Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 2, 2015

Original Article

ACUTE TOXICITY OF DIFFERENT SIZES OF SILVER NANOPARTICLES INTRAPERITONALLY INJECTED IN BALB/C MICE USING TWO TOXICOLOGICAL METHODS

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Received: 01 Nov 2014 Revised and Accepted: 28 Nov 2014

ABSTRACT

Objective: This study aimed to evaluate the acute toxicity of intraperitoneally administrated silver nanoparticles (AgNPs) with different particle sizes in BALB/c mice.

Methods: Citrate-capped AgNPs were prepared by citrate reduction method and isolated into small particles (average size 20 nm) and large particles (average size 50 nm). The median lethal dose (LD_{50}) of 20 nm and 50 nm AgNPs was estimated using two toxicological methods, classical Dixon's up-and-down method and AOT425statPgm method for up-and-down procedure.

Results: The LD_{50} was evaluated at the dosage level of 169 and 213.8 mg/kg, respectively for 20 nm AgNPs and at the dosage level of 354 and 391.5 mg/kg, respectively for 50 nm AgNPs. The results showed that LD_{50} obtained by the AOT425statPgm method was in accord with that of the Dixon's method and no significant differences between them (P = 0.06). The size 20 nm AgNPs were more toxic than the size 50 nm AgNPs. The behavioural responses and deviations were dose dependent, increasing by increasing the dose. The anatomical examinations showed that AgNPs were mainly accumulated in liver and spleen of dosed mice.

Conclusion: The results suggested that the AOT425statPgm method was an efficient tool and a good alternative method for use in future acute toxicity studies.

Keywords: Silver nanoparticles, Median lethal dose, LD_{50}, Acute toxicity, Mice.

INTRODUCTION

Nanoparticles (NPs) have been known from centuries, where the word „nanoˮ comes from the Greek meaning dwarf and nanoparticles have at least one dimension that is between 1 and 100 nm. Nanotechnology encloses the design, characterization, production and application of nanometer scale objects (nanoparticles, nanotubes, nanorodes, nanosheet) by manipulating their shape and size [1]. Different types of nanoparticles have potential biomedical applications, not only as drug delivery systems, but also as novel diagnostic and therapeutic agents [2].

Biological interactions with NPs differ from their bulk materials, possibly due to their ability to pass through the cell wall [3]. Several studies suggest that nanoparticles have a different toxicity profile compared with bulk particles of the same materials [4-6]. The properties of NPs appear to vary noticeably around 30 nm qualitatively and quantitatively and many properties are extremely improved below 10-15 nm [7]. From an environmental health point of view, the specific chemical and physical properties of the nanoparticles result in increased reactivity with biological systems [8, 9].

Silver nanoparticles (AgNPs) are fine particles of metallic silver that have been known as colloidal silver for over 100 years [10]. Before penicillin in 1928, colloidal silver had been used to treat many infectious diseases [11]. Silver nanoparticles are widely applied in consumer products, food technology and textiles/fabrics due to its unique chemical and biological properties [12]. Silver nanoparticles are the first and most widely commercialized nanomaterial in medical and healthcare sectors. AgNPs has been used in a range of biomedical applications, owing to their antibacterial activity [13, 14, 15], antifungal properties [16] and antiviral properties [17, 18, 19]. The potential applications of AgNPs in therapy and drug delivery make it a necessary to investigate the possible toxicity associated with them and their bioaccumulation. Silver nanoparticles toxicity *in vitro* found to induce cytotoxicity and genotoxicity in human cells [19, 20]. The toxicity of AgNPs is related to the ability to release free silver ions in the biological system [21-23]. The extent of silver ion release from silver nanoparticles is controlled by many factors such as size, shape, surface coating and surface charge [23]. The small particle size and the high surface area per mass of the nanoparticle enhanced the interaction with the surroundings and the release of silver ions [24, 25].

Regarding toxicity and biodistribution of AgNPs *in vivo*, most studies reported so far have employed administration routes such as inhalation, oral and transdermal delivery [26-32]. Very few studies were conducted on intravenous injection of AgNPs [33, 34].

