Supporting information

Biochemical-, Biophysical- and Microarray-Based Antifungal Evaluation of the Buffer-Mediated Synthesized nano Zinc Oxide: An in Vivo and in Vitro Toxicity Study

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1. Materials and Methods

1.1. Materials.

Zinc acetate dihydrate [$Zn(OAc)_2,2H_2O$], Tris(hydroxymethyl)aminomethane [$(HOCH_2)_3CNH_2$] (TRIS buffer), Ethanol, Glutaraldehyde were purchased from Merck (India); Potato Dextrose Agar (PDA) and Agar were purchased from Himedia (India). All the chemicals were of analytical grade and used without further purification. Milli-Q grade water (Sartorious Stedim biotech) was used throughout the experiment with conductivity less than 0.1 μ S cm^{-1}.

2. Physicochemical Characterization of ZNPs.

Hydrodynamic radius and zeta potential of ZNPs was analyzed using dynamic light scattering (DLS) in Malvern Zetasizer. UV-VIS absorbance of ZNPs was measured in a Shimadzu (model no. 1760) UV-VIS spectrometer. Powder sample XRD of ZNPs was carried out in Bruker D8 instrument applying a primary beam monochromator to select the Kα1 component of the employed copper radiation (wavelength of 1.54056 Å). FTIR spectra were recorded in Perkin-Elmer Spectrum 100. Photoluminescence spectrum (PL) was recorded in Hitachi spectrofluorimeter (model no: F-7000). The topography of ZNPs was obtained by Atomic Force Microscope (AFM) of NTEGRA, NT-MDT (TS-150) in tapping mode. Morphology of the synthesized ZNPs was studied in SUPRA 40 Field Emission Scanning Electron Microscope (FESEM) of Carl Zeiss, operated at acceleration voltage of 5.0 kV; EDX analysis was carried out with the same instrument. Particle size of ZNPs was obtained from High Resolution Transmission Electron Microscope (HR-TEM) (FEI) at an operational voltage of 200 kV. Stability of the ZNPs with increase in temperature through a wide range was obtained from TG-DTA analysis carried out in Diamond TG/DTA pyris series with a heating rate of 10ºC per minute under nitrogen atmosphere. Confocal microscopic image was acquired by using Nikon A1 confocal mmicroscope at 540 nm excitation wave length. ICPMS analysis was carried out on Perkin Elmer ELAN DRC-e instrument.

3. Experimental

3.1. Determination of ROS: superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase activity assay.

Super oxide dismutase activity was measured by its ability to inhibit photochemical reduction of NBT at 560 nm following the method of Beauchamp *et al*., 1971[1]. One enzyme unit of SOD was defined as that amount of protein (in mg) causing a 50% inhibition of photoreduction. Catalase activity was estimated according to Bergmeyer, 1970[2] which checked out the initial rate of disappearance of H_2O_2

at 240 nm. Ascorbate peroxidase activity was measured following Nakano *et al*., 1981[3] which depended on the decrease in absorbance at 290 nm as ascorbate was oxidized. Glutathione reductase enzyme activity was evaluated spectrophotometrically at 25°C[4]. One enzyme unit of glutathione reductase was defined as the oxidation of 1 mmol NADPH per min under the assay condition.

3.2. Total thiol (–SH) content assay.

Total thiol content was determined by a modified method of Ellman, 1959[5]. Briefly 0.5 g of fungal mycelia (grown at different ZNPs concentrations along with control) for each sample was taken and ground using a cold mortar and pestle. Then 4mL of 50mM ice-cold phosphate buffer (pH 7.0) was added in each sample into the mortar and mixed well. The homogenate was centrifuged at 2000g for 20min at 4ºC. 3 mL of the supernatant was mixed with 2 mL of phosphate buffer (pH7.0) and 5mL distilled water and they were mixed well to get 10 mL reaction mixture. 20 µL of 0.01M DTNB solution was added to 3mL of the reaction mixture, shaken well and absorbance was recorded at 412 nm. Thiol content was calculated using extinction coefficient 13, 600 M^{-1} cm^{-1} for DTNB at 412 nm.

3.3. Lipid peroxidation assay.

Malondialdehyde (MDA) content, final product of lipid peroxidation was measured following the slightly modified method of Dhindsa *et al*.,1981[6]. Fungal extracts were prepared following the same method of extraction for thiol content assay. Then 0.5 mL supernatant of fungal extract was added to 1 mL of 20% (v/v) trichloroaceticacid and 0.5% (v/v) thiobarbituric acid. The mixture was heated in a water bath at 95°C for 30min followed by cooling at room temperature. After centrifugation at 10, 000g for 10min, the supernatant was used for absorbance measurements at 532 and 600 nm respectively. The absorbance for non specific absorption at 600nm was subtracted from the value at 532 nm. The amount of MDA was calculated using the adjusted absorbance and the extinction coefficient 155 mM^{-1} cm^{-1} [7].

3.4. Proline assay.

Proline assay was evaluated following the modified method of Chinard, 1952[8]. Control and ZNPs treated fresh mycelia were ground with an ice-cold mortar and pestle and extracted with 4 mL of 3 % sulfosalicylic acid. After homogenization and centrifugation at 2000 g for 20 min, 2 mL of the supernatants were pipetted out into a10 mL screw capped glass test tube. 2 mL glacial acetic acid and 2mL acid-ninhydrin reagent were added to the supernatants. After heating to 100°C for 45 min these screw capped tubes were placed in an ice bath to cool to room temperature. Then 4 mL toluene was added to each tube and shaken vigorously for 2 min. Then samples were allowed to stay for 15 min for complete phase separation. Upper toluene layer was separated and kept in room temperature for 10

min and the red colour intensity was read at 520 nm against toluene blank. The proline concentration was determined from a standard curve and calculated on a fresh weight basis (mM proline/gFW).

3.5. Measurement of protein oxidation by DNPH assay.

Protein carbonyl content was a marker of oxidative stress which was measured spectrophotometrically following slightly modified DNPH binding assay[9]. All fungal strains were cultured in control and ZNP treated PDB; then incubated at 28ºC-30ºC with vigorous shaking for 48 h to form mycelial balls. After 48 h of incubation mycellial balls were isolated from culture and washed with 50 mM phosphate buffer (pH 7.0). The sample balls were then freeze dried with liquid nitrogen and ground with an ice-cold mortar pestle using 8 mL of 50 mM phosphate buffer (pH 7.0). The homogenates were centrifuged at 5000 rpm for 20 minutes at 4°C. The supernatants were incubated with DNPH for 1 h at 37°C; proteins were precipitated in 10% cold TCA and washed with ethanol: ethyl acetate (1:1) to remove excess DNPH. Finally samples were dissolved in 6 (M) guanidine chloride (pH 2). The optical density was measured at 380 nm and the carbonyl content was calculated using a molar extinction coefficient of 21 mM^{-1} cm^{-1}, resulting in final measurement in nanomoles of DNPH incorporated (protein carbonyls) per mg of protein.

3.6. Fungal protein content study by FTIR spectra.

For FTIR analysis fungal strain (*A. niger*, MTCC -10180) was cultured as described in DNPH binding assay up to washing of mycellial ball with phosphate buffer (pH 6.8-7.0). Then mycelial balls were washed successively with deionized water and 40% ethanol. After that all the samples (both control and treated) were sonicated for 30 minutes to rupture the fungal cell wall. Finally FTIR data were taken from the liquid samples (2-3 µL) after successful air drying[10].

3.7. Microarray analysis of ZNPs treated *A. niger*.

Normalization (Percentile shift) was done using GeneSpring GX Version 11.5 using recommended Per Chip and Per Gene Normalization. Percentile shift normalization is a global normalization, where the locations of all the spot intensities in an array are adjusted. This normalization took each column in an experiment independently, and computed the percentile of the expression values for this array, across all spots (where n has a range from 0-100 and n=50 is the median). It subtracted this value from the expression value of each entity.

3.8. Detection of Protein Carbonylation by Westernblotting.

Control and treated fungal samples were ground in a chilled mortar (0.5 g fresh weight ml^{-1}) in protein extraction buffer (PEB) containing 100 mM Tris-HCL, pH 8.0, 2 %(v/v) 2 mercaptoethanol, 5 mM

EGTA, 1 mM EDTA, 1 mM AEBSF, 10 g ml^{-1} leupeptin, 1 mM *p*-amino-benzamidine, 5 mM 6-aminohexanoic acid, 2 µM E64, 10 µM NaF, 1 mM DPTA and 1 mM BHT. Fungal soluble protein extracts were mixed with two volumes of 10mM DNPH in 2 M HCl at room temperature for 30 minutes with gentle agitation. A control sample was mixed with two volumes of 2M HCl, five volumes of ice-cold phenol (Tris-buffered, pH 7.9) was added to each tube respectively. After vortexing for 1 min, the mixture was centrifuged for 10 min at 10,000g. The upper phase was removed and discarded leaving the interface intact and the phenol phase was re extracted twice with ice-cold Tris-HCl buffer (50 mM, pH 8.0). Five volumes of cold 0.1 M ammonium acetate in methanol was added to the lower phase and incubated at -20ºC overnight. In the following day, resulting pellets were washed three times with 1 ml 0.1 M ammonium acetate in methanol and once with 1 ml cold ethanol. To each pellet, IEF buffer [containing 7 M urea, 2 M thiourea, 4% v/v CHAPS, 65 mM DTT, 20 µl 0.1% bromophenol and 20µl IPG buffer, pH 4-7] was added. The samples were incubated for 2 h at room temperature. Insoluble material was removed by centrifugation at 10000 g for 20 min at 4ºC and the supernantent was transferred in to a fresh tube for gel electrophoresis[11]. SDS-PAGE was performed with 12% gels with a loading of 10µg protein samples in each lane. Resolved proteins were electrophoretically transferred to Immobilon-P (PVDF, Millipore) membranes and oxidized proteins were detected by using anti DNP antibodies [Oxiblot Protein detection kit (Millipore)]

3.9. Bioavailability/ uptake study of ZNPs.

3.9.1. SEM, HR-TEM and Confocal microscopic analysis.

For Scanning Electron Microscopic (SEM) and High Resolution Transmission Electron Microscopic (HR-TEM) the fungal samples (*A. niger*, MTCC-10180) were cultured in PDA medium and after 48 h of inoculation, fungal cultures (*A. niger*, MTCC-10180) were isolated and washed with deionized water several times and fixed with 2% glutaraldehyde solution at 4°C for 2 hrs followed by post fixing of specimen for 2 h with 1% osmium tetroxide solution, the samples were dehydrated with graded ethanol [12]. This sample was directly used for SEM and EDX analysis, gold and carbon coating was avoided. For HR-TEM dehydrated samples were sectioned using cryotome and then subjected to a carbon coated copper grid.

For confocal ZNPs were converted into its fluorescent counterpart by conjugation of rhodamine-B-isothiocyanate (RITC). Synthesis of RITC tagged ZNPs was described in paragraph below. *A. niger* cultured in potato dextrose broth (PDB) was incubated with 500 ppm of RITC tagged ZNPs for 72h and fungal samples were isolated and prepared using standard protocol as mentioned above[12]. Finally the samples were fixed with glue (Tissue-Tek, Sakura) and observed under confocal microscope.

3.9.2. Synthesis of RITC tagged ZNPs.

RITC tagged ZNPs were synthesized in two steps, first one involved the conversion of ZNPs into amine functionalized ZNPs while the second step involved conjugation of RITC to amine functionalized ZNPs.

(a) Synthesis of amine functionalized ZNPs.

Amine functionalization of ZnPs was carried out by using 3-aminopropyltriethoxysilane (APTES) via co-condensation reaction using our previously reported method[13]. In brief, about 0.5 g of ZNPs were dispersed in about 50 mL of DMSO in a sonication bath for about 1 hour. The resultant dispersion was transferred to a round bottom flask attached with a reflux condenser. To it 400 µL of APTES was added and the solution was refluxed at 120ºC for about 3 hours. After completion of the reaction the resulting nanoparticle was centrifuged at 12000 rpm for about 15 minutes and washed several times with ethanol to remove the unreacted APTES. Finally the product was dried at 60ºC for overnight to produce amine functionalized ZnO nanorod.

(b) Conjugation of RITC to amine functionalized ZNPs.

ZNPs of appropriate concentration were dispersed in 0.1 M $NaHCO_3$ solution, to it 1 mg of RITC dissolved in 2 mL of aqueous DMSO (1:1, v/v) was added instantly and the reaction mixture was stirred at room temperature for 24 h in a dark condition. RITC labelled ZNPs (ZNPs-RITC) were separated by centrifugation at 10000 rpm at 4 ºC. ZNPs-RITC were washed and redispersed in water repeatedly to remove excess of RITC.

3.10. Toxicity study of ZNPs using mice model.

3.10.1. Treatment of mice.

The healthy swiss albino mice, weight about 20–22 g (8-weeks old) were placed in clean polypropylene cages with access to food and water. These cages were maintained in an air-conditioned animal house at 20 ± 28°C, 50–70% relative humidity and 12 h light/dark cycle. The animals were provided with commercial rat pellet diet and deionized water. After one week acclimation, the mice were randomly divided into 4 groups, each group consisted of five female and five male mice. Short term toxicity (acute oral toxicity) and long term toxicity (through intravenous injection) study was carried out separately.

3.10.2. Acute oral toxicity study.

Mice were placed separately in polypropylene cages. 0.5, 1.0, 2.0 g/kg body weight were selected doses for ZNPs treatment. The ZNPs were suspended in deionized water and dispersed by ultrasonic vibration for 15 min with before treatment. 1mL of each ZNPs suspension was fed orally. Two weeks later, the animals were sacrificed and the blood was obtained from ophthalmic veins. The brain, heart, lung, liver, spleen, stomach, kidneys, testis or uterus were collected[14].

3.10.3. Long term toxicity study through intravenous injection.

Long term toxicity study was carried out using three different doses of ZNPs and MZnO (100 µg/mL, 250 µg/mL and 500 µg/mL). Mice were injected intravenously through tail vein with 100 µL of each doses of ZNPs and MZnO once in a week for two months time span. After two months mice were sacrificed and blood and major organs were collected separately for biochemical and histopathological analysis.

3.10.2. Biochemical assay of serum and Blood-element test.

The serum was obtained by centrifugation of whole blood at 3000 rpm for 15 min. The serum biochemical levels including lactate dehydrogenase, creatinine, alkaline phosphatase, total protein, total cholesterol, tri-glyceride, uric acid , Bun, S.G.O.T, S.G.P.T and phosphorus were examined [14].

The 0.1 mL of 15 g/L EDTA-Na was pre-added into 1 mL of blood sample and the anticoagulant blood sample was immediately used for the blood element test within 2 h. The blood-elements, including TC (Red blood cell, White blood cell) and DC in % (Neutrophils, lymphocytes, monocytes, eosinophils, basophils) and blood platelets were assayed. The data were expressed as mean ± standard deviation.

3.10.4. Histopathological study.

A small piece of brain, heart, lung, liver, spleen, stomach, kidneys, testis and uterus were fixed by 10% formalin and then embedded into paraffin, sectioned for 5–6-mm thick, and mounted on the glass microscope slides using standard histopathological techniques. The sections were stained with hematoxylin-eosin and examined by light microscopy [Carl Zeiss] [14].

3.10.5. *In vivo* Haemolysis measurement.

