

Kinetic profile of silver and zinc oxide nanoparticles by intraperitoneal injection in mice , a comparative study

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ABSTRACT

To investigate the kinetic profile (Absorption , distribution , and excretion) of intraperitoneal injected Ag NPs (24.52 nm) and ZnO NPs (25.16 nm) in Albino mice , a single dose of the two kinds of NPs (100 mg/kg) were used for comparison .Kinetic profile in blood clarified shorter time (T max) were spend by ZnO NPs to be absorbed compared with longer one recorded by Ag NPs as their smaller volume of distribution (Vd) to confirm their higher concentration (C max) with 95 µg/ml compared with Ag NPs which stands on 57.5 µg/ml , also Ag NPs needs longer time of elimination to their half values (t1/2 Elem.) in blood with lower clearance rate (CL) compared with ZnO NPs . This study discussed the distribution profile in different organs over time and pointed a considerable Ag and Zn concentrations specially in liver , spleen , intestine and kidney, also an important levels recorded in lung ,heart , testes and brain. Ag and ZnO NPs excretion manner noted a significant percentage of excretion via feces with 33 % and 83%for Ag NPs and ZnO NPs respectively compared with small percentage recorded with urine .

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1. Introduction

Nano industry has been described as the next industrial revolution as turning to be one of the fastest growing industries in the history of human beings [1] with expanding nanotechnology scope in continuous manner as using of nano materials in many implementations in our daily life [2]. Nanoparticles are defined as one or more dimension small objects in range of 1–100 nm. Due to their small size and large- scale surface area compared with micro scale , different degrees of biological toxic effects were demonstrated by large numbers of studies [3]-[6]. Silver nanoparticles (Ag NPs) with mean annual production of 55 tons, individually represent the highest used in many consumer products with the widely expansion in NPs commerce which are used as antimicrobial agent in different consumer products with range from clothing, cosmetics, shoes, respirators surface coating, detergents, water filters and house purification systems, laptops, and phones [7] . Among metal containing nanoparticles , zinc oxide (ZnO NPs) was the third highest production over the world with 550 tons, widely used in cosmetic products as UV light scatter specially in sun screens , and dermal ointments, and toothpastes [8]. ZnO NPs mostly used in solar cells production and LCDs pigments, electronics, rubber, textiles and chemical fiber production [9],[10]. Due to the high surface-to-volume ratio, greater oxidant capacity and bio persistence, nanoparticles became the primary source for toxicity, which can penetrate easily through epithelium and reach to interstitial pulmonary area. There are real needs to protect our public and environmental health and safety, specially where the standards or guidelines that can directly rule

nano materials effects are not exist in present time [11]. However, the tunable and varied physicochemical properties of Nanoparticles pose a new challenge for understanding their biological behavior, and bio distribution, so the in vivo and in vitro kinetics can be controlled to meet the requirements of efficient drug delivery and minimizing side-effects. In vivo porticokinetics refers to the absorption, distribution, metabolism, and excretion (ADME) of nanomaterials. the wide definition for Toxicokinetics described by [12] as the movement and fate, also referred to as the disposition, of toxicants. The term is most commonly used when describing the time course of absorption, distribution, and elimination (including biotransformation and excretion) of toxicants in an organism. blood concentrations analysis against time course after single dose administration of Ag NPs and ZnO NPs was efficient method for evaluate the amount of absorption and quantitative the availability, and helps in evaluation the amount of distribution and elimination steps [13]. Many studies described kinetic behavior of Ag and ZnO NPs followed oral or intravenous administration [14]-[17] and Only single study demonstrated the biodistribution of ZnO NPs over 27 h followed intraperitoneal injection. But no occurred any study demonstrate and analyze the kinetic parameters and behaves of NPs followed intraperitoneal injection of Ag or ZnO NPs in mice at world level, in addition to the comparison between them.

2. Materials and Methods

2.1. Nanoparticles

Un coated Ag NPs (20 nm as the specification sheet) were purchased as grey to black nano powder from NANOSHEL company - USA , with purity of 99 % , 10.5 g/cm³ density , and spherical morphology . ZnO NPs (10-30 nm as the specification sheet) were purchased as white to light yellow nano powder from Skyspring Nanomaterials incorporation –USA, with purity of 99.8%, 5.606 g/cm³ density and spherical morphology. Ag and ZnO NPs were characterized using scanning probe microscope (SPM) from FILIPS-Germany to determine size average for the two kinds of nanoparticles that used in this study. The surface morphology of the Ag NPs and ZnO NPs were displayed by atomic force microscope (AFM) under normal atmospheric conditions. The examined samples of nanoparticles were dispersed on glass slide and explored using the instrument.

2.2. Animals housing

Healthy adult male (8-10 week aged) Swiss albino mice with average weight 25±2 gm were purchased from the national center for drug control and research – Ministry of Health. All mice were housed in polypropylene cages under controlled conditions of temperature 25 ± 5°C , humidity of 50-60 % , and 12 ± 2 hours light/dark cycles. Standard diet pellet and water ad libitum were used for feeding . The animals were kept for 7 days before starting the experiments for acclimatization to laboratory conditions. All animals were dealt in accordance to the guidelines of the Care and Use of Laboratory Animals- National Research Council and in accordance with the guidelines of the international guidelines for animal experimentation.

2.3. Ag and ZnO NPs suspensions preparation

Concentration (100 mg/kg) of Ag NPs and ZnO NPs suspensions were prepared with deionized distilled water . The two suspensions were homogenized by vortex for 20 sec. , then exposed to probe ultrasound sonication from Soniprep 150 MES -UK (pulsed mode of 1min operation and 30 sec. stopping) in ice bath for 60 min [18], the prepared suspensions were immediately exposed to mice with single intraperitoneal injection.

2.4. Experimental design

Three groups of male albino mice with average weight 25±2 gm used for kinetic study , each group contain 34 mice , first group intraperitoneally injected with single dose of 100 mg/kg Ag NPs , the same dose of ZnO NPs for the second group , the third group was control. Samples of 1 ml blood, and 0.25-0.5 gm of organs tissues including (liver, spleen, kidney, intestine, brain, testis, lung, heart) collected from two mice at specific time points after injection (5, 10,15, 30, 60 min, 2, 4, 6, 24 h, 2, 4, 7, 9, 11, 14, 18 d) and kept in -20 C° until prepared for digestion. Feces and urine samples collected from each group daily.

2.5. Ag and ZnO NPs quantitative analysis

Sub samples 0.25-0.5 gm of tissue (liver, spleen, kidney, intestine, brain, testis, lung, heart), 1ml of whole blood, urine, and 1gm feces samples digest individually using microwave digestion system from Milestone – Italy as described in [19],[20] using 9 ml of 68-72% nitric acid HNO₃, 1ml of 37% hydrochloric acid HCl, and 2 ml of 30% hydrogen peroxide H₂O₂, the digested samples diluted to 25 ml using distilled water. The concentration of silver and zinc detect in each sample using flame or flameless atomic absorption spectrophotometer (AAS) from Shimadzu- Japan.

2.6. Kinetic analysis

The kinetic analysis was performed using a non compartmental model . The bio kinetic parameters including maximum concentration (C max), Time to achieve maximum concentration (T max), Half-life of absorption (T_{1/2} Abs) , Half-life of Elimination (T_{1/2} Elem) calculated using the equation $T_{1/2} = 0.693/k$, were the elimination rate constant (K Elem) estimated using the formula $k = -2.303 \times ((\log \text{conc.2} - \log \text{conc.1})/(\text{time2} - \text{time1}))$, Clearance rate (CL) = $K \times V_d$, and apparent Volume of distribution (Vd) was determined from the intercept of the curve. $V_d = \text{Dose}/\text{Intercept}$ as described by [21].