In the present study, the acute toxicity of 20 and 50 nm AgNPs intraperitoneally injected in BALB/c mice were evaluated. The LD_{50} of both sizes of AgNPs was estimated intraperitoneally using the classical Dixon's up-and-down method and the new AOT425statPgm method.

MATERIALS AND METHODS

Reagents, standards and reference materials

All chemicals used were of analytical grade or of high purity. The silver nitrate ($AgNO_3$, purity 99.9%) and trisodium citrate ($C_6H_5O_7Na_3 \cdot 2H_2O$ purity 99 %) were supplied by Sigma Aldrich. Double distilled water (DDW) was used for all preparations.

Synthesis and characterization of citrate capped silver nanoparticles

Silver nanoparticles (AgNPs) were prepared by using chemical reduction method [35]. All solutions of reacting materials were prepared in DDW. In typical experiment, 1000 ml of 0.001 M $AgNO_3$ was heated to boiling. To this solution, 20 ml of 2 % trisodium citrate was added drop wise. During the process, solution was mixed vigorously until color's change is evident (pale yellow). Heating was continued for an additional 30 minutes, and then the solution was cooled to room temperature. The prepared AgNPs were separated to different particle sizes using selective size-fractionation process by centrifugation at different times and speeds. Silver nanoparticles obtained in the same reaction batch were size-fractionated in two distinct populations. The large silver nanoparticles were separated

from the small particles through centrifugation at 6000 RPM for 6 minutes. The aggregated fraction redispersed in DDW afterward with ultrasonic treatment. Then the small particles separated through centrifugation at 9000 RPM for 30 minutes. The obtained pellet was re suspended in DDW to obtain the desired concentration. The AgNPs stock was stored in cool and dark place at temperature of 4 °C. AgNPs stock solution of both sizes is sonicated for 400 second for well dispersed particles before preparing subsequent doses.

Transmission electron microscopy (TEM) and UV-Visible absorption spectroscopy was used to investigate the shape and size of fractionated AgNPs. The synthesized silver nanoparticles were characterized by UV-Vis spectroscopy (Perkin Elmer, Lambda 25) instrument scanning in the range of 200-900 nm, at a resolution of 1 nm. All Samples were measured after 10-fold dilution using a quartz cell with a 1 cm path length. The shape and average size of the particles was performed by means of transmission electron microscopy (TEM) operated at 120 kV accelerating voltage (JTEM-1230, Japan, JEOL). Finally, the obtained images were processed using the software ImageJ developed at the National Institutes of Health (NIH), USA [36].

Animals

Healthy male BALB/c mice (weighing between 21 ± 2 g) were purchased from the Schistosome Biological Supply Centre (SBSC), Theodor Bilharz Research Institute (TBRI), Giza, Egypt. They had been bred under conventional conditions for research purposes. All animals were pathogen free. They were separated in plastic cages with stainless steel mesh lids in a ventilated room. The room was maintained at around 25 °C and 45% to 60% relative humidity with a 12 h light-dark cycle. All animals had free access to tap water and the same type of food, throughout the study. The animals were kept in their cages for at least 5 days prior to dosing, to allow for their acclimatization to the laboratory conditions. All animal experiments were conducted in accordance with the guidelines of the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council and in accordance with the guidelines of the international guidelines for animal experimentation.

The different selected doses of 20 nm and 50 nm AgNPs were intraperitoneally administrated as a single dose. Prior dosing, mice were weighed and each dose of AgNPs was calculated in accordance with the animal's weight. Then mice were weighed again at the end of the study at day 14. After dosing, animals were observed individually with special attention given during the first 4 hours and periodically during the first 48 hours, and at least once daily for 14 days for any deviation in their behaviour.

LD_{50} of AgNPs using dixon's up-and-down method

LD_{50} was estimated using Dixon's up-and-down method [37] which uses an iterative dose-selection algorithm; ($LD_{50} = X_f + kd$). Where, X_f is the final experimental dose for the last N samples. (N) is the nominal number of samples (total number of samples; N' minus one) or is less than the number of identical samples at the beginning of the trial (i. e. any series begins with three or more like responses will be reduced to reach the nominal number of ≤ 6). (k) is the value from Dixon tables of maximum likelihood solutions for LD_{50} using the resulting responses for each series of performed experiments [38] and (d) is the difference between log dose levels. Dixon also gives a method to calculate S. E. as S. E. = $\sigma \times \sqrt{(2/N)}$, where σ is the standard deviation in base 10 logarithmic units. For conducting the test, a series of test levels was selected with equal spacing between doses (on log-dose) equal to σ. Doses were increased and decreased incrementally by a dose progression factor of 1.6. The trials were carried out using the rule: increase the dose following a negative response [i. e. Survived (O)] and decrease the dose following a positive response [i. e. dead (X)].

