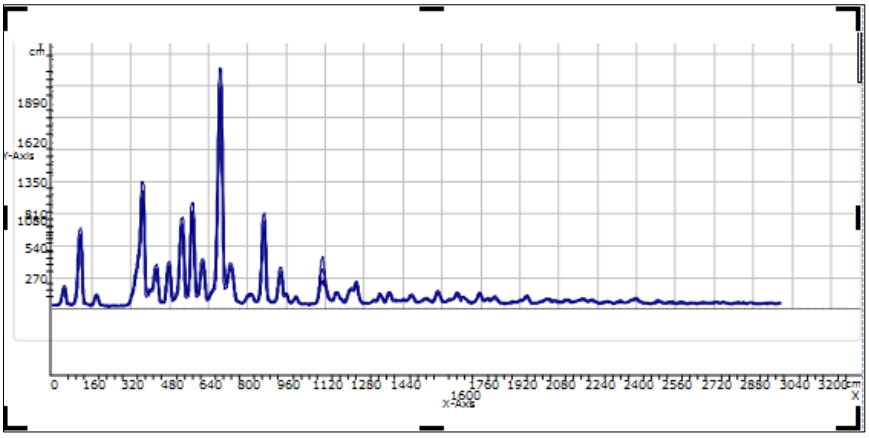


Figure 4: X-ray diffraction (XRD) of sulfamethaxazole

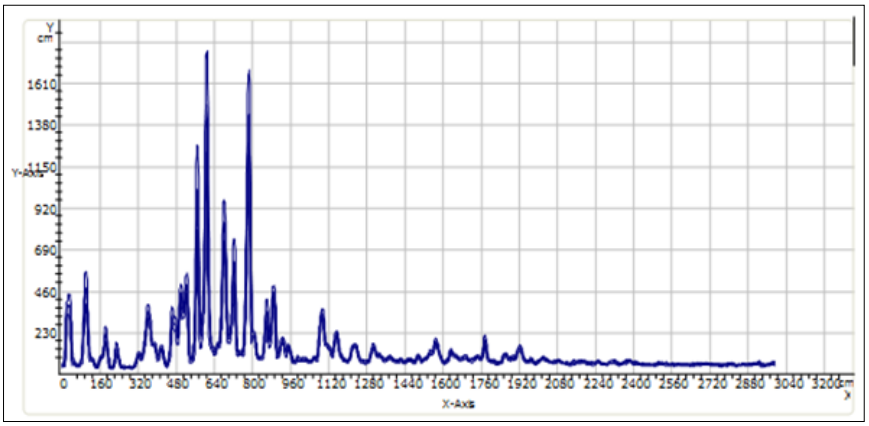


Figure 5: X-ray diffraction (XRD) for sulfamethaxazole nanomaterial

SEM Scanning Electron Microscopy

Scanning electron microscopy is used to view nanostructures and examine the morphology of the surfaces of samples. The samples were examined with Scanning Electron Microscope (Inspect) type S50 with a magnification power of X2000.It can be seen from Figure (6) that the grain size ranges between (37 and 50) nm.

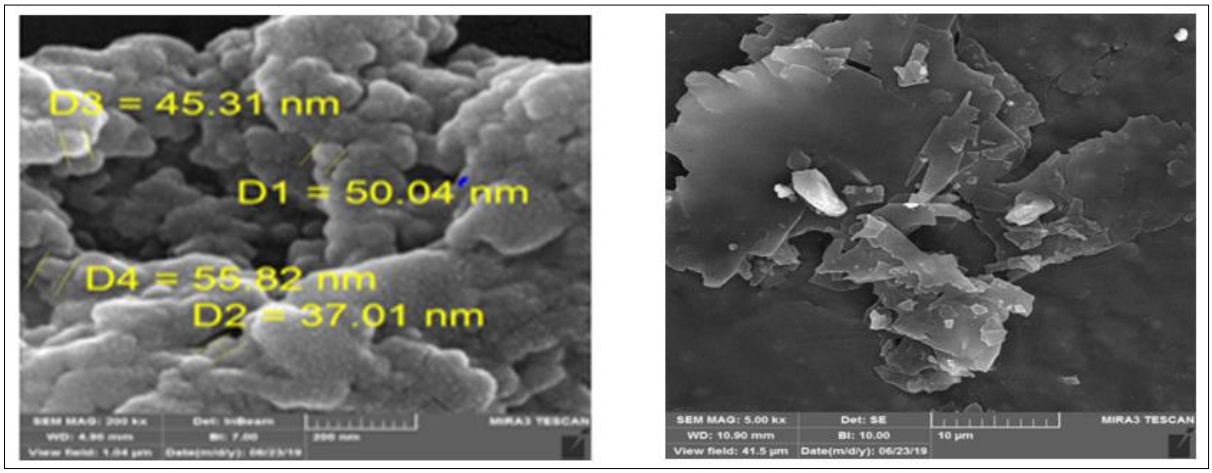


Figure 6: Shows sulfamethoxazole nanoparticles prepared by Sol Gel method and morphological size by scanning electron microscopy

Bacterial Examination

Bacterial testing of sulfamethaxazole nanomaterial was performed using several types of Cram-positive and negative bacteria as shown in Table 3, Figure 8.

