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DEVELOPMENT OF Se DOPED ZnO NANOPARTICLES: ANTIMICROBIAL ACTIVITY AND *IN VIVO* ACUTE NANOTOXICOLOGICAL IMPACT ON SWISS ALBINO MICE

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Keywords:

Chemical Synthesis, Se doped ZnO nanoparticles, characterization, Antimicrobial studies; *In vivo* studies, Biomedical applications

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ABSTRACT: Selenium nanoparticles are attracting more and more attention due to their excellent photoelectrical characteristics and high biological activity. Selenium is also found to be effective against cancer. ZnO nanoparticles are reported to inhibit the growth of a wide range of pathogenic bacteria under normal visible lighting conditions. In this research work, we report the preparation of a set of Selenium doped ZnO nanoparticles ($Zn_{1-x}Se_xO_{1-\delta}$; where x = 0.05, 0.10, 0.15 and 0.20) by simple chemical synthesis method. The materials were characterized systematically by X-ray Diffraction method, FT-IR spectroscopy, particle size analysis, SEM, EDAX analysis and TEM. The physical characterization studies have revealed the presence of fine particles in the prepared crystalline materials. The prepared materials were also subjected to antimicrobial assay by agar well diffusion method against both Gram positive *(Staphylococcus aureus* and *Bacillus subtilis)* and Gram negative bacteria *(Escherichia coli* and *Klebsiella pneumoniae).* Among studied, the material with the composition the materials $Zn_{0.80}Se_{0.20}O_{1-\delta}$ have exhibited excellent antibacterial activity against the bacteria such as, *Bacillus subtilis* and *Staphylococcus aureus.* Considering the superior antimicrobial activity of $Zn_{0.80}Se_{0.20}O_{1-\delta}$, the study was further extended to assess the potential toxic effect of these materials in Swiss Albino mice through *in vivo* studies (by oral administration). From the histopathological studies, it was found that the mice treated with 2000mg/kgbwt of $Zn_{0.80}Se_{0.20}O_{1-\delta}$ has shown mild central vein congestion in the hepatocytes due to the appropriate acute toxicity of Se doped ZnO nanoparticles at this high dosage level (LD_{50} ~ 2000 mg/kgbwt).

INTRODUCTION: Antibacterial activity of zinc oxide nanoparticles has been widely studied due to their advantages over conventional antimicrobial agents. Also, it is complimented with better stability at wider range of temperatures and pressures, higher self-life, re-usability, ease of storage and transportation [1]. Selenium is essential for the effective operation of the immune system in both animals and humans. It has received considerable attention because of its remarkable biological applications such as anti-cancer, anti-microbial, anti-diabetic and anti-oxidant activities [2]. Selenium nanoparticles are also known for their remarkable applications such as photo degradation, photoconductivity and waste water treatment. It was reported that selenium nanoparticles have good antimicrobial activity against *S. aureus* [4].

Therefore, development of nanostructures with antimicrobial properties are of extensive interest meanwhile doping in ZnO can alter the band-gap, optical, electrical, non-linear optical and magnetic properties. It was found that doping of ZnO with Ce and Fe by microwave method improved the antimicrobial activity [5].

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Apart from this, it was also reported that several doping ways have been used, such as, noble metal doping, transition metal doping, non-metal doping and double elements (simultaneous) doping in ZnO to effectively enhance the carrier concentration and antimicrobial efficiency of ZnO [6]. ZrO_2 doped ZnO has shown improvement in antimicrobial activity against *S. aureus* and *C albicans* [7]. It was found that Cu-doped ZnO nanorod samples have shown superior antibacterial action against gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes* and gram-negative bacteria such as *Escherichia coli* [8]. Among the various metal dopants studied, silver is one of the strongest bactericidal agents that exhibited a broad antibacterial spectrum,[3] and hence, Ag is one of the best candidate as a dopant for ZnO [9]. It was reported that Ag doped ZnO nanorod arrays have shown enhanced antimicrobial efficiency to gram-negative bacteria, *E. coli* and gram-positive bacteria, *S. aureus* [10].

The characteristics of magnetic nanopowders suitable for biomedical application mainly depend on particle size, shape and preparation conditions. Also, it was reported that the incorporation of transition metal impurities into the lattice of ZnO nanopowders can change the structural, optical, magnetic, morphological and antibacterial properties which makes it suitable for application in spintronic devices and biomedicines [11]. It was reported recently that silver nanoparticles can cause damage to human tissues in such cases selenium can replace silver [12].

Even though, good research studies have been reported elaborately regarding the anti-bacterial effect of ZnO nanoparticles doped with different dopants including silver but no clear reports are available based on the antimicrobial and *in vivo* toxicity studies with selenium doped ZnO nanoparticles. The amount of precursor materials used for the synthesis is given in **Table 1**. Hence, in this research work attempts have been made to develop a set of selenium doped ZnO nanoparticles by a simple chemical synthesis route and also to study their antimicrobial behavior against gram positive bacteria such as, *Staphylococcus aureus* and *Bacillus subtilis* and gram negative bacteria such as *Escherichia coli* and *Klebsiella pneumoniae*. Also, a detailed study has been carried out in order to evaluate the potential toxic effect of the selenium doped ZnO nanoparticles through *in vivo* acute toxicity tests in swiss albino mice.

MATERIALS AND METHODS:

Materials: To initiate the synthesis of Selenium doped ZnO nanoparticles ($Zn_{1-x}Se_xO_{1-\delta}$), analytical grade zinc nitrate hexahydrate $Zn(NO_3)_2.6H_2O$ (96-103%, Merck, India), selenium powder (99.5%, Loba, India), sodium hydroxide NaOH (≥ 97%, Merck, India) and ethanol (99.9%, Changshu Yangyuan, China) were used as starting materials for this research without any further purification.