2.7. Statistical analysis

The results are presented as the mean \pm standard error of means (SE) . Analysis of variance (ANOVA) and least significant difference (LSD) were used to explain the differences between means over time points in feces and urine samples at ($p \leq 0.05$).

3. Results and descussion

3.1. Characterization of Ag and ZnO NPs

According to the granularity distribution chart , the average diameter for Ag and ZnO NPs samples were 24.52 nm , 25.16 nm respectively with spherical shape determined for the two types of NPs using AFM (Figure 1)

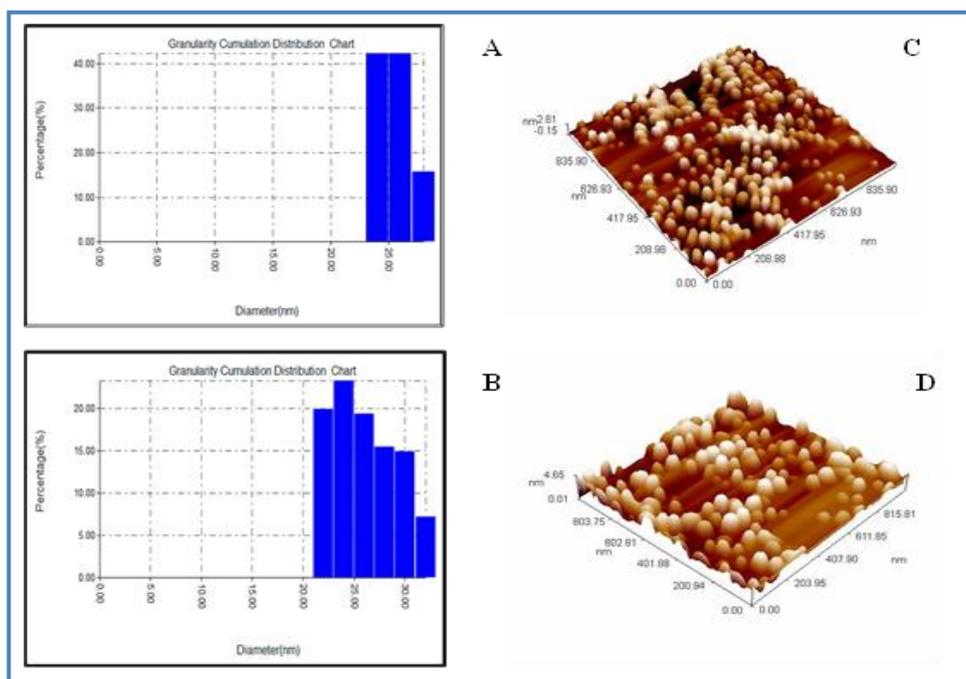


Figure 1. Characteristics of Ag and ZnO NPS , granularity distribution chart (left) for AgNPs (A) and ZnO NPs (B) , spherical shape using AFM (right) for Ag NPs (C) and ZnO NPs (D).

3.2. Ag and ZnO NPs absorption

Blood concentrations analysis against time course after single dose administration of Ag NPs and ZnO NPs was efficient method for evaluate the amount of absorption and quantitative the availability , and helps in evaluation the amount of distribution and elimination steps [13]. Highly significant difference ($p \leq 0.05$) in blood silver and Zn concentrations were illustrated over different time points (Fig 2 , table 1). The concentrations elevated within first few minutes followed injection with absorption half life ($T_{1/2}$ Abs) of 26.32 ± 0.57 min and 14.30 ± 0.00 min for Ag and Zn respectively , and recording maximum concentration C_{max} with 57.50 ± 2.50 $\mu\text{g/ml}$, 95 ± 0.00 $\mu\text{g/ml}$ after 1 h and 30 min respectively which indicate the shorter needed time for ZnO absorption in versus with Ag NPs by the effect of the low molecular weight of zinc, and the effect of lower apparent volume of distribution (V_d) compared with silver by which ZnO NPs could quickly reach distribution equilibrium between blood and tissues [22]. Lower value of maximum concentration detected in blood referred to silver compared with zinc which may caused by molecular weight variation [24], the probable aggregations of Ag NPs as the postponement in the peritoneal cavity leading to increase the surface area [23] and reduce the amount of silver that can pass through peritoneal membrane , in addition to and amount of generated ions [21], [25] and some considerable effects like lymphatic drainage , peritoneal permeability , and particle charge [26] , [27] .

The results of current study approach with those of previous one carried out by [29] showed the elevation of blood zinc level after 30 min. of intraperitoneal injected with 2.5g/kg ZnO NPs with >100 nm in diameter, the peak point recorded after 6 h , then kept with equilibrator scale at 72 h point . In the current study blood zinc concentrations need less time to reach their peak point as the effect of smaller particle size and lower concentration that play an important role in peritoneal particokinetic . Shorter half life of elimination ($T_{1/2}$ Elem) recorded for zinc oxide NPs 2.38 ± 0.08 d from blood compared with silver 8.39 ± 0.23 d which considered long and suggested that Ag NPs can not removed from the body in short time (table 2) , this slow elimination manner with low clearance rate (Cl) of 0.43 ± 0.03 mg/d.kg compared with higher recorded one with ZnO NPs 0.87 ± 0.03 mg/d.kg explained by formation silver protein complexes specially with sulfhydryl containing proteins as the high affinity of silver for sulfur group [14],[28] , also the insoluble part of two types of NPs may involved . The calculated apparent volume of distribution (V_d) were 5.16 ± 0.25 l/kg (129.00 ± 6.15 ml/25 gm) and 2.99 ± 0.02 l/kg (74.75 ± 0.56 ml/25gm) for Ag and ZnO NPs respectively , with taking into consideration the total body fluid volume was 15 ml in the maximum for mouse weighted 25 gm [15] , smaller volume of distribution of ZnO NPs illustrated the shorter needed time for distribution equilibrium [21] . It is worth to mention that the resulted values of kinetic parametersb of current study should not be compared with parameters in the other studies as the differentr mathematical models and animals weret used in the currents study.

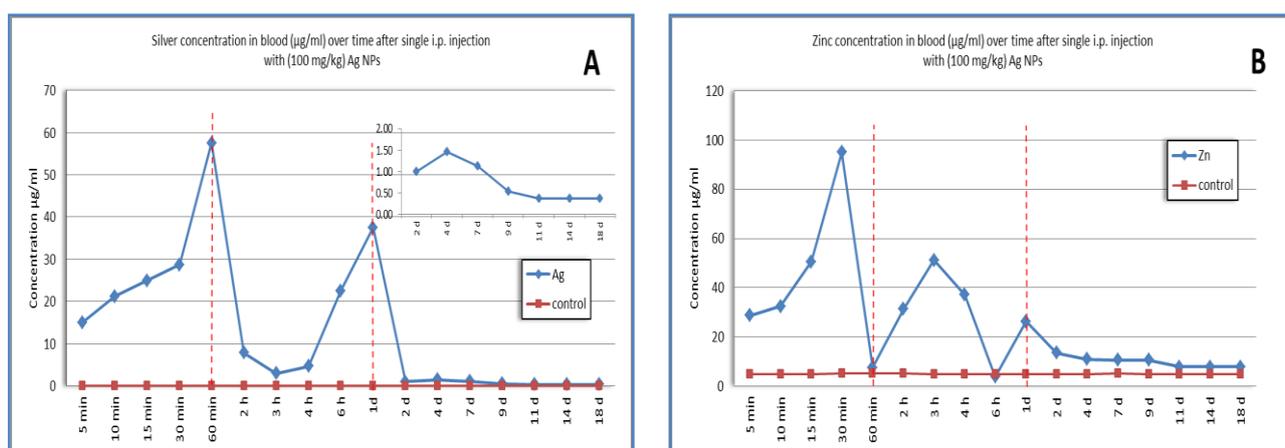


Figure 2. Silver (A) and Zinc (B) concentrations means in blood ($\mu\text{g/ml}$) over time followed single intraperitoneal (ip) injection with 100 mg/kg Ag and ZnO NPs after subtraction of the basal zinc level in the control group , no silver detected in control group .