For *in vivo* blood toxicity determination healthy young, non pregnant 8-weeks old healthy swiss albino mice about 20–22 g body weight was intravenously injected with 100µL of ZNPs and MZnO (500 µg/mL) through tail vein and the resultant blood was collected after 1hr of injection with respect to a control set. Erythrocyte enriched fraction was centrifuged twice (3,000 rpm, 15 min) at 4°C to

remove the residual plasma and buffy coat. RBCs were washed 3 times with phosphate buffer saline (PBS) (pH 7.4) and resuspended in same buffer to make packed cell volume of ~10% (w/v) as stock. Then haemolysis was carried out by mixing 690µL of the above RBC stock suspension making a total volume 1.5 mL by addition of PBS to maintain 5.5% cell volume for each and every set of experiment. Afterward, the erythrocyte cells were centrifuged (3,000 rpm, 15 min) at 4°C and washed thrice with PBS. Finally haemolysis was monitored UV-VIS spectrophotometrically at 540 nm [15].

3.11. Acetylcholine esterase assay of mice brain.

6-8 weeks old healthy swiss albino female mice (five for each treatment) were selected for this assay. 1 mL of ethanolic solution in ZNPs (2g/kg of body weight) along with an ethanolic control was fed orally to mice for 3 days after 7-10 days of acclimatization. Within 24 h of treatment, frontal cortex, hippocampus and septum of brain were quickly dissected out on a petridish chilled on crushed ice. Then 0.5 g of tissues were weighed and homogenized in 0.1M Phosphate buffer (pH 8). 0.4 mL aliquot of the homogenate was added to a cuvette containing 2.6 mL phosphate buffer (0.1M, pH 8) and 100µL of dithiobis 2 nitro-benzoic acid (DTNB) solution (39.6 mg in 10mL of 0.1 M phosphate buffer with 15 mg of $NaHCO_3$). 20 µL of 75 mM acetylthiocholine iodide was added and change in absorbance was recorded for a period of 10 minutes at intervals of 2 minutes. Change in the absorbance per minute was thus determined at 412 nm. The enzyme activity was calculated using the following formula; R = 5.74 x 10 x A/CO where, R = Rate in moles of substrate hydrolyzed /minute / gm tissue; A = Change in absorbance / min; CO = Original concentration of the tissue (mg / mL)[16].

3.12. Fertility study of mice.

This study was performed in highest dose of *in vivo* toxicity study *i.e.* 2g/kg to parental (F_0 generation) male and female mice. Five pairs of healthy swiss albino mice with uniform weight and age were used for both generations. The females were not pregnant and nulliparous. Animals were assigned to control and treated groups in a random manner to minimize bias. All treated and control animals were acclimated to the study conditions five days before treatment begins. Animals were exposed to the test substance through oral route during the entire study period.

For each mating, a female was placed with a randomly selected single male till the pregnancy occurs and throughout their whole gestational period. Near parturition, pregnant females were caged separately. One male and one female were randomly selected from each litter for mating with another pup of the same group to produce the next generation. The mating procedures for the F_1 males and females were carried out in the same manner as the F_0 parental animals. Each litter was examined as soon as possible after delivery for the number of pups, health of pups. The neonates were carefully observed on postnatal days 0 (day of birth), 4, 7, 14 and 21[17].

3.13. *In vitro* cytotoxicity study using MRC5 cell line.

Viability of MRC-5 cells after ZNPs and MZnO treatment was assessed in triplicate by WST-1 assay with proper controls. Briefly 10 µL of reconstituted WST-1 mixture (WST-1 assay kit: BioVision - K302-500) was added to each well of a 96-well plate. The cells were incubated for 4 h at 37ºC in a CO_2 incubator. Before reading the absorbance, the plates were kept on an orbital shaker for 1 min for gentle, homogeneous mixing of the colour. The absorbances of the treated and untreated cells were measured using a microtiter plate reader at 450 nm[18].

The extent of cytotoxicity of ZNPs and MZnO on MRC-5 cells was estimated in triplicate in 96-well plate by measurement of LDH released from damaged cells into the medium (LDH assay kit: Cayman chemical - 1008882). In brief, cell culture plates were centrifuged at 400 X g for 5 minutes. 100 µL of supernatant from each well of treated and control cells were transferred to corresponding wells in a new 96-well plate. 100 µL of reconstituted reaction mixture was added to each well using multi channel pipetman. The plate was incubated at room temperature with gentle shaking on an orbital shaker for 30 minutes. The absorbance was taken at 490 nm in a microtiter plate reader. In WST and LDH assay data were expressed as percentage of cells alive and percentage of cytotoxicity respectively in control and treated wells[18].

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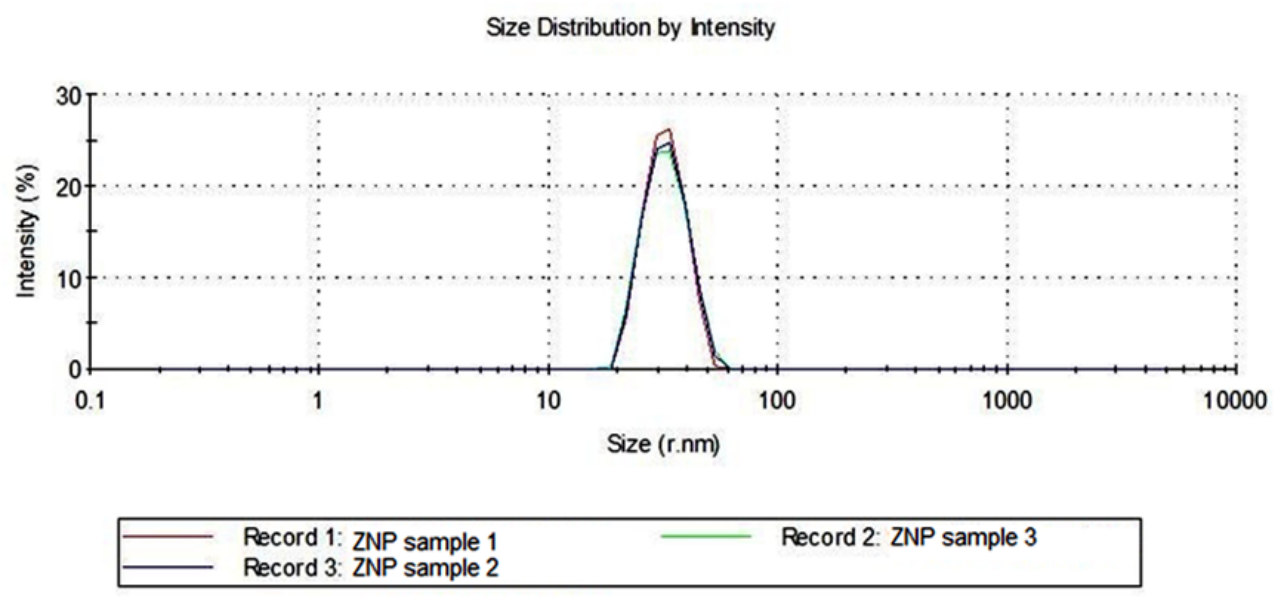
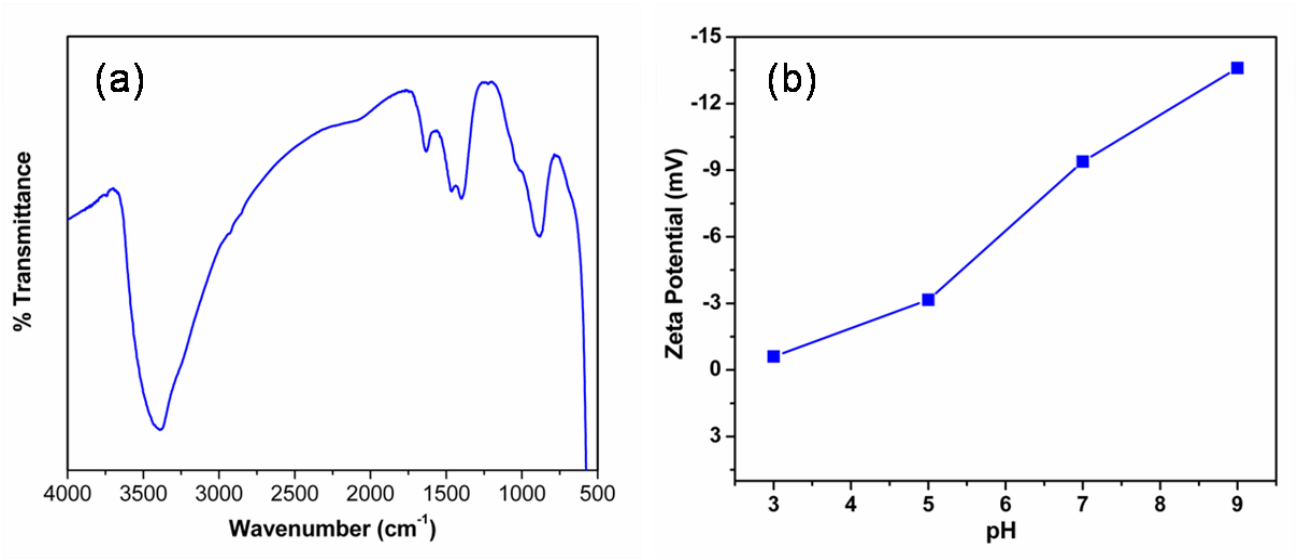
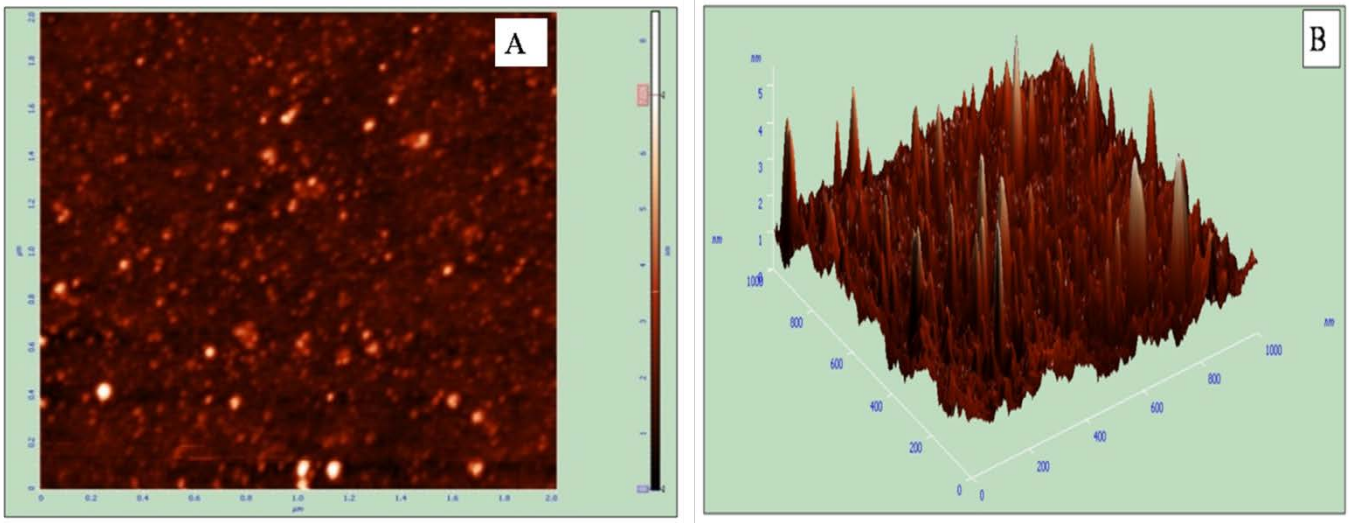


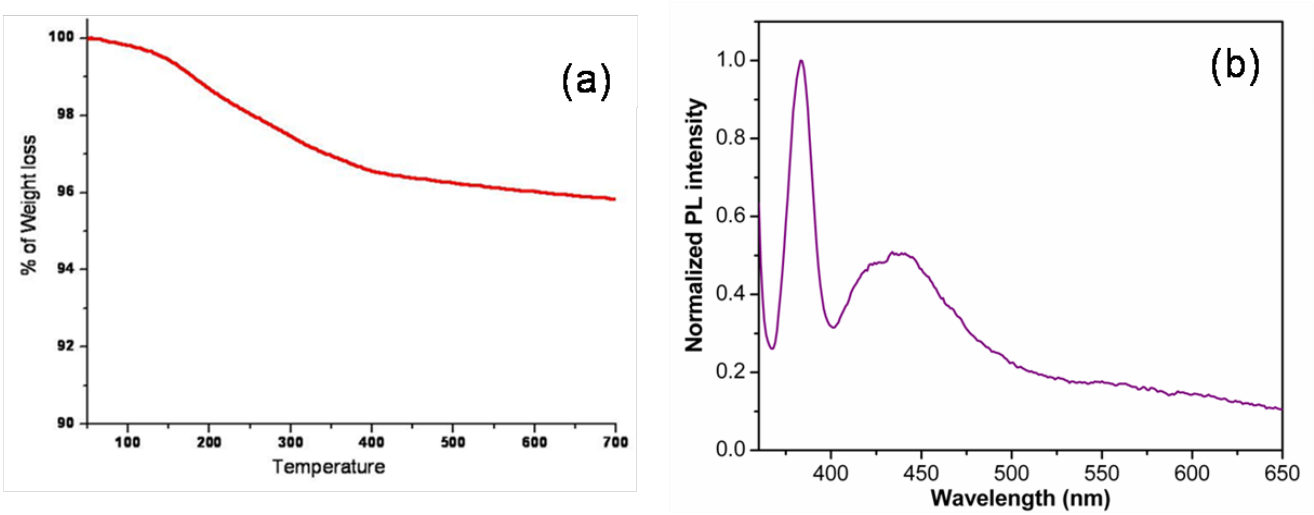
Supporting Figure S1. Measurements of hydrodynamic radius of ZNPs by using DLS.



Supporting Figure S2. (a) FTIR spectra of ZNPs; (b) Measurement of zeta potential of ZNPs as a function of pH.



Supporting Figure S3. Topological attributes of ZNPs as determined from AFM studies (a)1D and (b) 3D view.



Supporting Figure S4. (a) Thermal decomposition patterns of ZNPs using thermogravimetric analysis (TG), (b) PL spectral analysis of ZNPs.