Synthesis of Se doped ZnO nanoparticles: Selenium doped ZnO nanoparticles ($Zn_{1-x}Se_xO_{1-\delta}$; where x = 0.05, 0.10, 0.15 and 0.20) were synthesized using chemical synthesis method. The stoichiometric aqueous solution containing zinc nitrate and sodium hydroxide was prepared separately in double distilled water. Both the above solutions were mixed together using a magnetic stirrer. To which appropriate quantity of selenium powder was added. The entire mixture was mixed thoroughly at room temperature for about 3 hours in the same magnetic stirrer set-up. The resultant hydroxide having the selenium powder ($Zn(OH)_2$ + Se) was filtered off and washed thoroughly with double distilled water and ethanol (9:1 v/v). The obtained product was dried at 80 °C for about 10 hours in a hot air oven. The resultant dried product was calcined at 300, 450 and 650 ^oC for 2 hours each to get phase pure brick red coloured Se doped ZnO nanoparticles.

Materials characterization:

Physical Characterization of Materials: The heat treated selenium doped ZnO nanoparticles were characterized by Shimadzu XRD6000 X-ray diffractometer using CuK_α radiation. The lattice parameters were calculated by DOS computer programming. The crystallite sizes of the powder were calculated by Scherrer's formula. The theoretical density of the powders was calculated with the XRD data. FTIR spectrometer (Shimadzu IR Prestige – 21 model) was employed to record the FTIR spectra of materials in the range of 4000 - 400cm^{-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 TEM of the samples was measured by TEM model (The FEI Technai)

Antimicrobial Activity: The antimicrobial activity of the synthesized selenium doped ZnO nanoparticles was performed against Gram positive *(Staphylococcus aureus* and *Bacillus subtilis)* and Gram negative bacteria *(Escherichia coli* and *Klebsiella pneumoniae)* bacteria. The antibacterial activity was done by agar well diffusion method. In brief, the pure cultures of microorganisms were subcultured in nutrient broth at 37 °C on a rotary incubator shaker at 120 rpm overnight. For bacterial growth, about 20 ml of sterile molten nutrient agar was poured in to the sterile petriplates. The plates were swabbed with the overnight culture of concerned pathogenic bacteria. The solid agar medium was gently punctured with the help of cork borer to make a well. Finally, the nanoparticle suspension (100 µl) were added from the stock (10 mg/ml) into each well and incubated for 24 hr at 37 ^{o}C. After 24 hr the zone of inhibition was measured and expressed as millimeter in diameter. Distilled water was used as a blank or negative control.

***In vivo* Acute Oral Toxicological Studies:** Male Swiss Albino mice with 6 to 8 weeks old and average weight of 22 – 30 g were obtained from in-house animal facility, Kerala Veterinary and Animal science University, India and used as experimental animals. The mice were housed in plastic cages, six mice per cage, and maintained in the animal house of Department of Biosciences and Technology, Karunya Institute of Technology and Sciences, India. They were maintained under standard conditions of temperature 23± 2 ^{o}C, relative humidity 50 - 70 % and 12 - h light-dark cycle. Feeding of the animals and water intake were done with standard mouse pellets and water *ad libitum* throughout the experiments as reported [13]. In general, animal handling, from the beginning to the end of the study, was ethically done according to the agreed guidelines of Karunya Institute of Technology and Sciences, India (Ethical clearance number: IAEC/KU/BT/14/17).

Animals were kept for 7 days to allow for acclimatization to the laboratory conditions before commencement of the study. After acclimatizing, the mice were randomly divided into 4 groups:

Group 1 (control group) consists of 6 mice (received water and given no dosage of Se doped ZnO nanoparticles)

Group 2 consists of 6 mice given Se doped ZnO nanoparticles at a dose of 500 mg/kg

Group 3 consists of 6 mice given Se doped ZnO nanoparticles at a dose of 1000 mg/kg

Group 4 consists of 6 mice given Se doped ZnO nanoparticles at a dose of 2000 mg/kg

The dosage level was maintained as per Organization for Economic Cooperation and Development (OECD 425) guidelines for investigating the toxicity of nanoparticles in mice. The selenium doped ZnO nanoparticles ($Zn_{0.80}Se_{0.20}O_{1-\delta}$) were suspended in distilled water and administered orally once to the animals as per the details mentioned above. At the beginning of the study, the weight of animals was recorded; thereafter the body weight was finally recorded at day 14 before the sacrifice. Animals were monitored at intervals of 30 min, 1 h, 2 h, 4 h, 6 h and 12 h on the first day and subsequently twice daily during the course of the treatment, observing any clinical signs of toxicity and possible mortality. At the end of 14 days of observation, animals were sacrificed *via* cervical dislocation. The liver and kidney were harvested, weighed and macro-scopically examined for lesions and / or abnormalities. Both 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 Se doped ZnO ($Zn_{0.80}Se_{0.20}O_{1-\delta}$) nanoparticles for swiss albino mice by a single oral ingestion.

RESULTS AND DISCUSSION:

XRD Studies: The X-ray diffraction (**Fig. 1**) of the as-synthesized Se doped ZnO nanoparticles ($Zn_{1-x}Se_xO_{1-\delta}$; where x = 0.05, 0.10, 0.15 and 0.20) revealed the characteristic peaks at (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), (202), (104) and (203) planes [20] corresponding to the hexagonal wurtzite phase of ZnO (JCPDS card No: 89-1397) as reported [14, 18].

