

# Studies on fate and toxicity of nanoalumina in male albino rats: lethality, bioaccumulation and genotoxicity

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## Abstract

The purpose of this study is to follow-up the distribution, lethality percentile doses ( $LD_{50}$ ) and bioaccumulation of aluminium oxide nanoparticles ( $Al_2O_3$ -NPs, average diameter  $9.83 \pm 1.61$  nm) in some tissues of male albino rats, and to evaluate its genotoxicity to the brain tissues, during acute and sublethal experiments. The  $LD_{50}$  of  $Al_2O_3$ -NPs, including median lethal dose ( $LD_{50}$ ), were estimated after intraperitoneal injection. The computed  $LD_{50}$  at 24 and 48 h were 15.10 and 12.88 g/kg body weight (b.w.), respectively. For acute experiments, the bioaccumulation of aluminium (Al) in the brain, liver, kidneys, intestine and spleen was estimated after 48 h of injection with a single acute dose (3.9, 6.4 and 8.5 g/kg b.w.), while for sublethal experiments it was after 1, 3, 7, 14 and 28 days of injection with 1.3 g/kg b.w. once in 2 days. Multi-way analysis of variance affirmed that Al uptake, in acute experiments, was significantly affected by the injected doses, organs (brain, liver, kidneys, intestine and spleen) and their interactions, while for sublethal experiments an altogether effect based on time (1, 3, 7, 14, 28 days), doses (0 and 1.3 g), organs and their interactions was reported. In addition, Al accumulated in the brain, liver, kidney, intestine and spleen of rats administered with  $Al_2O_3$ -NPs were significantly higher than the corresponding controls, during acute and sublethal experiments. The uptake of Al by the spleen of rats injected with acute doses was greater than that accumulated by kidney>brain>intestine>liver, whereas the brain of rats injected with sublethal dose accumulated lesser amount of Al followed by the kidney<intestine<spleen<liver. Bioaccumulation of Al, in all studied tissues, was positively correlated with the injected doses (in acute term) and the experimental periods (in sublethal term). In the acute and sublethal experiments, comet assay parameters such as the tail intensity (i.e. DNA percentage), tail extent moment and olive tail moment were estimated using a single cell gel electrophoresis/comet assay. The results showed significant increase in DNA percentage damage in the brain cells. The obtained results indicate that bioaccumulation of Al was associated with significantly increased levels of comet parameters that depended on the doses and the experimental periods. In conclusion, Al has a high affinity to get accumulated in tissues to a level that is able to induce genotoxicity. Therefore, bioaccumulation is time, dose and organ dependant.

## Keywords

Nanoparticles, nanoalumina, rats, lethality, accumulation, genotoxicity

## Introduction

Nanomaterials are defined by the United States National Nanotechnology Initiative as materials that have at least one dimension in 1–100 nm range. In the past decade, nanotechnology has become one of the leading technologies (Stix, 2001), and consequently, public concern about the environmental and health effects of nanomaterials is growing rapidly (Li et al., 2012; Popat et al., 2004). Humans may be exposed to nanoparticles (NPs) via several possible

routes, including inhalation, dermal absorption and gastrointestinal tract absorption (Oberdörster et al.,

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2005). Due to their unique properties such as small size and corresponding large specific surface area, nanomaterials may impose different biological effects from their micro-scale material counterparts (Nel et al., 2006). However, to date, toxicological and environmental effects of nanomaterials remain largely unknown (Hamad, 2012).

As one of the widely studied and used nanomaterials, aluminum oxide nanoparticles ( $\text{Al}_2\text{O}_3$ -NPs) have been applied in catalysis (Jodin et al., 2006), structural ceramics for reinforcements (Bertsch et al., 2004), polymer modification (Cho et al., 2006), functionalization of textiles (Textor et al., 2006), heat transfer fluids (You et al., 2003; Zhang et al., 2011) and waste water treatment (Pacheco et al., 2006). In addition,  $\text{Al}_2\text{O}_3$ -NPs have shown wide biological applications in biosensors (Li et al., 2001), biofiltration (Popat et al., 2004) and antigen delivery for immunization purposes (Hamad, 2012). Thus, the environmental and health impact of  $\text{Al}_2\text{O}_3$ -NPs is of great interest.  $\text{Al}_2\text{O}_3$ -NPs have been widely used as abrasive, wear-resistant coatings, in solid rocket fuel and as drug delivery systems (Tyner et al., 2004).  $\text{Al}_2\text{O}_3$ -NPs have gained importance in the last decade because of their increased reactivity as compared to traditional micron-sized particle.

A few studies have demonstrated that aluminium (Al) and Al compounds are genotoxic (Balasubramanyam et al., 2010). Aluminium chloride evaluated with the micronucleus test, comet assay and chromosomal aberrations analysis in human peripheral blood lymphocytes showed significant genotoxicity *in vitro* (Lima et al., 2007). In the recent past, toxicity studies of NPs have mainly focused on cell culture systems and these could be misleading and need verification from animal experiments. However, *in vivo* response to the NPs of unique physicochemical properties could be a more predictive tool for assessing toxicity (Fischer and Chen, 2007). Comet assay is a simple and an inexpensive technique that evaluates chemicals for their ability to cause DNA strand breaks and alkali-labile sites under *in vivo* and *in vitro* conditions (Tice et al., 2000).

Due to the scarcity of information about the lethality, bioaccumulation and toxicity of  $\text{Al}_2\text{O}_3$ -NPs to mammals, the present study was designed to evaluate the processes of the lethality, uptake, distribution, accumulation and the genotoxicity of  $\text{Al}_2\text{O}_3$ -NPs in some tissues of male albino rats.

## Materials and methods

### Experimental animal

Healthy adult male albino rats, weighing  $115 \pm 5$  g, were used as experimental model for the present work. Rats were purchased from the animal house of National Research Center, Giza, Arab Republic of Egypt (ARE). Rats were acclimatised to the laboratory conditions for 2 weeks before starting the experiments and housed in polyethylene cages in air-conditioned animal house (temperature:  $23 \pm 1^\circ\text{C}$ , relative humidity: 20.37% and cyclic day light for  $12 \text{ h day}^{-1}$ ). The experimental animals had access *ad libitum* to deionised water and a balanced commercial pelleted diet. The food debris and faeces were removed daily, to keep sawdust dry throughout the course of experiments. The present experimental procedures were conducted, in accordance with the general international guidelines principles on the use of living laboratory animals in scientific research (Council of European Communities, 1986) and approved by the Ethical Committee of Cairo University, Faculty of Sciences, Giza, Egypt.

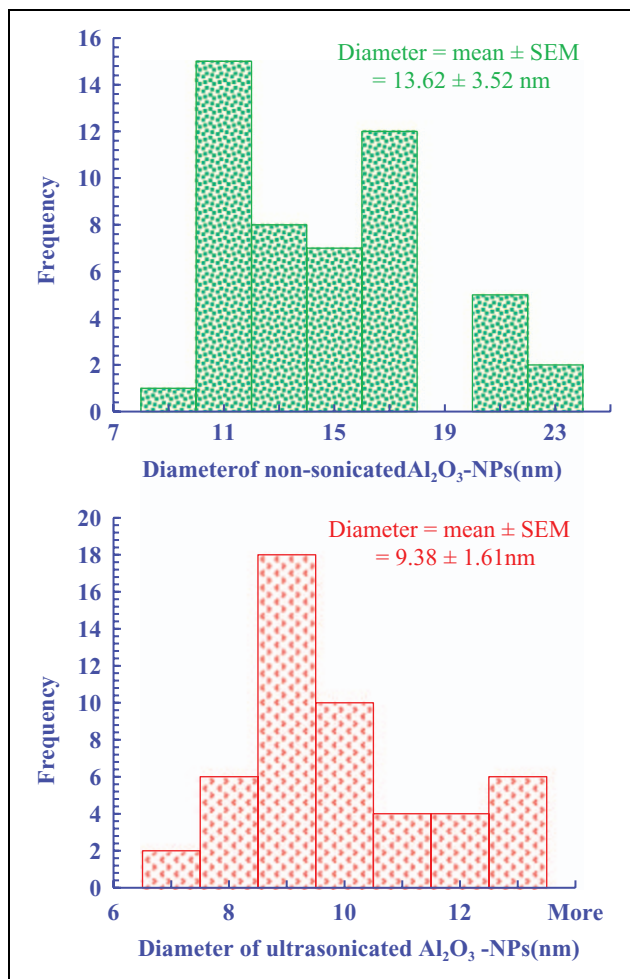
### Chemicals

$\text{Al}_2\text{O}_3$ -NPs, with diameter  $<13$  nm, were used in the present study. Nanoalumina was purchased from Sigma-Aldrich (Ward Hill, Massachusetts, USA; 99.98% purity, product number 718475, CAS number 1344-28-1, pH 9.4–10.1, boiling point:  $2980^\circ\text{C}$ , melting point:  $2040^\circ\text{C}$  and density:  $4.0 \text{ g cm}^{-3}$ ). Nitric acid ( $\text{HNO}_3$ ), with 99% purity, was purchased from Chemical Company of El-Gomhouria (Cairo, ARE).