Table 1. Ag and Zn means concentration($\mu\text{g/ml}$) \pm ESM in blood over time points after single intraperitoneal injection with 100 mg/kg Ag and ZnO NPs

Time	Ag NPs experimental group		ZnO NPs experimental group	
	Control	Ag NPs (100mg/kg)	Control	ZnO NPs (100 mg/kg)
5 min	0.00 \pm 0.00	15.00 \pm 0.00 ^a	5.00 \pm 0.00 ^a	28.75 \pm 1.53 ^f
10 min	0.00 \pm 0.00	21.25 \pm 1.02 ^f	5.00 \pm 0.00 ^a	32.50 \pm 1.02 ^h
15 min	0.00 \pm 0.00	25.00 \pm 0.00 ^g	5.00 \pm 0.51 ^a	50.63 \pm 0.51 ^j
30 min	0.00 \pm 0.00	28.75 \pm 1.02 ^h	5.31 \pm 0.26 ^a	95.00 \pm 0.00 ^k
60 min	0.00 \pm 0.00	57.50 \pm 2.04 ^j	5.31 \pm 0.26 ^a	7.50 \pm 0.00 ^b
2 h	0.00 \pm 0.00	7.88 \pm 0.31 ^d	5.31 \pm 0.26 ^a	31.25 \pm 0.00 ^g
3 h	0.00 \pm 0.00	3.00 \pm 0.00 ^{b,c}	5.00 \pm 0.00 ^a	51.25 \pm 0.00 ^j
4 h	0.00 \pm 0.00	4.63 \pm 0.31 ^c	5.00 \pm 0.00 ^a	37.19 \pm 0.26 ⁱ
6 h	0.00 \pm 0.00	22.50 \pm 2.04 ^f	5.00 \pm 0.51 ^a	3.75 \pm 0.51 ^a
1d	0.00 \pm 0.00	37.50 \pm 2.04 ⁱ	5.00 \pm 0.00 ^a	20.63 \pm 0.51 ^e
2 d	0.00 \pm 0.00	1.00 \pm 0.03 ^a	5.00 \pm 0.51 ^a	13.44 \pm 0.51 ^d
4 d	0.00 \pm 0.00	1.46 \pm 0.03 ^{a,b}	5.00 \pm 0.00 ^a	10.94 \pm 0.26 ^c
7 d	0.00 \pm 0.00	1.13 \pm 0.03 ^{a,b}	5.31 \pm 0.26 ^a	10.63 \pm 0.00 ^c
9 d	0.00 \pm 0.00	0.54 \pm 0.03 ^a	5.00 \pm 0.00 ^a	10.63 \pm 0.26 ^c
11 d	0.00 \pm 0.00	0.38 \pm 0.01 ^a	5.00 \pm 0.00 ^a	7.81 \pm 0.26 ^b
14 d	0.00 \pm 0.00	0.38 \pm 0.01 ^a	5.00 \pm 0.00 ^a	7.81 \pm 0.26 ^b
18 d	0.00 \pm 0.00	0.38 \pm 0.01 ^a	5.00 \pm 0.00 ^a	7.81 \pm 0.26 ^b
LSD		1.90	0.53	1.03

All data expressed with mean \pm standard error of means (SEM), similar small letters means no significant difference between means values on $p < 0.05$

Table 2. Ag and ZnO NPs blood kinetic parameters means \pm ESM after single intraperitoneal injection with 100 mg/kg Ag and ZnO NPs

dose	C Max.	T Max.	T1/2 Abs.	Last T1/2 Elem.	Clarence	k Elem.	Apparent Vd
mg/kg	$\mu\text{g/ml}$	min.	min.	d	l/kg.d	1/d	l.kg
Ag NPs 100	57.50 \pm 2.50	60.00 \pm 0.00	26.32 \pm 0.57	8.39 \pm 0.23	0.43 \pm 0.03	0.08 \pm 0.00	5.16 \pm 0.25
ZnO NPs 100	95.0 \pm 0.00	30.00 \pm 0.00	14.30 \pm 0.00	2.38 \pm 0.08	0.87 \pm 0.03	0.29 \pm 0.00	2.99 \pm 0.02

3.3. Ag and ZnO NPs distribution

Regardless the route of administration, nanoparticles may become available systematically and start potential interaction with different portions in plasma, blood contents, and coagulation factors [30], this special interaction have important influence on further steps of distribution addition to nano excretion [31]. Figures 3 and 4, Tables 3 and 4 showed the distribution profile of Ag and ZnO NPs after single intraperitoneal injected dose of 100 mg/Kg. With an overview, Ag and Zn were distributed to all studied organs. The highest levels of silver and zinc showed after 60 and 30 min of injection respectively with the same manner with their levels in blood depending in their speed on some factors starting from the half life of absorption which illustrated by increasing retention time of Ag NPs in peritoneal cavity compared with ZnO NPs by the effect of high molecular weight as discussed before and the amount of ions released from each kind of nanoparticles [26]. At time period between 1 h to 1 day the translocation appeared to began in different studied organs which may referred to the redistribution of monocytes with remaining in spleen as a reservoir [32] where silver and zinc in particulate, ionic, or complex binding forms may be trans located from the initial uptake tissues to other parts of the body through the circulatory system [33]-[36]. After time point of 1-2 days gradual dropping were became obvious for both of Ag and Zn concentrations in various studied organs with faster rate for Zn as larger clearance rate which recorded for ZnO NPs in blood. Equilibrium become clear after 7 days for Ag and not quite cleared for Zn concentrations.

Liver and spleen which have the dominant role in immune system [37], hold more nanoparticles pending systematic inflammation that led to shorthand retention of nanoparticles by other organs [38], and their clearing role by the interaction of itself protein effectively with nanoparticles with altering the antigenicity and inducing the autoimmune responses, resulting complex of nanoparticle-protein that can facilitate dendritic cells antigen up taking [39]. But as effect of masses different between liver and spleen which have smaller one [40], making spleen have grater accumulation per unit of mass specially during the first hour after injection. Interesting high concentrations of Zn were observed in intestine specially at early time of injection (10 min-1 h) which may be related to the direct absorption of ZnO NPs that resides initially in the visceral tissues surrounding area from intrperitoneal cavity to intestine [41], in addition to excreted nanoparticles

