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SOFT CHEMICAL SYNTHESIS OF Ag DOPED ZnO NANOPARTICLES: ANTIMICROBIAL ACTIVITY AND *IN VIVO* ACUTE NANOTOXICOLOGICAL IMPACT ON SWISS ALBINO MICE

P. Suresh Kumar and A. Samson Nesaraj *

Department of Science and Humanities, (Chemistry), Karunya University, Karunya Nagar, Coimbatore - 641114, Tamil Nadu, India.

Keywords:

Chemical synthesis, Ag doped ZnO nanoparticles, Characterization, Antimicrobial studies, Acute oral nanotoxicological studies

Correspondence to Author:
Dr. A. Samson Nesaraj

Professor of Chemistry,
Karunya University, Karunya Nagar,
Coimbatore - 641 114, Tamil Nadu,
India.

E-mail: drsamson@karunya.edu

ABSTRACT: In this research work $Zn_{1-x}Ag_xO_{1-\delta}$ (where x = 0.05, 0.10, 0.15 and 0.20) nano powders were synthesized *via* simple soft chemical method using corresponding metal nitrates and alkali precipitant. Sodium hydroxide was used as a precipitant during the synthesis. The prepared samples were characterized using X-ray diffraction, FTIR spectroscopy, particle size analysis, SEM, EDAX analysis and TEM. The antimicrobial activity of the silver doped zinc oxide nanoparticles were studied against both Gram positive *(Staphylococcus aureus* and *Bacillus subtilis)* and Gram negative *(Escherichia coli* and *Klebsiella pneumoniae)* bacteria by agar well diffusion method. Considering the better antimicrobial properties obtained with $Zn_{0.80}Ag_{0.20}O_{1-\delta}$, these materials were subjected for *in vivo* acute oral nanotoxicological studies in Swiss Albino mice. The histopathological changes in livers and kidneys of Swiss Albino mice due to the oral ingestion of the above material ($Zn_{0.80}Ag_{0.20}O_{1-\delta}$) at different dosage levels on day 14 administration have shown the LD_{50} as greater than 2000 mg/kg body weight.

INTRODUCTION: Nanoparticles are materials with dimensions in the nanoscale, *i.e.* under 100 nm. Recently these particles have great attention in research field of modern medicine due to their unique properties like chemical reactivity, energy absorption, and biological mobility. The nanoparticles possess unique physico-chemical, optical and biological properties which can be manipulated suitably for desired applications [1]. Metal-oxides semiconductor nano-composites have been extensively explored because of their potential applications in wide fields.

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Among them, Ag/ZnO nano-composites have large attention, not only because ZnO is one of the most important wide-band gap semiconductors and has various applications, including use in sensors, electronics, solar cells and photo electronics, but also because silver nanoparticles display some unique features in chemical and biological sensing[2].

Nano-sized ZnO is a bactericide and inhibits both Gram-positive and Gram-negative bacteria [3]. Also, ZnO nanoparticles have been received considerable attention in recent years, because of their stability under harsh processing conditions and moreover they are safe materials to human beings and animals [4]. Furthermore, doped Ag reduces the ionization energy of acceptors in ZnO and consequently enhances the emission. Therefore, Ag ions can enhance the antimicrobial ability of ZnO [5].

The first part of the present study reports that Ag doped ZnO nanoparticles can be applied effectively for the control of microorganisms and the prevention of infections caused by Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative (*Escherichia coli* and *Klebsiella pneumonia*) bacteria.

Considering the unique physicochemical properties, including small size effect, large specific surface area, extremely high biological surface reactivity and so forth, metal oxide nanoparticles might affect the toxicological behaviour of materials in organisms, therefore, it needs to further investigate whether the toxicity of nano - scaled materials is related to their particle size. The acute toxicity of oral exposure to nano-scaled zinc metal powder in mice and found that nano - scaled Zn powder could induce severe anaemia and renal damage than mico-scaled Zn [6]. Also, researchers have evaluated and reported the toxicity of nano - scaled ZnO (~ 20 nm) and sub micro - scaled ZnO ((~120 nm) powder at different doses in mice [7].

The toxicological evaluation of zinc oxide reported by National Institute for Occupational Safety and Health (NIOSH) have been showed that Lethal Dose 50 (LD_{50}) of normal ZnO for rats is more than 8 g/kg body weight and belongs to non-toxic chemicals demonstrated by a single oral ingestion [8]. However, the information on the basic toxicity of Ag doped ZnO nanoparticles especially with respect to health implications, occupational risks and hazards is not yet reported. Thus, the objective of the second part of this study was to investigate the acute toxicity of Ag doped ZnO ($Zn_{0.80}Ag_{0.20}O_{1-\delta}$) by *in vivo* experiments with male Swiss Albino Mice. Tests for acute oral toxicity were conducted using recommended OECD guidelines for testing of chemical for safety evaluation. Furthermore, LD_{50} in acute oral toxicity test was evaluated.

Experimental:

MATERIALS: To initiate the synthesis of Ag doped ZnO nanoparticles, analytical grade zinc nitrate hexahydrate, $Zn(NO_3)_2.6H_2O$ (96-103%, Merck), silver nitrate, $AgNO_3$ (99.9%, Himedia) and sodium hydroxide, NaOH (≥ 97%, Merck), ethanol (99.9%, Changshu Yangyuan) and distilled water were used as starting materials.

Synthesis of Ag Doped with ZnO Nanoparticles: The aqueous solution containing a known amount of zinc nitrate hexa hydrate, silver nitrate and sodium hydroxide were prepared in distilled water. The amount of precursor materials used for the preparation of different nanocrystalline materials are indicated in **Table 1**. Initially, the precipitating solution (sodium hydroxide) was taken in the 250 ml beaker. To this solution, $Zn(NO_3)_2$ and $AgNO_3$ solutions were added drop wise. They were mixed thoroughly with continuous stirring by a magnetic stirring apparatus at room temperature for about 3 hours at room temperature. The resultant metal hydroxide precipitate ($Zn(OH)_2$ + AgOH) was filtered off and washed thoroughly with double distilled water and ethanol in the ratio of 9:1 (v/v). The product was dried at 80 °C for about 10 hours in a hot air oven. The resultant grey coloured material was calcined at 300, 450 and 600 °C for 2 hours each to get a phase pure material.