The peaks' intensity is very sharp and narrow, indicating the materials are of high quality and good crystallinity and fine grain size [15]. Furthermore, no impurity phases were observed in the XRD patterns, as all of the four samples showed single-phase sample formation. The average crystallite size of the nanomaterials is estimated according to Debye-Scherer formula [19]. The crystallite size of the Se doped ZnO nanoparticles is found in the range of ~ 17 nm. However, the crystallite size of Ag doped ZnO is reported as 33 nm [6]. The lattice parameters 'a' and 'c', volume of the unit cell and theoretical density were also calculated. The calculated structural parameters are given in **Table 2**. It is found that the calculated values of lattice constants 'a' and 'c', volume of the unit cell and theoretical density are almost consistent with that of standard JCPDS card of ZnO. Also, it is found that the doping of Se in ZnO is not influencing the crystallographic parameters much.

TABLE 1: AMOUNT OF PRECURSOR MATERIALS (DISSOLVED IN 100 ML OF WATER EACH EXCEPT Se) USED FOR THE PREPARATION OF Se DOPED ZnO NANOPARTICLES BY CHEMICAL SYNTHESIS METHOD

Sample	Concentration of $Zn(NO_3)_2$(M) / Weight (g)	Concentration of NaOH (M) / Weight (g)	Weight of Se (g)
$Zn_{0.95}Se_{0.05}O_{1-\delta}$	0.95 /28.25	1.90/7.6	0.39
$Zn_{0.90}Se_{0.10}O_{1-\delta}$	0.90 /26.77	1.80 /7.2	0.78
$Zn_{0.85}Se_{0.15}O_{1-\delta}$	0.85 /25.28	1.70/6.8	1.18
$Zn_{0.80}Se_{0.20}O_{1-\delta}$	0.80 /23.79	1.60 /6.4	1.57

TABLE 2: CRYSTALLOGRAPHIC PARAMETERS OBTAINED ON Se DOPED ZnO NANOPARTICLES PREPARED BY CHEMICAL SYNTHESIS 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 c = 5.213	47.77	--	16.97
$Zn_{0.95}Se_{0.05}O_{1-\delta}$	Hexagonal	a = 3.257 c = 5.214	47.89	16.26	17.06
$Zn_{0.90}Se_{0.10}O_{1-\delta}$	Hexagonal	a = 3.251 c = 5.210	47.68	17.79	17.28
$Zn_{0.85}Se_{0.15}O_{1-\delta}$	Hexagonal	a = 3.256 c = 5.215	47.87	17.28	17.35
$Zn_{0.80}Se_{0.20}O_{1-\delta}$	Hexagonal	a = 3.253 c = 5.211	47.75	17.92	17.54

TABLE 3: ELEMENTAL COMPOSITION DATA OBTAINED ON Se DOPED ZnO NANOPARTICLES BY EDAX ANALYSIS

Samples	Atomic wt.% of elements
$Zn_{0.95}Se_{0.05}O_{1-\delta}$	Zn – 55.96 O – 43.72 Se – 0.32
$Zn_{0.90}Se_{0.10}O_{1-\delta}$	Zn – 48.71 O – 50.87 Se – 0.42
$Zn_{0.85}Se_{0.15}O_{1-\delta}$	Zn – 54.43 O – 45.08 Se – 0.50
$Zn_{0.80}Se_{0.20}O_{1-\delta}$	Zn – 49.28 O – 50.11 Se – 0.61

FTIR Studies: In order to know the vibrational bands present in our samples, we have recorded the FTIR spectra at room temperature. As already mentioned FTIR spectra were recorded in solid phase using KBr pellet as reference in the wave length region 4000 - 400 cm^{-1}. **Fig. 2** is a typical FTIR spectra of the prepared Se doped ZnO nanopowders. We have observed a strong absorption band at 433 - 527 cm^{-1} which may be assigned to the M-O stretching band which is consistent with that reported elsewhere [16]. The peaks observed at 1020 and 1625 cm^{-1} can be

attributed to aromatic C=C stretching mode. The peak appeared at 1383 cm^{-1} may be attributed to the symmetric stretching (C-O) of carbonyl group. The adsorption at 871 cm^{-1} is due to the formation of tetrahedral co-ordination of Zn [17].

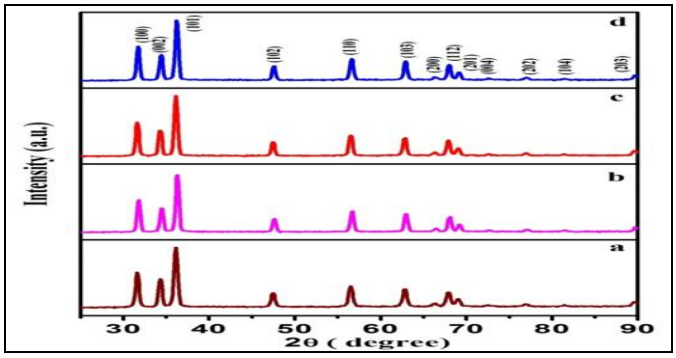

FIG. 1: X-RAY DIFFRACTION OF THE AS-SYNTHESIZED Se DOPED ZnO NANOPARTICLES PREPARED BY CHEMICAL SYNTHESIS METHOD (a) $Zn_{0.95}Se_{0.05}O_{1-\delta}$; (b) $Zn_{0.90}Se_{0.10}O_{1-\delta}$; (c) $Zn_{0.85}Se_{0.15}O_{1-\delta}$; (d) $Zn_{0.80}Se_{0.20}O_{1-\delta}$