### Ultrasonication of $\text{Al}_2\text{O}_3$ -NPs

$\text{Al}_2\text{O}_3$ -NPs were ultrasonicated to prepare the metal oxide NPs for the processes of characterization (the shape, size and aggregation) and administration of  $\text{Al}_2\text{O}_3$ -NPs to the experimental rats during the lethality, acute and sublethal experiments. A considerable weight of  $\text{Al}_2\text{O}_3$ -NPs was ultrasonicated in deionised water using the BioLogics ultrasonic homogeniser (Model 150VT; BioLogics, Inc., Manassas, VA, USA) immediately before the processes of characterization and administration, following the vibration at 20 kHz with continuous pulse of 40% of the total pulse power resulting in power output of 40 W, for 5-min preadministration.

Characterization of non- and ultrasonicated  $\text{Al}_2\text{O}_3$ -NPs were estimated by the negative stain transmission



**Figure 1.** The diameters (in nanometre) of non- and ultrasonicated aluminium oxide nanoparticles.

electron microscopy (TEM) technique (Model JEM-1400, JEOL, Japan), according to method described by Balasubramanyam et al. (2009a). A drop of non- or ultrasonicated nanoalumina suspension was poured on a sheet of parafilm, and the electron microscope copper grids were made directly off on the specimens. Each grid was placed on a drop of 2% filtered stain of phosphotungstic acid (PTA), at pH 7.0, and then incubated in petridish with the specimen side up, until examination by TEM. Photos of non- and ultrasonicated Al<sub>2</sub>O<sub>3</sub>-NPs were captured by charge-coupled device (CCD) model automated manual transmission, optronics camera with a resolution of 1632 × 1632 pixels. According to the results of characterization, the average diameters of non- and ultrasonicated Al<sub>2</sub>O<sub>3</sub>-NPs were  $13.62 \pm 3.52$  and  $9.38 \pm 1.61$  nm respectively (Figure 1), and the aggregation of non-sonicated (Figure 2(a)) NPs was greater than ultrasonicated NPs (Figure 2(b)).

### Estimation of LD<sub>5</sub>

In the present work, all the experiments of the lethality percentile doses (LD<sub>1</sub>, LD<sub>5</sub>, LD<sub>50</sub>, LD<sub>95</sub>, LD<sub>99</sub>) of Al<sub>2</sub>O<sub>3</sub>-NPs for male albino rats, following intraperitoneal (i.p.) injection with ultrasonicated Al<sub>2</sub>O<sub>3</sub>-NPs were estimated. Rats were divided randomly into seven groups with five rats in each group. Groups 1–7 were injected with 0, 12, 13, 14, 15, 16 and 18 g of Al<sub>2</sub>O<sub>3</sub>-NPs per kilogram b.w., respectively. The number of dead rats, in each group, was counted every 24 h. The lethality percentiles doses of Al<sub>2</sub>O<sub>3</sub>-NPs were computed at 24 and 48 h on the basis of probit analysis with the aid of Number Cruncher Statistical System package software (version 2007). The computed median lethal doses (LD<sub>50</sub>) at 24 and 48 h were 15.10 and 12.88 g kg<sup>-1</sup> b.w., respectively. The full results of computed LDs at 24 and 48 h are recorded in Table 2.

### Experimental design

The present study was designed to estimate the bioaccumulation of Al in some tissues, and its potential genotoxic effects, during acute and sublethal experiments.

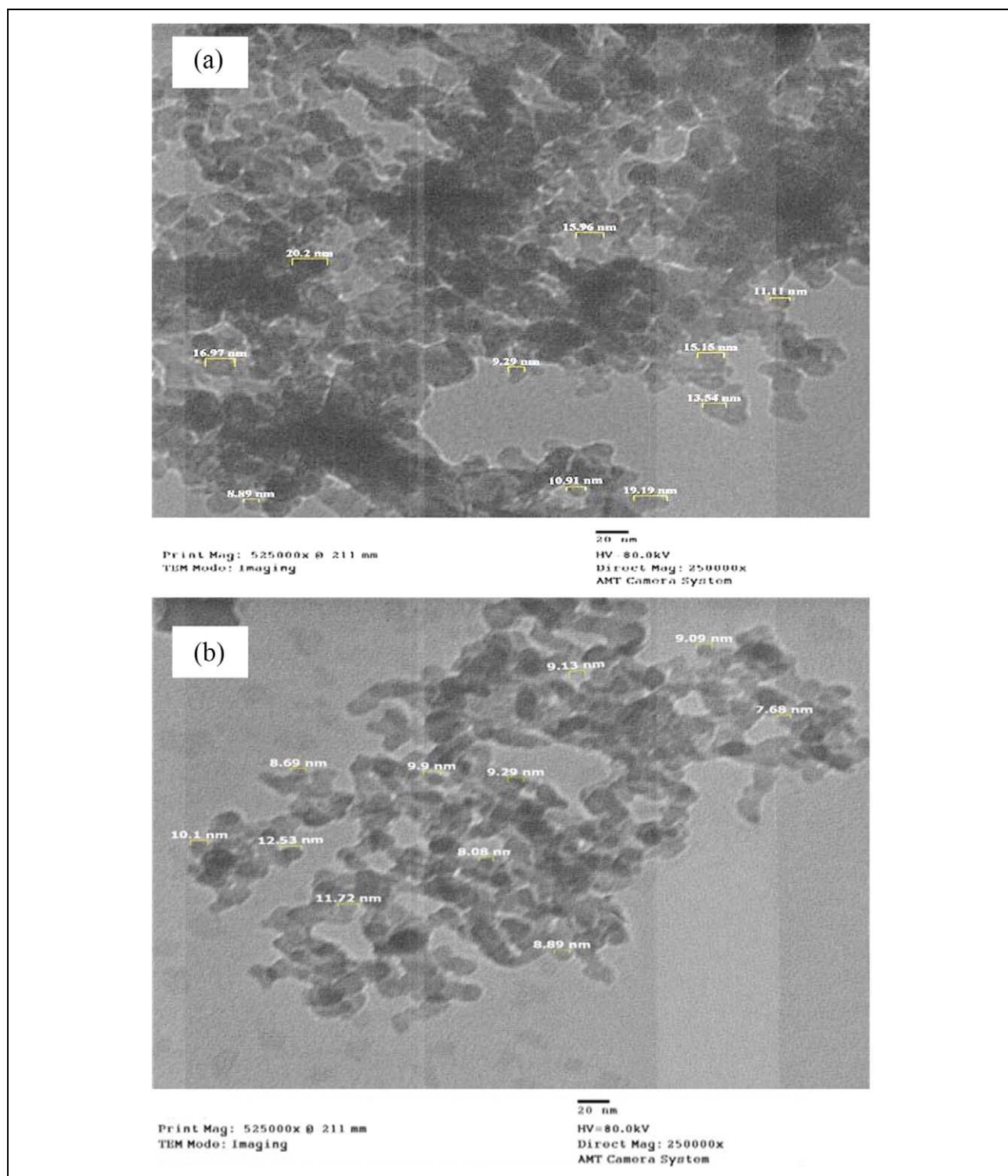
For acute experiments, 20 rats were divided randomly into 4 groups with 5 rats in each group. The experimental rats of the group 1 were given i.p. injection with saline (i.e. group I: control), whereas groups 2–4 were injected with a single low (3.9 g, group II), medium (6.4 g, group III) and high (8.5 g, group IV) dose of Al<sub>2</sub>O<sub>3</sub>-NPs, respectively.