Reaction Mechanism: The main reaction involved in the preparation of Ag doped ZnO nanoparticles can be written briefly as follows:

$$(1-x)\ Zn(NO_3)_2.6H_2O + x\ AgNO_3 + (2-x)\ NaOH \rightarrow Zn_{1-x}Ag_xO_{1-\delta} + (2-x)\ NaNO_{3\ (aq)} + yH_2O_{(aq)} \text{ ------ } (1)$$

(Where x = 0.05, 0.10, 0.15 and 0.20)

TABLE 1: AMOUNT OF PRECURSOR MATERIALS (DISSOLVED IN 100 ml OF WATER EACH) USED FOR THE PREPARATION OF Ag DOPED ZnO NANOPARTICLES BY CHEMICAL PRECIPITATION METHOD

Sample	Conc. of $Zn(NO_3)_2$ / Wt. (g)	Conc. Of $AgNO_3$ / Wt. (g)	Conc. of NaOH / Wt. (g)
$Zn_{0\cdot95}Ag_{0\cdot05}O_{1-\delta}$	0.95M / 5.652g	1.95M / 1.56g	0.05M / 0.169g
$Zn_{0\cdot90}Ag_{0\cdot10}O_{1-\delta}$	0.90M / 5.35g	1.90M / 1.52g	0.10M / 0.339g
$Zn_{0\cdot85}Ag_{0\cdot15}O_{1-\delta}$	0.85M / 5.057g	1.85M / 1.40g	0.15M / 0.509g
$Zn_{0\cdot80}Ag_{0\cdot20}O_{1-\delta}$	0.80M / 4.759 g	1.80M / 1.44g	0.20M / 0.679 g

Materials Characterization:

Physical Characterization of Materials: The heat treated Ag doped ZnO nanoparticles were characterized by Shimadzu XRD6000 X-ray diffractometer using CuKα radiation. The lattice parameters were calculated by least square fitting method using DOS computer programming. The theoretical density of the powders was calculated with the obtained XRD data. The crystallite sizes of the powder were calculated by Scherrer's formula. Shimadzu IR Prestige - 21 model. FTIR spectrometer was employed to record the FTIR spectra of materials in the range of 4000 - 400 cm^{-1}. The particle size of the powder was measured using Malvern Particle Size Analyzer using triple distilled water as medium. The surface morphology of the particles was studied by means of JEOL Model JSM - 6360 scanning electron microscope. EDAX analysis was also performed with JEOL Model JSM - 6360 to find out the atomic weight percentage of elements present in the samples. The HR TEM of the samples was measured by HR TEM - JEOL JEM 2100 model.

Antimicrobial Studies: Agar well diffusion method was used to determine the antimicrobial activity of the Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative (*Escherichia coli* and *Klebsiella pneumoniae*) bacteria towards Ag doped ZnO nanoparticles. Well diffusion is very cost-effective, facile and useful method for assessing the antibacterial properties. Initially, the nanoparticle suspension was prepared by dispersing 10mg of Ag doped ZnO nanoparticles (each type) individually in 1 ml of double distilled water and homogenizing well in an ultrasonic bath for about 20 minutes. The sterile nutrient agar was prepared and these agars were poured into the sterilized petri dishes. The micro-organisms were sub cultured in a nutrient broth medium at 37 °C on a rotary shacker (shacked at 120 rpm overnight) for the bacterial growth. Using sterile cotton swab the test organisms were swabbed over the surface of the agar plates.

The plates were allowed for 5 minutes to dry and then wells of uniform sizes (8 mm diameter) were made with a cork-borer from the agar plates. Subsequently, a 100 µl of the nanoparticle suspension was introduced into the wells of inoculated nutrient agar plates. The plates were incubated at 37 °C for 24 hours and diameter of the inhibitory zones was measured in millimeter (mm).

***In vivo* Acute Oral Nanotoxicological Studies:** After obtaining a clearance from the Institutional Animal Ethics Committee (Ethical clearance number: IAEC/KU/BT/14/17) of Karunya University, Coimbatore, India, thirty numbers Swiss albino mice of age 8 weeks were procured from Tamil Nadu Veterinary and Animal Science University, Chennai, India. The animals were housed in clean polypropylene cages and maintained in an air-conditioned animal house at 23 ± 2 °C, 50% - 70% relative humidity and 12h light / dark cycle. All the animals were provided with commercial mice pellet diet and water *ad libitum*. After one week acclimation, the mice were randomly divided into five groups. The Ag doped ZnO nanoparticles (Zn0.80Ag0.20O1-δ) were suspended in distilled water and administered through oral gavage once at dose levels (per group) of (control), 175, 500, 1000 and 2000 mg/kg body weight (b.w.) based on OECD guidelines(425) for testing acute oral toxicity chemical for the period of 14 days.

The test solution was prepared shortly prior to the administration. The dose volume maintained for all the groups was maximum (2 ml / kg b.w.). On 14^{th} day, the animals were sacrificed and the blood was collected through cardiac puncture (from each group). The organs such as liver and kidney were collected and the organs were kept in 10% buffer formalin for histopathological examination. The above study was carried out to find out the LD_{50} of the Ag doped ZnO (Zn0.80Ag0.20O1-δ) nanoparticles for Swiss Albino Mice by a single oral ingestion.

RESULTS AND DISCUSSION:

XRD Studies: Fig. 1 (**a, b, c** and **d**) shows XRD pattern of the Ag doped ZnO nanoparticles (Zn0.95Ag0.05O1-δ, Zn0.90Ag0.10O1-δ, Zn0.85 Ag0.15O1-δ and Zn0.80Ag0.20O1-δ) prepared by chemical precipitation method. All peaks can be assigned to the standard hexagonal phase of zinc oxide (JCPDS No. 89 - 1397) and (111), (200) and (220) crystallographic planes of the cubic phase of silver (JCPDS No. 89 - 3722). No other crystalline phase was formed. It is clearly observed that the diffraction peaks become sharper and stronger due to the high temperature treatment (600 °C for 2 hours) which suggests that the crystalline quality of the materials is improved [9].

Also, it was found that the intensity of peaks relevant to Ag phase is increased with respect to the increase in the % of Ag content in the materials. The average crystallite size of the Ag doped ZnO nanoparticles with various dopant concentrations of Ag have been calculated from XRD using Scherrer's formula. The crystallite size of the materials was found to be in the range of 24 - 27 nm, which is in good agreement with the reported data [10]. The crystallographic parameters obtained on the Ag doped ZnO nanoparticles materials are indicated in **Table 2**. Theoretical density values were also agreed well for all the compositions of Ag doped ZnO nanoparticles.

TABLE 2: CRYSTALLOGRAPHIC PARAMETERS OBTAINED ON Ag DOPED ZnO NANOPARTICLE SPREPARED BY CHEMICAL PRECIPITATION METHOD

Sample	Crystal structure	Unit Cell parameters (Å)	Unit cell volume ($Å^3$)	Crystallite Size (nm)	Theoretical density (g/cc)
Standard ZnO (JCPDS No. 89-1397)	Hexagonal	a = 3.253 b = 5.213	47.77	-	16.97
$Zn_{0.95}Ag_{0.05}O_{1-\delta}$	Hexagonal	a = 3.232 b = 5.168	46.75	27.3	14.71
$Zn_{0.90}Ag_{0.10}O_{1-\delta}$	Hexagonal	a = 3.235 b = 5.182	47.00	24.6	17.98
$Zn_{0.85}Ag_{0.15}O_{1-\delta}$	Hexagonal	a = 3.238 b = 5.184	47.09	25.7	20.42
$Zn_{0.80}Ag_{0.20}O_{1-\delta}$	Hexagonal	a = 3.237 b = 5.181	47.03	25.6	18.69