Table 3: Shows the bacterial activity of sulfamethoxazole nanomaterial using several types of bacteria

E. coli ATCC 10536	St. aureus ATCC 6538	Pseudomonas ATCC 15442	Candida albicans ATCC 10231	prototype
55	60	62	25	Standard sulfamethaxazole
56	52	62	30	Sulfampethexazole Nanomaterial

Figure 8: Inhibitory activity of sulfamethaxazole nanoparticles using *St. aureus bacteria.*
Standard Sulfamethoxazole1- H =High concentration; 2-Nano Sulfamethoxazole Concentration L= Low

Determination of (MIC) of sulfamethoxazole nanomaterial for Cram-negative and positive bacteria inhibitor . Through the study shows that the nanosubstance sulfamethaxazole has inhibitory activity of the positive and negative bacteria of the Cram dye, Table (4) shows that the minimum inhibitory concentration of sulfamethaxazole nanomaterial against (E.coli) is equivalent to 0.25 mg / ml, (Staph.) is equivalent to 0.125 mg/ml, (Pseudo.) is equivalent to 0.25 mg/ml, (Candida) is equivalent to 0.25 mg/ml.

Table 4: Shows the minimum inhibitory concentrations MIC ofsulfamethoxazole nanomaterial for an inhibitor of negative and positive bacteria of Cram dye

Concentrations mg/ml	E. coli ATCC 10536	St. aureus ATCC 6538	Ps.aeruginosa ATCC 15442	Candida albicans ATCC 10231
0.01	-	-	-	-
0.005	-	-	-	-
0.0025	-	-	-	-
0.00125	-	-	+	+
(+) presence of bacterial growth; (−) absence of bacterial growth				

Minimum Lethal Concentration (MBC) of Sulfamethoxazole Nanoscaleagainst Gram-Positive and Negative Bacteria

In Table 5, MBC was found to be four times lower than MIC and fewer thantencolonies in the dish and MBC value for sulfamethoxazole nanoparticles was between 99.9% to 100% against positive and negative bacteria.

Table 5: Shows the minimumlethal concentration (MBC) of sulfamethoxazole nanoscale against gram-positive and gram-negative bacteria

Concentrations mg/ml	E. coli ATCC 10536 (%)	St. aureus ATCC 6538 (%)	Ps.aeruginosa ATCC 15442 (%)	Candida albicans ATCC 10231 (%)
	5x105 colony \ml			
0.01	99.9	100	99.9	99.8
0.005	99.9	100	99.9	99.8
0.0025	99.9	99.9	99.9	99.8
0.00125	99.9	99.9	99.8	99.8

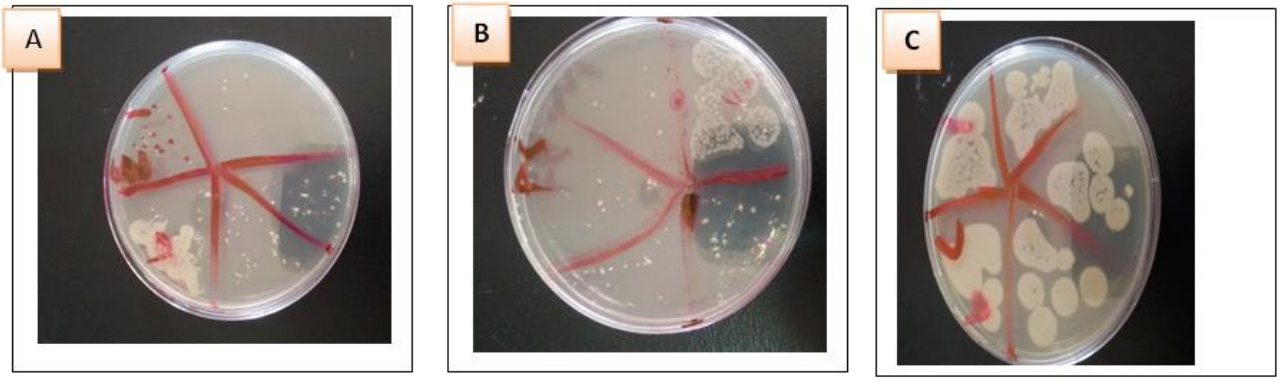


Figure 9: Shows the prepared concentrations of the nanomaterial, namely 0.125% c =, 0.25% b = and 0.5% a = to know the minimum inhibitory concentrations MIC of sulfamethaxazole nano-negative and positive bacteria inhibitor of the dye Cram (a: E.coli), (b: St. aureus), (c:candida albicans)