from the liver via the biliary pathway [42]. In lung a considerable concentration of Ag and Zn were noted in this study which confirmed the translocation [43], also redistribution of nanoparticles over time which indicated before by Dziendzikowska [44] using TEM in rat alveolar macrophages after 7 days of intravenous injection with 5 mg/kg Ag NPs. The noted concentrations decreased over time to record their lowest levels after 9 days and 11 days for Ag and Zn respectively. This study remarked a considerable Ag and Zn levels in brain with $2.26 \pm 0.00 \mu\text{g/gm}$ and $32.81 \pm 0.31 \mu\text{g/gm}$ respectively, these results corroborate the ability of both types of nanoparticles to cross the blood brain barrier (BBB) as proved in previous studies of [45],[16],[46],[44],[47]. The equilibrium were not well clarified at the end time points specially between 7-18 days as some rising in Ag and Zn concentrations in brain which may related to improved absorption by endothelial cells and as a result facilitate their uptake and translocation through blood vessels wall by process of transcytosis [48]. These results came with those of [44] in increasing silver concentration in brain at the end point 28 d of their experiment after single intravenous injection with 5 mg/kg of 20 and 200 nm Ag NPs. There is an essential evidence proposing that Ag NPs can pass the blood testis barrier BTB as the occurrence of silver in the tissue of testicles which confirmed by exposing animals to Ag NPs through different routs of administration [36],[49],[50], in current study the maximum concentrations of Ag and ZnO were 9.57 ± 0.43 and $48.45 \pm 0.39 \mu\text{g/gm}$ after 60 and 30 min of injection respectively with slow rate of elimination over experimental time points.

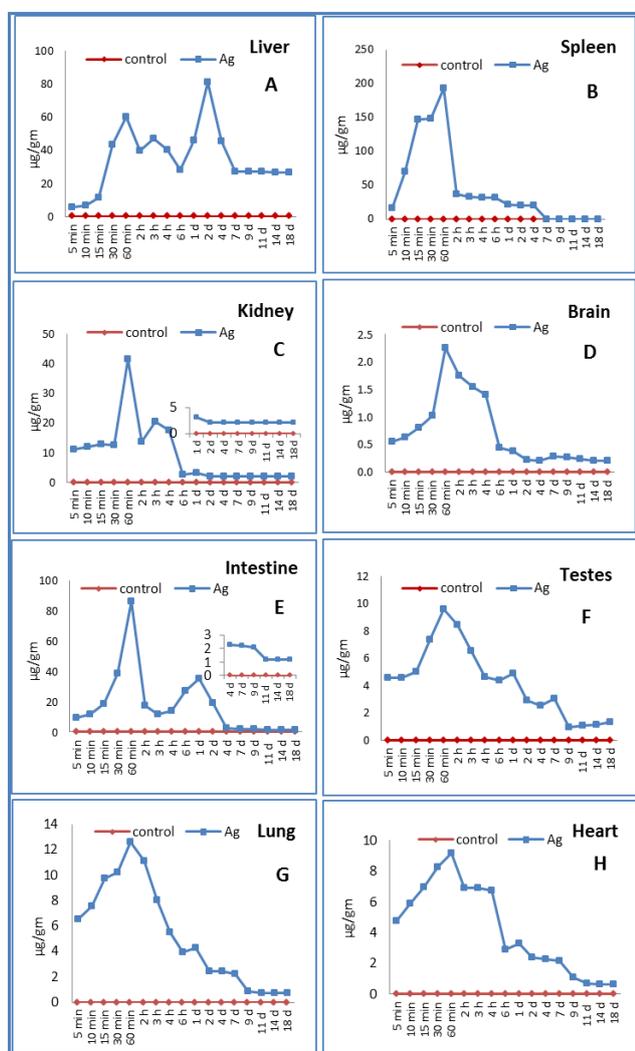


Figure 3. Silver concentrations means ($\mu\text{g/gm}$) in (A) Liver, (B) Spleen, (C) Kidney, (D) Brain, (E) Intestine, (F) Testes, (G) Lung, and (H) Heart over time following single intraperitoneal injection with 100 mg/kg Ag NPs.

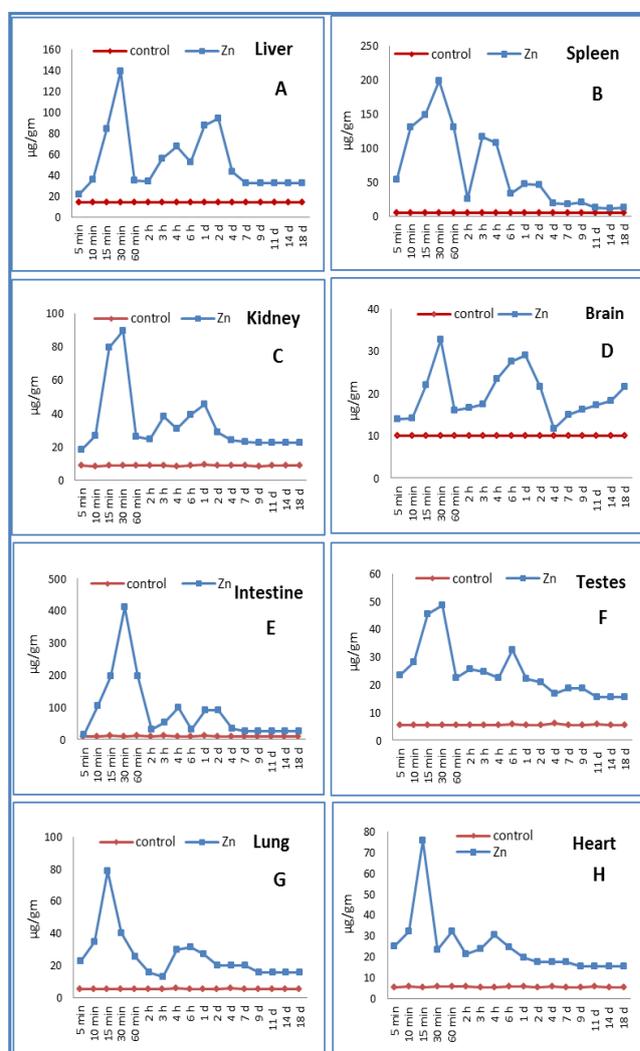


Figure 4. Zinc concentrations means ($\mu\text{g/gm}$) in (A) Liver, (B) Spleen, (C) Kidney, (D) Brain, (E) Intestine, (F) Testes, (G) Lung, and (H) Heart over time following single intraperitoneal injection with 100 mg/kg ZnO NPs.

Table 3. Silver concentrations means ($\mu\text{g}/\text{gm}$) \pm SEM in liver, spleen, kidney, brain, intestine, testes, lung, heart over experimental time points following single intraperitoneal injection with 100 mg/kg Ag NPs.