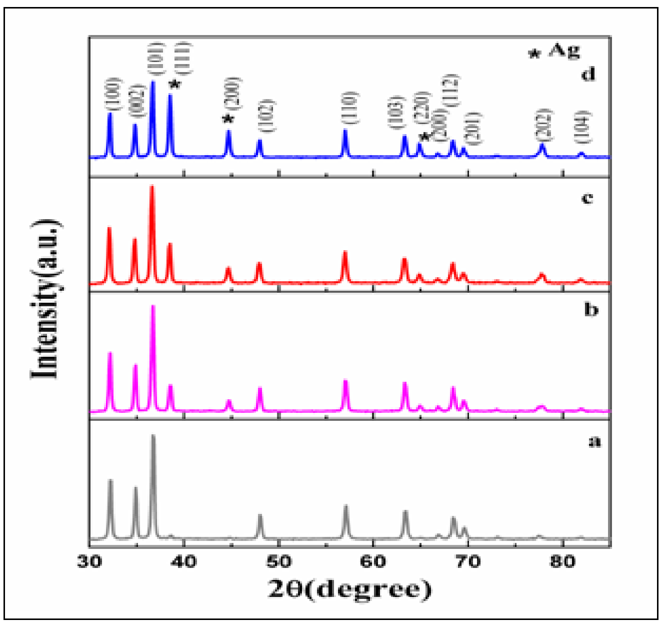

FIG. 1: XRD PATTERNS OBTAINED ON THE Ag DOPED ZnO NANOPARTICLES PREPARED BY CHEMICAL PRECIPITATION METHOD (A) $Zn_{0.95}Ag_{0.05}O_{1-\delta}$; (B) $Zn_{0.90}Ag_{0.10}O_{1-\delta}$; (C) $Zn_{0.85}Ag_{0.15}O_{1-\delta}$; (D) $Zn_{0.80}Ag_{0.20}O_{1-\delta}$

FTIR Studies: Fig. 2 (**a, b, c** and **d**) shows the FTIR spectra obtained on Zn0.95Ag0.05O1-δ, Zn0.90Ag0.10O1-δ, Zn0.85Ag0.15O1-δ and Zn0.80Ag0.20O1-δ nanoparticles prepared by the chemical precipitation method. FTIR measurements were done using KBr method at room temperature (RT). In the FTIR spectra, the peaks appeared at 448.47, 412.77, 472.56 and 441.7 cm^{-1} in samples are assigned to the characteristic stretching mode of Ag-O and Zn-O bond [11]. The band in the region within 3000 cm^{-1} to 3800 cm^{-1} corresponds to the OH^{-1} band, which is due to absorption of water molecules during exposure to atmospheric air [12]. The band near 2360 cm^{-1} is because of the absorption of atmospheric CO_2 on the metallic cations [9]. Bands appeared at 1504.48 and 1560.41 cm^{-1} correspond to O-H bending vibrations as reported [13].

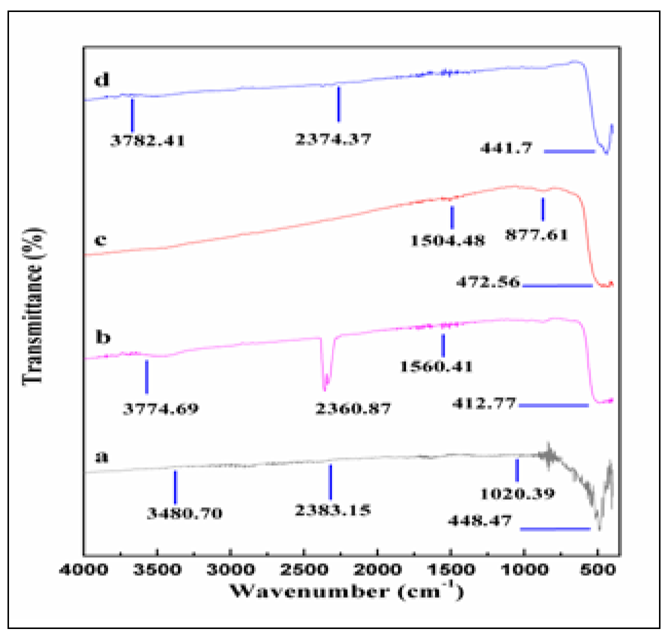

FIG. 2: FTIR SPECTRA OBTAINED ON THE Ag DOPED ZnO NANOPARTICLES PREPARED BY CHEMICAL PRECIPITATION METHOD (A) $Zn_{0.95}Ag_{0.05}O_{1-\delta}$; (B) $Zn_{0.90}Ag_{0.10}O_{1-\delta}$; (C) $Zn_{0.85}Ag_{0.15}O_{1-\delta}$; (D) $Zn_{0.80}Ag_{0.20}O_{1-\delta}$

Particle Size Measurements: The particle size characteristic curves obtained on Zn0.95Ag0.05O1-δ, Zn0.90Ag0.10O1-δ, Zn0.85Ag0.15O1-δ and Zn0.80Ag0.20O1-δ nanoparticles prepared by the chemical precipitation method are indicated in **Fig. 3** (**a, b, c** and **d**). For all the measurements, 0.010 g of nanoparticles was sonicated in about 10 ml of double distilled water for about 20 minutes and after which the sample was subjected for particle size analysis. The particle characteristics data obtained on Ag doped ZnO nanoparticles are indicated in **Table 3.**

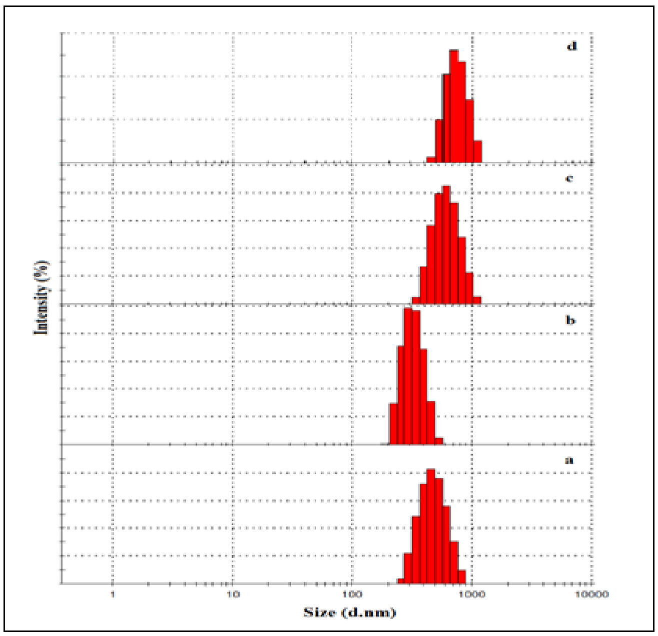

FIG. 3: PARTICLE SIZE CHARACTERISTIC CURVES OBTAINED ON THE Ag DOPED ZnO NANOPARTICLES PREPARED BY CHEMICAL PRECIPITATION METHOD (A) $Zn_{0.95}Ag_{0.05}O_{1-\delta}$; (B) $Zn_{0.90}Ag_{0.10}O_{1-\delta}$; (C) $Zn_{0.85}Ag_{0.15}O_{1-\delta}$; (D) $Zn_{0.80}Ag_{0.20}O_{1-\delta}$

TABLE 3: PARTICLE CHARACTERISTICS DATA OBTAINED ON Ag DOPED ZnO NANOPARTICLES PREPARED BY CHEMICAL PRECIPITATION METHOD

Sample	Peak 1 % Intensity	Peak 1 Diameter (nm)	Average particle size (nm)
$Zn_{0.95}Ag_{0.05}O_{1-\delta}$	100	486.1	456.9
$Zn_{0.90}Ag_{0.10}O_{1-\delta}$	100	326.1	324.6
$Zn_{0.85}Ag_{0.15}O_{1-\delta}$	100	626.6	580.5
$Zn_{0.80}Ag_{0.20}O_{1-\delta}$	100	752.8	721.4

From the particle size data (**Table 3**), it was found that the average size of the particles was present between 324.6 to 721.4 nm. Also, it was understood that the particle size of the materials was dependant on the concentration of the dopant ions. The results inferred that the particle size gets increased with increase in the concentration of silver ions.