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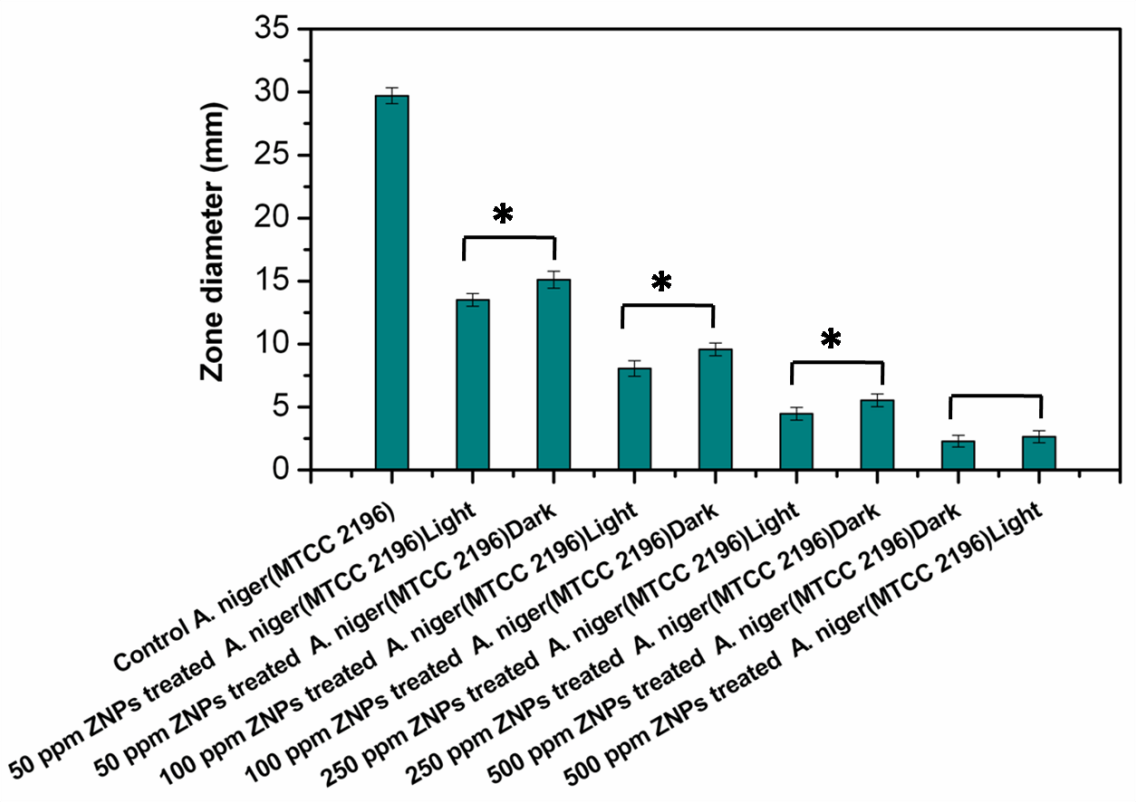
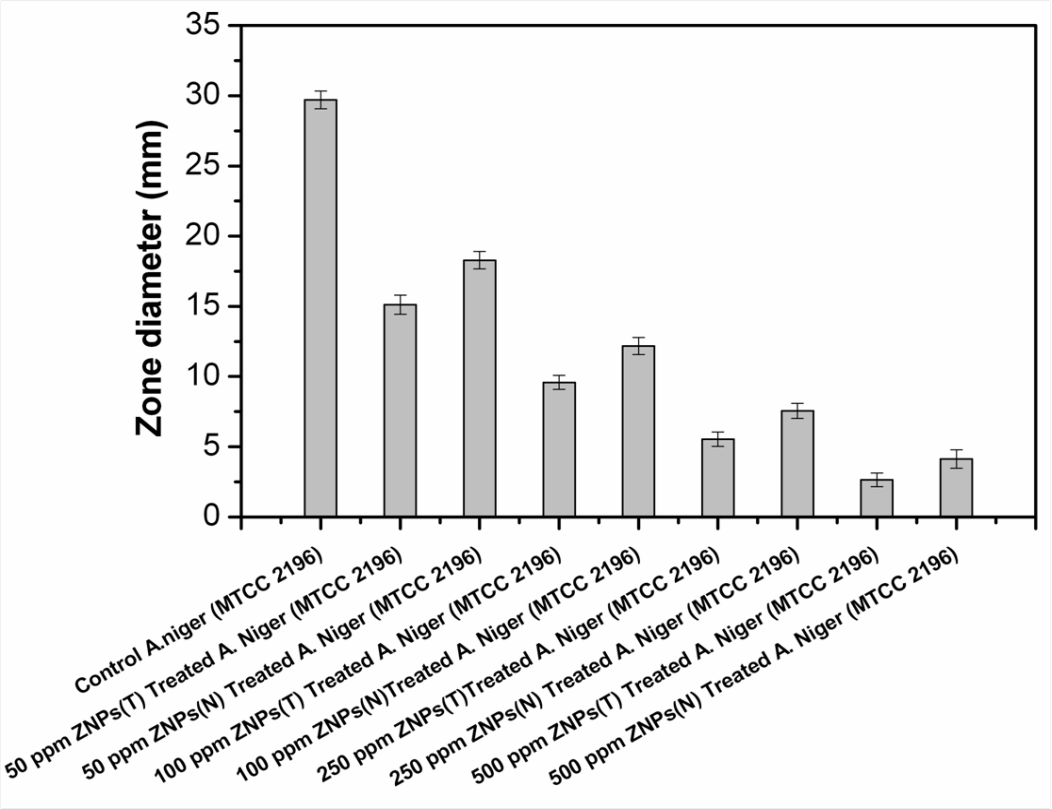


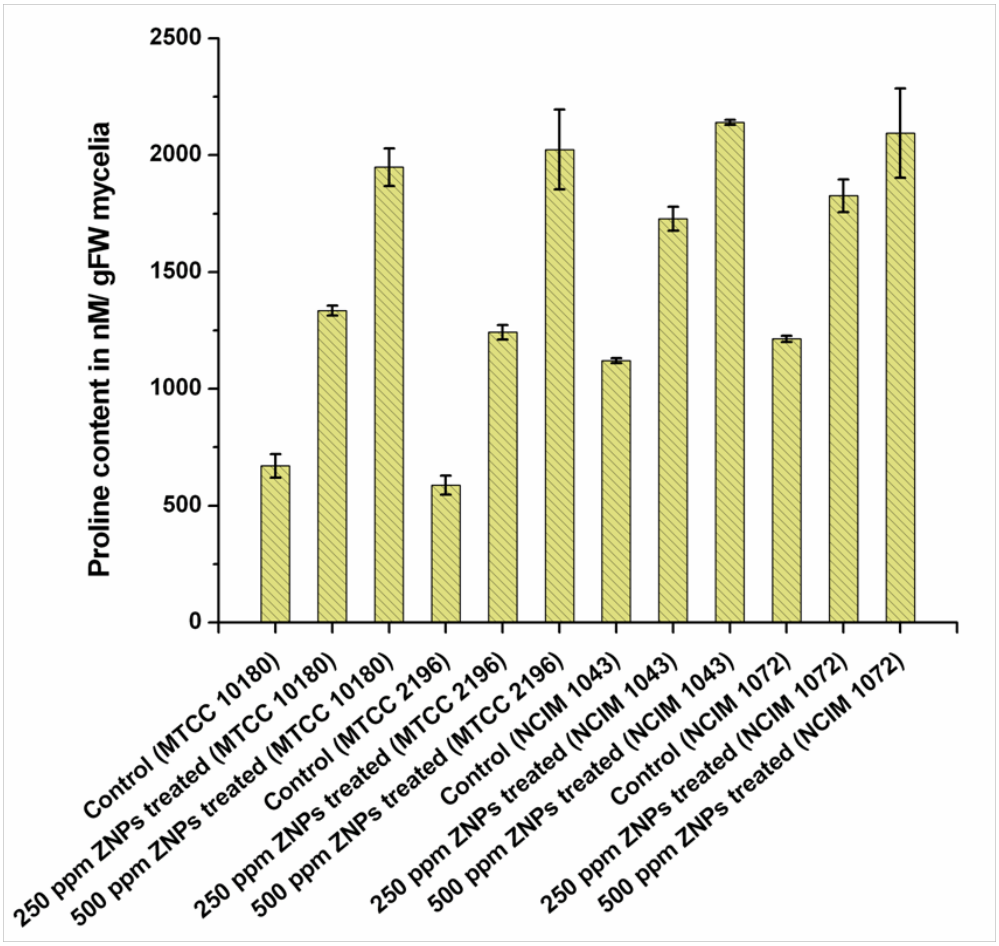
Supporting Figure S5. Light and dark dependent antifungal effect of microwave synthesized ZNPs. ZNPs exhibited more fungitoxic effect under light condition in comparison with dark condition, except 500 ppm dosage [* denoted significant difference, $P < 0.05$].



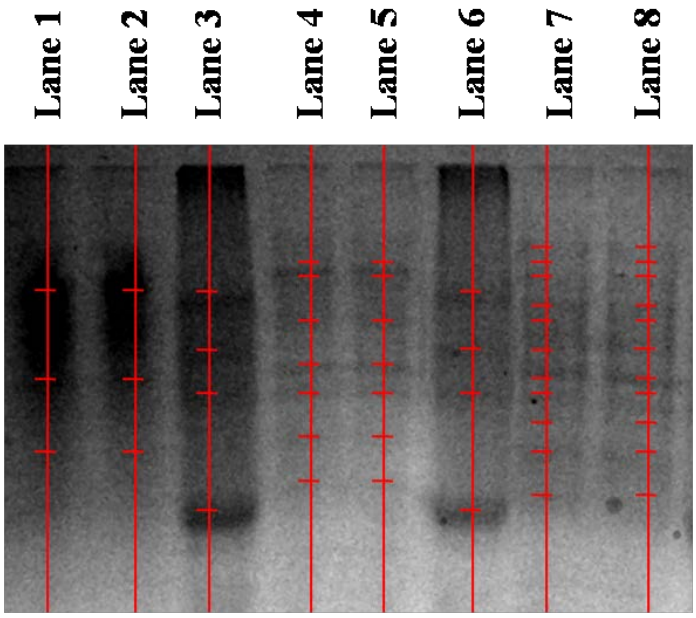
Supporting Figure S6. A comparison of antifungal activity between microwave synthesized ZNPs [ZNP (T)] and ZNPs synthesized by conventional chemical method [ZNP (N)]. Interestingly it was noted that microwave synthesized ZNPs exhibited greater antifungal effect in contrast to ZNPs synthesized by conventional chemical method. [F=12502; $P < 0.001$].

Table 1. Number of germination spores among *A.niger* (MTCC 10180 and MTCC 2196) and *F. oxysporum* (NCIM 1043 and NCIM 1072) strains as obtained from slide bioassay.

Fungal Strains: No. of applied spores (spores/mL)	*Concentration of M ZnO and ZNPs*	*No of germination spores*	
		M ZnO(spores/mL)	ZNPs(spores/mL)
A. niger (MTCC-10180): 58×10^4	50 ppm	27×10^4	15×10^4
	100 ppm	25×10^4	7×10^4
	250 ppm	23×10^4	3×10^4
	500 ppm	14×10^4	No spores found
A. niger (MTCC-2196): 62×10^4	50 ppm	32×10^4	20×10^4
	100 ppm	27×10^4	12×10^4
	250 ppm	24×10^4	3×10^4
	500 ppm	13×10^4	No spores found
F. oxysporum (NCIM-1043): 67×10^4	50 ppm	34×10^4	18×10^4
	100 ppm	29×10^4	11×10^4
	250 ppm	24×10^4	4×10^4
	500 ppm	17×10^4	No spores found
F. oxysporum (NCIM-1072): 65×10^4	50 ppm	35×10^4	20×10^4
	100 ppm	29×10^4	13×10^4
	250 ppm	25×10^4	4×10^4
	500 ppm	16×10^4	No spores found

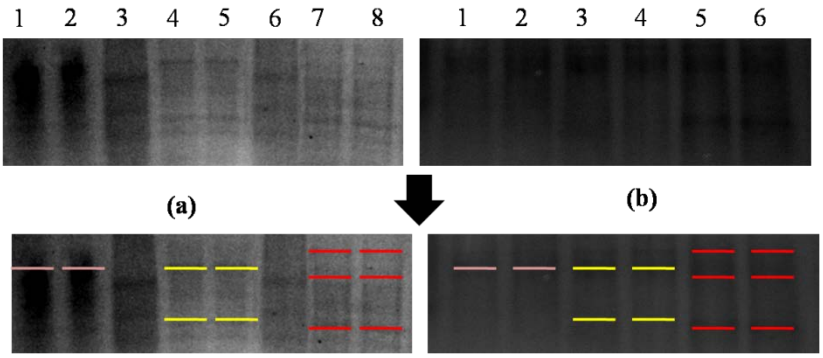


Supporting Figure S7. Change in proline content in fungal strains (*A.niger and F. oxysporum*) treated with different ZNPs concentrations (250 ppm and 500 ppm).



Supporting Figure S8. SDS PAGE gel electrophoresis of control and ZNPs treated samples of *A. niger*.

Increase of protein carbonylation (marker of oxidative stress) was shown in Lane 4 and 5 (250 ppm ZNPs treated *A. niger* sample) and Lane 7 and 8 (250 ppm ZNPs treated *A. niger* sample) with respect to control (Lane 1 and 2). Lane 3 and 6 denoted molecular marker (Protein marker, BLM003, 14-95 kDa, SRL).



Supporting Figure S9. Western blot analysis of control and ZNPs treated samples of *A. niger*.

Immuno blot analysis of control and ZNPs treated (250 ppm and 500 ppm) *A. niger* samples with oxyblot detection kit (Millipore). Figure S9 (a) showed SDS PAGE of protein carbonylation (Lane 1,2 denoted control, Lane 3, 6 denoted molecular marker, Lane 4, 5 denoted 250 ppm ZNPs treated *A. niger* sample and Lane 7, 8 denoted 500 ppm ZNPs treated *A. niger* samples). Figure S9 (b) showed corresponding western blot analysis of corresponding protein where Lane 1,2 denoted control, Lane 3, 4 denoted 250 ppm ZNPs treated *A. niger* sample and Lane 5, 6 denoted 500 ppm ZNPs treated *A. niger* samples.

Gene wise microarray analysis of MZnO and ZNPs treated A. niger compared to control related to oxidative stress of different pathways.

1. Alanine and aspartate activity.

Gene ID	Expression ratio (MZnO)	Expression ratio (NZnO)	Presumed function
AN_specific_010786	1.694	2.101	Alanine and aspartate activity
AN_specific_009570	1.363	1.879	
AN_specific_005964	1.283	1.509	
AN_specific_002008	1.225	0.968	
AN_specific_007073	1.219	1.630	
AN_specific_003009	1.036	1.781	
AN_specific_008036	1.026	0.902	
AN_specific_001812	1.024	2.304	
AN_specific_001664	0.673	1.412	
AN_specific_009633	0.797	1.005	

2. Amino acid biosynthesis.

Gene ID	Expression ratio (MZnO)	Expression ratio (NZnO)	Presumed function
AN_specific_004737	3.829	4.275	Amino acid Biosynthesis
AN_specific_005433	3.239	4.110	
AN_specific_005064	2.310	3.085	
AN_specific_003878	1.212	0.838	
AN_specific_005172	1.088	1.305	
AN_specific_010296	0.382	0.253	
AN_specific_005319	2.477	2.804	
AN_specific_007785	0.959	2.137	
AN_specific_010482	0.771	1.543	
AN_specific_000339	0.351	1.302	
AN_specific_003569	0.755	1.284	
AN_specific_010397	0.423	1.026	

3. Amino acid metabolism.

Gene ID	Expression ratio (MZnO)	Expression ratio (NZnO)	Presumed function
AN_specific_007897	3.107	0.779	Amino acid metabolism
AN_specific_002717	2.457	2.817	
AN_specific_008950	2.400	3.630	
AN_specific_001565	2.254	3.158	
AN_specific_009807	1.574	2.998	
AN_specific_009992	1.450	1.305	
AN_specific_009373	1.247	1.812	
AN_specific_008145	1.236	1.111	
AN_specific_005172	1.088	1.305	
AN_specific_002624	1.051	1.523	
AN_specific_003009	1.036	1.781	
AN_specific_001812	1.024	2.304	
AN_specific_005484	1.004	0.433	
AN_specific_010690	3.500	4.068	
AN_specific_004869	2.639	3.142	
AN_specific_003492	2.469	3.345	
AN_specific_004208	2.095	2.442	
AN_specific_003500	2.094	2.047	
AN_specific_009994	1.953	3.088	
AN_specific_010845	1.401	2.036	
AN_specific_003494	1.246	1.657	
AN_specific_004869	2.639	3.142	
AN_specific_004329	0.397	1.314	
AN_specific_006844	2.053	1.419	
AN_specific_002086	2.081	0.669	
AN_specific_004329	0.397	1.314	
AN_specific_002086	2.081	0.669	
AN_specific_010690	3.500	4.068	
AN_specific_003492	2.469	3.345	
AN_specific_001565	2.254	3.158	