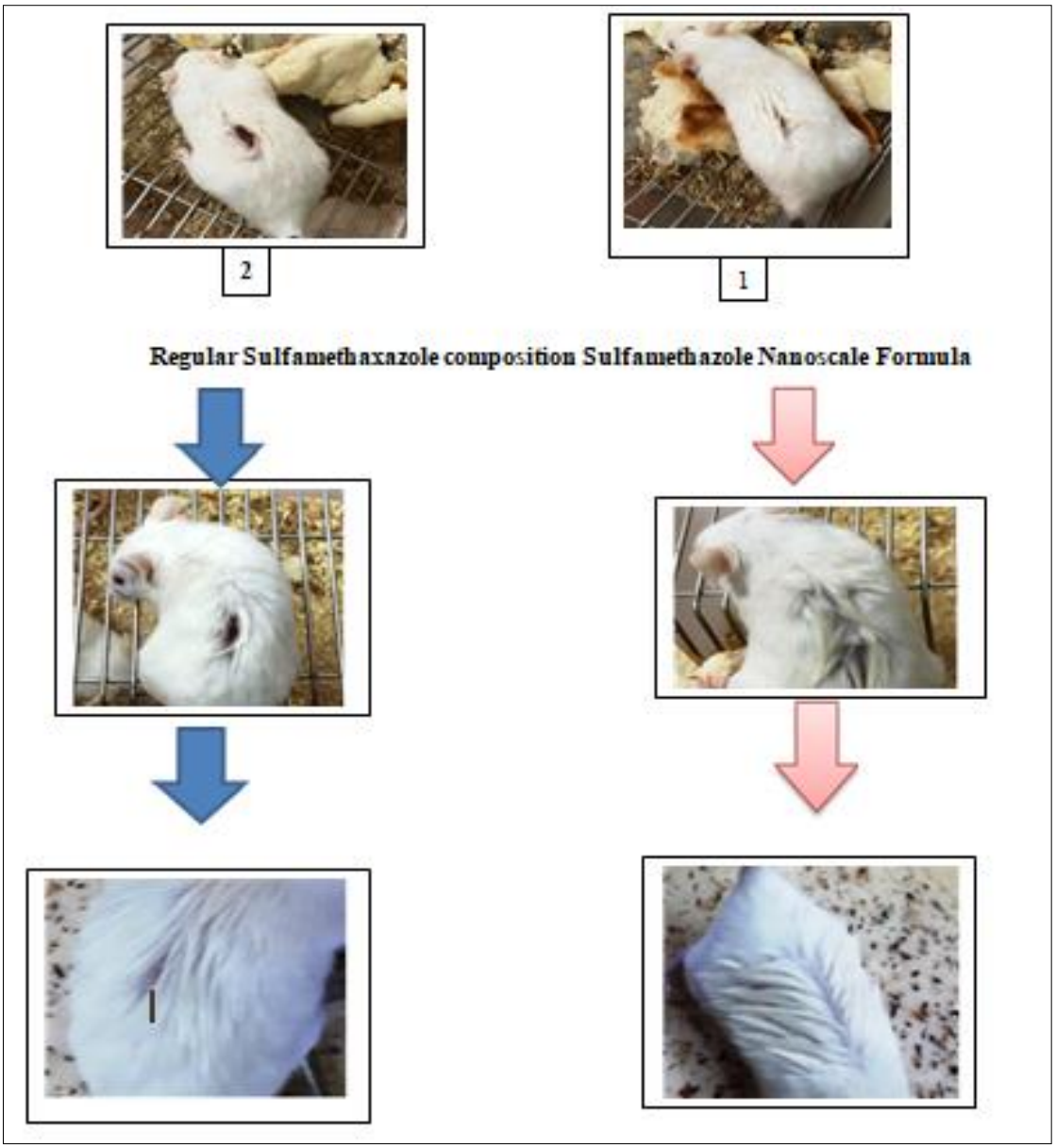
Toxicity Screening

Table 6 shows the results of acute toxicity screening and clinical signs that appeared in white mice after two weeks of oral dosing with standard and nano-sulfamethaxazole.

Table 6: Shows the results of acute toxicity

Clinical signs	Number of dead mice	Number of mice in each group	Dosage material type	t
No clinical signs	5/0	5	Distilled water (group control)	Group A
			Dosed with standard svamethaxazole	Group B
Rapid breathing with rat disorder begins at once after administration and ends after several minutes	5/0	5	0.125gm dissolved in 100ml of distilled water	Group (1)
	5/0	5	0.25gm dissolved in 100ml of distilled water	Groups (2)
	5/0	5	0.5gm dissolved in 100ml of distilled water	Set (3)
			Dosed with sulfamethaxazole nanoparticles	Group C
Rapid breathing and increased severity of rat disorder begin at once after administration and end after a brief period.	5/0	5	12.5ml of distilled water is added	Set (1)
	5/0	5	2.5ml add 100ml of distilled water	Set (2)
	5/0	5	50ml add 100ml of distilled water	Set (3)

It is noted through acute toxicity examination as shown in Table 6, that oral administration of sulfamethaxazole shows that most of the clinical signs shown in white mice were mild and appeared immediately after administration and ended after a period of administration and not a single death during the administration period (after two weeks), indicating that the concentrations used of the standard and nanomaterial are non-toxic or slightly toxic [8-10].



Photos represent Stages of treatment of laboratory animals for three weeks compared to the effect of the nanopreparation material with the normal non-nanomaterial of the same material.

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