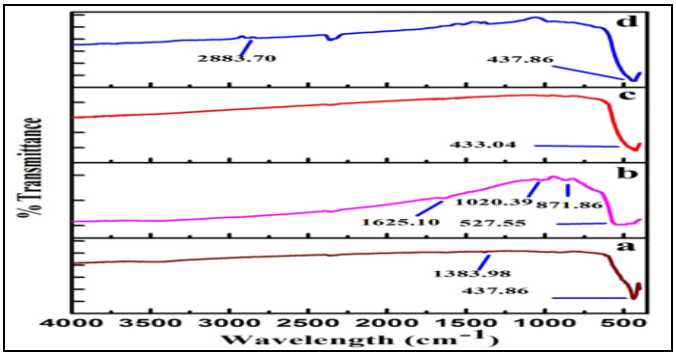

FIG. 2: FTIR SPECTRA OBTAINED ON THE Se DOPED ZnO NANOPARTICLES PREPARED BY CHEMICAL SYNTHESIS METHOD (a) $Zn_{0.95}Se_{0.05}O_{1-\delta}$; (b) $Zn_{0.90}Se_{0.10}O_{1-\delta}$; (c) $Zn_{0.85}Se_{0.15}O_{1-\delta}$; (d) $Zn_{0.80}Se_{0.20}O_{1-\delta}$

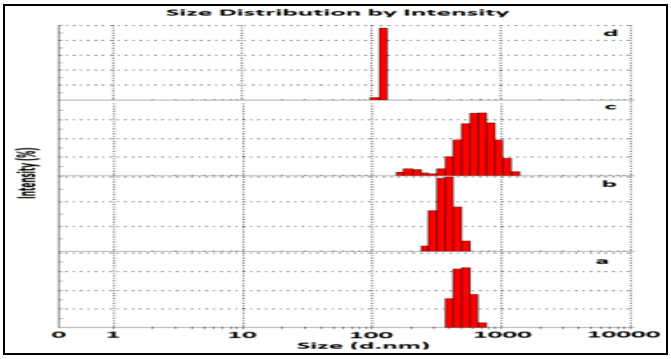

FIG. 3: PARTICLE SIZE CHARACTERISTIC CURVES OBTAINED ON THE Se DOPED ZnO NANOPARTICLES PREPARED BY CHEMICAL SYNTHESIS METHOD (a) $Zn_{0.95}Se_{0.05}O_{1-\delta}$; (b) $Zn_{0.90}Se_{0.10}O_{1-\delta}$; (c) $Zn_{0.85}Se_{0.15}O_{1-\delta}$; (d) $Zn_{0.80}Se_{0.20}O_{1-\delta}$

Particle Size Analysis: Fig. 3 shows the particle size characteristic curves obtained on Se doped ZnO nanoparticles. Particle size measurements based on dynamic light scattering is the powerful tool to characterize the nanomaterials in solutions, because of Brownian motion of particles. The as synthesized particles were dispersed in triple distilled water in a sonicator before subjecting them for the particle size distribution analysis. The particle size distribution was recorded in the different parameters, in which the prepared nanoparticles distributed in the range of 100 - 500 nm. The average particle size obtained in this techniques correlates well with the SEM analysis.

SEM Measurements: Fig. 4 exhibits the SEM images of the Se doped ZnO nanoparticles. From the SEM results, it was noted that the presence of grains in different sizes and shapes in the samples irrespective of dopant concentration levels in ZnO. The grain size of the samples was found in between the range of 50 -150 nm. Also, it was found that the grains are mostly spherical in shape and aggregates into bigger particles with different morphologies. The presence of different shapes and bigger grains may be due to the agglomeration effect of particles by the absorption of moisture present in the environment.

EDAX Analysis: Fig. 5 shows the energy-dispersive X-ray spectroscopy (EDAX) spectra of Se doped ZnO nanoparticles. The spectra of all the samples have shown the signal peaks corresponding to Zn, Se and O elements only. The appropriate atomic weight % of Zn, Se and O present in each sample is indicated in **Table 3**. From the results, it was confirmed that no other impurity element present in the samples. Also, the purity of the samples is confirmed by EDAX analysis.