AN_specific_004869	2.639	3.142	
AN_specific_010612	2.112	3.090	
AN_specific_009807	1.574	2.998	
AN_specific_001812	1.024	2.304	
AN_specific_003500	2.094	2.047	
AN_specific_009350	0.769	1.785	
AN_specific_003009	1.036	1.781	
AN_specific_003494	1.246	1.657	
AN_specific_003785	0.223	1.542	
AN_specific_002624	1.051	1.523	
AN_specific_009113	0.884	1.399	
AN_specific_001451	0.647	1.313	
AN_specific_005748	0.440	1.185	

4. Carbohydrate metabolism.

Gene ID	Expression ratio (MZnO)	Expression ratio (NZnO)	Presumed function
AN_specific_002637	3.158	2.983	Carbohydrate metabolism
AN_specific_003368	2.313	1.285	
AN_specific_010337	2.040	2.530	
AN_specific_010005	1.903	2.687	
AN_specific_003520	1.792	1.957	
AN_specific_008945	1.651	1.761	
AN_specific_001212	1.593	1.136	
AN_specific_001204	1.543	2.653	
AN_specific_003579	1.307	2.144	
AN_specific_009544	1.217	1.469	
AN_specific_001649	1.161	1.106	
AN_specific_002483	1.154	3.695	
AN_specific_002624	1.051	1.523	
AN_specific_000057	2.313	2.132	
AN_specific_006767	0.326	3.180	
AN_specific_010194	0.453	0.721	
AN_specific_009440	0.693	0.291	
AN_specific_009775	0.639	0.393	
AN_specific_006610	0.672	0.180	
AN_specific_005258	1.217	1.668	
AN_specific_009398	0.740	0.986	
AN_specific_004090	0.318	0.365	
AN_specific_006844	2.053	1.419	
AN_specific_006767	0.326	3.180	
AN_specific_002086	2.081	0.669	

AN_specific_009974	0.618	0.728	
AN_specific_009646	1.717	0.822	
AN_specific_006365	0.373	0.023	
AN_specific_002086	2.081	0.669	
AN_specific_008150	0.159	0.944	
AN_specific_006365	0.373	0.023	
AN_specific_006714	0.321	0.630	
AN_specific_001659	0.932	0.721	
AN_specific_006610	0.672	0.180	
AN_specific_000298	0.386	4.280	
AN_specific_002483	1.154	3.695	
AN_specific_003579	1.307	2.144	
AN_specific_009529	0.547	1.942	
AN_specific_005258	1.217	1.668	
AN_specific_010179	0.669	1.558	
AN_specific_007287	0.876	1.539	
AN_specific_005286	0.941	1.411	
AN_specific_004817	0.906	1.411	
AN_specific_005401	0.745	1.203	
AN_specific_009696	0.641	1.186	
AN_specific_010766	0.380	1.156	
AN_specific_000088	0.660	1.119	
AN_specific_002880	0.934	1.001	

5. Catalytic activity.

Gene ID	Expression ratio (MZnO)	Expression ratio (NZnO)	Presumed function
AN_specific_001910	1.543	2.653	Catalytic activity
AN_specific_004654	9.046	9.756	
AN_specific_010178	3.035	3.170	
AN_specific_003009	2.313	2.132	
AN_specific_010879	4.757	1.775	
AN_specific_005133	3.464	4.069	
AN_specific_008843	1.683	1.241	
AN_specific_002404	0.373	0.023	
AN_specific_000057	0.693	1.043	
AN_specific_001432	0.662	0.500	
AN_specific_010471	3.069	1.266	
AN_specific_009474	1.476	0.152	
AN_specific_002372	1.202	1.044	
AN_specific_002958	2.150	0.331	
AN_specific_010296	3.174	4.896	
AN_specific_010090	1.912	1.761	
AN_specific_006744	0.809	0.612	
AN_specific_004005	0.902	0.205	
AN_specific_004415	0.829	0.761	
AN_specific_002042	0.749	0.658	
AN_specific_004037	1.339	0.608	
AN_specific_010825	0.467	0.342	
AN_specific_006023	0.321	0.533	
AN_specific_009219	1.141	0.975	
AN_specific_008376	1.189	1.152	
AN_specific_006744	1.683	1.241	
AN_specific_002669	1.104	0.438	
AN_specific_009142	0.120	1.167	
AN_specific_011003	4.757	1.775	
AN_specific_010676	0.667	1.448	

AN_specific_002294	0.693	1.043	
AN_specific_000057	2.313	2.132	
AN_specific_009270	9.046	9.756	
AN_specific_009142	0.120	1.167	
AN_specific_001204	1.543	2.653	
AN_specific_000057	2.313	2.132	
AN_specific_008843	3.464	4.069	
AN_specific_006365	0.373	0.023	
AN_specific_002294	0.693	1.043	
AN_specific_003436	0.662	0.500	
AN_specific_008304	3.069	1.266	
AN_specific_003845	1.476	0.152	
AN_specific_000360	1.202	1.044	
AN_specific_003495	1.434	0.749	
AN_specific_005243	2.150	0.331	
AN_specific_000708	3.174	4.896	
AN_specific_002523	1.912	1.761	
AN_specific_003248	0.809	0.612	
AN_specific_008584	0.902	0.205	
AN_specific_006276	0.829	0.761	
AN_specific_006294	0.749	0.658	
AN_specific_010666	0.467	0.342	
AN_specific_007251	0.321	0.533	
AN_specific_003541	1.141	0.975	
AN_specific_007608	1.189	1.152	
AN_specific_006657	0.344	1.042	

6. Electron transport.

Gene ID	Expression ratio (MZnO)	Expression ratio (NZnO)	Presumed function
AN_specific_005733	9.371	8.750	Electron transport
AN_specific_008288	7.691	5.508	
AN_specific_009572	7.322	7.183	
AN_specific_005734	5.968	4.347	
AN_specific_007119	4.922	5.376	
AN_specific_004858	4.100	5.610	
AN_specific_005028	3.799	4.586	
AN_specific_007657	3.778	1.965	
AN_specific_009121	2.950	1.883	
AN_specific_010463	2.830	3.764	
AN_specific_004762	2.579	3.068	
AN_specific_000956	2.549	3.861	
AN_specific_007871	2.541	2.089	
AN_specific_002534	2.372	2.425	
AN_specific_007289	2.322	3.528	
AN_specific_003037	2.307	1.459	
AN_specific_006999	2.244	1.140	
AN_specific_007599	2.213	1.053	
AN_specific_001801	2.204	1.524	
AN_specific_005667	2.164	1.585	
AN_specific_004126	2.104	2.103	
AN_specific_006071	2.061	2.113	
AN_specific_005997	1.947	2.127	
AN_specific_008376	1.881	1.923	
AN_specific_010729	1.856	1.511	
AN_specific_004335	1.833	0.783	
AN_specific_001756	1.818	1.805	
AN_specific_010663	1.784	1.614	
AN_specific_005969	1.761	0.971	
AN_specific_009257	1.699	0.798	
AN_specific_004137	1.679	1.230	
AN_specific_002852	1.628	2.639	

AN_specific_010127	1.586	2.127	
AN_specific_001504	1.530	0.586	
AN_specific_002510	1.523	0.785	
AN_specific_001635	1.517	0.859	
AN_specific_005149	1.491	1.127	
AN_specific_004515	1.435	1.787	
AN_specific_005322	1.414	1.769	
AN_specific_009291	1.391	2.226	
AN_specific_002345	1.391	2.005	
AN_specific_007423	1.361	2.382	
AN_specific_008647	1.325	1.360	
AN_specific_010052	1.309	1.686	
AN_specific_009414	1.307	1.300	
AN_specific_011037	1.277	1.320	
AN_specific_008149	1.277	1.281	
AN_specific_010448	1.274	0.769	
AN_specific_009774	1.239	1.527	
AN_specific_005567	1.229	1.037	
AN_specific_005422	1.205	0.430	
AN_specific_003649	1.180	1.515	
AN_specific_004751	1.175	2.086	
AN_specific_010623	1.164	2.418	
AN_specific_001558	1.163	0.034	
AN_specific_010086	1.158	2.060	
AN_specific_010503	1.157	1.527	
AN_specific_002694	1.105	1.533	
AN_specific_003001	1.099	0.987	
AN_specific_006984	1.092	0.503	
AN_specific_003661	1.076	0.653	
AN_specific_007934	1.075	1.403	
AN_specific_004055	1.072	0.499	
AN_specific_008938	1.023	2.177	
AN_specific_007601	1.012	1.039	
AN_specific_005733	9.371	8.750	
AN_specific_005734	5.968	4.347	
AN_specific_004060	0.859	0.300	

AN_specific_006999	2.244	1.140	
AN_specific_003661	1.076	0.653	
AN_specific_008647	1.325	1.360	
AN_specific_002345	1.391	2.005	
AN_specific_002852	1.628	2.639	
AN_specific_001635	1.517	0.859	
AN_specific_001756	1.818	1.805	
AN_specific_010663	1.784	1.614	
AN_specific_010052	1.309	1.686	
AN_specific_010448	1.274	0.769	
AN_specific_008376	1.881	1.923	
AN_specific_010503	1.157	1.527	
AN_specific_008149	1.277	1.281	
AN_specific_008149	1.277	1.281	
AN_specific_005733	9.371	8.750	
AN_specific_004060	0.859	0.300	
AN_specific_007119	4.922	5.376	
AN_specific_006999	2.244	1.140	
AN_specific_005733	9.371	8.750	
AN_specific_000343	0.991	2.562	
AN_specific_004060	0.859	0.300	
AN_specific_007119	4.922	5.376	
AN_specific_006999	2.244	1.140	
AN_specific_005734	5.968	4.347	
AN_specific_003661	1.076	0.653	
AN_specific_009572	7.322	7.183	
AN_specific_007601	1.012	1.039	
AN_specific_005149	1.491	1.127	
AN_specific_004335	1.833	0.783	
AN_specific_004853	0.256	1.234	
AN_specific_006552	0.966	0.380	
AN_specific_008605	0.711	0.489	
AN_specific_006751	0.191	0.788	
AN_specific_001635	1.517	0.859	
AN_specific_007001	0.065	0.119	
AN_specific_007492	0.426	0.788	

AN_specific_002949	0.656	0.597	
AN_specific_002510	1.523	0.785	
AN_specific_006635	0.468	0.578	
AN_specific_001561	0.714	0.543	
AN_specific_001228	0.567	1.689	
AN_specific_005733	9.371	8.750	
AN_specific_009572	7.322	7.183	
AN_specific_007119	4.922	5.376	
AN_specific_005734	5.968	4.347	
AN_specific_010463	2.830	3.764	
AN_specific_005997	1.947	2.127	
AN_specific_006071	2.061	2.113	
AN_specific_007871	2.541	2.089	
AN_specific_010086	1.158	2.060	
AN_specific_002345	1.391	2.005	
AN_specific_006982	1.951	1.947	
AN_specific_010291	1.493	1.923	
AN_specific_004515	1.435	1.787	
AN_specific_005322	1.414	1.769	
AN_specific_010052	1.309	1.686	
AN_specific_010663	1.784	1.614	
AN_specific_005667	2.164	1.585	
AN_specific_002694	1.105	1.533	
AN_specific_009774	1.239	1.527	
AN_specific_010503	1.157	1.527	
AN_specific_003649	1.180	1.515	
AN_specific_010729	1.856	1.511	
AN_specific_005445	0.838	1.489	
AN_specific_005068	0.830	1.470	
AN_specific_001314	0.312	1.433	
AN_specific_003588	0.960	1.416	
AN_specific_007934	1.075	1.403	

7. Fatty acid biosynthesis.

Gene ID	Expression ratio (MZnO)	Expression ratio (NZnO)	Presumed function
AN_specific_004534	2.641	2.638	Fatty acid
AN_specific_010629	1.721	1.745	biosynthasis
AN_specific_003898	1.368	1.359	
AN_specific_004983	1.341	1.118	
AN_specific_002612	1.136	0.437	
AN_specific_006726	1.136	2.274	
AN_specific_000014	1.770	0.578	
AN_specific_002294	0.693	1.043	
AN_specific_001856	0.569	0.126	
AN_specific_003549	0.139	0.959	
AN_specific_001856	0.569	0.126	
AN_specific_002819	0.593	0.684	
AN_specific_009831	0.943	1.105	
AN_specific_007972	0.385	1.101	
AN_specific_003815	1.338	3.650	
AN_specific_010220	0.774	1.255	

8. Fatty acid metabolism.

Gene ID	Expression ratio (MZnO)	Expression ratio (NZnO)	Presumed function
AN_specific_005733	9.371	8.750	Fatty acid metabolism
AN_specific_011003	4.757	1.775	
AN_specific_002382	2.156	2.841	
AN_specific_003429	1.550	0.942	
AN_specific_001052	1.385	0.313	
AN_specific_010448	1.274	0.769	
AN_specific_007934	1.075	1.403	
AN_specific_001469	10.897	10.088	

9. Glutathione activity.

Gene ID	Expression ratio (MZnO)	Expression ratio (NZnO)	Presumed function
AN_specific_001618	3.474	3.522	Glutathione activity
AN_specific_008843	3.464	4.069	
AN_specific_008945	1.651	1.761	
AN_specific_010101	1.092	1.254	
AN_specific_001634	1.082	0.199	
AN_specific_006610	0.672	0.180	
AN_specific_009401	0.077	0.858	
AN_specific_001634	1.082	0.199	

10. Glycine, serine and threonine metabolism.

Gene ID	Expression ratio (MZnO)	Expression ratio (NZnO)	Presumed function
AN_specific_009572	7.322	7.183	glycine serine and threonine metabolism
AN_specific_002803	5.829	4.870	
AN_specific_005198	3.709	1.965	
AN_specific_010090	2.337	3.144	
AN_specific_005969	1.761	0.971	
AN_specific_001949	1.728	1.807	
AN_specific_011021	1.688	1.968	
AN_specific_010581	1.684	1.896	
AN_specific_009660	1.613	1.599	
AN_specific_009493	1.569	1.535	
AN_specific_010845	1.401	2.036	
AN_specific_010052	1.309	1.686	
AN_specific_002992	1.292	2.539	
AN_specific_002951	1.041	0.889	
AN_specific_009493	1.569	1.535	
AN_specific_010052	1.309	1.686	
AN_specific_002372	1.339	0.608	
AN_specific_005490	2.565	2.399	
AN_specific_010844	0.536	0.634	
AN_specific_009455	0.931	2.138	
AN_specific_002365	1.799	2.212	
AN_specific_007125	0.960	1.959	
AN_specific_010047	2.526	1.109	
AN_specific_005121	1.332	1.357	
AN_specific_005503	2.089	2.017	
AN_specific_005286	0.941	1.411	
AN_specific_009667	1.178	1.308	
AN_specific_001818	0.222	0.429	
AN_specific_002803	5.829	4.870	

AN_specific_007119	4.922	5.376	
AN_specific_010839	1.062	1.781	
AN_specific_005243	2.150	0.331	
AN_specific_003176	1.646	2.607	
AN_specific_006294	0.749	0.658	
AN_specific_009423	0.644	0.176	
AN_specific_004738	0.862	2.324	
AN_specific_010645	0.763	0.604	
AN_specific_010845	1.401	2.036	
AN_specific_005198	3.709	1.965	
AN_specific_002992	1.292	2.539	
AN_specific_011021	1.688	1.968	
AN_specific_010581	1.684	1.896	
AN_specific_001949	1.728	1.807	
AN_specific_009350	0.769	1.785	
AN_specific_010482	0.771	1.543	
AN_specific_005640	0.880	1.342	