For sublethal experiments, 50 rats were divided randomly into 2 main groups, controls (group V) and Al<sub>2</sub>O<sub>3</sub>-NPs-injected rats (group VI) with 25 rats in each group. Rats of group V were given i.p. injection with saline, whereas those of group VI were injected with a sublethal dose of 1.3 g kg<sup>-1</sup> b.w once in 2 days, (equivalent to one-tenth of LD<sub>50</sub> of Al<sub>2</sub>O<sub>3</sub>-NPs at 48 h i.e.  $1/10 \text{ LD}_{50} = 12.88/10 = 1.3$  g), over a period of 28 days. The design of the acute and sublethal experiments is summarised in Table 1.

Rats were fasted 12 h prior to the sampling (48 h for acute and 1, 3, 7, 14 and 28 days for sublethal experiments) with free access to water. The experimental animals were anaesthetised and were dissected immediately to obtain the brain, liver, kidneys, intestine and spleen for Al bioaccumulation and comet assays.

### Al assay

The concentrations of Al in the brain, liver, kidney, intestine and spleen of male albino rats were estimated



**Figure 2.** Characterization (the shape, dispersion, aggregation and diameters) of (a) non- and (b) ultra-sonicated Al<sub>2</sub>O<sub>3</sub>-NPs. Al<sub>2</sub>O<sub>3</sub>-NPs: aluminium oxide nanoparticles.

**Table 1.** Design for acute and sublethal study to clarify the experimental conditions throughout the course of the experiments.

Conditions	Experimental groups					
	Acute				Sublethal	
	I	II	III	IV	V	VI
Saline	+++	---	---	---	+++	---
Al <sub>2</sub> O <sub>3</sub> -NP doses (g kg <sup>-1</sup> b.w.)	---	3.9 <sup>a</sup>	6.4 <sup>b</sup>	8.5 <sup>c</sup>	---	1.3 <sup>d</sup>
Dosing		Single			Repeated once in 2 days	
Sample size per group		5 rats			25 rats	
Sampling (days)		2			1, 3, 7, 14 and 28	

Al<sub>2</sub>O<sub>3</sub>-NPs: aluminium oxide nanoparticles; b.w.: body weight; LD<sub>50</sub>: median lethal dose.

<sup>a</sup>Equivalent to 30% of LD<sub>50</sub> after 48 h post injection.

<sup>b</sup>Equivalent to 50% of LD<sub>50</sub> after 48 h post injection.

<sup>c</sup>Equivalent to 65% of LD<sub>50</sub> after 48 h post injection.

<sup>d</sup>Equivalent to 10% of LD<sub>50</sub> after 48 h post injection.

**Table 2.** The computed LD<sub>s</sub> (in gram per kilogram b.w.) of Al<sub>2</sub>O<sub>3</sub>-NPs to male albino rats after 24 and 48 h of i.p. injection.<sup>a</sup>

At LD <sub>s</sub>	24 h	48 h
LD <sub>1</sub>	10.03 ± 1.49	8.35 ± 1.87
LD <sub>5</sub>	11.30 ± 1.21	9.48 ± 1.63
LD <sub>10</sub>	12.05 ± 1.03	10.14 ± 1.46
LD <sub>20</sub>	13.02 ± 0.82	11.01 ± 1.23
LD <sub>25</sub>	13.41 ± 0.75	11.36 ± 1.14
LD <sub>30</sub>	13.77 ± 0.70	11.68 ± 1.05
LD <sub>40</sub>	14.44 ± 0.65	12.28 ± 0.90
LD <sub>50</sub>	15.10 ± 0.69	12.88 ± 0.77
LD <sub>60</sub>	15.79 ± 0.82	13.50 ± 0.69
LD <sub>70</sub>	16.56 ± 1.04	14.20 ± 0.70
LD <sub>75</sub>	17.00 ± 1.19	14.60 ± 0.77
LD <sub>80</sub>	17.51 ± 1.38	15.06 ± 0.88
LD <sub>90</sub>	18.92 ± 1.97	16.35 ± 1.36
LD <sub>95</sub>	20.17 ± 2.53	17.49 ± 1.88
LD <sub>99</sub>	22.74 ± 3.82	19.85 ± 3.13

LD<sub>s</sub>: lethality percentile doses; Al<sub>2</sub>O<sub>3</sub>-NPs: aluminium oxide nanoparticles; b.w.: body weight; i.p.: intraperitoneal.

<sup>a</sup>Data represented as a mean ± standard error of mean.

during acute and sublethal experiments. Reagents were checked for Al contamination. The glassware was acid washed and rinsed in deionised water before use. A considerable weight of the desired organs were dried, overnight, at 60°C in microwave oven (Model Fisher iso-TEMP oven, 200 series, 215G; Thermo Fisher Scientific Inc., Mount Holly, NJ, USA), and then water was evaporated at 105°C, producing dry tissues that were weighed and then converted to ash at 3000°C in a muffle furnace (Model NEY M-525; Ney Co., USA). The ash residue of Al was dissolved in 15% HNO<sub>3</sub>.

Al, in the diluted HNO<sub>3</sub>, was analysed two times against standards in a linear range by inductively coupled plasma atomic emission spectrometer (ICP-AES, model iCAP 6000 series; Thermo Electron Ltd., Cambridge, UK) with a fast multi-elements technique with moderate–low detection limits according to the method described by Smichowski et al. (2005). A stock solution of Al with concentration 1 g L<sup>-1</sup> was prepared. The working fresh standards of Al (0.5, 1.0, 2.0, 4.0 and 6 mg L<sup>-1</sup>) were aspirated by diluted HNO<sub>3</sub>-containing Al samples. Argon plasma was used for excitation of Al atoms. The lines for Al were selected at minimal interference with other elements. The blank values were subtracted from each sample value. The concentrations of Al in tissues were expressed as milligram of Al per gram dry weight.

### Comet assay

The alkaline (pH > 13) comet assay for the brain was performed according to the method described by (Recio et al., 2010; Tice et al., 2000). A small piece of brain was immersed in 1 mL of cold Hank's balanced salt solution free from calcium and magnesium ions (containing 20 mM ethylenediaminetetraacetic acid (EDTA) and 10% dimethyl sulphoxide (DMSO)), slightly homogenised to mince the tissue to fine pieces, and the produced suspension was left to allow the precipitation of large pieces of tissues to settle down, forming aliquot suspension of brain cells. Then, 10 µL of aliquot cell suspension, containing approximately 10,000 brain cells, was mixed with 80 µL of 0.5% low-melting point agarose and spread



on a fully frosted slide predipped in normal melting agarose (1%). Then, the slides were allowed to harden and solidify on a cold surface. The hard slides were immersed in cold lysis buffer (2.5 M sodium chloride, 100 mM EDTA and 10 mM Tris; pH 10 with freshly added 10% DMSO and 1% Triton X-100) for 24 h at 4°C in darkness. Subsequently, the slides were incubated in fresh alkaline buffer (300 mM sodium hydroxide and 1 mM EDTA, pH > 13) for 20 min. The unwinding DNA on the slides was electrophoresed for 20 min at 300 mA and 25 V (0.90 V cm<sup>-1</sup>), and then the slides were immersed in an excessive amount of 0.4 M Trizma base (pH 7.5) to neutralise the alkali. Finally, the slides were fixed in 100% cold ethanol, dried in air and stored at room temperature in a desiccator until the scoring process. Prior to scoring, slides were stained with ethidium bromide (2 µg mL<sup>-1</sup>). A computerised image analysis system (Comet 5.0, Kinetic Imaging Ltd., Liverpool, UK), with simultaneous image capture and scoring of 50 cells at 400× magnification, was used to measure the selected comet parameters. Triplicate samples, each containing 50 cells, were quantified for each condition. The parameters selected for presentation were: (1) tail intensity (TI, i.e. percentage of DNA in tail and defined as the intensity of all tail pixels divided by the total intensity of all pixels in the comet, expressed as percentage), (2) the tail moment (TM, equivalent to the integrated value of density multiplied by migration distance, is considered to be the most sensitive measurement, and it was automatically generated by the computer.) and (3) the olive tail moment (OTM, defined as the output of multiplication of TM and TI). The comet parameters were computed according to the following formula:

$$\text{TI} = \text{DNA}(\%) = \frac{\text{Comet intensity} - \text{Nucleoid intensity}}{\text{Comet intensity}} \times 100$$

$$\text{TM} = \text{Tail length} \times \text{Percentage of DNA in tail}$$

$$\text{OTM} = \text{TI} \times \text{TM}$$

### Statistical analysis

In the present study, data were statistically analysed using Statistical Package for the Social Sciences version 20 package software (SPSS<sup>®</sup> Statistics 20, copyright IBM Corporation 1989, 2011, USA). Multivariate analyses of variance (MANOVA) was used to test the effect of doses, type of organs (brain, liver, kidneys, intestine

and spleen) and their interactions (acute experiments), in addition to the experimental periods (1, 3, 7, 14, 28 days), during the sublethal experiments, on Al bioaccumulation. One-way analysis of variance (ANOVA) was applied to test the effect of experimental periods on the studied parameters of the controls or/and Al<sub>2</sub>O<sub>3</sub>-NPs-injected rats. Least significant difference was used to compare the studied parameters of rats treated with Al<sub>2</sub>O<sub>3</sub>-NPs and their corresponding controls. In addition, Duncan's test of homogeneity was applied to estimate the similarities among the studied experimental groups. Regression analyses and correlation coefficient were applied to fit the relationships between the different studied variables. Data were represented as mean ± standard error of mean.

## Results

### Lethality

In the present work, the lethality percentile doses (LD<sub>s</sub>) including LD<sub>1</sub>, LD<sub>5</sub>, LD<sub>50</sub>, LD<sub>95</sub>, LD<sub>99</sub> of Al<sub>2</sub>O<sub>3</sub>-NPs were estimated for male albino rats, after 24 h and 48 h following i.p. injection with the metal oxide NPs (Table 2). The LD<sub>s</sub> were increased with increasing metal dose with the minimal dose showed at LD<sub>1</sub>, whereas the maximum was at LD<sub>99</sub> (Table 2). The computed LD<sub>50</sub> of Al<sub>2</sub>O<sub>3</sub>-NPs at 24 and 48 h were 15.10 and 12.88 g kg<sup>-1</sup> b.w., respectively (Table 2).

### Al accumulation

Al bioaccumulation in the brain, liver, kidneys, intestine and spleen of male albino rats were estimated during acute and sublethal experiments. During the short-term experiments, Al bioaccumulation was significantly affected by the administered doses ( $F_{3,80} = 236.6, p < 0.0001$ ), type of organs ( $F_{4,80} = 398.7, p < 0.0001$ ) and their interaction ( $F_{12,80} = 46.9, p < 0.0001$ ). The liver of group II accumulated Al less than the brain < intestine < kidney < spleen (Table 3). In the rats of groups III and IV, the uptake of Al by the spleen was greater than that accumulated by kidney > brain > intestine > liver (Table 3). In all the studied organs of groups II, III and IV, the concentrations of Al were significantly greater than the corresponding controls at all the experimental periods (Table 3). The administered acute doses of nanoalumina (3.9, 6.4 and 8.5 g) were positively correlated with the Al accumulated in the brain, liver, kidneys, intestine and spleen of rats with correlation coefficients of +0.99, +0.91, +0.91, +0.89 and +0.54, respectively (Table 3).

**Table 3.** Al bioaccumulated (in milligram per gram dry weight) in the brain, liver, kidneys, intestine and spleen of control male albino rats (group I) and those injected with a single low (group II), medium (group III) and high (group IV) dose of Al<sub>2</sub>O<sub>3</sub>-NPs, after 48 h of injection.<sup>a</sup>

Experimental groups	Al accumulated (mg g <sup>-1</sup> dry weight)				
	Brain	Liver	Kidney	Intestine	Spleen
Group I	0.09 ± 0.01 <sup>b</sup>	0.06 ± 0.01 <sup>b</sup>	0.12 ± 0.03 <sup>bc</sup>	0.16 ± 0.01 <sup>c</sup>	0.12 ± 0.00 <sup>bc</sup>
Group II	0.14 ± 0.02 <sup>b;§</sup>	0.13 ± 0.01 <sup>b;§</sup>	0.86 ± 0.06 <sup>c;§</sup>	0.19 ± 0.01 <sup>b*</sup>	1.54 ± 0.11 <sup>d;§</sup>
(Percentage change) <sup>§</sup>	(+56%)	(+117%)	(+617%)	(+19%)	(+1183%)
Group III	0.33 ± 0.02 <sup>c;§</sup>	0.15 ± 0.01 <sup>b;§</sup>	1.22 ± 0.03 <sup>d;§</sup>	0.22 ± 0.01 <sup>bc*</sup>	1.58 ± 0.07 <sup>e;§</sup>
(Percentage change) <sup>§</sup>	(+267%)	(+150%)	(+917%)	(+38%)	(+1217%)
Group IV	0.60 ± 0.05 <sup>c;§</sup>	0.16 ± 0.00 <sup>b;§</sup>	1.42 ± 0.07 <sup>d;§</sup>	0.55 ± 0.07 <sup>c;§</sup>	1.66 ± 0.10 <sup>d;§</sup>
(Percentage change) <sup>§</sup>	(+567%)	(+167%)	(+1083%)	(+244%)	(+1283%)
Effect of doses (One way ANOVA)	F <sub>3,16</sub> = 67.98, P < 0.000	F <sub>3,16</sub> = 19.99, P < 0.000	F <sub>3,16</sub> = 124.52, P < 0.000	F <sub>3,16</sub> = 23.44, P < 0.000	F <sub>3,16</sub> = 84.62, P < 0.000
Correlation coefficient (r)	+0.99	+0.91	+0.91	+0.89	+0.54

Al: aluminium; ANOVA: analysis of variance; Al<sub>2</sub>O<sub>3</sub>-NPs: aluminium oxide nanoparticles.

<sup>a</sup>Each value is a mean of five rats ± standard error mean (SEM).

In the row, mean values marked with the same superscript letters are similar (insignificant, P>0.05), whereas others are not (significant, P<0.05).

\*Significant difference (P<0.01) in comparison with the corresponding control at α = 0.01.

\*\*Significant difference (P<0.000) in comparison with the corresponding control at α = 0.0001.

<sup>§</sup>Percentage change in comparison with the corresponding controls.

r is the correlation coefficient between Al accumulated in tissues and the injected doses of Al<sub>2</sub>O<sub>3</sub>-NPs.

In the sublethal experiments, MANOVA revealed that Al bioaccumulation was markedly affected by the experimental time ( $F_{4,200} = 112.6, p < 0.0001$ ), doses ( $F_{1,200} = 3475.4, p < 0.0001$ ), organs ( $F_{4,200} = 190.5, p < 0.0001$ ) and their interactions ( $F_{16,200} = 16.5, p < 0.0001$ ). In rats of group VI, the brain accumulated Al lesser than kidney < intestine < spleen < liver from day 3 till the end of experiments (Table 4). Al accumulated in tissues of group VI was significantly higher than the corresponding controls, at all the experimental periods, over 28 days of sublethal experiments (Table 4). The experimental periods (1, 3, 7, 14 and 28 days) exhibited a positive correlation with Al accumulated in the brain, liver, kidneys, intestine and spleen of group VI with correlation coefficients of +0.88, +0.69, +0.97, +0.88 and +0.97, respectively (Table 4).