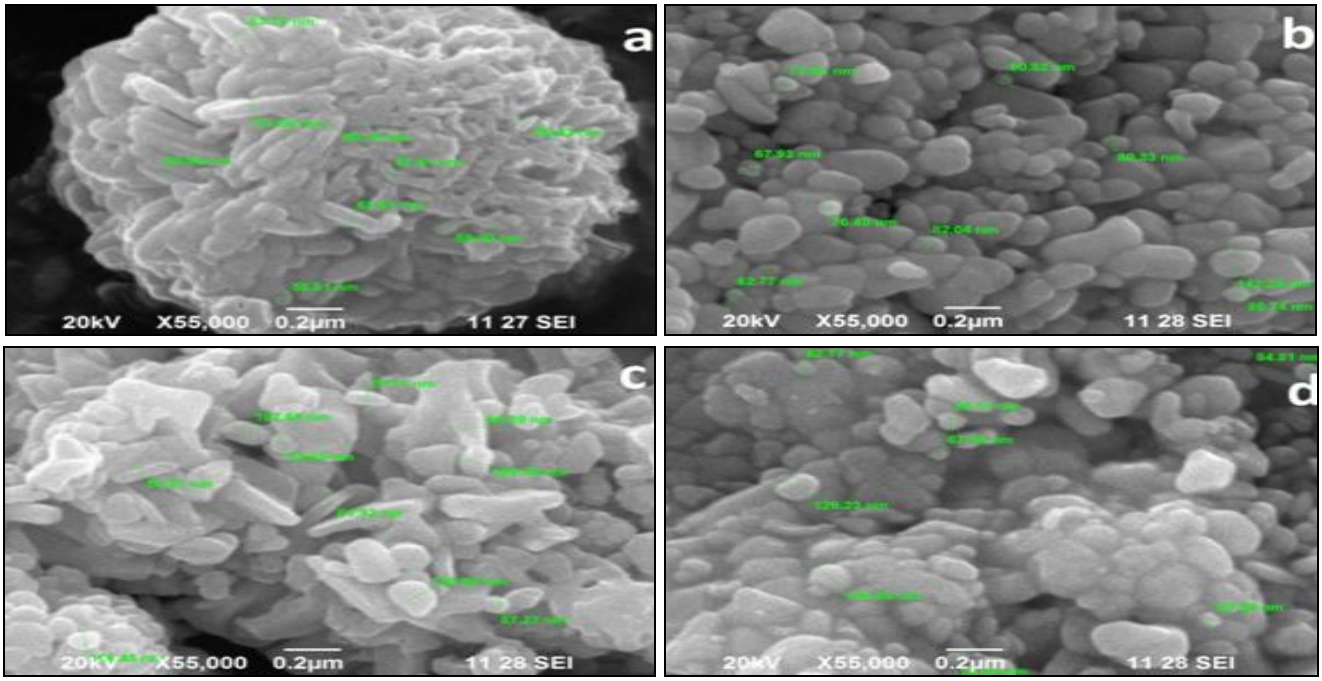

FIG. 4: SEM PHOTOGRAPHS OBTAINED ON THE Se DOPED ZnO NANOPARTICLES PREPARED BY CHEMICAL SYNTHESIS METHOD (a) $Zn_{0.95}Se_{0.05}O_{1-\delta}$; (b) $Zn_{0.90}Se_{0.10}O_{1-\delta}$; (c) $Zn_{0.85}Se_{0.15}O_{1-\delta}$; (d) $Zn_{0.80}Se_{0.20}O_{1-\delta}$

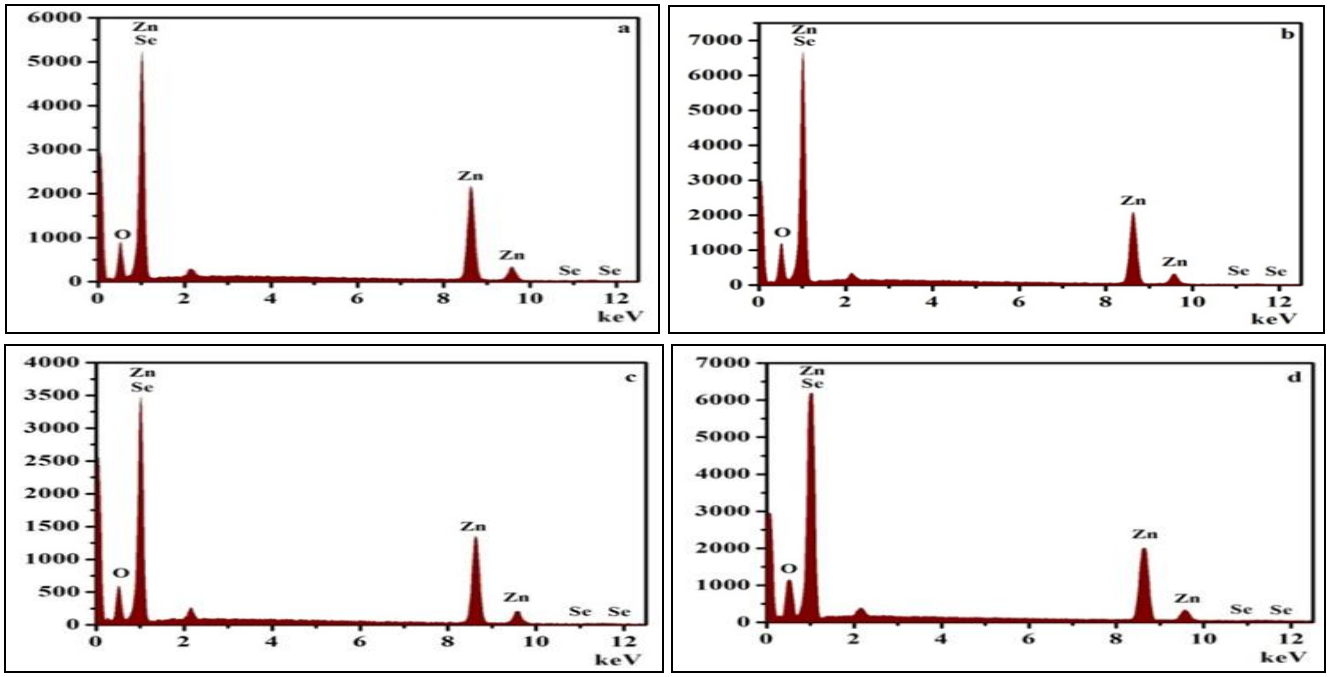

FIG. 5: EDAX SPECTRA OBTAINED ON THE Se DOPED ZnO NANOPARTICLES PREPARED BY CHEMICAL SYNTHESIS METHOD (a) $Zn_{0.95}Se_{0.05}O_{1-\delta}$; (b) $Zn_{0.90}Se_{0.10}O_{1-\delta}$; (c) $Zn_{0.85}Se_{0.15}O_{1-\delta}$; (d) $Zn_{0.80}Se_{0.20}O_{1-\delta}$

TEM Studies: The TEM images of the $Zn_{0.80}Se_{0.20}O_{1-\delta}$ nanoparticles are shown in **Fig. 6(a, b, c** and **d**). The TEM images exhibited sphere like grains in the sample. Also, the grains are present jointly with other grains. The grain size of the sample is found to be in the range of 50 - 200 nm.