LD_{50} of AgNPs using AOT 425 stat Pgm method

The AOT425statPgm "Acute Oral Toxicity statistical program" (version 1.0) is a new statistically up-and-down procedure prepared by "US Environmental Protection Agency" and approved by OECD (1998) [39] under the test guideline 425 (OECD, TG 425). The program consists of two tests, the main test and the limit test.

The main test is performed when the chemical is expected to be toxic. When little or no information about chemical toxicity is available, the program suggests a starting dose of 175 mg/kg with typical dose progression factor of 3.2 (*sigma* of 0.5). The subsequent doses to be administrated were suggested by the computer program and should not normally exceed 2000 mg/kg body weight.

However, based on the pilot study, a log dose or sigma of 0.124 with a dose progression factor of 1.33 was used for 20 nm AgNPs and a log dose or sigma of 0.2 was used with a dose progression of 1.58 for 50 nm AgNPs.

According to these sigma and dose progression, the first mouse received a dose suggested by the computer program and then monitored for up to 48 hours. The subsequent doses suggested by the computer were increased and decreased based on the resulted responses. A combination of stopping criteria is used to keep the number of animals low and the study is finally ended when one of the stopping criteria were met. At that time, the program will then generate a printout of the doses that were administrated, the outcomes and an estimate of the LD_{50}, and its 95% confidence interval [39].

Anatomical examination

All animals including those died during the test and those survived until day 14 were monitored carefully and were used in a macroscopic inspection of the visceral organs for any abnormalities to obtain more information on AgNPs toxicity.

RESULTS

AgNPs characterization

The prepared AgNPs were isolated to different particle sizes by size-fractionation process by centrifugation at different times and speeds. Visual observation of the separated AgNPs is shown in Fig. 1. The small AgNPs particle size are light yellow to yellowish brown (Fig. 1 A), whereas the large AgNPs particle size are dark brown to gray (Fig. 1 B).

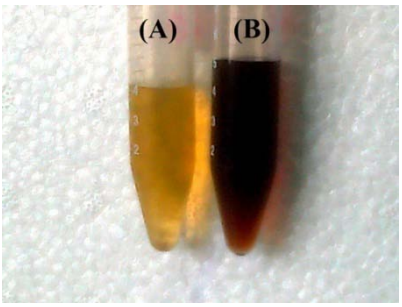


Fig. 1: Digital images of diluted colloidal of AgNPs; (A) small particle size and (B) large particle size

The UV–Vis spectrum illustrated in fig. 2, show a well-defined absorption peaks for both particle sizes. These correspond to the wavelength of the surface plasmon resonance (SPR) of AgNPs. The small particle size shows the maximum SPR peak at 426 nm while the large particle size shows the maximum SPR peak at 445 nm.

AgNPs was further analyzed by TEM analysis. TEM images of small and large AgNPs are shown in Fig. 3 (A) and (B) respectively. Both samples showed mainly spherical, mono dispersed nanoparticles without any agglomeration. Quantitative analysis of AgNPs size was performed by measuring the diameter of individual particles using image processing and analysis program (ImageJ). The particle size histograms of both particle sizes are shown in Fig. 4. The average particle size for the small particles is 20 nm (Fig. 4 A) and for the large particles is 50 nm (Fig. 4 B).