### Comet assay

Comet assay was determined to assess DNA integrity in the brain's nerve cells of control rats and those administered with Al<sub>2</sub>O<sub>3</sub>-NPs. The studied comet parameters (TI, TM and OTM) were investigated throughout the course of acute and sublethal experiments.

One-way ANOVA revealed that the administered single dose of the studied metal oxide NPs (0 or 3.9 or 6.4 or 8.5 g kg<sup>-1</sup> b.w.) and the experimental periods (1, 3, 7, 14 and 28 days), during the acute and sublethal experiments, respectively, caused significant alterations for all the studied comet parameters in brain's neuron (Tables 5 and 6). In acute experiments, the measured TI, TM and OTM parameters of rats injected with a single acute dose were significantly higher than the corresponding controls, except for TI and TM of rats administered with 3.9 g (Table 5), throughout the course of the experiments. As a response to the metal oxide NPs, in acute experiments, Figure 3 showed a gradual spreading of DNA percentage in the developed comet that increased with increasing the administered acute doses. TI, TM and OTM, in the brain's neuron, exhibited a positive correlation with administered acute doses of Al<sub>2</sub>O<sub>3</sub>-NPs (Table 5) as well as with Al accumulated in the brain tissues (Figure 4).

In sublethal experiments, MANOVA results confirmed that TI, TM and OTM were significantly affected by the experimental time ( $F_{4,20} = 626.9, p < 0.0001$ ;  $F_{4,20} = 7594.2, p < 0.0001$ ;  $8498.5, p < 0.0001$ ), administered doses ( $F_{1,20} = 2277.4, p < 0.0001$ ;  $F_{1,20} = 10,901, p < 0.0001$ ;  $F_{1,20} = 82,158,$

$p < 0.0001$ ) and their interaction ( $F_{4,20} = 626.9, p < 0.0001$ ;  $F_{4,20} = 7594.2, p < 0.0001$ ;  $F_{4,20} = 8498.5, p < 0.0001$ ), respectively. In addition, the determined comet parameters of controls did not get affected by the experimental time (Table 6). In rats of group VI, all the studied comet parameters were significantly higher than the corresponding controls, at all the experimental periods, except TI after 1 day of injection (Table 6). Regression analysis revealed that the TI, TM and OTM, in rats of group VI, were positively correlated with either the experimental periods (Table 6) and Al accumulated in the brain tissue (Figure 5). A steady increase in the TI (percentage of DNA damage) parameter was observed from the first day till 28th day of sublethal administration (Figure 6).

## Discussion

### Lethality percentile doses

In the present work, the LD<sub>5</sub> of Al<sub>2</sub>O<sub>3</sub>-NPs via i.p. injection were estimated at 24 and 48 h to discover the accurate LD<sub>50</sub> of Al<sub>2</sub>O<sub>3</sub>-NPs, for male albino rats, that was not reported in the previous or published literature, as well as to determine the administered doses of Al<sub>2</sub>O<sub>3</sub>-NPs necessary for the acute and sublethal experiments. The computed LD<sub>50</sub> at 24 and 48 h were 15.10 and 12.88 g kg<sup>-1</sup> b.w., respectively. These were higher than the mean values of LD<sub>50</sub> for micro-sized Al salt (0.025–0.082 g kg<sup>-1</sup> b.w. per rat), following i.p. administration (EFSA, 2008), that is, the dose necessary to kill 50% of Al<sub>2</sub>O<sub>3</sub>-NPs, is higher than micro-sized Al compounds. Balasubramanyam et al. (2009a) stated that the oral LD<sub>50</sub> of Al<sub>2</sub>O<sub>3</sub>-NPs (30 and 40 nm) for rats was >2000 mg kg<sup>-1</sup> b.w., that is, not accurate and did not estimate the definite various LD<sub>5</sub>. This may be attributed to the hydrophobicity of administered NPs and their interactions with the gastrointestinal mucosa, forming adhesive physical bonds, leading to marked reduction in the amount of NPs absorbed via villi to the circulation (Irache et al., 2005).

### Bioaccumulation of Al

In the present results, we demonstrated that the i.p. injection of either a single acute (3.9, 6.4 and 8.5 g) or repeated sublethal dose (1.3 g) of Al<sub>2</sub>O<sub>3</sub>-NPs caused the uptake and accumulation of significant amounts of Al, in a variety of tissues in rats, as compared to the corresponding controls. Moreover, Al concentrations in tissues increased with increasing dose and time, that is, Al bioaccumulation by tissues



**Table 4.** Al accumulated (in milligram per gram dry weight) in the brain, liver, kidney, intestine and spleen of the control male albino rats (group V) and those injected with 1.3 g Al<sub>2</sub>O<sub>3</sub>-NP per kilogram body weight once in 2 days (group VI).<sup>a</sup>

Tissues	Experimental Periods (Days)					Effect of time (one way ANOVA)		r
	1	3	7	14	28			
Brain								
Group V	0.11 ± 0.006 <sup>aB</sup>	0.09 ± 0.012 <sup>aB</sup>	0.10 ± 0.005 <sup>aB</sup>	0.11 ± 0.007 <sup>aB</sup>	0.12 ± 0.005 <sup>aB</sup>	F <sub>4,20</sub> = 1.38, P > 0.05		
Group VI	0.20 ± 0.009 <sup>aA*</sup>	0.93 ± 0.041 <sup>bA*</sup>	1.30 ± 0.090 <sup>cA*</sup>	1.48 ± 0.022 <sup>cA*</sup>	1.94 ± 0.143 <sup>dA*</sup>	F <sub>4,20</sub> = 69.47, P < 0.000		+0.88
(Percentage change) <sup>g</sup>	(+82%)	(+933%)	(+1200%)	(+1246%)	(+1517%)			
Liver								
Group V	0.08 ± 0.002 <sup>aA</sup>	0.07 ± 0.004 <sup>aA</sup>	0.07 ± 0.005 <sup>aA</sup>	0.07 ± 0.002 <sup>aA</sup>	0.07 ± 0.005 <sup>aA</sup>	F <sub>4,20</sub> = 1.67, P > 0.05		
Group VI	0.24 ± 0.014 <sup>aA*</sup>	4.81 ± 0.130 <sup>bC*</sup>	5.27 ± 0.123 <sup>bCD*</sup>	6.37 ± 0.136 <sup>cC*</sup>	6.51 ± 0.768 <sup>cC*</sup>	F <sub>4,20</sub> = 51.38, P < 0.000		+0.69
(Percentage change) <sup>g</sup>	(+200%)	(+6771%)	(+7429%)	(+9000%)	(+9200%)			
Kidney								
Group V	0.12 ± 0.005 <sup>aB</sup>	0.11 ± 0.0037 <sup>aB</sup>	0.12 ± 0.002 <sup>aB</sup>	0.11 ± 0.004 <sup>aB</sup>	0.12 ± 0.006 <sup>aB</sup>	F <sub>4,20</sub> = 1.10, P > 0.05		
Group VI	0.91 ± 0.012 <sup>aB*</sup>	0.97 ± 0.038 <sup>abA*</sup>	1.47 ± 0.004 <sup>abA*</sup>	1.56 ± 0.057 <sup>bA*</sup>	3.56 ± 0.336 <sup>cB*</sup>	F <sub>4,20</sub> = 49.56, P < 0.000		+0.97
(Percentage change) <sup>g</sup>	(+658%)	(+782%)	(+1125%)	(+1318%)	(+2867%)			
Intestine								
Group V	0.17 ± 0.004 <sup>aC</sup>	0.17 ± 0.0009 <sup>aC</sup>	0.17 ± 0.0015 <sup>aC</sup>	0.17 ± 0.0037 <sup>aC</sup>	0.17 ± 0.0007 <sup>aC</sup>	F <sub>4,20</sub> = 0.26, P > 0.05		
Group VI	1.96 ± 0.136 <sup>aC*</sup>	2.78 ± 0.271 <sup>abB*</sup>	2.83 ± 0.023 <sup>abB*</sup>	3.52 ± 0.273 <sup>bb*</sup>	3.72 ± 0.473 <sup>bb*</sup>	F <sub>4,20</sub> = 6.19, P < 0.01		+0.88
(Percentage change) <sup>g</sup>	(+1052%)	(+1535%)	(+1565%)	(+1971%)	(+2088%)			
Spleen								
Group V	0.12 ± 0.005 <sup>aB</sup>	0.11 ± 0.010 <sup>aB</sup>	0.11 ± 0.010 <sup>aB</sup>	0.12 ± 0.008 <sup>aB</sup>	0.10 ± 0.008 <sup>aB</sup>	F <sub>4,20</sub> = 0.99, P > 0.05		
Group VI	2.97 ± 0.211 <sup>aD*</sup>	2.98 ± 0.205 <sup>aB*</sup>	3.93 ± 0.150 <sup>bC*</sup>	4.01 ± 0.207 <sup>bb*</sup>	5.51 ± 0.177 <sup>cC*</sup>	F <sub>4,20</sub> = 29.49, P < 0.000		+0.97
(Percentage change) <sup>g</sup>	(+2375%)	(+2609%)	(+3473%)	(+3242%)	(+5410%)			