Time	Liver		Spleen		Kidney		Brain	
	Control	Ag NPs (100mg/kg)	Control	Ag NPs (100 mg/kg)	Control	Ag NPs (100mg/kg)	Control	Ag NPs (100 mg/kg)
5 min	0.00 \pm 0.00	6.00 \pm 0.00 ^a	0.00 \pm 0.00	15.74 \pm 0.93 ^b	0.00 \pm 0.00	11.09 \pm 0.22 ^c	0.00 \pm 0.00	0.54 \pm 0.07 ^d
10 min	0.00 \pm 0.00	6.75 \pm 0.25 ^a	0.00 \pm 0.00	69.44 \pm 0.93 ^h	0.00 \pm 0.00	11.96 \pm 0.22 ^d	0.00 \pm 0.00	0.63 \pm 0.02 ^e
15 min	0.00 \pm 0.00	11.75 \pm 0.25 ^b	0.00 \pm 0.00	147.22 \pm 0.93 ⁱ	0.00 \pm 0.00	12.83 \pm 0.22 ^e	0.00 \pm 0.00	0.80 \pm 0.02 ^f
30 min	0.00 \pm 0.00	43.52 \pm 0.93 ^{ef}	0.00 \pm 0.00	148.15 \pm 1.85 ^j	0.00 \pm 0.00	12.61 \pm 0.43 ^d	0.00 \pm 0.00	1.02 \pm 0.07 ^g
60 min	0.00 \pm 0.00	60.19 \pm 0.93 ^h	0.00 \pm 0.00	193.52 \pm 0.93 ^j	0.00 \pm 0.00	41.67 \pm 0.93 ⁱ	0.00 \pm 0.00	2.26 \pm 0.00 ^k
2 h	0.00 \pm 0.00	39.81 \pm 0.93 ^d	0.00 \pm 0.00	36.11 \pm 0.93 ^g	0.00 \pm 0.00	13.89 \pm 0.93 ^f	0.00 \pm 0.00	1.75 \pm 0.05 ^j
3 h	0.00 \pm 0.00	47.22 \pm 0.93 ^e	0.00 \pm 0.00	33.33 \pm 1.85 ^g	0.00 \pm 0.00	20.50 \pm 0.50 ^h	0.00 \pm 0.00	1.55 \pm 0.05 ⁱ
4 h	0.00 \pm 0.00	40.74 \pm 1.85 ^{de}	0.00 \pm 0.00	31.48 \pm 0.00 ^e	0.00 \pm 0.00	17.50 \pm 0.50 ^g	0.00 \pm 0.00	1.40 \pm 0.10 ^h
6 h	0.00 \pm 0.00	28.70 \pm 0.93 ^c	0.00 \pm 0.00	31.48 \pm 1.85 ^e	0.00 \pm 0.00	2.57 \pm 0.00 ^{ab}	0.00 \pm 0.00	0.44 \pm 0.04 ^c
1 d	0.00 \pm 0.00	46.43 \pm 1.19 ^{fg}	0.00 \pm 0.00	21.30 \pm 0.93 ^d	0.00 \pm 0.00	3.14 \pm 0.14 ^b	0.00 \pm 0.00	0.38 \pm 0.05 ^c
2 d	0.00 \pm 0.00	81.25 \pm 2.08 ⁱ	0.00 \pm 0.00	19.44 \pm 0.93 ^c	0.00 \pm 0.00	2.21 \pm 0.07 ^a	0.00 \pm 0.00	0.22 \pm 0.02 ^a
4 d	0.00 \pm 0.00	45.83 \pm 4.17 ^f	0.00 \pm 0.00	19.44 \pm 0.93 ^c	0.00 \pm 0.00	2.14 \pm 0.14 ^a	0.00 \pm 0.00	0.20 \pm 0.01 ^a
7 d	0.00 \pm 0.00	27.50 \pm 2.50 ^c	0.00 \pm 0.00	0.07 \pm 0.00 ^a	0.00 \pm 0.00	2.21 \pm 0.07 ^a	0.00 \pm 0.00	0.28 \pm 0.01 ^b
9 d	0.00 \pm 0.00	27.50 \pm 1.67 ^c	0.00 \pm 0.00	0.07 \pm 0.00 ^a	0.00 \pm 0.00	2.21 \pm 0.07 ^a	0.00 \pm 0.00	0.26 \pm 0.01 ^{ab}
11 d	0.00 \pm 0.00	27.29 \pm 2.29 ^c	0.00 \pm 0.00	0.06 \pm 0.00 ^a	0.00 \pm 0.00	2.21 \pm 0.07 ^a	0.00 \pm 0.00	0.23 \pm 0.01 ^a
14 d	0.00 \pm 0.00	27.08 \pm 2.08 ^c	0.00 \pm 0.00	0.06 \pm 0.00 ^a	0.00 \pm 0.00	2.21 \pm 0.07 ^a	0.00 \pm 0.00	0.21 \pm 0.01 ^a
18 d	0.00 \pm 0.00	27.08 \pm 2.08 ^c	0.00 \pm 0.00	0.06 \pm 0.00 ^a	0.00 \pm 0.00	2.21 \pm 0.07 ^a	0.00 \pm 0.00	0.21 \pm 0.01 ^a
LSD	NO	3.00	NO	1.70	NO	0.70	NO	0.07

Time	Small intestine		Testes		Lung		Heart	
	Control	Ag NPs (100mg/kg)	Control	Ag NPs (100 mg/kg)	Control	Ag NPs (100mg/kg)	Control	Ag NPs (100 mg/kg)
5 min	0.00 \pm 0.00	9.25 \pm 0.75 ^b	0.00 \pm 0.00	4.57 \pm 0.22 ^{cd}	0.00 \pm 0.00	6.52 \pm 0.43 ^e	0.00 \pm 0.00	4.78 \pm 0.00 ^f
10 min	0.00 \pm 0.00	11.50 \pm 0.50 ^c	0.00 \pm 0.00	4.57 \pm 0.22 ^{cd}	0.00 \pm 0.00	7.61 \pm 0.22 ^f	0.00 \pm 0.00	5.87 \pm 0.22 ^g
15 min	0.00 \pm 0.00	18.75 \pm 0.75 ^e	0.00 \pm 0.00	5.00 \pm 0.22 ^d	0.00 \pm 0.00	9.78 \pm 0.22 ^h	0.00 \pm 0.00	6.96 \pm 0.00 ^h
30 min	0.00 \pm 0.00	38.89 \pm 1.85 ^h	0.00 \pm 0.00	7.39 \pm 0.43 ^f	0.00 \pm 0.00	10.22 \pm 0.65 ^h	0.00 \pm 0.00	8.26 \pm 0.00 ⁱ
60 min	0.00 \pm 0.00	86.11 \pm 0.93 ⁱ	0.00 \pm 0.00	9.57 \pm 0.43 ^h	0.00 \pm 0.00	12.61 \pm 0.00 ^j	0.00 \pm 0.00	9.15 \pm 0.02 ^j
2 h	0.00 \pm 0.00	17.50 \pm 0.50 ^d	0.00 \pm 0.00	8.46 \pm 0.77 ^g	0.00 \pm 0.00	11.15 \pm 0.38 ⁱ	0.00 \pm 0.00	6.92 \pm 0.00 ^h
3 h	0.00 \pm 0.00	11.50 \pm 0.50 ^c	0.00 \pm 0.00	6.54 \pm 0.38 ^e	0.00 \pm 0.00	8.08 \pm 0.38 ^g	0.00 \pm 0.00	6.92 \pm 0.00 ^h
4 h	0.00 \pm 0.00	14.00 \pm 0.00 ^d	0.00 \pm 0.00	4.62 \pm 0.38 ^{cd}	0.00 \pm 0.00	5.50 \pm 0.00 ^d	0.00 \pm 0.00	6.75 \pm 0.75 ^h
6 h	0.00 \pm 0.00	27.00 \pm 3.00 ^f	0.00 \pm 0.00	4.38 \pm 0.38 ^{cd}	0.00 \pm 0.00	3.95 \pm 0.47 ^c	0.00 \pm 0.00	2.91 \pm 0.12 ^d
1 d	0.00 \pm 0.00	35.00 \pm 3.00 ^g	0.00 \pm 0.00	4.88 \pm 0.38 ^{cd}	0.00 \pm 0.00	4.30 \pm 0.35 ^c	0.00 \pm 0.00	3.26 \pm 0.23 ^e
2 d	0.00 \pm 0.00	19.00 \pm 1.00 ^e	0.00 \pm 0.00	2.93 \pm 0.07 ^b	0.00 \pm 0.00	2.43 \pm 0.00 ^b	0.00 \pm 0.00	2.36 \pm 0.07 ^c
4 d	0.00 \pm 0.00	2.29 \pm 0.14 ^a	0.00 \pm 0.00	2.57 \pm 0.14 ^b	0.00 \pm 0.00	2.43 \pm 0.14 ^b	0.00 \pm 0.00	2.29 \pm 0.00 ^c
7 d	0.00 \pm 0.00	2.21 \pm 0.07 ^a	0.00 \pm 0.00	3.07 \pm 0.07 ^b	0.00 \pm 0.00	2.21 \pm 0.07 ^b	0.00 \pm 0.00	2.14 \pm 0.00 ^c
9 d	0.00 \pm 0.00	2.07 \pm 0.07 ^a	0.00 \pm 0.00	0.97 \pm 0.03 ^a	0.00 \pm 0.00	0.89 \pm 0.04 ^a	0.00 \pm 0.00	1.07 \pm 0.07 ^b
11 d	0.00 \pm 0.00	1.21 \pm 0.07 ^a	0.00 \pm 0.00	1.07 \pm 0.07 ^a	0.00 \pm 0.00	0.75 \pm 0.04 ^a	0.00 \pm 0.00	0.68 \pm 0.04 ^a
14 d	0.00 \pm 0.00	1.21 \pm 0.07 ^a	0.00 \pm 0.00	1.17 \pm 0.03 ^a	0.00 \pm 0.00	0.75 \pm 0.04 ^a	0.00 \pm 0.00	0.61 \pm 0.04 ^a
18 d	0.00 \pm 0.00	1.21 \pm 0.07 ^a	0.00 \pm 0.00	1.37 \pm 0.03 ^a	0.00 \pm 0.00	0.75 \pm 0.04 ^a	0.00 \pm 0.00	0.64 \pm 0.07 ^a
LSD	NO	2.03	NO	0.53	NO	0.47	NO	0.34