Antimicrobial Studies: Comparative analysis of the antibacterial efficiency of Se doped ZnO nanoparticles ($Zn_{0.95}Se_{0.05}O_{1-\delta}$, $Zn_{0.90}Se_{0.10}O_{1-\delta}$, $Zn_{0.85}Se_{0.15}O_{1-\delta}$ and $Zn_{0.80}Se_{0.20}O_{1-\delta}$) carried out using agar well diffusion method is shown in **Fig.7**. It reveals that all the samples are effective antibacterial agents on both Gram positive *(Staphylococcus aureus* and *Bacillus subtilis)* and Gram negative bacteria *(Escherichia coli* and *Klebsiella pneumoniae) bacteria*.

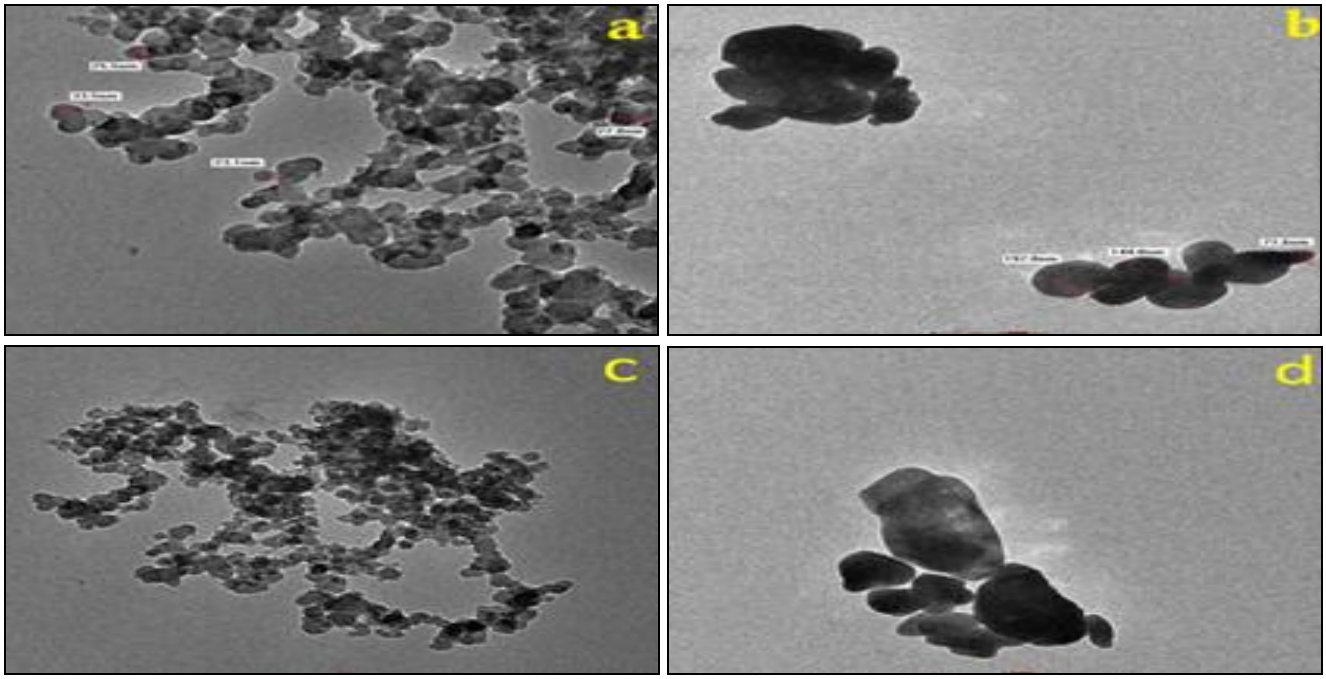

FIG. 6: (a, b, c AND d) TEM IMAGES OBTAINED ON $Zn_{0.80}Se_{0.20}O_{1-\delta}$ NANOPARTICLES PREPARED BY CHEMICAL SYNTHESIS METHOD

However, the percentage of reduction in bacterial growth was found to be significantly higher for the sample, $Zn_{0.80}Se_{0.20}O_{1-\delta}$. Among the four samples studied, $Zn_{0.80}Se_{0.20}$ $O_{1-\delta}$ maintained considerably higher anti-microbial activity against bacteria, such as, *Bacillus subtilis* and *Staphylococcus aureus*. Such good performance of $Zn_{0.80}Se_{0.20}O_{1-\delta}$ can possibly be attributed due to the presence of higher concentration of Se(20 mole%) in the sample.

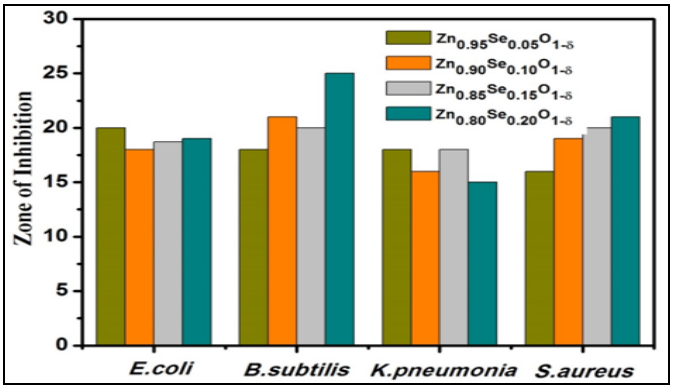

FIG. 7: ANTIMICROBIAL ACTIVITY OF Se DOPED ZnO NANOPARTICLES AGAINST BACTERIA: *E. COLI* (a); *B. SUBTILIS* (b); *K. PNEUMONIAE* (c); AND *S. AUREUS* (d)

However, the same sample did not show effective antibacterial activity against *Escherichia coli* and *Klebsiella pneumoniae.* It may be inferred that the reduction of antibacterial activity of $Zn_{0.80}Se_{0.20}O_{1-\delta}$ against *Escherichia coli* and *Klebsiella pneumoniae* may be due to the possibility of higher concentration of Se acting as additional micronutrients for further bacterial growth and this resulted in poor performance in the test [1]. However, it was found that the sample, $Zn_{0.80}Se_{0.20}O_{1-\delta}$ could show maximum zone of inhibition against the bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) than the other samples studied. Hence, further study was explored using this sample in our next phase of research.