Al: aluminium; ANOVA: analysis of variance; Al<sub>2</sub>O<sub>3</sub>-NPs: aluminium oxide nanoparticles.

<sup>a</sup>Each value is a mean of five rats ± standard error mean (SEM).

For each group, mean values marked with the same superscript capital (in the column) or small (in the row) letters are similar (insignificant, P > 0.05) whereas others are not (significant, P < 0.05).

<sup>\*</sup>Significant difference (P < 0.000) in comparison with the corresponding control at α = 0.0001.

<sup>g</sup>Percentage change in comparison with the corresponding controls.

r: is the correlation coefficient between the experimental periods and Al accumulated in the studies tissues.

**Table 5.** The TI (DNA percentage), TM and OTM of DNA, in the brain's nerve cells, of control male albino rats (group I) and those injected with a single low (group II), medium (group III) and high (group IV) dose of Al<sub>2</sub>O<sub>3</sub>-NPs per kilogram body weight.<sup>a</sup>

Experimental groups	Comet Parameters		
	TI	TM	OTM
Group I	18.31 ± 0.003	0.98 ± 0.031	56.67 ± 4.28
Group II (Percentage change) <sup>g</sup>	17.87 ± 0.437 (-2.40 %)	0.88 ± 0.083 (-10.2 %)	3198.59 ± 175.66* (+5544 %)
Group III (Percentage change) <sup>g</sup>	25.47 ± 0.012* (+39.10 %)	16.77 ± 0.237* (+1611%)	15036.31 ± 250.61* (+26432 %)
Group IV (Percentage change) <sup>g</sup>	193.61 ± 0.431* (+ 957 %)	23.72 ± 0.136* (+2320 %)	17723.20 ± 534.71* (+31174 %)
Effect of doses (One way ANOVA)	F <sub>3,8</sub> = 79567.04, P < 0.000	F <sub>3,8</sub> = 6423.48, P < 0.000	F <sub>3,8</sub> = 794.44, P < 0.000
Correlation coefficient (r)	+0.89	+0.97	+0.94

TI: tail intensity; TM: tail moment; OTM: olive tail moment; Al<sub>2</sub>O<sub>3</sub>-NPs: aluminium oxide nanoparticles; ANOVA: analysis of variance.

<sup>a</sup>Each value is a mean of three rats ± standard error mean (SEM).

\*Significant difference (P < 0.000) in comparison with the corresponding control at  $\alpha = 0.0001$ .

<sup>g</sup>Percentage of change in comparison with the corresponding controls.

r is correlation coefficient between the administered acute doses and the comet parameters.

was dose and time dependent. In the same manner, Balasubramanyam et al. (2009b) and Fathy (2013) concluded that Al and Ni bioaccumulation respectively, in tissues of male albino rats, were dose and time-dependent, following administration of nano-sized Al and nickel (Ni).

It is well known that the injection of a chemical or/and a drug into the peritoneal cavity will directly pour into the intrathoracic lymph nodes, via transdiaphragmatic lymphatic vessels, to the blood circulation, within a short time (Parungo et al., 2007). Once Al is in the bloodstream, it distributes widely to the various body tissues in a pattern that may parallel the density of transferrin receptors within those tissues (Walton, 2011). Systemic Al binds to serum proteins or anions and is distributed rapidly to other tissues throughout the body (Rawy et al., 2013).

In the present results, the highest concentration of accumulated Al was recorded in the spleen of rats administered with acute high dose of Al<sub>2</sub>O<sub>3</sub>-NPs. This is consistent with many previous studies on the accumulation of NPs (Chen et al., 2009). Lasagna-Reeves et al. (2010) found that after injection with 400 µg gold-NPs per kilogram b.w., the highest concentrations of accumulated gold were reported in the spleen of mice. This was attributed to the reticuloendothelial system (RES) in the spleen, which is a part of the

immune system that is involved in the uptake and metabolism of exogenous molecules and particles. In the present results of acute experiments, kidneys of group IV accumulated a considerable amount of Al 48 h post-injection. This may be explained by the size of Al-NPs (<13 nm), which is greater than the diameter of glomerular pores (5.5 nm), leading to a continuous trapping of Al-NPs in the kidneys (Lasagna-Reeves et al., 2010). In addition, the contribution of liver in Al excretion, through the bile, is less than the kidney, which is the main pathway for excretion of Al via urine (Exley, 2009). This may explain the lowest Al content in liver of rats administered with acute doses of Al<sub>2</sub>O<sub>3</sub>-NPs, in our data of acute experiment.

On the other hand, the present results of sublethal experiments revealed that the liver and spleen retained the greatest mean concentrations of Al, over a period of 28 days of experiments. This is consistent with several previous studies showing that the injected metal NPs are mainly accumulated in the liver and spleen for long durations regardless of their size, shape, dose, types of materials and their toxicokinetics. After 60 days of intravenous injection with gold-NPs, the highest uptake of this metal was recorded by the liver, as detoxifying organ, followed by the spleen and kidney in the experimental rats (Balasubramanian et al., 2010). They attributed that

**Table 6.** The TI (DNA percentage), TM and OTM of DNA, in the brain's nerve cells, of control male albino rats (group V) and those injected with 1.3 g Al<sub>2</sub>O<sub>3</sub>-NPs per kilogram body weight once in 2 days (group VI).<sup>a</sup>

Comet Parameters	Experimental periods (Days)					Effect of time (one way ANOVA)	r
	1	3	7	14	28		
<b>TI</b>							
Group V	19.24 ± 1.19 <sup>b</sup>	19.23 ± 1.18 <sup>b</sup>	19.22 ± 1.19 <sup>b</sup>	19.18 ± 1.16 <sup>b</sup>	19.22 ± 1.19 <sup>b</sup>	F <sub>4,10</sub> = 0.0003, P>0.05	—
Group VI	17.78 ± 0.11 <sup>b</sup>	30.92 ± 0.04 <sup>c*</sup>	31.46 ± 0.06 <sup>c*</sup>	50.87 ± 0.36 <sup>d*</sup>	96.45 ± 0.69 <sup>e*</sup>	F <sub>4,10</sub> = 7659.4, P<0.000	+0.87
(Percentage change) <sup>g</sup>	(- 7.59%)	(+60.79%)	(+63.68%)	(+ 165%)	(+ 402%)		
<b>TM</b>							
Group V	0.97 ± 0.203 <sup>b</sup>	1.06 ± 0.120 <sup>b</sup>	1.00 ± 0.173 <sup>b</sup>	1.00 ± 0.173 <sup>b</sup>	1.03 ± 0.145 <sup>b</sup>	F <sub>4,10</sub> = 0.05, P>0.05	—
Group VI	133.48 ± 1.68 <sup>b*</sup>	152.60 ± 0.63 <sup>b*</sup>	165.29 ± 1.22 <sup>b*</sup>	175.08 ± 7.72 <sup>b*</sup>	4082.69 ± 44.31 <sup>c*</sup>	F <sub>4,10</sub> = 7594.6, P<0.000	+0.89
(Percentage change) <sup>g</sup>	(+13660%)	(+14296%)	(+16429%)	(+ 17408%)	(+ 396277%)		
<b>OTM</b>							
Group V	49.33 ± 5.93 <sup>b</sup>	43.33 ± 4.91 <sup>b</sup>	46.67 ± 4.48 <sup>b</sup>	51.33 ± 6.64 <sup>b</sup>	44.67 ± 6.23 <sup>b</sup>	F <sub>4,10</sub> = 0.33, P>0.05	—
Group VI	655.82 ± 4.54 <sup>b*</sup>	780.47 ± 6.47 <sup>c*</sup>	2402.82 ± 6.42 <sup>d*</sup>	3752.17 ± 18.12 <sup>e*</sup>	4369.00 ± 20.55 <sup>f*</sup>	F <sub>4,10</sub> = 9405.6, P<0.000	+0.92
(Percentage change) <sup>g</sup>	(+ 1229%)	(+ 1701%)	(+ 5049%)	(+ 7210%)	(+ 9680%)		