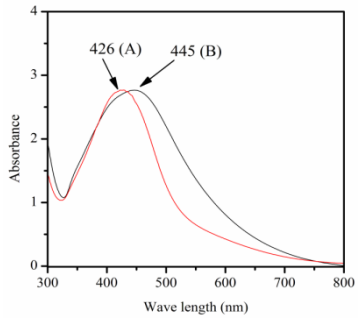
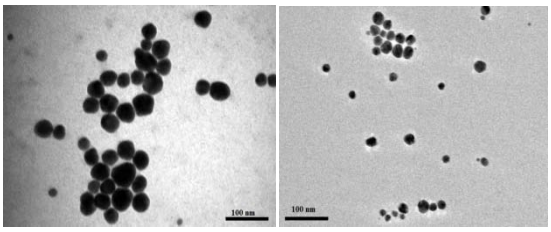
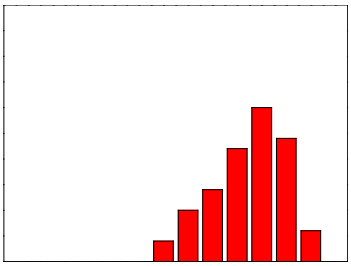


Fig. 2: UV–Vis absorption spectra of AgNPs; (A) small particle size, (B) large particle size

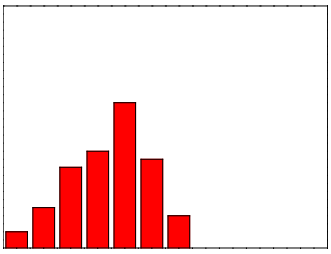


(A) (B)

Fig. 3: TEM micrograph of AgNPs suspensions; (A) small particle size, (B) large particle size



(A)



(B)

Fig. 4: Particle size distributions of the silver nanoparticles; (A) small particle size, (B) large particle size

LD_{50} of AgNPs using Dixon's Up-and-Down method

LD_{50} for 20 nm silver nanoparticles

Eight animals were used in the test. From table 1, the total number of tests performed (N') equals 8 with the series OOOXOXOX, while the nominal sample size (N) equals 6 with series OXOXOX. The resulting responses for each series are referred to the table of maximum likelihood solutions for the LD_{50}.

The LD_{50} of 20 nm AgNPs was calculated using the formula provided by Dixon's up-and-down method:

$LD_{50} = 2.3304 + [-0.500 * (\log 214 - \log 134)]$

$LD_{50} = 169.39 \pm 1.31$ mg/kg

As showed in table 2, mice injected with 214 mg/kg AgNPs died before 48 hours after injection. These mice showed obvious symptoms of passive behavior, piloerection, labored breathing, impaired movement, arching of back, loss appetite and diarrhea. Whereas none of the mice injected with 134, 84 and 52.5 mg/kg AgNPs died until day 14. These animals showed passive behavior, piloerection and hypopnea for a period not exceeding one day depending on the administrated dose. Then animals recovered from previous symptoms acting normally to the end of the experiment (Table 2). It was observed that weight gain decreased in a dose-dependent manner. Weight of animals administrated 134 mg/kg decreased by 38%, whereas that administrated 84 mg/kg decreased by 16.2 %.

Table 1: The series of resulting responses for each test for 20 nm AgNPs according to Dixon's up-and-down procedure

Dose mg/kg	Log dose	Results of test							
52.5	1.720	O							
84	1.924		O						
134	2.127			O		O		O	
214	2.330				X		X		X

X = Died, O = Survived

Table 2: Observations of mice injected with 20 nm AgNPs

Observations	Dose (mg/kg)				
	Control	214	134	84	52.5
Skin & Fur	N	+	+	+	+
Eyes	N	N	N	N	N
Mucous membranes	N	N	N	N	N
Salivation	Nil	Nil	Nil	Nil	Nil
Hypoactivity	Nil	+	+	+	+
Sleep & Coma	Nil	+	Nil	Nil	Nil
Tremors	Nil	+	Nil	Nil	Nil
Diarrhea	Nil	+	Nil	Nil	Nil
Death	Nil	+	Nil	Nil	Nil

N=normal, + = occurred

LD_{50} for 50 nm silver nanoparticles

A series of doses of 110, 175, 280, 448 mg/kg were used for LD_{50} determination for 50 nm AgNPs as shown in table 3. The total number of tests performed (N') equals 8 with the series OOOXOXOX, while the nominal sample size (N) equals 6 with series OXOXOX. The resulting responses (negative or positive) for the series were referred to the table of maximum likelihood solutions for the LD_{50} as in case of 20 nm AgNPs.