11. Lysine biosynthesis.

Gene ID	Expression ratio (MZnO)	Expression ratio (NZnO)	Presumed function
AN_specific_010505	2.832	4.000	Lysine biosynthesis
AN_specific_002317	2.703	2.016	
AN_specific_009064	2.490	3.422	
AN_specific_005653	2.089	1.167	
AN_specific_010804	2.081	2.551	
AN_specific_003495	1.434	0.749	
AN_specific_009941	1.375	0.562	
AN_specific_003541	1.141	0.975	
AN_specific_009780	1.078	1.303	
AN_specific_003896	1.076	0.878	
AN_specific_003436	0.662	0.500	
AN_specific_008584	0.902	0.205	
AN_specific_009064	2.490	3.422	
AN_specific_010482	0.771	1.543	
AN_specific_009780	1.078	1.303	

12. Methyl transferase activity.

Gene ID	Expression ratio (MZnO)	Expression ratio (NZnO)	Presumed function
AN_specific_001039	1.476	1.672	Methyl transferase activity
AN_specific_009992	1.450	1.305	
AN_specific_008026	1.421	2.189	
AN_specific_010845	1.401	2.036	
AN_specific_009098	1.371	0.784	
AN_specific_009513	1.301	1.414	
AN_specific_010904	1.293	1.406	
AN_specific_002992	1.292	2.539	
AN_specific_005462	1.254	2.080	
AN_specific_010925	1.111	2.007	
AN_specific_009780	1.078	1.303	
AN_specific_000720	1.058	1.563	
AN_specific_002951	1.041	0.889	
AN_specific_004741	1.026	1.498	
AN_specific_003430	1.024	1.600	
AN_specific_010714	1.009	1.407	
AN_specific_009001	1.009	1.925	

13. Mitochondrial activity.

Gene ID	Expression ratio (MZnO)	Expression ratio (NZnO)	Presumed function
AN_specific_009079	3.624	0.564	Mitochondrial activity
AN_specific_002895	2.948	3.619	
AN_specific_009946	2.067	2.415	
AN_specific_005436	1.996	2.571	
AN_specific_001923	1.990	2.646	
AN_specific_004828	1.615	1.777	
AN_specific_007156	1.526	1.434	
AN_specific_010691	1.503	1.990	
AN_specific_009635	1.355	1.608	
AN_specific_004994	1.280	1.992	
AN_specific_011037	1.277	1.320	
AN_specific_001197	1.253	1.137	
AN_specific_004066	1.239	1.438	
AN_specific_003996	1.208	1.813	
AN_specific_009743	1.200	1.512	
AN_specific_010503	1.157	1.527	
AN_specific_010155	1.145	1.335	
AN_specific_004975	1.132	1.332	
AN_specific_001372	1.097	1.145	
AN_specific_002641	1.086	1.309	
AN_specific_010392	1.050	1.930	
AN_specific_006691	0.980	1.040	
AN_specific_010503	1.157	1.527	
AN_specific_008113	0.640	0.067	
AN_specific_006691	0.980	1.040	
AN_specific_009079	3.624	0.564	
AN_specific_002895	2.948	3.619	
AN_specific_001372	1.097	1.145	
AN_specific_005436	1.996	2.571	
AN_specific_001804	0.805	2.419	
AN_specific_009946	2.067	2.415	
AN_specific_004581	0.927	2.319	

AN_specific_004994	1.280	1.992	
AN_specific_010691	1.503	1.990	
AN_specific_009635	1.355	1.608	
AN_specific_010503	1.157	1.527	
AN_specific_004066	1.239	1.438	
AN_specific_007156	1.526	1.434	
AN_specific_004975	1.132	1.332	
AN_specific_002641	1.086	1.309	
AN_specific_005244	0.948	1.284	
AN_specific_010011	0.572	1.218	
AN_specific_001372	1.097	1.145	
AN_specific_004474	0.599	1.141	
AN_specific_004992	0.932	1.053	
AN_specific_006691	0.980	1.040	
AN_specific_005848	0.518	1.013	

14. Oxidoreductase activity.

Gene ID	Expression ratio (MZnO)	Expression ratio (NZnO)	Presumed function
AN_specific_001469	10.897	10.088	Oxidoreductase activity
AN_specific_009572	7.322	7.183	
AN_specific_005734	5.968	4.347	
AN_specific_002803	5.829	4.870	
AN_specific_007119	4.922	5.376	
AN_specific_010244	4.325	3.945	
AN_specific_009277	4.232	3.288	
AN_specific_004858	4.100	5.610	
AN_specific_005028	3.799	4.586	
AN_specific_000796	3.693	4.575	
AN_specific_009079	3.624	0.564	
AN_specific_001473	3.572	3.467	
AN_specific_008843	3.464	4.069	
AN_specific_009127	3.108	3.266	
AN_specific_009121	2.950	1.883	
AN_specific_010505	2.832	4.000	
AN_specific_010463	2.830	3.764	
AN_specific_007740	2.681	1.467	
AN_specific_004534	2.641	2.638	
AN_specific_004869	2.639	3.142	
AN_specific_005543	2.582	2.701	
AN_specific_004762	2.579	3.068	
AN_specific_000956	2.549	3.861	
AN_specific_002534	2.372	2.425	
AN_specific_004749	2.372	0.735	
AN_specific_010751	2.342	3.207	
AN_specific_003037	2.307	1.459	
AN_specific_006703	2.250	1.834	
AN_specific_006999	2.244	1.140	
AN_specific_007599	2.213	1.053	
AN_specific_001801	2.204	1.524	
AN_specific_008812	2.203	3.163	

AN_specific_005667	2.164	1.585	
AN_specific_009972	2.160	2.320	
AN_specific_002382	2.156	2.841	
AN_specific_005370	2.145	1.969	
AN_specific_004126	2.104	2.103	
AN_specific_006071	2.061	2.113	
AN_specific_003628	2.025	1.005	
AN_specific_010816	1.981	2.697	
AN_specific_001698	1.965	1.641	
AN_specific_006982	1.951	1.947	
AN_specific_008046	1.844	0.231	
AN_specific_004335	1.833	0.783	
AN_specific_010663	1.784	1.614	
AN_specific_004752	1.771	1.992	
AN_specific_005969	1.761	0.971	
AN_specific_009331	1.730	2.102	
AN_specific_009257	1.699	0.798	
AN_specific_005279	1.686	1.754	
AN_specific_008945	1.651	1.761	
AN_specific_010736	1.638	1.931	
AN_specific_010809	1.622	0.602	
AN_specific_004755	1.592	1.904	
AN_specific_009493	1.569	1.535	
AN_specific_000450	1.550	0.999	
AN_specific_003429	1.550	0.942	
AN_specific_001078	1.535	2.822	
AN_specific_002510	1.523	0.785	
AN_specific_001635	1.517	0.859	
AN_specific_003388	1.463	2.173	
AN_specific_009761	1.444	0.920	
AN_specific_001458	1.432	0.969	
AN_specific_005322	1.414	1.769	
AN_specific_005608	1.411	1.355	
AN_specific_009291	1.391	2.226	
AN_specific_002345	1.391	2.005	
AN_specific_001052	1.385	0.313	

AN_specific_010187	1.384	2.692	
AN_specific_000980	1.381	1.705	
AN_specific_003898	1.368	1.359	
AN_specific_007423	1.361	2.382	
AN_specific_004983	1.341	1.118	
AN_specific_009737	1.331	1.792	
AN_specific_010052	1.309	1.686	
AN_specific_009414	1.307	1.300	
AN_specific_009774	1.239	1.527	
AN_specific_005422	1.205	0.430	
AN_specific_007739	1.195	2.379	
AN_specific_004405	1.177	1.395	
AN_specific_004751	1.175	2.086	
AN_specific_001558	1.163	0.034	
AN_specific_002748	1.162	1.695	
AN_specific_001649	1.161	1.106	
AN_specific_010086	1.158	2.060	
AN_specific_010503	1.157	1.527	
AN_specific_005440	1.144	1.603	
AN_specific_007011	1.123	1.557	
AN_specific_009778	1.122	0.736	
AN_specific_002694	1.105	1.533	
AN_specific_003001	1.099	0.987	
AN_specific_010101	1.092	1.254	
AN_specific_006984	1.092	0.503	
AN_specific_005172	1.088	1.305	
AN_specific_001634	1.082	0.199	
AN_specific_003661	1.076	0.653	
AN_specific_004055	1.072	0.499	
AN_specific_008943	1.058	2.242	
AN_specific_004946	1.054	0.107	
AN_specific_002897	1.040	0.756	
AN_specific_007601	1.012	1.039	
AN_specific_005402	1.001	0.044	
AN_specific_008843	3.464	4.069	
AN_specific_009121	2.950	1.883	

AN_specific_004060	0.859	0.300	
AN_specific_005608	1.411	1.355	
AN_specific_006999	2.244	1.140	
AN_specific_002382	2.156	2.841	
AN_specific_005440	1.144	1.603	
AN_specific_002709	0.988	0.519	
AN_specific_003628	2.025	1.005	
AN_specific_002294	0.693	1.043	
AN_specific_003501	0.148	0.432	
AN_specific_003661	1.076	0.653	
AN_specific_009127	3.108	3.266	
AN_specific_002345	1.391	2.005	
AN_specific_002935	0.545	1.131	
AN_specific_004751	1.175	2.086	
AN_specific_000956	2.549	3.861	
AN_specific_010663	1.784	1.614	
AN_specific_009493	1.569	1.535	
AN_specific_001856	0.569	0.126	
AN_specific_007125	0.960	1.959	
AN_specific_002377	0.766	1.686	
AN_specific_010052	1.309	1.686	
AN_specific_008945	1.651	1.761	
AN_specific_005370	2.145	1.969	
AN_specific_004752	1.771	1.992	
AN_specific_004869	2.639	3.142	
AN_specific_004329	0.397	1.314	
AN_specific_004904	0.956	0.128	
AN_specific_010503	1.157	1.527	
AN_specific_006703	2.250	1.834	
AN_specific_010244	4.325	3.945	
AN_specific_004060	0.859	0.300	
AN_specific_003628	2.025	1.005	
AN_specific_002382	2.156	2.841	
AN_specific_008843	3.464	4.069	
AN_specific_006999	2.244	1.140	
AN_specific_002294	0.693	1.043	

AN_specific_002510	1.523	0.785	
AN_specific_002345	1.391	2.005	
AN_specific_010736	1.638	1.931	
AN_specific_005734	5.968	4.347	
AN_specific_002819	0.593	0.684	
AN_specific_003661	1.076	0.653	
AN_specific_001801	2.204	1.524	
AN_specific_009127	3.108	3.266	
AN_specific_009572	7.322	7.183	
AN_specific_002382	2.156	2.841	
AN_specific_007208	0.001	0.072	
AN_specific_007601	1.012	1.039	
AN_specific_003037	2.307	1.459	
AN_specific_003628	2.025	1.005	
AN_specific_004335	1.833	0.783	
AN_specific_001634	1.082	0.199	
AN_specific_009257	1.699	0.798	
AN_specific_004755	1.592	1.904	
AN_specific_004853	0.256	1.234	
AN_specific_005172	1.088	1.305	
AN_specific_000980	1.381	1.705	
AN_specific_007599	2.213	1.053	
AN_specific_008605	0.711	0.489	
AN_specific_001635	1.517	0.859	
AN_specific_006703	2.250	1.834	
AN_specific_007001	0.065	0.119	
AN_specific_003805	0.480	0.679	
AN_specific_007492	0.426	0.788	
AN_specific_004858	4.100	5.610	
AN_specific_002949	0.656	0.597	
AN_specific_002510	1.523	0.785	
AN_specific_001228	0.567	1.689	
AN_specific_002935	0.545	1.131	
AN_specific_004738	0.862	2.324	
AN_specific_007009	0.074	0.115	
AN_specific_009493	1.569	1.535	

AN_specific_005028	3.799	4.586	
AN_specific_005734	5.968	4.347	
AN_specific_008843	3.464	4.069	
AN_specific_010505	2.832	4.000	
AN_specific_010244	4.325	3.945	
AN_specific_000956	2.549	3.861	
AN_specific_005734	5.968	4.347	
AN_specific_008843	3.464	4.069	
AN_specific_010505	2.832	4.000	
AN_specific_010244	4.325	3.945	
AN_specific_000956	2.549	3.861	
AN_specific_009127	3.108	3.266	
AN_specific_010751	2.342	3.207	
AN_specific_008812	2.203	3.163	
AN_specific_004869	2.639	3.142	
AN_specific_004762	2.579	3.068	
AN_specific_002382	2.156	2.841	
AN_specific_005543	2.582	2.701	
AN_specific_010816	1.981	2.697	
AN_specific_004534	2.641	2.638	
AN_specific_002534	2.372	2.425	
AN_specific_007423	1.361	2.382	
AN_specific_003963	2.487	2.373	
AN_specific_004738	0.862	2.324	
AN_specific_009972	2.160	2.320	
AN_specific_003948	0.515	2.306	
AN_specific_008943	1.058	2.242	
AN_specific_009291	1.391	2.226	
AN_specific_003388	1.463	2.173	
AN_specific_006071	2.061	2.113	
AN_specific_004126	2.104	2.103	
AN_specific_009331	1.730	2.102	
AN_specific_004751	1.175	2.086	
AN_specific_010086	1.158	2.060	
AN_specific_002345	1.391	2.005	
AN_specific_004752	1.771	1.992	

AN_specific_005370	2.145	1.969	
AN_specific_007125	0.960	1.959	
AN_specific_006982	1.951	1.947	
AN_specific_010736	1.638	1.931	
AN_specific_004755	1.592	1.904	
AN_specific_009121	2.950	1.883	
AN_specific_006703	2.250	1.834	
AN_specific_009737	1.331	1.792	
AN_specific_005322	1.414	1.769	
AN_specific_008945	1.651	1.761	
AN_specific_005279	1.686	1.754	
AN_specific_000980	1.381	1.705	
AN_specific_002748	1.162	1.695	
AN_specific_003695	0.031	1.693	
AN_specific_001228	0.567	1.689	
AN_specific_002377	0.766	1.686	
AN_specific_010052	1.309	1.686	
AN_specific_001698	1.965	1.641	
AN_specific_010663	1.784	1.614	
AN_specific_005440	1.144	1.603	
AN_specific_008797	0.386	1.596	
AN_specific_005667	2.164	1.585	
AN_specific_009493	1.569	1.535	
AN_specific_002694	1.105	1.533	
AN_specific_009774	1.239	1.527	
AN_specific_010503	1.157	1.527	
AN_specific_001801	2.204	1.524	
AN_specific_004914	0.952	1.487	
AN_specific_007740	2.681	1.467	
AN_specific_003037	2.307	1.459	
AN_specific_004405	1.177	1.395	
AN_specific_006626	0.640	1.389	
AN_specific_008070	0.645	1.369	
AN_specific_003898	1.368	1.359	
AN_specific_005608	1.411	1.355	
AN_specific_004329	0.397	1.314	

AN_specific_005172	1.088	1.305	
AN_specific_009414	1.307	1.300	
AN_specific_001585	0.414	1.282	
AN_specific_010101	1.092	1.254	
AN_specific_007113	0.084	1.249	
AN_specific_004853	0.256	1.234	
AN_specific_010011	0.572	1.218	
AN_specific_009304	0.164	1.213	
AN_specific_009676	0.262	1.175	
AN_specific_009671	0.753	1.144	
AN_specific_006999	2.244	1.140	
AN_specific_002935	0.545	1.131	
AN_specific_001649	1.161	1.106	
AN_specific_004569	0.808	1.102	
AN_specific_007972	0.385	1.101	
AN_specific_009669	0.960	1.086	
AN_specific_007599	2.213	1.053	
AN_specific_002294	0.693	1.043	
AN_specific_003530	0.847	1.016	
AN_specific_003628	2.025	1.005	