All data expressed with mean \pm standard error of means (SEM), similar small letters means no significant difference between means values on $p \leq 0.05$ **Table 4.** Zinc concentrations means ($\mu\text{g}/\text{gm}$) \pm SEM in liver, spleen, kidney, brain, intestine, testes, lung and heart over experimental time points following single intraperitoneal injection with 100 mg/kg Ag NPs.

Time	Liver		Spleen		Kidney		Brain	
	Control	ZnO NPs (100mg/kg)	Control	ZnO NPs (100 mg/kg)	Control	ZnO NPs (100mg/kg)	Control	ZnO NPs (100 mg/kg)
5 min	14.39 \pm 0.24 ^a	22.22 \pm 1.85 ^a	4.88 \pm 0.49 ^a	52.86 \pm 0.48 ^h	9.02 \pm 0.24 ^a	18.57 \pm 0.48 ^a	10.00 \pm 0.24 ^a	13.84 \pm 0.45 ^b
10 min	14.15 \pm 0.49 ^a	36.19 \pm 0.95 ^c	4.63 \pm 0.73 ^a	130.95 \pm 2.38 ^{bc}	8.78 \pm 0.49 ^a	27.14 \pm 0.48 ^f	10.00 \pm 0.24 ^a	14.23 \pm 0.38 ^{bc}
15 min	14.39 \pm 0.24 ^a	84.29 \pm 0.48 ^h	4.63 \pm 0.24 ^a	148.57 \pm 0.95 ⁱ	9.02 \pm 0.24 ^a	79.52 \pm 0.48 ^k	10.00 \pm 0.24 ^a	21.92 \pm 0.38 ^g
30 min	14.39 \pm 0.24 ^a	139.05 \pm 0.95 ^k	4.88 \pm 0.49 ^a	198.10 \pm 0.95 ^m	9.27 \pm 0.49 ^a	89.52 \pm 0.95 ⁱ	10.00 \pm 0.24 ^a	32.81 \pm 0.31 ^k
60 min	14.39 \pm 0.24 ^a	35.24 \pm 0.95 ^c	4.63 \pm 0.73 ^a	130.00 \pm 2.38 ^k	9.02 \pm 0.24 ^a	26.19 \pm 0.48 ^{ef}	10.00 \pm 0.24 ^a	16.09 \pm 0.43 ^d
2 h	14.15 \pm 0.49 ^a	34.35 \pm 0.43 ^{bc}	4.63 \pm 0.73 ^a	25.22 \pm 0.00 ^d	9.27 \pm 0.49 ^a	24.78 \pm 0.43 ^{de}	10.00 \pm 0.24 ^a	16.52 \pm 0.87 ^{de}
3 h	14.39 \pm 0.24 ^a	56.09 \pm 0.43 ^f	4.63 \pm 1.22 ^a	116.52 \pm 0.87 ^j	9.02 \pm 0.24 ^a	38.26 \pm 0.00 ⁱ	10.00 \pm 0.24 ^a	17.39 \pm 0.00 ^e
4 h	14.15 \pm 0.49 ^a	67.83 \pm 0.87 ^g	4.88 \pm 0.49 ^a	106.96 \pm 0.00 ⁱ	8.78 \pm 0.49 ^a	30.87 \pm 0.43 ^h	10.00 \pm 0.24 ^a	23.48 \pm 0.00 ^h
6 h	14.15 \pm 0.49 ^a	52.53 \pm 2.02 ^e	4.63 \pm 0.73 ^a	33.33 \pm 1.01 ^e	9.02 \pm 0.24 ^a	39.39 \pm 1.01 ⁱ	10.00 \pm 0.24 ^a	27.50 \pm 0.50 ⁱ
1 d	14.15 \pm 0.49 ^a	87.88 \pm 1.01 ⁱ	4.88 \pm 0.98 ^a	47.47 \pm 1.01 ^g	9.51 \pm 0.24 ^a	45.96 \pm 1.52 ^j	10.00 \pm 0.24 ^a	29.00 \pm 1.00 ^j
2 d	14.39 \pm 0.24 ^a	93.89 \pm 0.56 ^j	4.63 \pm 0.24 ^a	45.70 \pm 0.54 ^f	9.02 \pm 0.24 ^a	29.03 \pm 2.15 ^g	10.00 \pm 0.24 ^a	21.67 \pm 0.56 ^g
4 d	14.39 \pm 0.24 ^a	43.33 \pm 4.44 ^d	4.63 \pm 0.73 ^a	18.82 \pm 0.54 ^{bc}	9.27 \pm 0.49 ^a	24.19 \pm 0.54 ^{cd}	10.00 \pm 0.24 ^a	11.67 \pm 0.56 ^a
7 d	14.15 \pm 0.49 ^a	32.78 \pm 0.56 ^b	4.63 \pm 0.24 ^a	17.74 \pm 0.54 ^b	9.02 \pm 0.24 ^a	23.12 \pm 0.54 ^{bc}	10.00 \pm 0.24 ^a	15.00 \pm 0.56 ^c
9 d	14.39 \pm 0.24 ^a	32.78 \pm 0.56 ^b	4.88 \pm 0.98 ^a	19.89 \pm 0.54 ^c	8.78 \pm 0.49 ^a	22.58 \pm 0.00 ^b	10.00 \pm 0.24 ^a	16.11 \pm 0.56 ^d
11 d	14.15 \pm 0.49 ^a	32.78 \pm 0.56 ^b	4.88 \pm 0.49 ^a	12.37 \pm 0.54 ^a	9.02 \pm 0.24 ^a	22.58 \pm 1.08 ^b	10.00 \pm 0.24 ^a	17.22 \pm 0.56 ^e
14 d	14.39 \pm 0.24 ^a	32.78 \pm 0.56 ^b	4.63 \pm 0.73 ^a	11.29 \pm 0.54 ^a	9.02 \pm 0.24 ^a	22.58 \pm 1.08 ^b	10.00 \pm 0.24 ^a	18.33 \pm 0.56 ^f
18 d	14.39 \pm 0.24 ^a	32.78 \pm 0.56 ^b	4.88 \pm 0.49 ^a	11.83 \pm 0.00 ^a	9.02 \pm 0.24 ^a	22.58 \pm 0.00 ^b	10.00 \pm 0.24 ^a	21.67 \pm 0.56 ^g
LSD	0.61	2.36	1.10	1.71	0.81	1.46	0.40	0.90