TI: tail intensity; TM: tail moment; OTM: olive tail moment; Al<sub>2</sub>O<sub>3</sub>-NPs: aluminium oxide nanoparticles; ANOVA: analysis of variance.

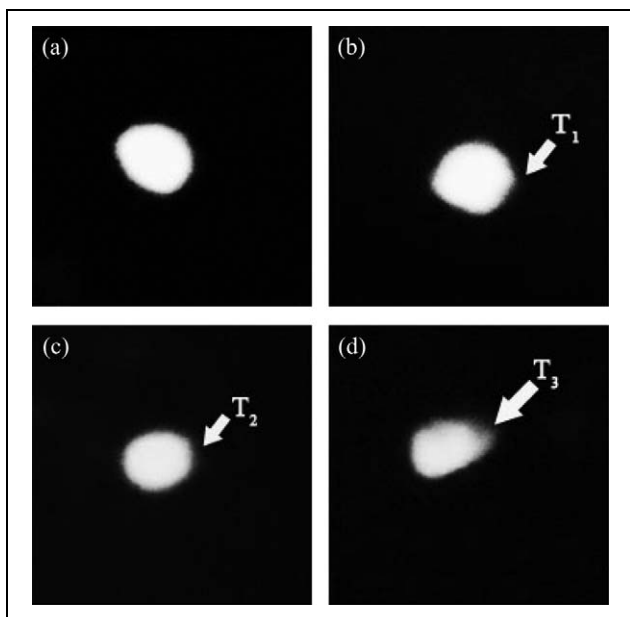
<sup>a</sup>Each value is a mean of three rats ± standard error mean (SEM).

In the row, mean values marked with the same superscript letters are similar (insignificant, P > 0.05), whereas others are not (significant, P < 0.05).

\*Significant difference (P < 0.000) in comparison with the corresponding control at α = 0.0001.

<sup>g</sup>Percentage of change in comparison with the corresponding controls.

r is the correlation coefficient between the experimental periods and the comet parameters.



**Figure 3.** DNA damage of a single neuron isolated from the brain of controls (a) and those administered with a single dose of 3.9 g ((b), T<sub>1</sub>), 6.4 g ((c), T<sub>2</sub>) and 8.5 g ((d), T<sub>3</sub>) of Al<sub>2</sub>O<sub>3</sub>-NPs, after 2 days of injection. Al<sub>2</sub>O<sub>3</sub>-NPs: aluminium oxide nanoparticles.

the fenestrated, discontinuous endothelia of liver and spleen allow the passage of NPs up to 100 nm in diameter from the bloodstream into the parenchyma. In addition, organs of the RES, including the liver and spleen, can efficiently accumulate high amounts of NPs via opsonisation, that is, NPs could bind to antibody in the plasma and are subsequently recognised by Kupffer cells in the liver and then by macrophages in other places, regardless of the particle size (Sadauskas et al., 2007, 2009).

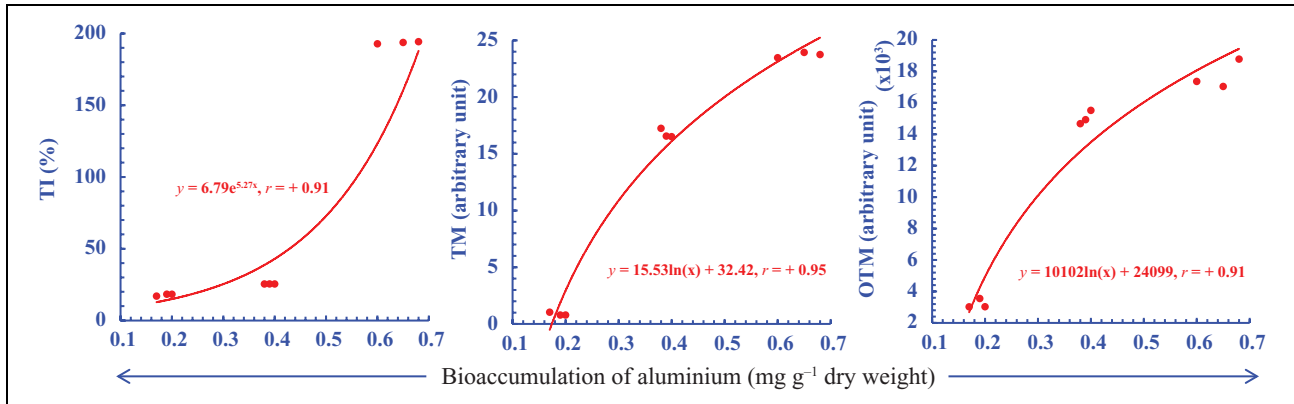
In the present work, the intestine and kidney of rats of group VI accumulated significant concentrations of Al, during the course of sublethal experiments. The same results were obtained by Yeh et al. (2005) who reported longer retention and higher accumulation of paclitaxel NPs in the small intestine and kidney after 24 h of intravenous injection. They attributed the redistribution of the NPs to the gastrointestinal tract for further excretion. Chen et al. (2009) reported that nanosized particles could cross the small intestine by persorption and further transfer to the blood, brain, lung, heart, kidney, spleen and liver. Our data revealed that, throughout the course of acute and sublethal experiment, the Al accumulated by the brain was very small, although significantly higher than the corresponding controls. Balasubramanian et al. (2010) reported that the gold particles, 20 nm in size, intravenously injected in rats were not

detected in the brain. However, De Jong et al. (2008) found that the 10 nm gold particles were detected in the brain. This phenomenon suggests that passage through the blood–brain barrier (BBB) is size dependent and that the particles smaller than 20 nm may be able to cross the barrier. The mechanisms underlying nanoparticle passage through the BBB and the potential particle size limits merit further research (Almeida et al., 2011).