15. Pentosephosphate pathway.

Gene ID	Expression ratio (MZnO)	Expression ratio (NZnO)	Presumed function
AN_specific_009816	3.335	3.858	Pentose Phosphate Pathway
AN_specific_001801	2.204	1.524	
AN_specific_002345	1.391	2.005	
AN_specific_003661	1.076	0.653	
AN_specific_009398	0.740	0.986	

16. SOD activity.

Gene ID	Expression ratio (MZnO)	Expression ratio (NZnO)	Presumed function
AN_specific_006855	1.272	1.933	Super oxide dismutase activity
AN_specific_004967	1.182	1.286	

17. Serine activity.

Gene ID	Expression ratio (MZnO)	Expression ratio (NZnO)	Presumed function
AN_specific_009688	1.290	1.759	Serine activity
AN_specific_004596	1.259	1.861	
AN_specific_006478	1.234	1.205	
AN_specific_004764	1.212	0.029	
AN_specific_009445	1.207	0.394	
AN_specific_009667	1.178	1.308	
AN_specific_008212	1.131	1.923	
AN_specific_010261	1.062	1.913	
AN_specific_010839	1.062	1.781	
AN_specific_004654	1.059	1.467	
AN_specific_002542	1.043	1.718	
AN_specific_005231	1.043	0.639	
AN_specific_004844	1.027	0.691	
AN_specific_008938	1.023	2.177	
AN_specific_001060	1.017	1.312	
AN_specific_002803	5.829	4.870	
AN_specific_009493	1.569	1.535	
AN_specific_004738	0.862	2.324	
AN_specific_002372	1.339	0.608	
AN_specific_010666	0.467	0.342	
AN_specific_010844	0.536	0.634	
AN_specific_002317	2.703	2.016	
AN_specific_005198	3.709	1.965	
AN_specific_007125	0.960	1.959	
AN_specific_010581	1.684	1.896	
AN_specific_004596	1.259	1.861	
AN_specific_001949	1.728	1.807	
AN_specific_009350	0.769	1.785	
AN_specific_003475	1.615	1.756	
AN_specific_004850	0.426	1.725	
AN_specific_010052	1.309	1.686	
AN_specific_009660	1.613	1.599	
AN_specific_009685	1.531	1.590	

AN_specific_006933	2.022	1.550	
AN_specific_010482	0.771	1.543	
AN_specific_009493	1.569	1.535	
AN_specific_008626	1.456	1.505	
AN_specific_004173	1.423	1.490	
AN_specific_004554	0.823	1.432	
AN_specific_005286	0.941	1.411	
AN_specific_005121	1.332	1.357	
AN_specific_004573	1.576	1.345	
AN_specific_005640	0.880	1.342	
AN_specific_004951	1.439	1.339	
AN_specific_010773	0.779	1.334	
AN_specific_001060	1.017	1.312	
AN_specific_000122	0.942	1.301	
AN_specific_002957	0.630	1.251	
AN_specific_001499	0.782	1.206	
AN_specific_002096	0.621	1.187	
AN_specific_009696	0.641	1.186	
AN_specific_004820	0.720	1.162	
AN_specific_004547	0.281	1.150	
AN_specific_002185	0.979	1.141	
AN_specific_000275	0.244	1.109	
AN_specific_010047	2.526	1.109	
AN_specific_003285	0.735	1.063	
AN_specific_009296	0.499	1.028	
AN_specific_003585	0.387	1.010	
AN_specific_009762	0.138	1.008	
AN_specific_003204	2.850	1.005	

18. Starch and sucrose metabolism.

Gene ID	Expression ratio (MZnO)	Expression ratio (NZnO)	Presumed function
AN_specific_003204	2.850	1.005	Starch and Sucrose metabolism
AN_specific_008579	2.749	3.563	
AN_specific_010047	2.526	1.109	
AN_specific_003368	2.313	1.285	
AN_specific_008670	2.127	1.893	
AN_specific_006825	2.003	2.657	
AN_specific_010478	2.001	2.083	
AN_specific_010829	1.954	2.755	
AN_specific_009681	1.792	1.557	
AN_specific_001891	1.656	1.740	
AN_specific_003475	1.615	1.756	
AN_specific_001105	1.606	0.676	
AN_specific_010000	1.605	2.177	
AN_specific_009177	1.470	1.890	
AN_specific_002584	1.385	1.582	
AN_specific_010018	1.382	0.829	
AN_specific_009382	1.291	1.644	
AN_specific_009667	1.178	1.308	
AN_specific_002483	1.154	3.695	
AN_specific_009778	1.122	0.736	
AN_specific_010261	1.062	1.913	
AN_specific_010839	1.062	1.781	
AN_specific_002624	1.051	1.523	
AN_specific_006767	0.326	3.180	
AN_specific_005745	1.291	1.644	
AN_specific_009382	0.580	0.943	
AN_specific_010004	2.526	1.109	
AN_specific_010047	0.639	0.393	
AN_specific_009775	1.178	1.308	
AN_specific_009667	2.053	1.419	

AN_specific_006844	1.154	3.695	
AN_specific_006767	2.081	0.669	
AN_specific_002086	0.618	0.728	
AN_specific_009974	0.377	0.045	
AN_specific_005745	0.580	0.943	
AN_specific_010004	2.081	0.669	
AN_specific_002086	0.618	0.728	
AN_specific_009974	0.159	0.944	
AN_specific_008150	1.062	1.781	
AN_specific_010839	0.685	1.651	
AN_specific_006000	0.606	0.285	
AN_specific_001554	1.178	1.308	
AN_specific_009667	1.154	3.695	
AN_specific_008579	2.749	3.563	
AN_specific_010478	2.001	2.083	
AN_specific_003520	1.792	1.957	
AN_specific_010261	1.062	1.913	
AN_specific_010839	1.062	1.781	
AN_specific_006000	0.685	1.651	
AN_specific_009382	1.291	1.644	
AN_specific_002584	1.385	1.582	
AN_specific_002624	1.051	1.523	
AN_specific_006411	0.829	1.474	
AN_specific_009667	1.178	1.308	
AN_specific_001499	0.782	1.206	
AN_specific_003308	0.608	1.179	
AN_specific_009669	0.960	1.086	
AN_specific_003285	0.735	1.063	
AN_specific_011044	0.963	1.037	

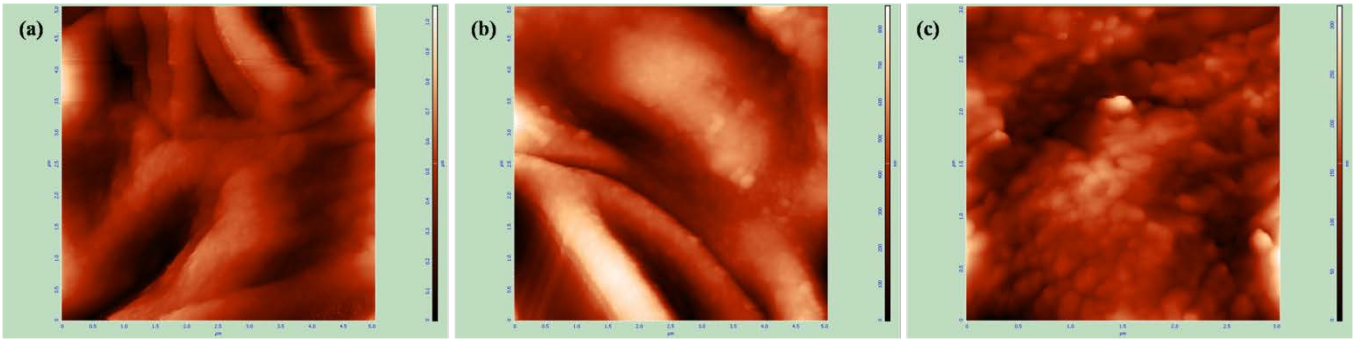
19. Threonine.

Gene ID	Expression ratio (MZnO)	Expression ratio (NZnO)	Presumed function
AN_specific_009572	7.322	7.183	Threonine activity
AN_specific_002803	5.829	4.870	
AN_specific_003204	2.850	1.005	
AN_specific_004869	2.639	3.142	
AN_specific_010047	2.526	1.109	
AN_specific_003492	2.469	3.345	
AN_specific_002717	2.457	2.817	
AN_specific_005243	2.150	0.331	
AN_specific_005503	2.089	2.017	
AN_specific_006933	2.022	1.550	
AN_specific_005438	1.843	2.190	
AN_specific_010300	1.774	1.757	
AN_specific_005969	1.761	0.971	
AN_specific_009908	1.738	0.840	
AN_specific_001949	1.728	1.807	
AN_specific_011021	1.688	1.968	
AN_specific_010581	1.684	1.896	
AN_specific_003176	1.646	2.607	
AN_specific_003475	1.615	1.756	
AN_specific_009660	1.613	1.599	
AN_specific_007119	4.922	5.376	
AN_specific_000318	0.403	0.615	
AN_specific_009436	0.334	2.713	
AN_specific_001269	0.536	0.445	
AN_specific_002992	1.292	2.539	
AN_specific_010000	1.605	2.177	
AN_specific_008938	1.023	2.177	
AN_specific_010845	1.401	2.036	
AN_specific_008212	1.131	1.923	
AN_specific_010261	1.062	1.913	

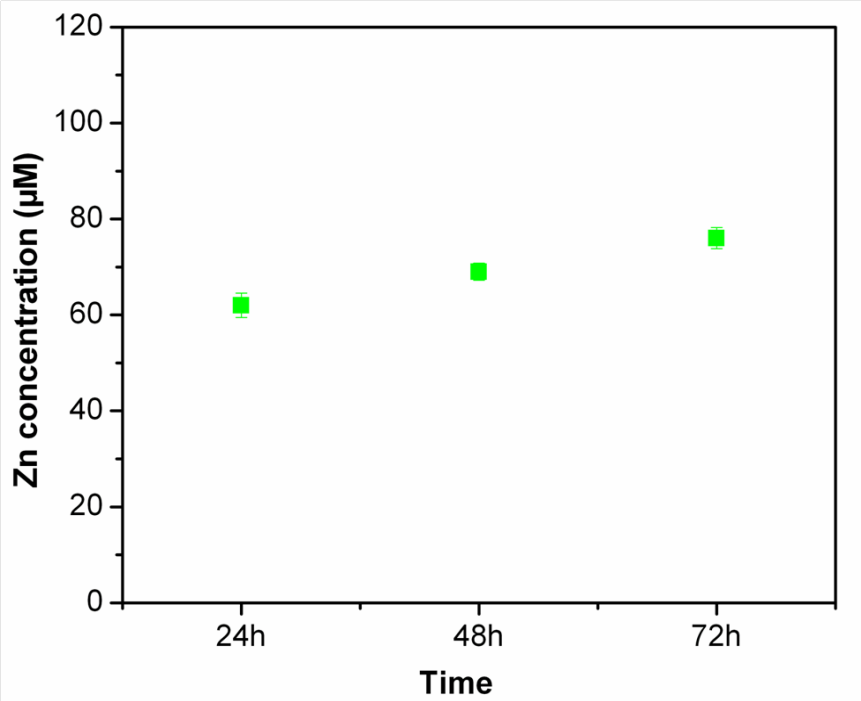
AN_specific_010581	1.684	1.896	
AN_specific_004596	1.259	1.861	
AN_specific_001949	1.728	1.807	
AN_specific_009350	0.769	1.785	
AN_specific_010839	1.062	1.781	
AN_specific_009688	1.290	1.759	
AN_specific_003475	1.615	1.756	
AN_specific_004850	0.426	1.725	
AN_specific_002542	1.043	1.718	
AN_specific_010052	1.309	1.686	
AN_specific_009660	1.613	1.599	
AN_specific_005504	1.358	1.590	
AN_specific_010482	0.771	1.543	
AN_specific_009493	1.569	1.535	
AN_specific_004173	1.423	1.490	
AN_specific_004554	0.823	1.432	
AN_specific_005286	0.941	1.411	
AN_specific_005121	1.332	1.357	
AN_specific_005640	0.880	1.342	
AN_specific_004951	1.439	1.339	
AN_specific_010773	0.779	1.334	
AN_specific_009667	1.178	1.308	
AN_specific_000122	0.942	1.301	
AN_specific_002957	0.630	1.251	
AN_specific_001499	0.782	1.206	
AN_specific_006478	1.234	1.205	
AN_specific_009696	0.641	1.186	
AN_specific_002185	0.979	1.141	
AN_specific_003285	0.735	1.063	
AN_specific_003585	0.387	1.010	
AN_specific_009762	0.138	1.008	

20. Tryptophan metabolism.

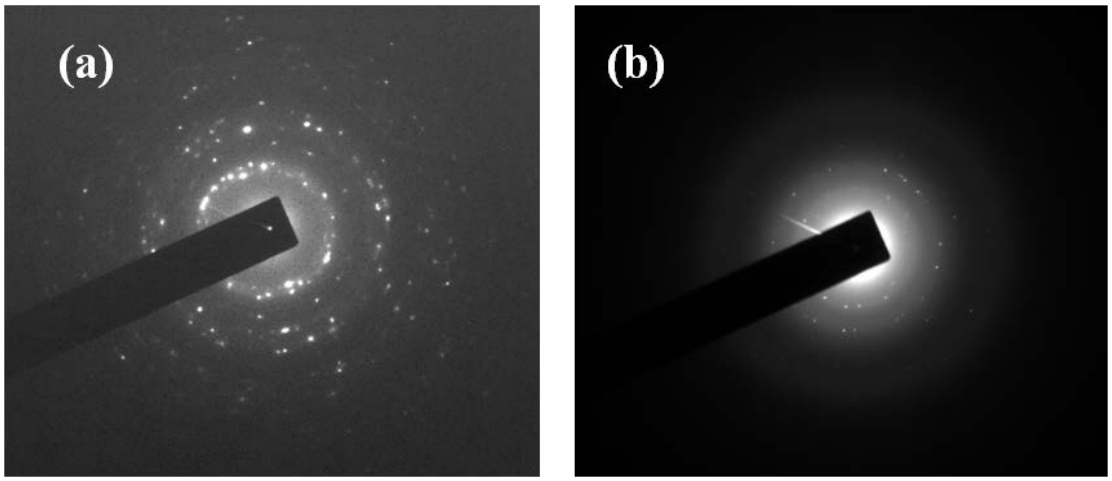
Gene ID	Expression ratio (MZnO)	Expression ratio (NZnO)	Presumed function
AN_specific_005733	9.371	8.750	Tryptophan
AN_specific_009648	3.689	0.184	metabolism
AN_specific_008950	2.400	3.630	
AN_specific_001565	2.254	3.158	
AN_specific_009751	2.102	2.700	
AN_specific_007571	2.008	1.228	
AN_specific_009994	1.953	3.088	
AN_specific_002852	1.628	2.639	
AN_specific_009807	1.574	2.998	
AN_specific_009992	1.450	1.305	
AN_specific_010448	1.274	0.769	
AN_specific_007934	1.075	1.403	
AN_specific_010333	0.949	1.621	
AN_specific_010448	1.274	0.769	
AN_specific_009751	2.102	2.700	
AN_specific_009648	3.689	0.184	
AN_specific_005733	9.371	8.750	
AN_specific_003271	0.052	0.605	
AN_specific_006347	4.133	4.183	
AN_specific_001565	2.254	3.158	
AN_specific_009994	1.953	3.088	
AN_specific_009807	1.574	2.998	
AN_specific_001919	0.870	1.892	
AN_specific_003588	0.960	1.416	
AN_specific_010220	0.774	1.255	
AN_specific_005748	0.440	1.185	



Supporting Figure S10. AFM study of Fungal hypha (*A. niger*, MTCC 10180): (a) Control (b) 250 ppm ZNPs treated (c) 500 ppm ZNPs treated.