SEM Measurements: Fig. 4 (**a-d**) exhibits the SEM images of the $Zn_{0.95}Ag_{0.05}O_{1-\delta}$, $Zn_{0.90}Ag_{0.10}O_{1-\delta}$, $Zn_{0.85}Ag_{0.15}O_{1-\delta}$ and $Zn_{0.80}Ag_{0.20}O_{1-\delta}$ nanoparticles prepared by the chemical precipitation method.

The SEM photographs exhibited homogeneous and relatively agglomerated particles with average size of 90 to 320 nm. The presence of agglomerates may be due the densification at higher temperature (600 °C). Generally, the grain size of the particles gets increased with increase in concentration of Ag ions. It was noticed that $Zn_{0.95}Ag_{0.05}O_{1-\delta}$; and $Zn_{0.90}Ag_{0.10}O_{1-\delta}$ particles show smooth grains when compared with $Zn_{0.85}Ag_{0.15}O_{1-\delta}$ and $Zn_{0.80}Ag_{0.20}O_{1-\delta}$particles. Further, the morphology of the particles clearly changed with doping.

EDAX Analysis: Fig. 5 (**a, b, c** and **d**) show the energy dispersive X-ray microanalysis (EDAX) spectra of the $Zn_{0.95}Ag_{0.05}O_{1-\delta}$, $Zn_{0.90}Ag_{0.10}O_{1-\delta}$, $Zn_{0.85}Ag_{0.15}O_{1-\delta}$ and $Zn_{0.80}Ag_{0.20}O_{1-\delta}$ nanoparticles prepared by the chemical precipitation method. EDAX spectra of Ag doped ZnO nanoparticles show the composition of different elements present in the systems. The spectra indicate the presence of small amount of Ag in all the systems which are also confirmed by the phase purity of XRD.

The elemental composition data obtained on nano-materials by EDAX analysis is given in **Table 4**. The data confirmed the presence of appropriate elements in all the samples.

TABLE 4: ELEMENTAL COMPOSITION DATA OBTAINED ON Ag DOPED ZnO NANOPARTICLES BY EDAX ANALYSIS

Samples	Atomic Wt. % of Elements
$Zn_{0.95}Ag_{0.05}O_{1-\delta}$	Zn – 49.18
	O – 50.17
	Ag – 0.65
$Zn_{0.90}Ag_{0.10}O_{1-\delta}$	Zn – 58.29
	O – 39.89
	Ag – 1.82
$Zn_{0.85}Ag_{0.15}O_{1-\delta}$	Zn – 48.42
	O – 48.56
	Ag – 3.02
$Zn_{0.80}Ag_{0.20}O_{1-\delta}$	Zn – 42.38
	O – 52.18
	Ag – 5.44

Fig. 5 (**a, b, c** and **d**) shows two strong peaks corresponding to Zn as well as two peaks corresponding to Ag and O elements which confirms the high purity of Ag doped ZnO nanoparticles.

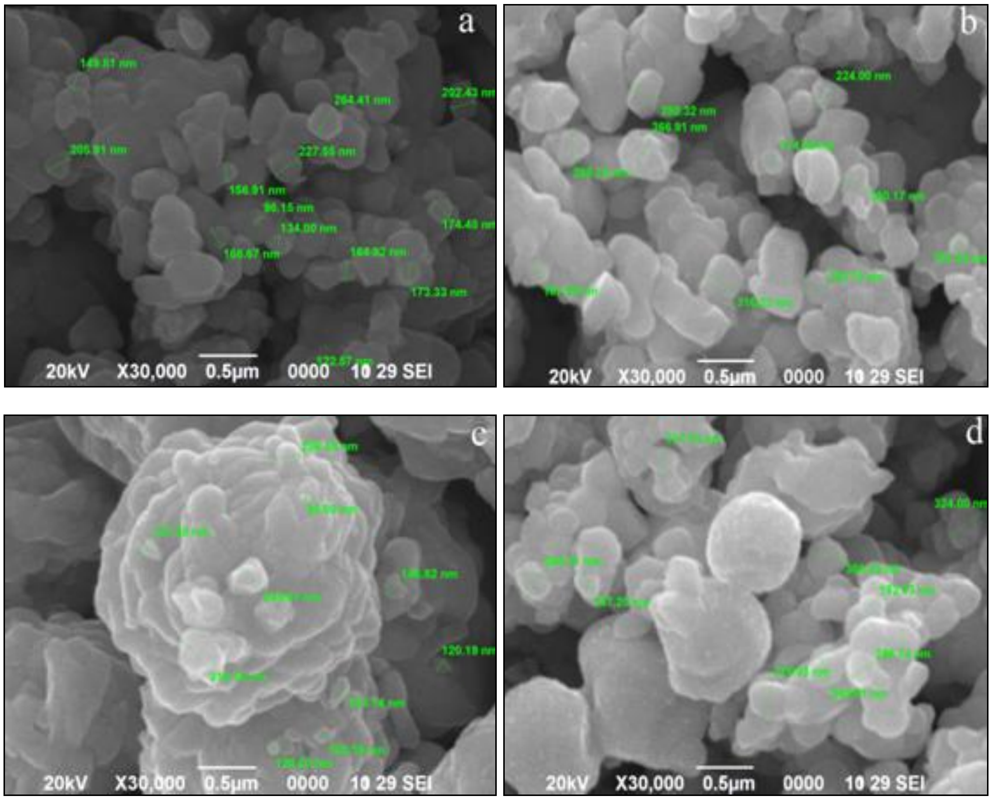

FIG. 4: SEM PHOTOGRAPHS OBTAINED ON THE Ag DOPED ZnO NANOPARTICLES PREPARED BY CHEMICAL PRECIPITATION METHOD (A) $Zn_{0.95}Ag_{0.05}O_{1-\delta}$; (B) $Zn_{0.90}Ag_{0.10}O_{1-\delta}$; (C) $Zn_{0.85}Ag_{0.15}O_{1-\delta}$; (D) $Zn_{0.80}Ag_{0.20}O_{1-\delta}$