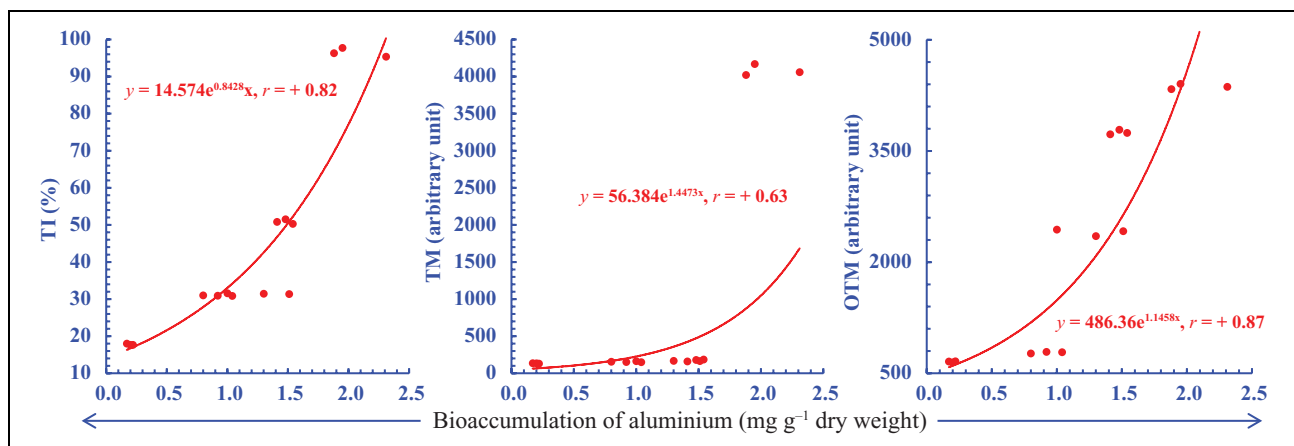
### Comet assay

Due to the scarcity about the biological information for Al<sub>2</sub>O<sub>3</sub>-NPs to mammals, the present study try to assess the toxicity and the possible risk of accidental, acute and sublethal exposure to this nanometal oxide. Comet assay was used to study DNA damage in neurons of the brain that has high sensitivity and protection against xenobiotic by the BBB. Previous studies confirmed the genotoxic effect of some NPs such as nanosized titanium oxide (TiO<sub>2</sub>) on the bone marrow, brain, liver and kidney (Hamad, 2012; Trouiller et al., 2009). Data of comet assay, in the present work, indicated that accumulation of Al<sub>2</sub>O<sub>3</sub>-NPs, in the brain, is in fact genotoxic during acute and subacute experiments, and this was confirmed by the significant increase in the TI, TM and OTM in the brain cells. These results are parallel to previous studies on TiO<sub>2</sub>-NPs (Hamad, 2012; Reeves et al., 2008; Wang et al., 2007).

Metal oxide NPs might damage DNA directly or indirectly via oxidative stress and/or inflammatory responses (Zhu et al., 2007). A direct chemical interaction between TiO<sub>2</sub>-NPs and DNA, through the DNA phosphate group has been proven (Li et al., 2008). Also, Al<sub>2</sub>O<sub>3</sub>-NPs could also directly bind to DNA or repair enzymes leading to the generation of strand breaks (Grassian et al., 2007). Similar to TiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>-NPs can cause DNA damage indirectly through inflammation (Chen et al., 2006) and the generation of reactive oxygen species (ROS) (Federici et al., 2007; Gurr et al., 2005; Kang et al., 2008). Ali (2013) found that i.p. injection with nanosized Al<sub>2</sub>O<sub>3</sub> caused severe oxidative stress to the brain tissue, as a result of Al accumulation, leading to generation of ROS, significant increase in lipid peroxidation of the brain cells that is associated with marked severe depletion of glutathione. Oxidative stress is generally accepted to be an important mechanism for cell damage induced by nano-, micro- and ultrafine particles (Ayres et al., 2008; Oberdörster et al., 2005), and a wide range of nanomaterial species have been shown to create ROS both *in vivo* and *in vitro* (Nel et al., 2006; Oberdörster, 2004). Enhanced production of ROS



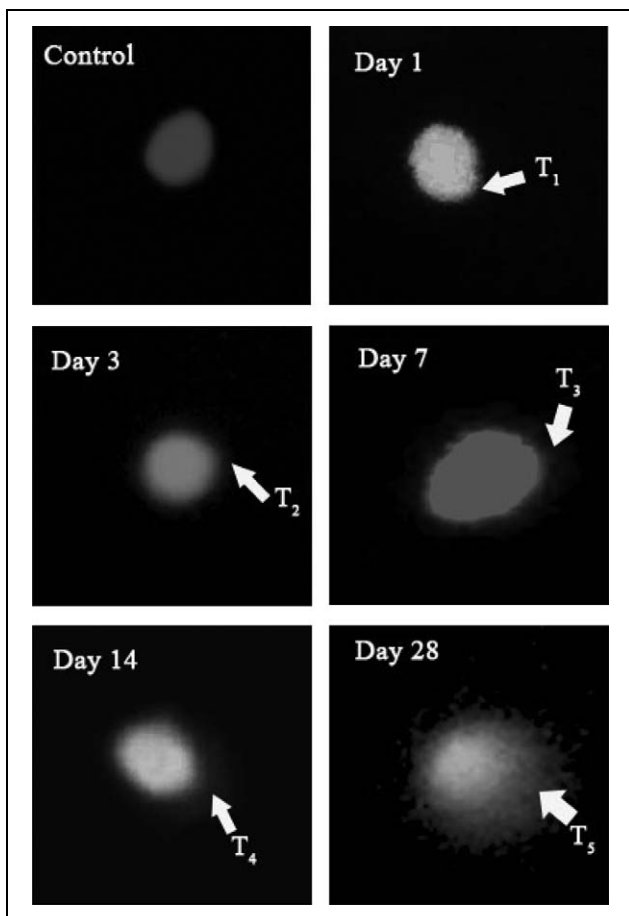
**Figure 4.** The relationships of Al accumulated in the brain tissues (in milligram per gram dry weight) with the TI, TM and OTM in the brain of male albino rats after 48 h of administration a single acute dose of  $\text{Al}_2\text{O}_3$ -NPs. Bioaccumulation of Al (in milligram per gram dry weight). Al: aluminium; TI: tail intensity; TM: tail moment; OTM: olive tail moment;  $\text{Al}_2\text{O}_3$ -NPs: aluminium oxide nanoparticles.



**Figure 5.** The relationships of Al accumulated in the brain tissues (in milligram per gram dry weight) and the TI, TM and OTM of the brain cells of male albino rats injected with 1.3 g  $\text{Al}_2\text{O}_3$ -NPs per kilogram b. w. once in 2 days over a period of 28 days post-injection. Al: aluminium; TI: tail intensity; TM: tail moment; OTM: olive tail moment;  $\text{Al}_2\text{O}_3$ -NPs: aluminium oxide nanoparticles; b.w.: body weight.

caused oxidative damage to the cellular macromolecules such as lipids, proteins and DNA (Hamad, 2012) leading to neurodegeneration of the brain tissues (Dizdaroglu, 2003). The reactive oxygen radical is highly capable of oxidising the single base and sugar phosphate of DNA and breaks its strand (Bjelland and Seeberg, 2003; Cadet et al., 2003). Ali (2013) stated that  $\text{Al}_2\text{O}_3$ -NPs, accumulated in the brain, caused significant inhibition of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) leading to increased ROS. In addition, the recorded positive correlation between Al accumulated in the brain and the TI (DNA % damage), TM and OTM confirmed that the Al accumulated by the brain cells was probably the main key route by which nano-sized Al induces cytotoxicity.

DNA damage response is triggered by the detection of DNA lesions, and this response consists of an orderly sequence of signal transduction events that can induce the accumulation of tumour protein 53, which plays a critical role in responding to various stresses that cause DNA damage; especially ROS as differential phosphorylation modulate its stability as well as induction of its downstream gene products (Zhang et al., 2005). The observed stability of  $\text{Al}_2\text{O}_3$ -NPs genotoxicity, during acute and subacute experiments, in the brain may be attributed to the long retention half-life time of Al leading to an increase in the time necessary for its clearance from the tissues of the brain and consequently increase its deposition and accumulation in brain causing neurotoxicity.



**Figure 6.** DNA damage of a single neuron isolated from the brain of control male albino rats and those injected with  $1.3 \text{ g kg}^{-1}$  b.w. once in 2 days after 1 ( $T_1$ ), 3 ( $T_2$ ), 7 ( $T_3$ ), 14 ( $T_4$ ) and 28 ( $T_5$ ) days of injection with  $\text{Al}_2\text{O}_3$ -NPs. Bioaccumulation of Al (in milligram per gram dry weight).  $\text{Al}_2\text{O}_3$ -NPs: aluminium oxide nanoparticles; Al: aluminium.

## Conclusion

Bioaccumulation of Al, in male albino rats, was dose, time and organ dependent. In addition, comet assay confirmed that Al accumulated in the brain tissues, induced genotoxicity to the brain cells, that is, comet assay can be used a sensitive test to evaluate the neurotoxicity of NPs.

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