Supporting Figure S11. Zn^{2+} release from microwave synthesized ZNPs by ICPMS analysis at three different interval of times 24h, 48h and 72h respectively. A small amount of Zn^{2+} was released from ZNPs at the pH of fungal culture medium which could simultaneously contribute to the fungitoxic effect of ZNPs.



Supporting Figure S12. Selected area diffraction pattern (SAED) of (a) ZNPs and (b) ZNPs after internalization on fungal hyphae. The first one i.e. native ZNPs exhibited distinct crystalline structure but after internalization on to fungal hyphae its crystalline pattern was partially lost but still present.

Table 2: Comparative study of mice blood biochemical parameters of ZNPS treated mice at different doses compare to control in acute oral toxicity assay.

Parameters		Control	ZNPs		
			0.5 g/ kg	1 g/ kg	2g/ kg
Hemoglobin (g/dl)		12.1±0.36056	13±0.2	12.67±0.12	13.2±0.62
TC	**RBC (million/ mm^3)**	4.1± 0.2	2.67±0.12	4.23±0.12	4.5±0.2
	WBC/ mm^3	5658.33±62.9153	5790±36.05	5770±36.06	5686.67±80.83
DC (%)	**Neutrophils**	40±2	36±1	41±1.73	40±2
	Lymphocytes	56.6667±3.51188	59±1	57±1	70±1
	Monocytes	1.66667±0.57735	1±0	1.67±0.58	1.33±0.58
	Eosinophils	2.66667±0.57735	2.33±0.58	2±0	2.67±0.58
	Basophils	0±0	0±0	0±0	0±0
Platelets (lakh/ mm^3)		1.51±0.03606	1.5±0.02	1.53±0.01	1.52±0.06
LDH (IU/L)		240±2	237±3.06	243.33±1.15	239.33±1.53
Creatinine (mg/dL)		0.73±0.02	0.73±0.02	0.74±0.02	0.78±0.02
Alkaline Phosphate (U/L)		60±2	57±1	57±1.73	58.67±2.52
Total Protein (gm/dL)		6.1±0.1	6.03±0.058	6.17±0.15	6.2±0.26
Cholesterol (mg/dL)		136±2	133.67±1.53	134.33±3.06	140.67±3.06
Triglyceride (mg/dL)		70.3333±0.57735	71±1	70.67±2.52	71.67±1.53
Uric Acid (mg/dL)		2.8±0.1	2.67±0.15	2.9±0.1	3.07±0.153
BUN (mg/dL)		11±1	11±1	11±1	12.33±0.58
S.G.O.T. (IU/L)		12±1	12±1	12±1	13±1
S.G.P.T. (IU/L)		11±1	11.67±0.58	12.33±1.53	10±1
Phosphorous (mg/dL)		2.9±0.1	2.73±0.15	2.63±0.15	3.03±0.153

Table 3. Comparison of blood biochemical parameters between control and ZNPs treated mice by i.v. injection.

Parameters		**Control**	**ZNPs**		
			100 µg/mL	**250 µg/mL**	**500 µg/mL**
Haemoglobin (g/dL)		13.15 ± 0.1	13.2 ± 0.15	13.25 ± 0.058	13.4 ± 0.26
TC	**RBC (million/ mm^3)**	4.2 ± 0.1	4.2 ± 0.208	4.25 ± 0.15	4.35 ± 0.25
	WBC/ mm^3	5785 ± 37.86	5860 ± 75	5825 ± 36.05	5835 ± 25.17
DC (%)	**Neutrophils**	41.5 ± 1.53	40 ± 2	41 ± 1	42.5 ± 1
	Lymphocytes	66.5 ± 1.53	68.5 ± 1.53	68.5 ± 1	70 ± 1.15
	Monocytes	2.5 ± 0.58	2.5 ± 0.58	3 ± 0.58	2.5 ± 0.58
	Eosinophils	2.5 ± 0.58	2 ± 0.58	2.5 ± 0.58	2.5 ± 0.58
	Basophils	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Platelets (lakh/ mm^3)		1.6 ± 0.1	1.85 ± 0.15	1.9 ± 0.058	1.85 ± 0.1
LDH (IU/L)		240 ± 2.08	250 ± 1.53	254.5 ± 0.58	258.5 ± 1.53
Creatinine (mg/dL)		0.785 ± 0.015	0.785 ± 0.01	0.79 ± 0.01	0.79 ± 0.01
Alkaline Phosphate (IU/L)		59.5 ± 1.53	64.5 ± 1.53	65 ± 1.53	69±1
Total Protein (gm/dL)		6.95 ± 0.1	7.1 ± 0.15	7 ± 0.1	7.05 ± 0.21
Cholesterol (mg/dL)		138.5 ± 1	138.5 ± 1.53	141.5 ± 1	140.5 ± 1
Triglyceride (mg/dL)		73.5 ± 1.53	68.5 ± 1.53	69 ± 1	70 ± 1
Uric Acid (mg/dL)		3.05 ± 0.058	3.05 ± 0.1	2.95 ± 0.058	2.95 ± 0.1
BUN (mg/dL)		11 ± 0.58	12.5 ± 1	13 ± 1	13.5 ± 0.58
SGOT (IU/L)		13 ± 0.58	15.5 ± 1.52	17.5 ± 0.58	19 ± 1
SGPT (IU/L)		11.5 ± 1	13 ± 0	12.5 ± 0.58	12.5 ± 0.58
Phosphorous (mg/dL)		3 ± 0.15	3.25 ± 0.15	3.25 ± 0.15	3.3 ± 0.1

Table 4. Comparison of blood biochemical parameters between control and MZnO treated mice by i.v. injection.

Parameters		**Control**	**MZnO**		
			100 µg/mL	**250 µg/mL**	**500 µg/mL**
Haemoglobin (g/dL)		13.15 ± 0.1	13.33 ± 0.15	13.3 ± 0.35	13.6 ±0.26
TC	**RBC (million/ mm^3)**	4.2 ± 0.1	4.46 ± 0.15	4.53 ± 0.15	4.7 ± 0.2
	WBC/ mm^3	5785 ± 37.86	5870 ± 26.46	5776.66± 32.15	5776.67 ± 25.17
DC (%)	**Neutrophils**	41.5 ± 1.53	39.33 ± 2.08	39.67 ± 1.58	40 ± 2.08
	Lymphocytes	66.5 ± 1.53	67.33 ± 2.08	70.5 ± 2.08	68.33 ± 2.08
	Monocytes	2.5 ± 0.58	2.33 ± 0.58	3 ± 0.58	2.33 ± 0.58
	Eosinophils	2.5 ± 0.58	2.33 ± 0.58	2 ± 0.58	2.33 ± 0.58
	Basophils	0±0	0±0	0±0	0±0
Platelets (lakh/ mm^3)		1.6 ± 0.1	1.67 ± 0.058	1.7 ± 0.21	1.73 ± 0.058
LDH (IU/L)		240 ± 2.08	252.33 ± 2.51	249 ± 1.15	257.33 ± 2.52
Creatinine (mg/dL)		0.785 ± 0.015	0.77 ± 0.02	0.765 ± 0.01	0.77 ± 0.021
Alkaline Phosphate (IU/L)		59.5 ± 1.53	70.33 ± 1.53	68.67 ± 2.08	72.5 ± 1.52
Total Protein (gm/dL)		6.95 ± 0.1	6.7 ± 0.265	6.85 ± 0.15	7.1 ± 0.1
Cholesterol (mg/dL)		138.5 ± 1	139 ± 2	137.5 ± 2.64	138.67 ± 1.58
Triglyceride (mg/dL)		73.5 ± 1.53	73.33 ± 1.52	70.5 ± 1	69.67 ± 2.52
Uric Acid (mg/dL)		3.05 ± 0.058	2.93 ± 0.115	3.05 ± 0.1	3 ± 0.2
BUN (mg/dL)		11 ± 0.58	11.33 ± 0.58	12.5 ± 0.58	12.67 ± 0.58
SGOT (IU/L)		13 ± 0.58	16.67 ± 1.15	16.5 ± 0.58	18.33 ± 0.58
SGPT (IU/L)		11.5 ± 1	12.5 ± 1.53	12.67 ± 1.52	13 ± 1
Phosphorous (mg/dL)		3 ± 0.15	3.17 ± 0.15	3.26 ± 0.15	3.35 ± 0.1

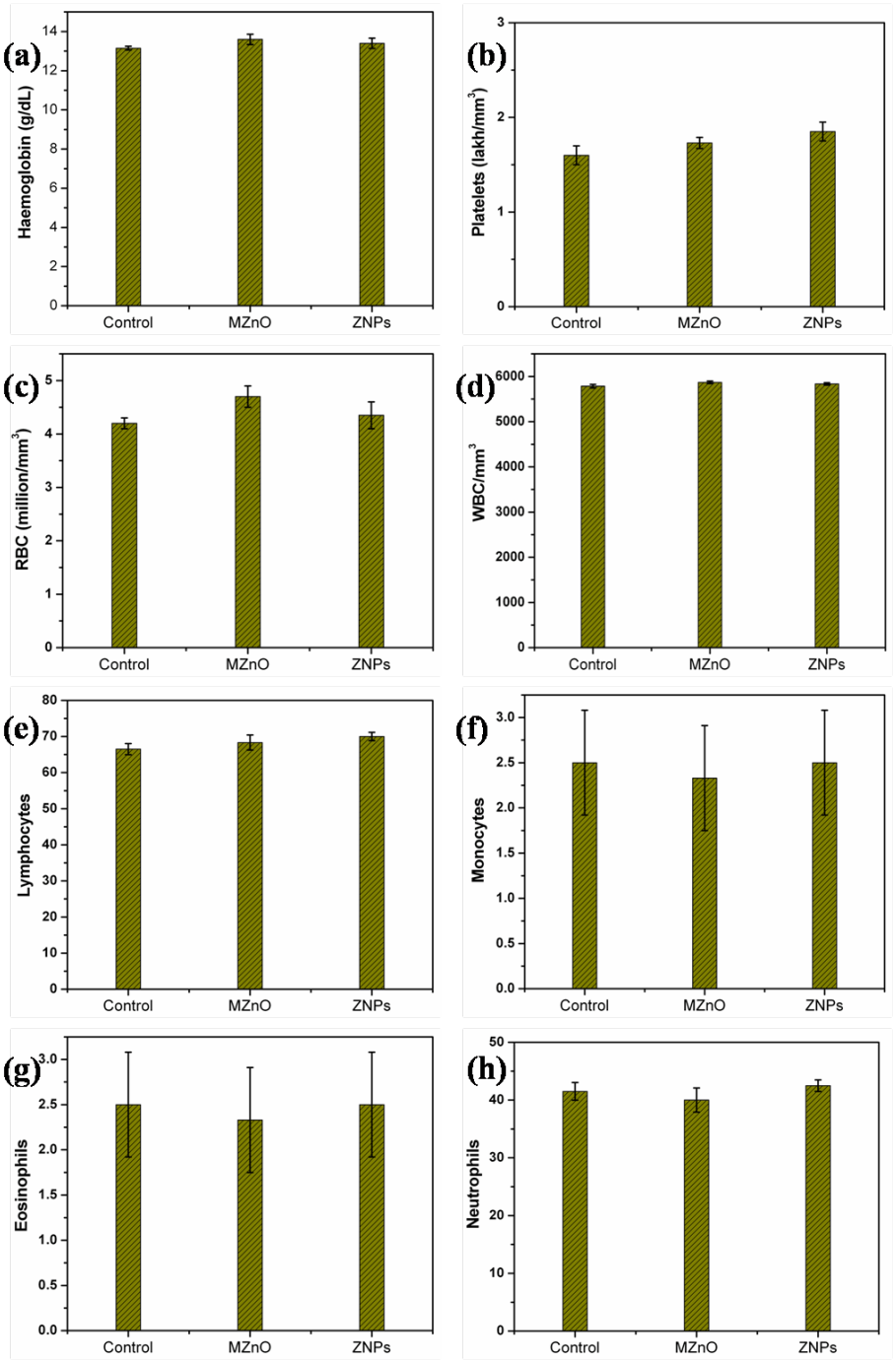


Figure S13A. Diagrammatic representation of relative hematology indicators such as (a) Haemoglobin, (b) Platelets count, (c) Red blood cell count (RBC), (d) White blood cell count (WBC), (e) Lymphocytes, (f) Monocytes, (g) Eosinophils and (h) Neutrophils of Control samples, MZnO treated mice at maximum concentration after 2 months (intravenous injection) and ZNPs treated mice at maximum concentration after 2 months (intravenous injection). Systematic toxicity study on hematology parameters suggested that no significant toxicity or any significant changes were observed in post intravenous injected mice, therefore MZnO and ZNPs both were biocompatible in nature.

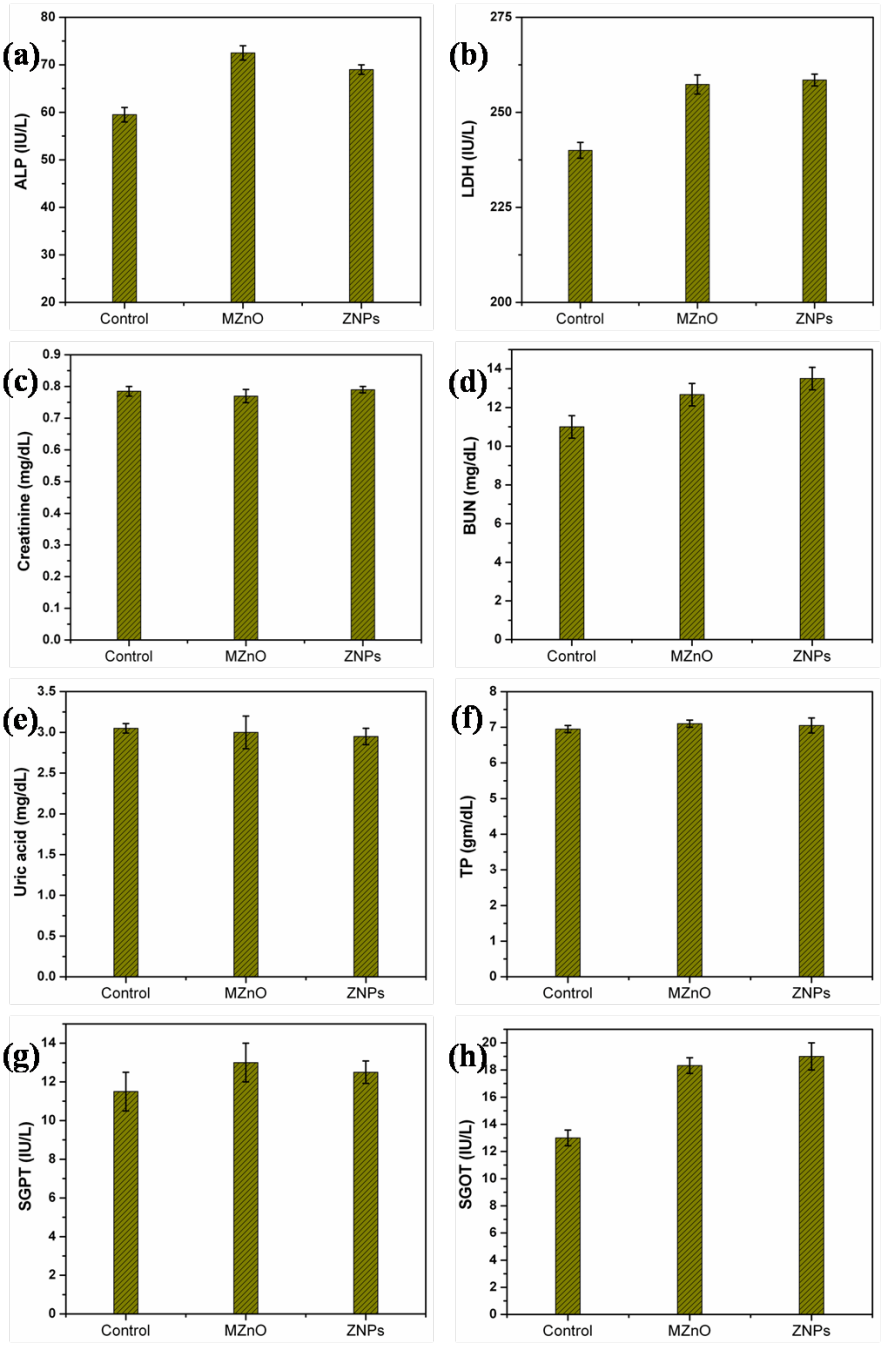


Figure S13B. Diagrammatic representation of relative serum biochemical indicators such as (a) Alkaline phosphatase (ALP), (b) Lactate dehydrogenase (LDH), (c) Creatinine, (d) Blood urea nitrogen (BUN), (e) Uric acid, (f) Total protein (TP), (g) Serum glutamic pyruvic transaminase (SGPT) and (h) Serum glutamic oxaloacetic acid (SGOT) of Control samples, MZnO treated mice at maximum concentration after 2 months (intravenous injection) and ZNPs treated mice at maximum concentration after 2 months (intravenous injection). Systematic toxicity study on serum biochemical parameters suggested that no significant toxicity or any significant changes were observed in post intravenous injected mice (for both MZnO and ZNPs). Only minor alterations were observed in SGOT, LDH and ALP levels.