Acute Histopathological Studies: As indicated in the experimental section, the mice were administered with Se doped ZnO nanoparticles (Zn0.80Se0.20O1-δ) in order to study the nanotoxicological effect in a systematic manner. After oral administration, their clinical signs and mortality were monitored on daily basis. The treated mice didn't exhibit any mortality, body weight or behavioral change or toxicity compared to the control group until 14 days of post-oral injection. From the studies, it was found that there was no sign of tremor, convulsion, salivation, diarrhea, lethargy or skin symptoms in the treated mice. However, a slight abnormal behaviour was noticed in the mice treated with 2000 mg/kg body weight (b.w.). On the 15th day, the mice were sacrificed and their organs such as liver and kidney were taken from them by cervical dislocation process for histopathological assessment.

Then representative blocks of liver and kidney tissues from each lob were taken and possessed for paraffin embedding using the standard microtechnique. Sections of (~5 µm) of livers and kidneys stained with hemotoxylin and eosin were observed microscopically for the evaluation of acute histopathological changes. The acute histo-pathological observations of liver and kidney of mice after single oral administration at different dosage levels, such as, control, 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 respectively.

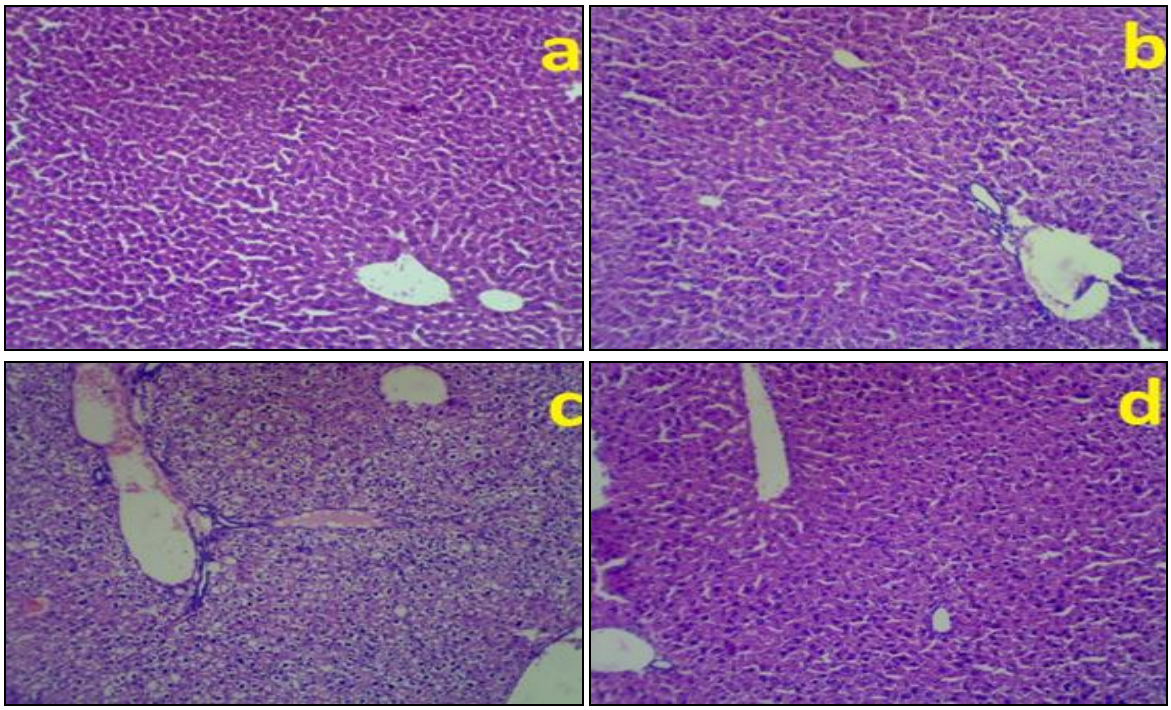

FIG. 8: ACUTE HISTOPATHOLOGICAL FINDINGS OF LIVER IN MICE AFTER ORAL ADMINISTRATION OF Se DOPED ZnO NANOPARTICLES ($Zn_{0.80}Se_{0.20}O_{1-\delta}$); (a) WITH CONTROL I.E., WITH NO DOSAGE OF NANOPARTICLES (SHOWS NORMAL MORPHOLOGY OF HEPATOCYTES); (b) TREATED WITH 500 mg/kg BODY WEIGHT (B.W.) (SHOWS ALTERED HEPATOCYTES ARCHITECTURE); (c) TREATED WITH 1000 mg/kg BODY WEIGHT (B.W.) (SHOWS MICROVESICULAR STEATOSIS); (d) WITH 2000 mg/kg BODY WEIGHT (B.W.) (SHOWS CENTRAL VEIN CONGESTION)