Time	Small intestine		Testes		Lung		Heart	
	Control	ZnO NPs (100mg/kg)	Control	ZnO NPs (100 mg/kg)	Control	ZnO NPs (100mg/kg)	Control	ZnO NPs (100 mg/kg)
5 min	10.73 \pm 0.00 ^a	15.24 \pm 0.95 ^a	5.61 \pm 0.24 ^a	23.33 \pm 0.48 ^e	5.12 \pm 0.24 ^a	22.77 \pm 0.45 ^d	5.61 \pm 0.24 ^a	25.00 \pm 0.00 ^g
10 min	10.24 \pm 0.49 ^a	103.						

3.4. Ag and ZnO NPs excretion

The excretion kinetics of Ag and ZnO nanoparticles were evaluated after 1 day of intraperitoneal injection by measuring daily silver and zinc concentrations in feces and urine which collected over 18 days. A considerable mean values of silver and zinc levels in feces and urine were noted after one day of injection (Figure 5, table 5). with $131.67 \pm 1.36 \mu\text{g/gm}$ in feces observe to $2.89 \pm 0.03 \mu\text{g/ml}$ in urine samples for silver, $214.00 \pm 1.63 \mu\text{g/gm}$ and $15.00 \pm 0.00 \mu\text{g/ml}$ for Zinc in feces and urine respectively, these averages decreased with highly statistical significant difference ($p \leq 0.05$) reaching to the lowest concentrations values after 13 days and stand on $18.33 \pm 1.36 \mu\text{g/gm}$ in feces sample and extremely low concentration in urine samples with $0.54 \pm 0.03 \mu\text{g/ml}$, this profile of reducing concentrations over days may be refers to silver binding with the intestinal surfaces which led to decreasing the concentrations in the feces. Silver excretion percentage via urine appeared extremely low $0.8 \pm 0.019\%$ compared with their percentage in feces $33 \pm 0.17\%$ (Table 5) as the excretion of Ag NPs via renal system could not be the main path of elimination, furthermore small amount of Ag detected in feces pointed that Ag NPs were excreted slowly by the biliary pathway or deposited in different organs over long time [51]. These results approached to the demonstrations of [17] in the recording the fecal excretion percentage of $35.87 \pm 9.94\%$, also [35] mentioned the recovering of 50% of silver of infused dose in bile of rat after non oral administration. ZnO NPs excretion kinetic take the same manner as in Ag NPs with the high percentage of fecal excretion $83 \pm 0.180\%$ against urinary one with $7.5 \pm 0.020\%$. As mentioned with silver, the highest excretion averages of zinc in feces and urine samples were recorded after one day of injection ($214 \pm 1.63 \mu\text{g/gm}$, $15 \pm 0.00 \mu\text{g/ml}$) respectively, and decrees with remark significant differences ($p \leq 0.05$) to record their lowest levels in feces and urine samples after 16 days of injection with $65.5 \pm 0.41 \mu\text{g/gm}$, $6.5 \pm 0.41 \mu\text{g/ml}$ respectively (Figure 4). These high scales of Zn in feces compared with their equivalents in urine referred to vastly elimination of ZnO NPs were take placed by the bile into feces [16],[52] which play a crucial roles in the elimination of nanoparticles regardless the factors of experimental animal type and their sex, routes of exposure, particulate size and charge [53].

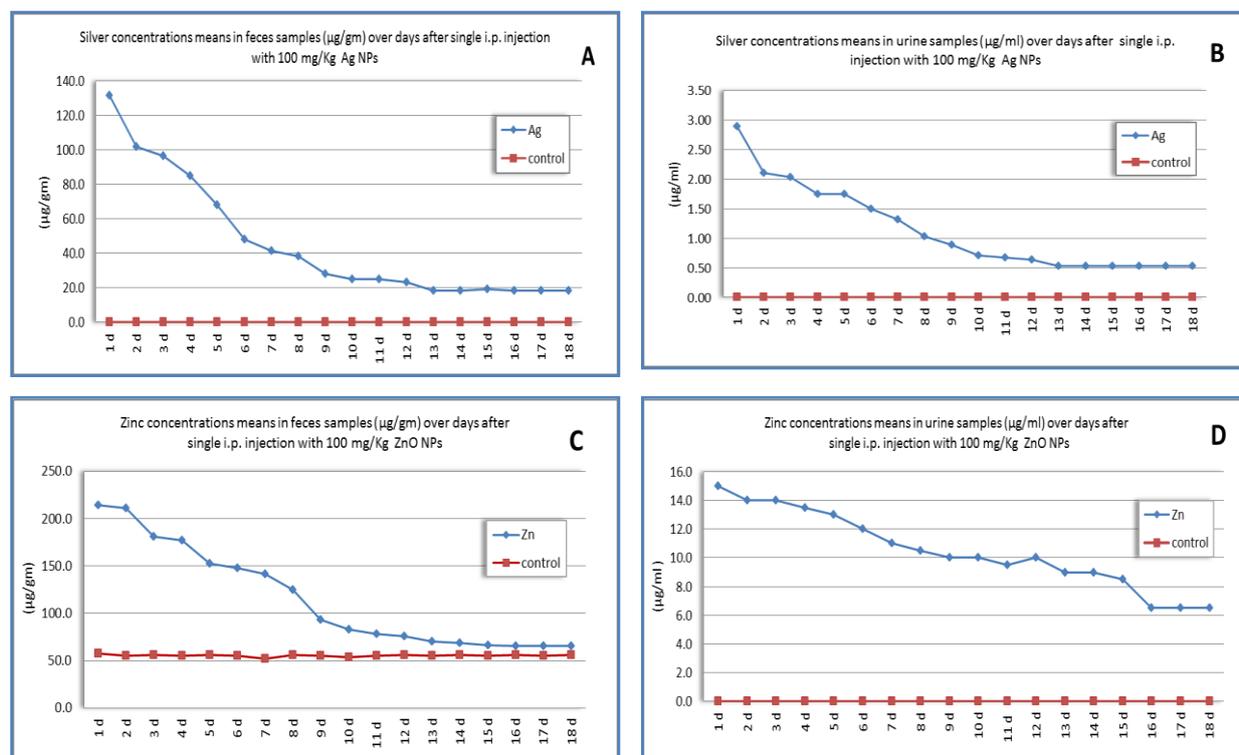


Figure 5. Silver (A,B) and Zinc (C,D) concentrations means \pm SE in feces ($\mu\text{g/gm}$) and urine ($\mu\text{g/ml}$) samples over days after single intraperitoneal (ip) injection with 100 mg/kg Ag and ZnO NPs

Table 5. silver and zinc concentrations means \pm SE in feces (mg/kg) and urine (mg/ml) over experimental time points after single intraperitoneal injection with 100 mg/kg Ag and ZnO NPs and The excretion amount (mg) and percentage (%) of Ag and ZnO NPs via feces and urine