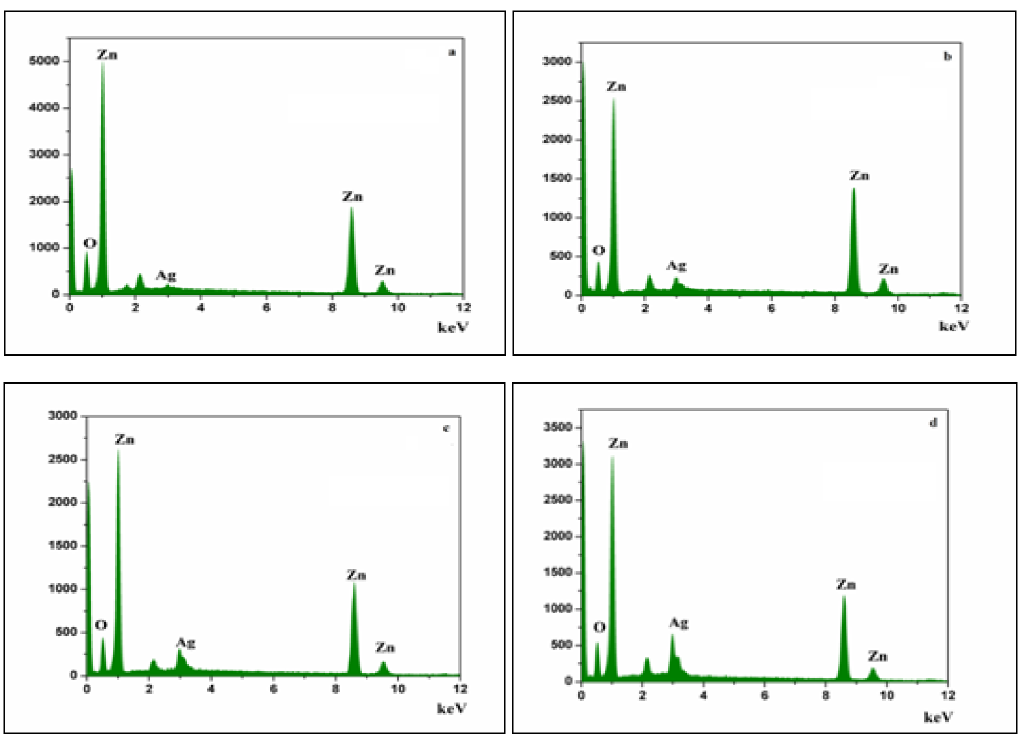

FIG. 5: EDAX SPECTRA OBTAINED ON THE Ag DOPED ZnO NANOPARTICLES PREPARED BY CHEMICAL PRECIPITATION METHOD (A) $Zn_{0.95}Ag_{0.05}O_{1-\delta}$; (B) $Zn_{0.90}Ag_{0.10}O_{1-\delta}$; (C) $Zn_{0.85}Ag_{0.15}O_{1-\delta}$; (D) $Zn_{0.80}Ag_{0.20}O_{1-\delta}$

HRTEM Studies: The HRTEM images of the $Zn_{0.80}Ag_{0.20}O_{1-\delta}$ nanoparticles are shown in **Fig. 6** (**a, b, c** and **d**). **Fig. 6** (**a, b** and **c**) displays the TEM images of $Zn_{0.80}Ag_{0.20}O_{1-\delta}$ which indicate the presence of nano rod like a structure in the samples. From the TEM micrograph, the diameter of the Ag doped zinc oxide was found to be approximately 50 to 150 nm length and 200 to 100 nm with one dimension of rod. As reported in the literature, the doping of silver with zinc oxide nanomaterial changes from spherical to rod shaped Ag: ZnO particles [13]. **Fig. 6c** clearly shows the presence of doped silver in the ZnO phase. From the single electron diffraction (SAED) pattern (**Fig. 6d**) the measured d-spacing values (0.26 and 0.20 nm as indicated in the figure) correspond to the single crystal planes of hexagonal ZnO (002) and cubic silver (200). The obtained results from the TEM are in good agreement with the XRD data.

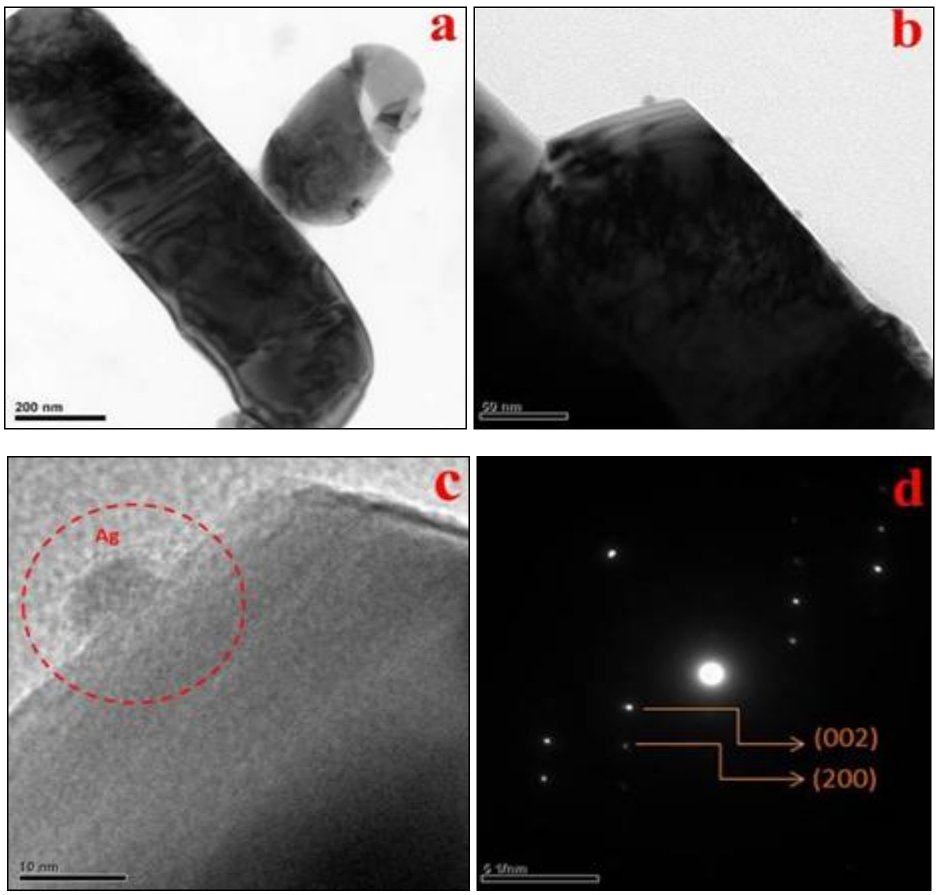

FIG. 6: HRTEM IMAGES (A, B AND C) OF OBTAINED ON $Zn_{0.80}Ag_{0.20}O_{1-\delta}$ AND (D) SELECTED AREA ELECTRON DIFFRACTION PATTERN OBTAINED ON $Zn_{0.80}Ag_{0.20}O_{1-\delta}$

Antimicrobial Studies: The antimicrobial activity of Ag doped ZnO nanoparticles ($Zn_{0.95}Ag_{0.05}O_{1-\delta}$, $Zn_{0.90}Ag_{0.10}O_{1-\delta}$, $Zn_{0.85}Ag_{0.15}O_{1-\delta}$ and $Zn_{0.80}Ag_{0.20}O_{1-\delta}$) have been tested for the inhibition of *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. The antimicrobial activities against the above bacteria were qualitatively and quantitatively assessed by determining the presence of inhibition zones. Antibacterial effect in the form of inhibition zones, evaluated by agar well diffusion method, is shown in **Fig. 7**. The bactericidal activity against *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Staphylococcus aureus* shows a clear of zone of inhibition within and around the disc impregnated with all Ag doped ZnO nanoparticle samples. These results indicate that Ag doped ZnO nanoparticles have efficient antibacterial capability for both gram-negative (G^-) and gram-positive (G^+) bacteria as reported in the literature [14].