The LD_{50} of 50 nm AgNPs was calculated as mentioned previously:

LD_{50} = 2.651 + (- 0.500 * 2.651 – 2.447)

LD_{50} = 354 ± 1.31 mg/kg

As showed in Table 4, mice injected with 448 mg/kg AgNPs died within the first 24 hours after injection. These animals were inactive and non-responsive for any external stimuli (Table 4). Whereas none of the mice injected with 280, 175 and 109.4 mg/kg died until day 14. The latter mice showed signs of toxicity that disappeared after 48 hours and showed normal behaviour to the end of the experiment (Table 4). The treated mice experienced body weight loss, as weight of animals dosed 280 mg/kg decreased by 36.5% and animal injected with 175 mg/kg decreased by 21%.

Table 3: The series of resulting responses for each test for 50 nm AgNPs according to Dixon's up-and-down procedure

Dose mg/kg	Log dose	Results of test								
109.4	2.039	O								
175	2.243		O							
280	2.447			O			O		O	
448	2.651				X			X		X

X = Died, O = Survived

Table 4: Observations of mice injected with 50 nm AgNPs.

Observations	50 nm AgNPs				
	Control	448	280	175	109.4
Skin & Fur	N	+	+	+	+
Eyes	N	N	N	N	N
Mucous membranes	N	N	N	N	N
Salivation	Nil	Nil	Nil	Nil	Nil
Hypoactivity	Nil	+	+	+	+
Sleep & Coma	Nil	+	Nil	Nil	Nil
Tremors	Nil	+	Nil	Nil	Nil
Diarrhea	Nil	Nil	Nil	Nil	Nil
Death	Nil	+	Nil	Nil	Nil

N=normal, += occurred

LD_{50} of AgNPs using AOT425statPgm method

LD_{50} for 20 nm silver nanoparticles

For testing acute toxicity of intraperitoneally injected 20 nm AgNPs in mice, the dose progression (...., 132, 175, 233, 310, … mg/kg) was recommended by the computer program based on assumed sigma of 0.124. Only 5 mice were used during the test as showed in table 5. Dosing the animals was stopped based on the stopping criteria that states the dosing stop when the nominal sample size reaches five animals with 4 reversals between response (died or X) and non-response (survived or O), and the non-responses were at a dose lower than the responses. From the data sheet provided by the program, the estimated LD_{50} = 213.8 mg/kg (Based on an assumed sigma of 0.124) with the approximate 95% confidence interval from 175 to 233 mg/kg.

Table 6: Acute toxicity test of 20 nm AgNPs according to the "AOT425 statistical program"

Test Seq.	Animal ID	Dose (mg/kg)	Short-term Result	Long-term Result
1	1	175	O	O
2	2	233	X	X
3	3	175	O	O
4	4	233	X	X
5	5	175	O	O

X = Died, O = Survived

Mice dosed 233 mg/kg of 20 nm AgNPs killed within the first 12 hours after dosing and they suffered severe moribund state and showed generally passive behavior until their death. While mice dosed 175 mg/kg showed passive behaviour for the few hours and recovered after acting normally until day 14.

LD_{50} of 50 nm AgNPs

For testing acute toxicity of intraperitoneally injected 50 nm AgNPs in mice, dose progression from 1.75 mg/kg to 2000 mg kg was recommended by the computer program based on assumed sigma of 0.2, the dose 175 mg/kg was used as the starting dose. Only 6 mice were used in this test as showed in table 6. Dosing the animals was stopped based on meeting one of the stopping criteria that states the dosing stop when the nominal sample size reaches 6 with 5 reversals between response (died or X) and non-response (live or O) and the non-responses were at a dose lower than the responses. From the data sheet provided by the program, the estimated LD_{50} = 391.5 mg/kg (Based on an assumed sigma of 0.2) with the approximate 95% confidence interval from 280 to 440 mg/kg.