From **Fig. 8** (**a, b, c,** and **d**), the following observations were made. The acute histo-pathological examination of liver (with no dosage level) shows normal lobular architecture and portal traid shows normal morphology. The liver treated with 500mg/kg of dosage level shows altered hepatocytes and binucleation in the central vein when compared with control. The livers treated with 1000 and 2000 mg/kg dosage levels show micro vesicular steatosis and central vein congestion respectively when compared to control. From **Fig. 9** (**a, b, c,** and **d**), 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 treated with 500 mg/kg dosage level shows interstitium and shows normal when compared with control. The kidneys of mice treated with 1000 and 2000 mg/kg dosage levels exhibit mild interstitial lymphocytic infiltration and mild mesangial hyper cellularity when compared with control. From the acute histopathological studies, it was found that the orally administered Se 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 behavior or symptoms such as decrease in food and water intake, diarrhea, loss of movement and change in size of eyes were observed in mice treated with 500, 1000 mg/kg dosage levels of Se doped ZnO nanoparticles after 14 days of oral

injection indicating that LD_{50} values will be more than 2000 mg/kg of body weight (b.w.). However the mice treated with 2000mg/kg body weight resulted in abnormal characteristics when compared with control mice.

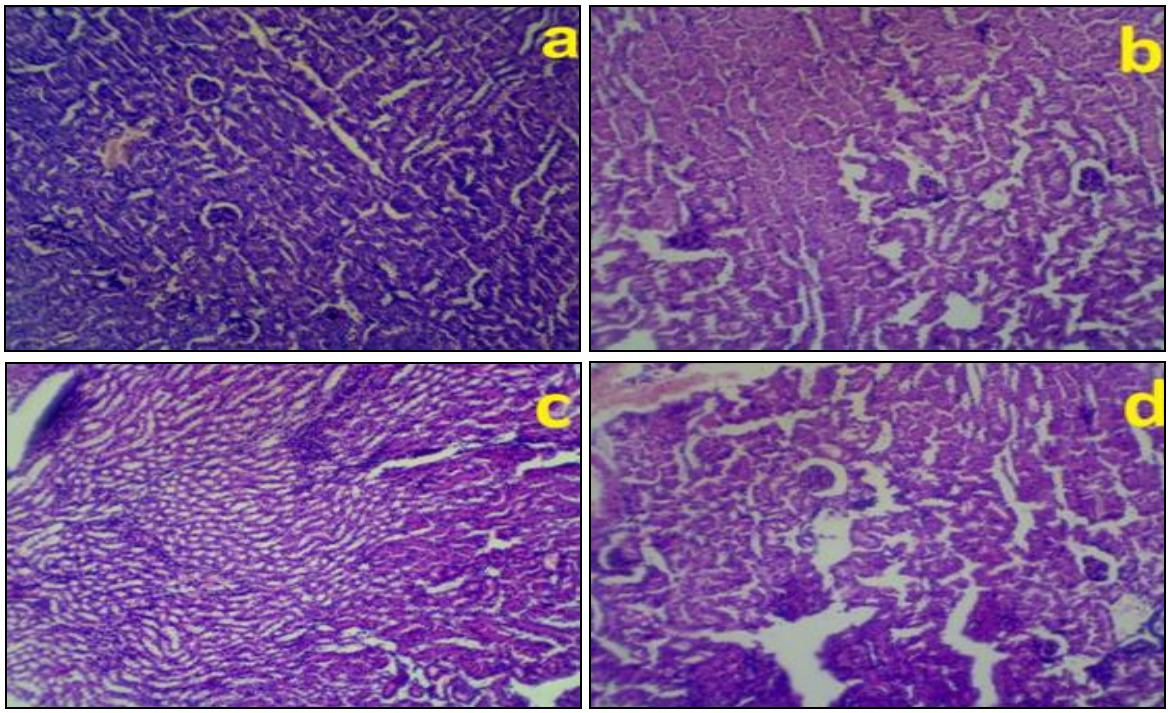

FIG. 9: ACUTE HISTOPATHOLOGICAL FINDINGS OF MICE KIDNEY AFTER ORAL ADMINISTRATION OF Se DOPED ZnO NANOPARTICLES ($Zn_{0.80}Se_{0.20}O_{1-\delta}$); (a) WITH CONTROL I.E. WITH NO DOSAGE OF NANOPARTICLES (SHOWS NORMAL CORTEX AND MEDULLA); (b) WITH 500 mg/kg BODY WEIGHT (B.W.) (SHOWS NORMAL INTERSTITIUM); (c) WITH 1000 mg/kg BODY WEIGHT (B.W.) (SHOWS MESANGIAL HYPERCELLULARITY AND MILD LYMPHOCYTIC INFILTRATION); (d) WITH 2000 mg/kg BODY WEIGHT (B.W.) (SHOWS MILD MESANGIAL HYPERCELLULARITY)

CONCLUSION: The present study revealed the preparation and characterization of Se doped ZnO nanoparticles by simple chemical synthesis method. The XRD results indicated that the synthesized materials are having hexagonal wurtzite structure The FTIR results exhibited the stretching mode of M-O in the samples. The particle size data inferred the presence of particles between 100 -500 nm. Higher particle size may be due to the agglomeration effect.

SEM microstructures confirmed the presence of grains in the range of 50 to 150 nm in all the samples. The EDAX spectra exhibited the presence of elements as per the stoichiometric composition in the samples. TEM images ($Zn_{0.80}Se_{0.20}O_{1-\delta}$) exhibited sphere like grains in the sample. Also, the grains were present jointly with other grains.

Among the samples studied, $Zn_{0.80}Se_{0.20}O_{1-\delta}$ maintained considerably higher anti-microbial activity than the other samples when tested with bacteria. From the histopathological studies, it was confirmed that there was no mortality and abnormal behavior in mice treated with $Zn_{0.80}Se_{0.20}O_{1-\delta}$ at different dosage levels after 14 days of oral injection indicating that LD_{50} values greater than 2000 mg/kg of body weight (b.w.).

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