Among the four samples studied, $Zn_{0.80}Ag_{0.20}O_{1-\delta}$ maintained considerably higher antimicrobial activity than the other samples. Such good performance of $Zn_{0.80}Ag_{0.20}O_{1-\delta}$ can possibly be attributed to the presence of higher concentration of silver (20 mole %) in the sample, leading to efficient antibacterial activity. It was reported that the bacterial susceptibility improved considerably with increase in the silver content for the silver doped ZnO nanoparticles [15]. The increment in antibacterial efficiency of $Zn_{0.80}Ag_{0.20}O_{1-\delta}$ nanoparticles can further be explained on the basis of synergism.

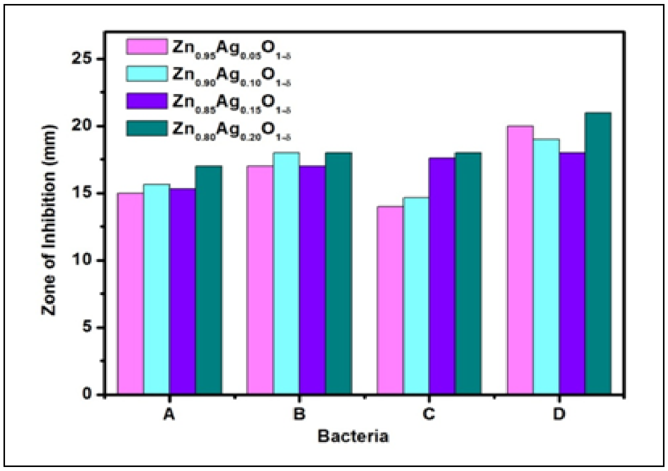

FIG. 7: ANTIMICROBIAL ACTIVITY OF Ag DOPED ZnO NANOPARTICLES AGAINST BACTERIA: *ESCHERICHIA COLI* (A), *BACILLUS SUBTILIS* (B), *KLEBSIELLA PNEUMONIAE* (C) AND *STAPHYLOCOCCUS AUREUS* (D)

It can be assumed that, as the amount of silver increases, the resultant bactericidal contribution further increases because of more attachment to the bacterial cell with a subsequent after attachment activity followed by a contribution from zinc oxide with the generation of H_2O_2, hence resulting a synergistic activity. Also, it was mentioned that silver has an important antimicrobial effect, which depends upon superficial contact, where in silver can inhibit enzymatic systems of the respiratory chain, there by altering the DNA synthesis [16].

Acute Histopathological Studies: The acute histopathological observations of mice liver and kidney after oral administration of ($Zn_{0.80}Ag_{0.20}O_{1-\delta}$) [since it was showing considerable good microbial activity] at different dosage levels, such as, control, 175 mg/kg body weight (b.w.), 500 mg/kg body weight (b.w.), 1000 mg/kg body weight (b.w.) and 2000 mg/kg body weight (b.w.) are shown in **Fig. 8** and **9**. The treated mice didn't exhibit any mortality, body weight or behavioural change or toxicity compared to the control group until 14 days of post-oral injection. The summary of the results obtained on toxicity studies after 14 days post-oral injection in mice is indicated in **Table 5**.

There was no sign of tremor, convulsion, salivation, diarrhoea, lethargy or unusual behaviour such as self mutilation or walking backward in the treated mice. However, slight abnormal behaviour was noticed in the mice treated with 1000 and 2000 mg/kg body weight (b.w.). On the 15^{th} day, the liver and kidney from each mouse were removed after dissection and preserved in 10% formalin. Then representative blocks of liver and kidney tissues from each lob were taken and possessed for paraffin embedding using the standard micro-technique [17]. Sections of (~5 µm) of livers and kidneys stained with haematoxylin and eosin were observed microscopically for the evaluation of acute histopathological changes.

TABLE 5: SUMMARY OF RESULTS OBTAINED ON ACUTE TOXICITY STUDIES FOR ALBINO MICE AFTER 14 DAYS OF ORAL INJECTION

Parameters monitored	Doses of $Zn_{0.80}Ag_{0.20}O_{1-\delta}$ (mg/kg body weight)			
	175	500	1000	2000
Changes in skin	-	-	+	+
Changes in fur	-	-	+	+
Changes in eyes	-	-	-	-
Changes in mucous membrane	-	-	-	-
Behaviour patterns	-	-	-	+
Tremors	-	-	-	-
Sleep	-	-	+	+
Coma	-	-	-	-
Death	-	-	-	-

Mice were monitored for 14 days post-oral injection: (+) abnormal, (-) normal

From **Fig. 8** (**a, b, c, d** and **e**), the following observations were made. The acute histopathological examination of liver (with no dosage level) shows normal lobular architecture and mild dilatation of sinusoids. The liver of the mice having the dosage of 175 mg/kg exhibits lesser extend of cytoplasmic vacuolation and interface hepatitis along with mild central vein congestion. However, the liver treated 500 mg/kg of dosage level shows periportal inflammation with micro-vesicular steatosis when compared with control. The livers treated with 1000 and 2000 mg/kg dosage levels show severe reactive a typia with diffuse parenchymal lymphocytic infiltration, extensive cytoplasmic vacuolation with dilatation and congestion in the mice hepatocytes when compared to control.