Table 6: Acute toxicity test of 50 nm AgNPs according to the "AOT425 statistical program"

Test Seq.	Animal ID	Dose (mg/kg)	Short-term Result	Long-term Result
1	1	175	O	O
2	2	280	O	O
3	3	440	X	X
4	4	280	O	O
5	5	440	X	X
6	6	280	O	O

X = Died, O = Survived

Mice dosed 440 mg/kg of 50 nm AgNPs killed within the first 24 hours after dosing and they generally showed passive behavior until their death as showed in mice dosed 448 mg/kg in Dixon's method. While mice dosed 175 and 280 mg/kg showed passive behavior for the first hour after dosing and recovered within 48 hours.

Anatomical examination

The anatomical examination of died mice injected with a single dose of 214 mg/kg 20 nm AgNPs and 448 mg/kg 50 nm AgNPs showed the heaviest deposition of AgNPs in mice body cavity and organs. The liver and spleen of these mice showed the heaviest accumulation of AgNPs. Whereas other animals that survived to the end of the experiment showed less accumulation of the black residue of AgNPs in their body cavity after 14 days from an injection. Animals injected with 84 mg/kg 20 nm AgNPs and 109.4 mg/kg 50 nm AgNPs showed remaining of AgNPs only at the mesenteries and healthier appearance of liver and spleen than higher doses. The animal dosed 52.5 mg/kg 20 nm AgNPs showed no obvious residual of AgNPs in its body cavity and showed the healthiest morphology of internal organs among the different dosed mice. Generally, animals injected with 50 nm AgNPs retained more silver inside their body cavities than animals injected with 20 nm.

The anatomical observations by AOT425statPgm method were similar to those in Dixon's method and they showed AgNPs accumulation in a dose dependent manner. The liver and spleen of dosed mice were the heaviest in AgNPs accumulation.

DISCUSSION

AgNPs consider one of the most widely applied nanomaterials in the biomedical and pharmacological fields and that lead to concern about the safety regulations and probable toxicity associated with releasing of biologically active Ag^+ into the human body [20]. Laboratory evaluations of the toxicity of AgNPs are not numerous and some have been done in relation to the cytotoxicity and genotoxicity of silver particles in cultured lung fibroblasts and glioblastoma cells [19]. Many *in vitro* studies suggest that the AgNPs-induced cytotoxic effects against tissue cells are particle size-dependent. Kim et al [40] showed that the small sized AgNPs (10 nm size) had a greater ability to induce apoptosis in the MC3T3-E1 cells than the large sized AgNPs (50 and 100 nm). In a recent study, Ivask et al [41] came to the same conclusion; for the size-dependent toxic effects of AgNPs to several microbial species, protozoans, algae, crustaceans and mammalian cells *in vitro* [41]. In this work, AgNPs were synthesized by reduction of $AgNO_3$ with sodium citrate according with the procedure described by Sileikaite and co-workers [35]. The prepared AgNPs was isolated into two sizes, the colour change from yellow to dark brown is in harmony with a previous reports [35], where the colour change from light yellow to yellowish brown then to gray with increasing particle size. The small particle size shows the maximum SPR peak at 426 nm while the large particle size shows the maximum SPR peak at 445 nm. It is a well known that the plasmon resonance peak shift to low wavelength with decreasing the particle size [42]. Various reports have established that the resonance peak of silver nanoparticles appears around this region, but the exact position depends on a number of factors such as particles size, and the surface-adsorbed species [42, 43]. The absence of absorbance at wavelengths greater than 550 nm indicated their well-dispersed state in solution. According to the Mie theory [44], only a single surface plasmon resonance band is expected in the absorption spectra of spherical nanoparticles [45]. In the present case, a single band was observed for both particles that give evidence for spherical shape of silver nanoparticles, which was confirmed by TEM image.

Most of the *in vivo* studies on AgNPs have selected routes of administration such as inhalation, oral and intratracheal instillation [27, 28, 31, 32]. Intraperitoneal route was selected in this study as the way of AgNPs administration because it is one of the most neglected routes for testing AgNPs toxicity. Although intraperitoneal delivery is considered a parenteral route of administration, the pharmacokinetics of substances administered intraperitoneally are more similar to those seen after oral administration. However, intraperitoneal route has the advantage of getting substances into the circulation faster than oral route [46]. The two toxicological methods, Dixon up-and-down method and AOT425statPgm method used in this study diminished the number of animals required to estimate LD_{50} values to less than 25% used by conventional methods. Although, fewer animals were used in AOT425statPgm method, it was found no significant difference between the two methods in animals number used. Comparing the LD_{50} of 20 nm and 50 nm obtained by Dixon up-and-down method with that of AOT425statPgm method showed also no significant differences in between estimated LD_{50} ($P = 0.06$).