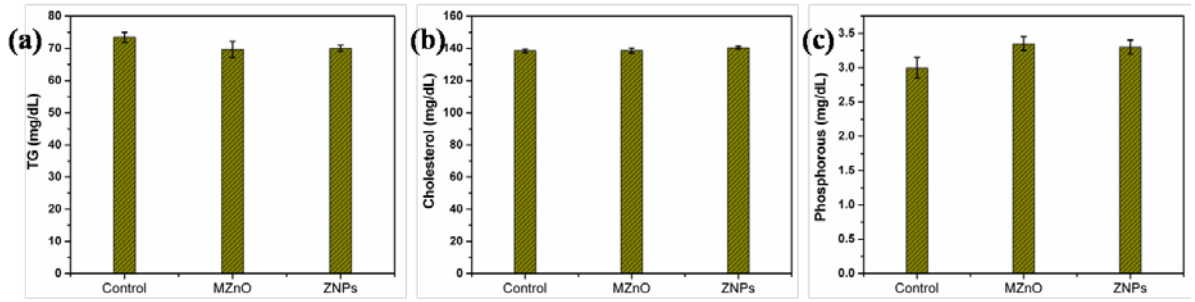
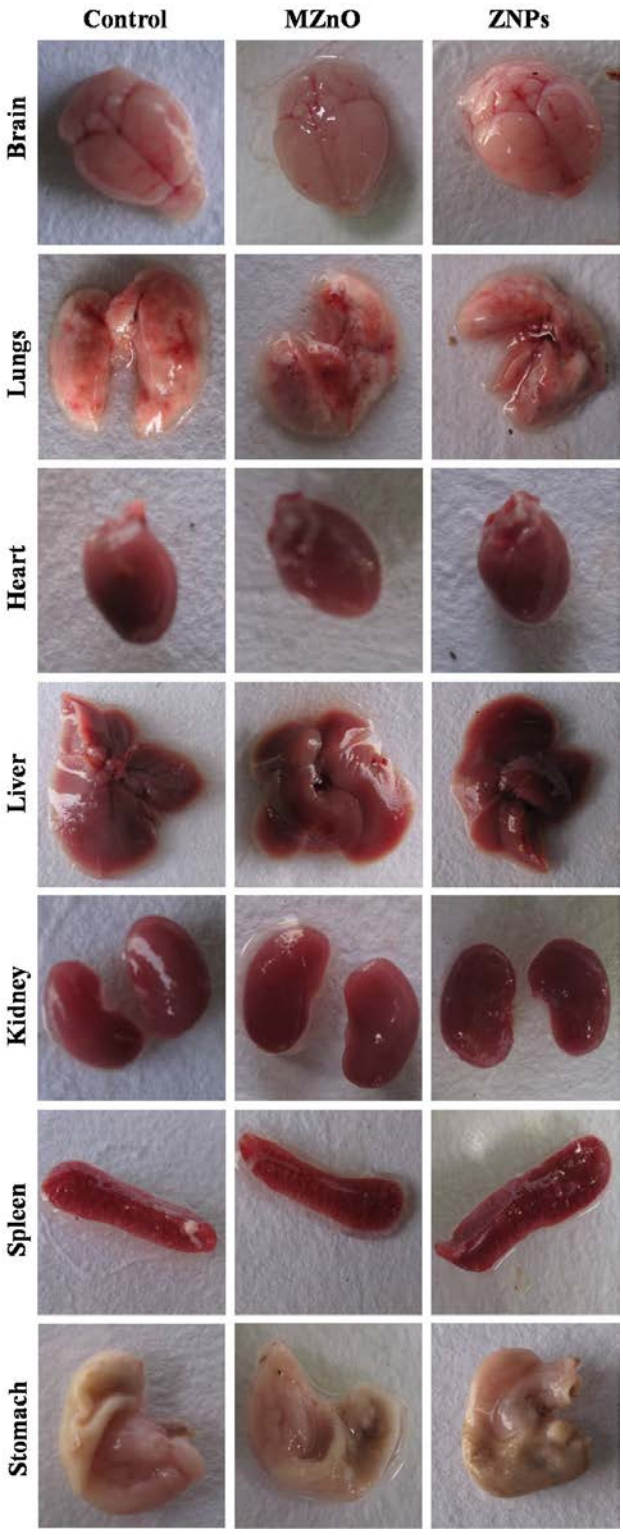
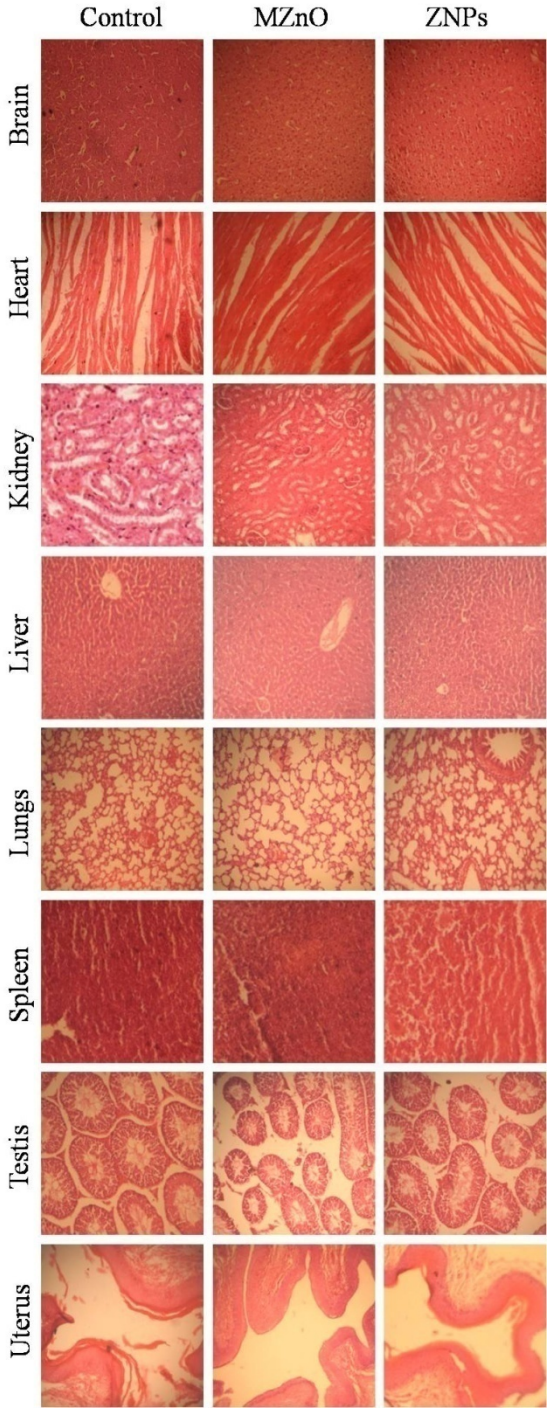


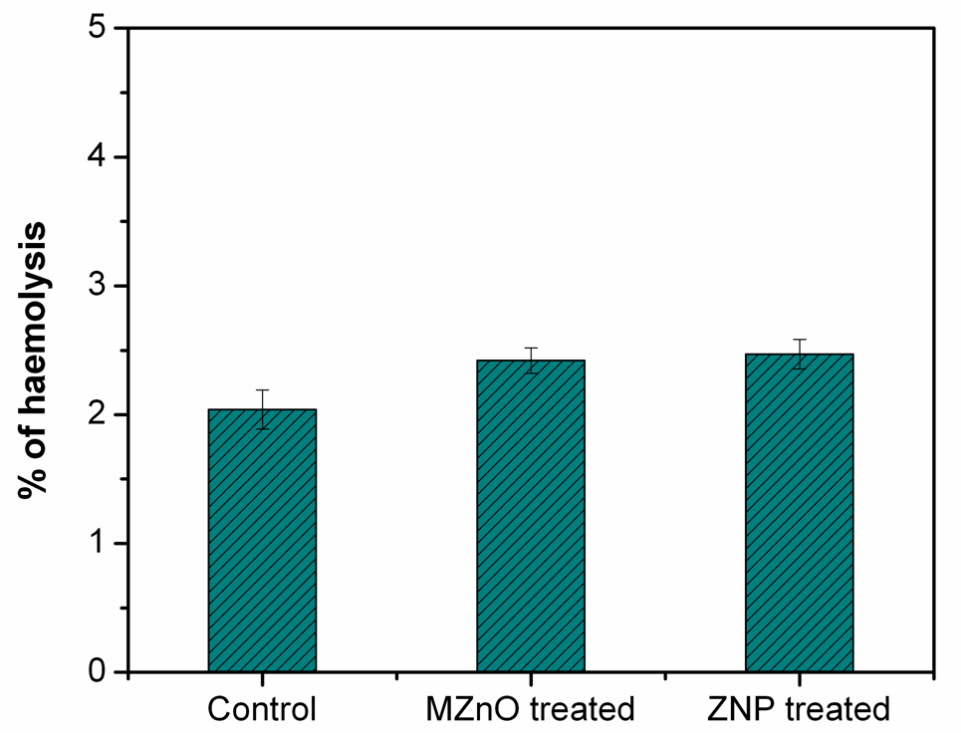
Figure S13C. Diagrammatic representation of other biochemical parameters such as (a) Triglyceride (TG), (b) Cholesterol and (c) Phosphorus of Control samples, MZnO treated mice at maximum concentration after 2 months (intravenous injection) and ZNPs treated mice at maximum concentration after 2 months (intravenous injection). No significant toxicity was observed in all the above cases for MZnO and ZNPs treatment by intravenous injection pathway during evaluation of long term toxicity (for 2 months). Therefore oral administration route as well as intravenous injection route justified biocompatibility of ZNPs.



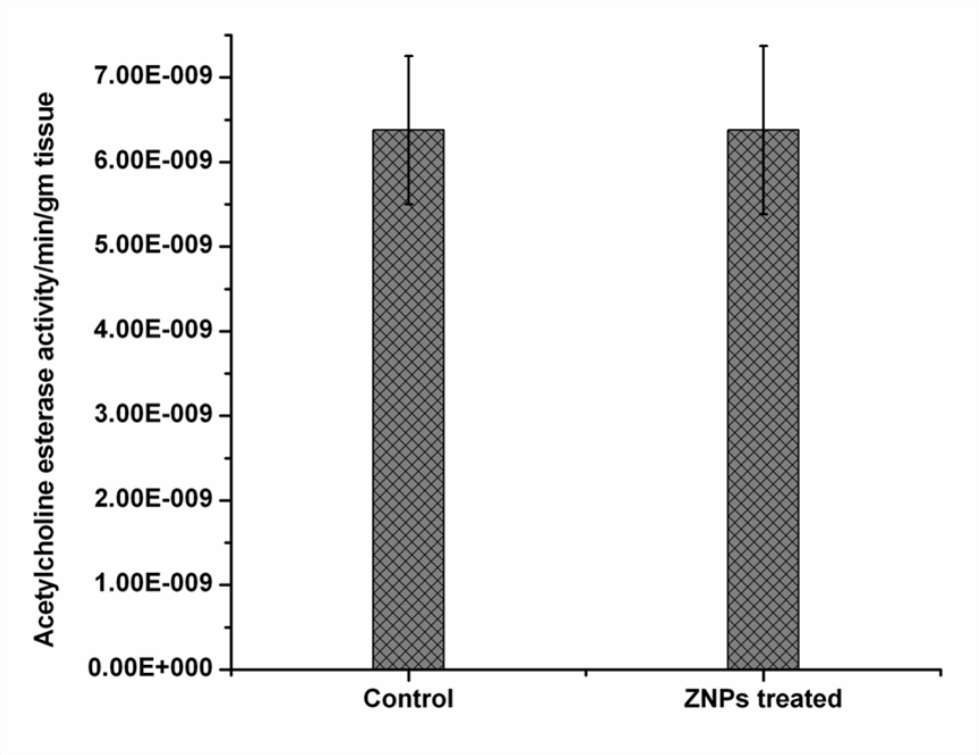
Supporting Figure S14. Major organs such as brain, lung, heart, liver, kidney, spleen and stomach of Control mice (left panel), MZnO (500 µg/mL) intravenously injected mice after 2 months (middle panel) and ZNPs (500 µg/mL) intravenously injected mice after 2 months (right panel). So significant alteration in the organs were was observed after intravenous injection of MZnO and ZNPs.



Supporting Figure S15. Histopathological analysis of major organs such as brain, heart, kidney, liver, lung, spleen, testis and uterus of Control mice (left panel), MZnO (500 µg/mL) intravenously injected mice after 2 months (middle panel) and ZNPs (500 µg/mL) intravenously injected mice after 2 months (right panel). No significant difference between control and treated mice was observed, only minor alteration was observed in liver and kidney due to ROS generation by MZnO and ZNPs. All other organs were unaltered after treatment with respect to control. Only the histopathological analysis at maximum concentration of MZnO and ZNPs were shown here.



Supporting Figure S16. *In vivo* haemolysis experiment on mice model. Maximum concentration used for long term toxicity determination was used for this haemolysis experiment. The mice were intravenous injected with 100µL MZnO and ZNPs of concentration 500 µg/mL through tail vein. After 1h of injection the blood was isolated and haemolysis measurement was carried out (as mentioned above). Interestingly no significant haemolysis was observed after intravenous injection; only 2.40% and 2.47% haemolysis was observed in MZnO and ZNPs treated mice while the control mice showed ~2% of haemolysis. Therefore no significant toxicity was observed in MZnO and ZNPs treated mice in blood stream.



Supporting Figure S17. Comparative acetylcholine esterase activity study of mice brain [control and ZNPs treated (2g/kg)].