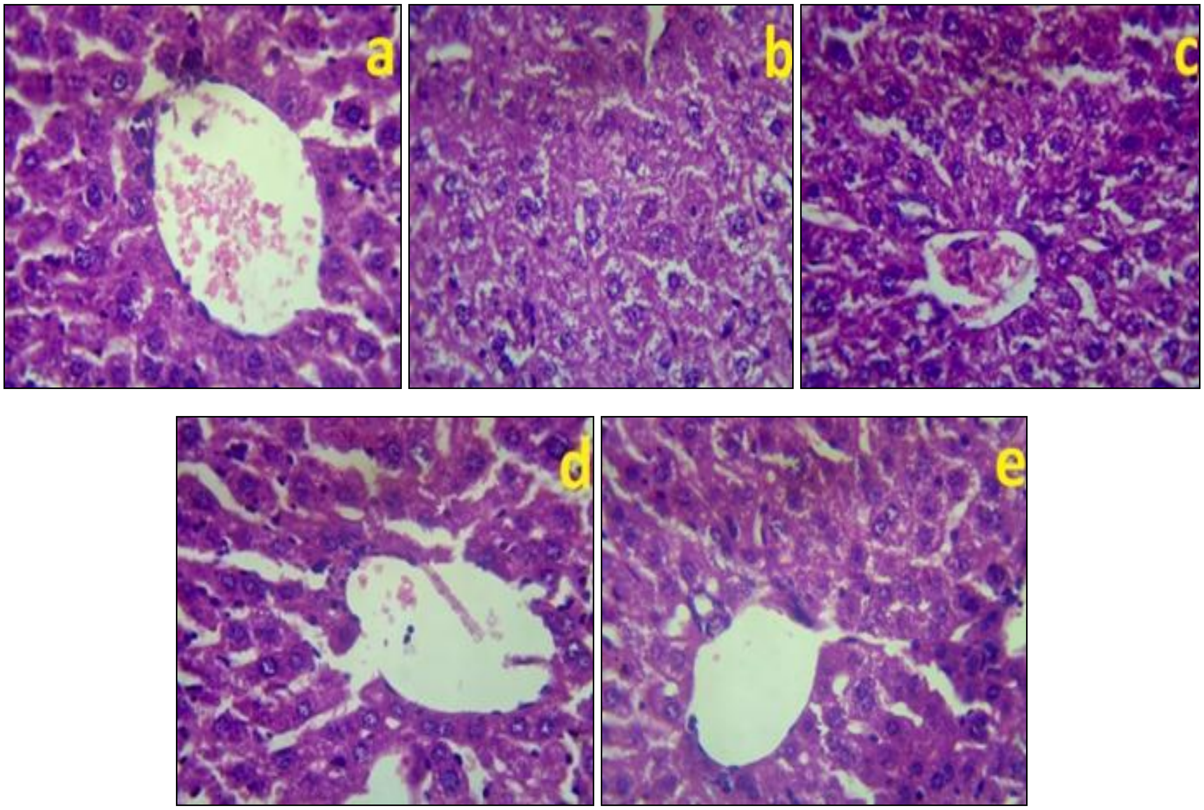

FIG. 8: ACUTE HISTOPATHOLOGICAL OBSERVATION OF MICE LIVER AFTER ORAL ADMINISTRATION OF Ag DOPED NANOPARTICLES ($Zn_{0.80}Ag_{0.20}O_{1-\delta}$); (A) WITH CONTROL *i.e.* WITH NO DOSAGE OF NANOPARTICLES (SHOWS NORMAL MORPHOLOGY OF HEPATOCYTES STRUCTURE); (B) WITH 175 mg/kg BODY WEIGHT (B.W.) (SHOWS CYTO-PLASMIC VACUOLATION AND INTERFACE HEPATITIS); (C) WITH 500 mg/kg BODY WEIGHT (B.W.) (SHOWS MICROVESICULAR STEATOSIS); (D) WITH 1000 mg/kg BODY WEIGHT (B.W.) (SHOWS REACTIVE ATYPIA WITH DIFFUSE PARENCHYMAL LYMPHOCYTIC INFILTRATION); (E) WITH 2000 mg/kg BODY WEIGHT (B.W.) (SHOWS CYTOPLASMIC VACUOLATION AND BINUCLEATION)

From **Fig. 9** (**a, b, c, d** and **e**), the following inferences were made. The acute histopathological examination of kidney (without any dosage of nanoparticles) shows normal morphology of cortex and medulla and shows no interstitium inflammation along with normal tubular architecture. The kidney with the dosage level of 175 mg/kg exhibits lesser extends of mesangial hyper-cellularity and shows mild congestion in the blood vessels. The kidney treated with 500 mg/kg dosage level shows mild mesangial hyper-cellularity along with proximal tubules and shows tubular necrosis when compared with control.

The kidneys of mice treated with 1000 and 2000 mg/kg dosage levels exhibit mild mesangial hyper-cellularity along with mild congestion, thickened blood vessels and the interstitium lymphotic infiltration when compared with control.

From the acute histopathological studies, it was found that the orally administered Ag doped nanoparticles did not cause acute toxicity in mice. However, the mice treated with 1000 and 2000 mg/kg body weight (b.w.) caused mild liver and kidney injury by gastrointestinal ingestion. Also, there was no mortality and abnormal behaviour or symptoms such as decrease in food and water intake, diarrhoea, loss of movement and change in size of eyes were observed in mice treated with silver doped ZnO nanoparticles at different dosage levels after 14th days of oral injection indicating that LD_{50} values will be more than 2000 mg/kg of body weight (b.w.)