This study may be considered a contribution in the estimation of median lethal dose (LD_{50} mg/kg) of 20 nm and 50 nm AgNPs intraperitoneally administrated in male BALB/c mice and in establishing safety regulations of AgNPs for biomedical use. Different LD_{50} of AgNPs obtained in this study were classified according to the ''Organization for Economic Cooperation and Development'' (OECD, 2001) [47] and the ''Globally Harmonized System of Classification and Labeling of Chemicals'' (GHS, 2005) [48]. It was found that LD_{50} of 20 nm AgNPs from both acute toxicological studies is likely to be classified as toxic substance and for 50 nm AgNPs; is likely to be classified as harmful. This classification is in accordance with this study as post-administration toxic symptoms produced by 20 nm were more obvious than those produced by 50 nm AgNPs. The toxic symptoms were dose-dependent, as increased by increasing the administrated dose.

In this study, liver and spleen were the main target organs of silver accumulation after intraperitoneal dosing of 20 nm and 50 nm AgNPs. The deposition of AgNPs inside mice body cavities was correlated to its concentration as it decreased by decreasing the concentration of the injected dose. The animals injected with 50 nm AgNPs retained more obvious silver residue in their body cavities than animals injected with 20 nm AgNPs. Liu and coworkers [49] concluded that smaller nanoparticles enter cells more easily than larger ones, which may be the cause of their higher toxicity. Burrell [24] also showed that AgNPs exhibits a six fold or higher solubility in water than other forms of silver and more than one-hundred-fold higher release of Ag^+ than that from other metallic silver forms. Thus, it is concluded that 20 nm AgNPs will have more solubility, more silver ions release and more efficient cell contact than in case of 50 nm AgNPs. Therefore, it is likely that 20 nm AgNPs had higher toxicity than 50 nm AgNPs.

Generally, the results estimated with the AOT425statPgm method were in accord with that obtained with the Dixon method. The AOT425statPgm method could be used as an alternative method for estimation of LD_{50} of various chemicals. It was found that mice were more sensitive to 20 nm AgNPs than 50 nm AgNPs and the toxic symptoms and the body weight change was size and dose-dependent. Although the latter conclusion is initially based on one test organism, it may lead to an explanation for "size and dose dependent" biological effects of AgNPs.

CONCLUSION

The aim of the current investigation was to determine LD_{50} of intraperitoneally injected 20 nm and 50 nm AgNPs using two toxicological methods, Dixon's Up-and-Down method and AOT425statPgm method. Citrate-capped AgNPs was synthesized using chemical reduction method and they were characterized using TEM and UV-Vis spectrum. The LD_{50} of 20 nm and 50 nm AgNPs were estimated at 169 and 354 mg/kg for Dixon's Up-and-Down method and at 213.8 and 391.5 mg/kg for AOT425statPgm method. The LD_{50} from AOT425statPgm method was in accord with that obtained from Dixon's method. The study clearly shows size-dependent LD_{50} of AgNPs. The small particle size (20 nm) exhibit more toxic effect than large particle size (50 nm). The difference in acute toxicity of 20 nm and 50 nm AgNPs can be explained by the difference in AgNPs solubility and Ag ions released upon contact with biological systems. The anatomical examination of animals revealed that AgNPs are mainly deposited and accumulated in the liver and spleen of intraperitoneally dosed mice. Thus, in synthesis of nanoparticles for biomedical uses, a special attention must be given to the particle size distribution and concentration. It was concluded that the AOT425statPgm method was an efficient tool and good alternative method for use in future acute toxicity studies.

ACKNOWLEDGEMENTS

We show gratitude to the financial support from Suez Canal University (SCU). The authors thank Prof. Nasser Y. Mostafa from

Suez Canal University for technical assistance in of AgNPs preparation and characterization.

CONFLICT OF INTERESTS

Declared None

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