Time	Feces samples				Urine samples			
	Ag NPs experimental group		ZnO NPs experimental group		Ag NPs experimental group		ZnO NPs experimental group	
	Control	Ag NPs (100mg/kg)	Control	ZnO NPs (100 mg/kg)	Control	Ag NPs (100mg/kg)	Control	ZnO NPs (100 mg/kg)
1 d	0.00 \pm 0.00 ^a	131.67 \pm 1.36 ^k	57.50 \pm 0.00 ^a	214.00 \pm 1.63 ⁿ	0.00 \pm 0.00 ^a	2.89 \pm 0.03 ^k	0.01 \pm 0.00 ^a	15.00 \pm 0.00 ^j
2 d	0.00 \pm 0.00 ^a	101.67 \pm 1.36 ^j	55.00 \pm 2.04 ^a	211.00 \pm 0.82 ^m	0.00 \pm 0.00 ^a	2.11 \pm 0.03 ^j	0.01 \pm 0.00 ^a	14.00 \pm 0.82 ⁱ
3 d	0.00 \pm 0.00 ^a	96.67 \pm 2.72 ⁱ	56.25 \pm 1.02 ^a	181.00 \pm 0.82 ^l	0.00 \pm 0.00 ^a	2.04 \pm 0.03 ⁱ	0.01 \pm 0.00 ^a	14.00 \pm 0.00 ⁱ
4 d	0.00 \pm 0.00 ^a	85.00 \pm 1.36 ^h	55.00 \pm 2.04 ^a	177.00 \pm 0.82 ^k	0.00 \pm 0.00 ^a	1.75 \pm 0.03 ^h	0.01 \pm 0.00 ^a	13.50 \pm 0.41 ^{hi}
5 d	0.00 \pm 0.00 ^a	68.33 \pm 1.36 ^g	56.25 \pm 1.02 ^a	152.50 \pm 2.04 ^j	0.00 \pm 0.00 ^a	1.75 \pm 0.03 ^h	0.01 \pm 0.00 ^a	13.00 \pm 0.82 ^h
6 d	0.00 \pm 0.00 ^a	48.33 \pm 1.36 ^f	55.00 \pm 2.04 ^a	147.50 \pm 0.41 ⁱ	0.00 \pm 0.00 ^a	1.50 \pm 0.06 ^g	0.01 \pm 0.00 ^a	12.00 \pm 0.00 ^g
7 d	0.00 \pm 0.00 ^a	41.67 \pm 1.36 ^e	52.50 \pm 2.04 ^a	141.50 \pm 1.22 ^h	0.00 \pm 0.00 ^a	1.32 \pm 0.03 ^f	0.01 \pm 0.00 ^a	11.00 \pm 0.82 ^f
8 d	0.00 \pm 0.00 ^a	38.33 \pm 1.36 ^d	56.25 \pm 1.02 ^a	124.50 \pm 1.22 ^g	0.00 \pm 0.00 ^a	1.04 \pm 0.03 ^e	0.01 \pm 0.00 ^a	10.50 \pm 0.41 ^{ef}
9 d	0.00 \pm 0.00 ^a	28.33 \pm 1.36 ^c	55.00 \pm 2.04 ^a	93.00 \pm 0.82 ^f	0.00 \pm 0.00 ^a	0.89 \pm 0.03 ^d	0.01 \pm 0.00 ^a	10.00 \pm 0.00 ^{de}
10 d	0.00 \pm 0.00 ^a	25.00 \pm 1.36 ^b	53.75 \pm 3.06 ^a	83.00 \pm 0.82 ^e	0.00 \pm 0.00 ^a	0.71 \pm 0.06 ^c	0.01 \pm 0.00 ^a	10.00 \pm 0.00 ^{de}
11 d	0.00 \pm 0.00 ^a	25.00 \pm 1.36 ^b	55.00 \pm 2.04 ^a	78.00 \pm 0.00 ^d	0.00 \pm 0.00 ^a	0.68 \pm 0.03 ^{bc}	0.01 \pm 0.00 ^a	9.50 \pm 0.41 ^{cd}
12 d	0.00 \pm 0.00 ^a	23.33 \pm 0.00 ^b	56.25 \pm 1.02 ^a	75.50 \pm 0.41 ^c	0.00 \pm 0.00 ^a	0.64 \pm 0.00 ^b	0.01 \pm 0.00 ^a	10.00 \pm 0.00 ^{de}
13 d	0.00 \pm 0.00 ^a	18.33 \pm 1.36 ^a	55.00 \pm 2.04 ^a	70.00 \pm 0.82 ^b	0.00 \pm 0.00 ^a	0.54 \pm 0.03 ^a	0.01 \pm 0.00 ^a	9.00 \pm 0.00 ^{bc}
14 d	0.00 \pm 0.00 ^a	18.33 \pm 1.36 ^a	56.25 \pm 1.02 ^a	69.00 \pm 0.00 ^b	0.00 \pm 0.00 ^a	0.54 \pm 0.03 ^a	0.01 \pm 0.00 ^a	9.00 \pm 0.00 ^{bc}
15 d	0.00 \pm 0.00 ^a	19.17 \pm 0.68 ^a	55.00 \pm 2.04 ^a	66.50 \pm 0.41 ^a	0.00 \pm 0.00 ^a	0.54 \pm 0.03 ^a	0.01 \pm 0.00 ^a	8.50 \pm 0.41 ^b
16 d	0.00 \pm 0.00 ^a	18.33 \pm 1.36 ^a	56.25 \pm 1.02 ^a	65.50 \pm 0.41 ^a	0.00 \pm 0.00 ^a	0.54 \pm 0.03 ^a	0.01 \pm 0.00 ^a	6.50 \pm 0.41 ^a
17 d	0.00 \pm 0.00 ^a	18.33 \pm 1.36 ^a	55.00 \pm 2.04 ^a	65.50 \pm 0.41 ^a	0.00 \pm 0.00 ^a	0.54 \pm 0.03 ^a	0.01 \pm 0.00 ^a	6.50 \pm 0.41 ^a
18 d	0.00 \pm 0.00 ^a	18.33 \pm 1.36 ^a	56.25 \pm 1.02 ^a	65.50 \pm 0.41 ^a	0.00 \pm 0.00 ^a	0.54 \pm 0.03 ^a	0.01 \pm 0.00 ^a	6.50 \pm 0.41 ^a

All data expressed with mean \pm standard error of means (SEM), similar small letters means no significant difference between means values on $p < 0.05$

	Ag NPs 100 mg/kg	ZnO NPs 100 mg/kg
Excretion amount via feces (mg)	0.82 \pm 0.004	2.08 \pm 0.004
Excretion via feces (%)	33 \pm 0.17	83 \pm 0.180
(mg)Excretion amount via urine	0.02 \pm 0.000	0.19 \pm 0.000
Excretion via urine (%)	0.8 \pm 0.019	7.5 \pm 0.020

4. Conclusions

The present study illustrate the kinetic profile of Ag and ZnO NPs followed the intraperitoneal injection with same dose of the two type of nano particles in mice which is not demonstrated in previous study . In summery ZnO NPs need 30 min for absorption into the blood and reaching to all studded organs facing to Ag NPs which need 1 h . Ag NPs spend more time for elimination (T1/2 Elem.) with over 8.39 days compared with ZnO NPs as their lower clearance rate (CL) and higher volume of distribution (Vd) . higher excretion percentage recorded with ZnO NPs in feces and less by urine compared with Ag NPs .

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