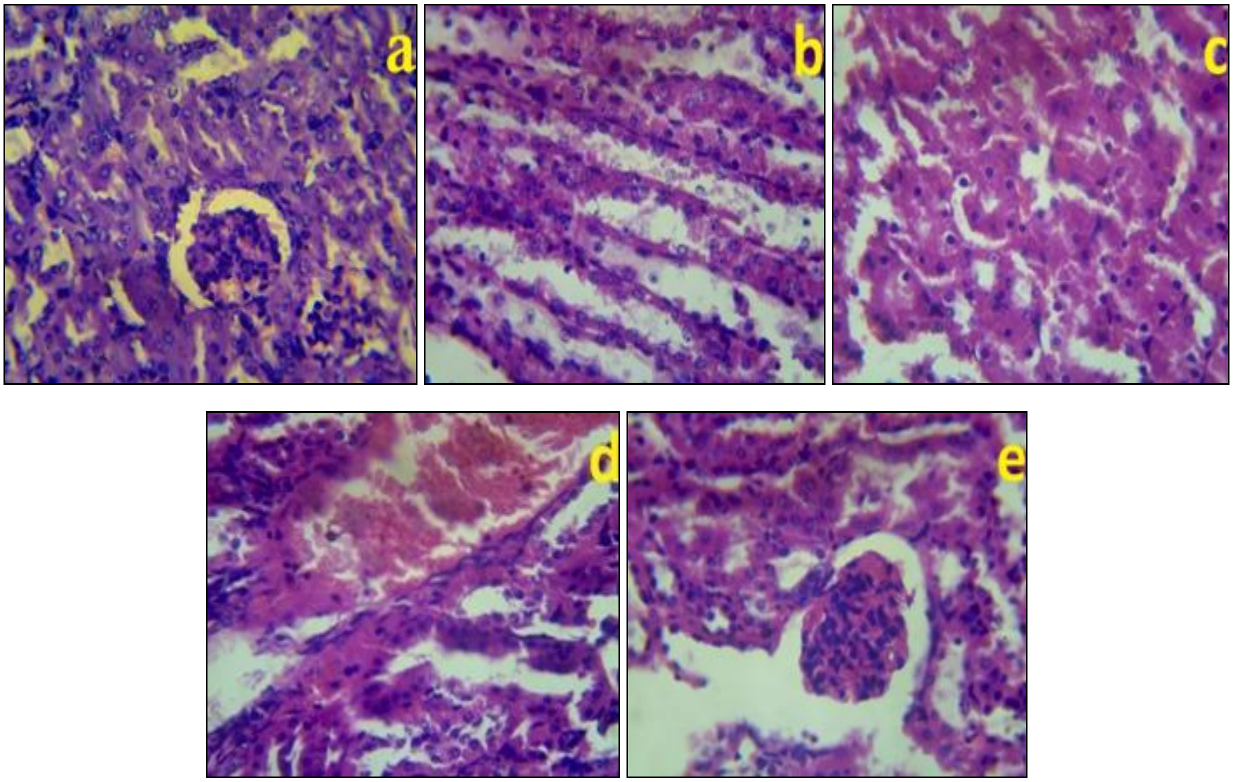

FIG. 9: ACUTE HISTOPATHOLOGICAL OBSERVATION OF MICE KIDNEY AFTER ORAL ADMINISTRATION OF Ag DOPED NANOPARTICLES ($Zn_{0.80}Ag_{0.20}O_{1-\delta}$); (A) WITH CONTROL *i.e.* WITH NO DOSAGE OF NANOPARTICLES (SHOWS NORMAL CORTEX AND MEDULLA); (B) WITH 175 mg/kg BODY WEIGHT (B.W.) (SHOWS MILD TUBULAR INJURY); (C) WITH 500 mg/kg BODY WEIGHT (B.W.) (SHOWS MESANGIAL HYPERCELLULARITY AND ACUTE TUBULAR NECROSIS); (D) WITH 1000 mg/kg BODY WEIGHT (B.W.) (SHOWS MILD CONGESTION OF BLOOD VESSELS AND THICKENED WALL); (E) WITH 2000 mg/kg BODY WEIGHT (B.W.) (SHOWS LYMPHOCYTIC INFILTRATION)

CONCLUSION: Chemical precipitation technique was effectively used to prepare different compositions of Ag doped ZnO nanoparticles. The XRD results indicated the presence of hexagonal phase of zinc oxide and cubic phase of silver in all the samples. The FTIR results exhibited the stretching mode of Ag-O and Zn-O in the samples. The particle size data inferred that the particle size gets increased with the increase in the dopant concentration of silver in ZnO. SEM micro structures confirmed presence of particles in the range of 90 to 320 nm. The EDAX spectra exhibited the presence of elements as per the stoichiometric composition in the samples.

The HRTEM results of $Zn_{0.80}Ag_{0.20}O_{1-\delta}$ indicated the presence of nano rod like a structure. Among the samples studied, $Zn_{0.80}Ag_{0.20}O_{1-\delta}$ maintained considerably higher antimicrobial activity than the other samples when tested with bacteria, such as, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. From the histopathological studies, it was confirmed that there was no mortality and abnormal behaviour in mice treated with $Zn_{0.80}Ag_{0.20}O_{1-\delta}$ at different dosage levels after 14th days of oral injection indicating that LD_{50} values will be greater than 2000 mg/kg of body weight (b.w). However, there is the need for further toxicity study, emphasizing on sub-chronic toxicity assessment and focusing more on possible liver and kidney damage.

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REFERENCES:

1. Ravishankar RV and Bai JA: Nanoparticles and their potential application as antimicrobials. Science against microbial pathogens. Communicating current Research and Technological Advances 2011; 197-209.
2. Xiao-Yun Ye, Zhou YM, Sun YQ, Chen J and Wang ZQ: Preparation and characterization of Ag / ZnO composites *via* a simple hydrothermal route. Journal Nanoparticle Research 2009; 11: 1159-1166.
3. Karunakran C, Rajeswari C and Gomathisankar P: Optical, electrical, photocatalytic and bactericidal properties of microwave synthesized nanocrystalline Ag-ZnO and ZnO. Solid State Sciences 2011; 13: 923-928.
4. Ramesh P, Rajendran A and Meenakshisundaram M: Green Synthesis of Zinc Oxide Nanoparticles Using Flower Extract *Cassia auriculata*. Journal of Nanoscience and Nanotechnology 2014; 2: 41-45.
5. Susan A, Mansor BA, Hussein MZ and Nor Azowa Ibrahim: Synthesis, antibacterial and thermal studies of cellulose nanocrystal stabilized ZnO-Ag hetero structure nanoparticles. Molecules 2013; 18: 6269-6280.
6. Bing Wang, Feng WY, Wang TC, Jia G, Wang M, Shi JW, Zhang F, Zhao YL and Chai ZF: Acute toxicity of nano and micro-scale zinc powder in healthy adult mice. Toxicology Letters 2006; 161: 115 - 123.
7. Bing W, Weiyue F, Meng W, Tiancheng W, Yiqun G, Motao Z, Hong O, Junwen S, Fang Z, Yuliang Z, Zhifang C, Haifang W and Jing W: Acute cological impact of nano and sub micro-scaled zinc oxide powder on healthy adult mice. Journal of Nanoparticle Research 2008; 10: 263-276.
8. Won-Chul Jung, Kim S, and Hu-Jang L: Acute toxicity nano-scale zinc oxide powder in ICR mice. Journal of Biomedical Research 2010; 11: 219-224.
9. Gayathri S, Sivraman O, Ghosh N, Sathiskumar S, Sudhakara P, Jayaramudu J, Ray SS and Annamraju KV: Investigation of physicochemical properties of Ag doped ZnO nanoparticles prepared by chemical route. Applied Science Letters 2015; 1: 8 - 13.
10. Asadi H: Effect of calcination temperature on antimicrobial properties of ZnO/Ag nanocomposite incorporated in architectural paints. Indian Journal of Fundamental Applied Life Sciences 2014; 3: 315 - 319.
11. Shah AH, Manikandan E, Basheer Ahmed M and Ganesan V: Enhanced bio-activity of Ag/ZnO nanorods - A comparative antibacterial study, Nanomedicine and Nanotechnology 2013; 4: 1-6.
12. Parvin T, Keerthiraj N, Ahmed I, Phanichphant S, and Byrappa K: Photocatalytic degradation of municipal wastewater and brilliant blue dye using hydrothermally synthesized surface-modified silver-doped ZnO designer particles. International Journal of Photoenergy 2012; 1-8.
13. Oleg L, Vasilii C, Vasile P , Mahdi A, Beatriz RC, Lee C, Ion T, Bruno V, Thierry P and Rainer A: Silver-doped zinc oxide single nano-wire multifunctional nano-sensor with a significant enhancement in response. Sensors and Actuators B 2016; 893–903.
14. Lu W, Liu G, Gao S, Xing S, and Wang J: Tyrosine-assisted preparation of Ag / ZnO nanocomposites with enhanced photocatalytic performance and synergistic antibacterial activities. Nanotechnology 2008; 19: 445711 - 445720.
15. Sharma N, Kumar J, Thakur S, Sharma S and Shrivastava V: Antibacterial study of silver doped zinc oxide nanoparticles against *Staphylococus aureus* and *Bacillus subtilis*. Drug Invention Today 2013; 5: 50 - 54.
16. Talari MK, Abdul B, Majeed A, Tripathi DK and Tripathy M: Synthesis, characterization and antimicrobial investigation of mechano-chemically processed silver doped ZnO nanoparticles. Chemical and pharmaceutical Bulletin 2012; 60: 818 - 824.
17. Kumar PS and Nesaraj AS: Chemical Precipitation of ZnO Nanoparticles: Antimicrobial activity and *in vivo* sub-acute Nano-toxicological impact on the liver and kidney of swiss albino mice. International Journal of Pharma and Biosciences 2016; 8(1): 232- 237.

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