Toxicology and Industrial Health

Studies on fate and toxicity of nanoalumina in male albino rats: 2. Oxidative stress in the brain, liver and kidney

Journal:	*Toxicology and Industrial Health*
Manuscript ID:	TIH-13-0187.R1
Manuscript Type:	Article
Keywords:	Acute Toxicity, Aluminum, Superoxide Dismutase, Oxidative Stress, Nanotoxicity, Hepatic Toxicity, Antioxidan

Abstract:	The present work aimed to evaluate the oxidative stress of nanoalumina (aluminium oxide nanoparticles, Al2O3-NPs) with a diameter <13nm (9.83±1.61 nm) as assessed by the perturbations in the enzymatic and non-enzymatic antioxidant as well as lipid peroxidation (LPO) in the brain, liver and kidney of male albino rats, after two days of injection of a single-acute dose (3.9g or 6.4g or 8.5g Al2O3/kg), and a sub-lethal dose of 1.3g Al2O3/kg/2day, over a periods of 28 days. According to two-ways ANOVA, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities as well as the levels of glutathione (GSH) and LPO were significantly affected by the injected-doses, organs and their interactions. On the other hand, in sub-lethal experiments, these parameters were affected by the experimental periods, organs and their interactions. Regression analysis confirmed that the activities of SOD, CAT, GPx and GSH levels in the brain, liver and kidney were inversely proportional with the acute doses, the experimental periods, and aluminium accumulated in these tissues, whereas levels of LPO exhibited a positive relationship. Correlation coefficient indicated that oxidative stress mainly depends on aluminium accumulated in the studied organs, followed by injected doses and the experimental periods. In comparison with the corresponding controls, the acute and sub-lethal doses of Al2O3-NPs caused significant inhibition of the brain, hepatic and renal SOD, CAT, GPx activities and a severe marked reduction in the concentrations of GSH that were associated with a significant elevation in the levels of malondialdehyde MDA (LPO). In conclusion, our data indicated that rats-injected with nanoalumina suffered from the oxidative stresses that were dose and time dependent. In addition, Al2O3-NPs released into the biospheres could be potentiating a risk to the environment and causing hazard effects on living organisms, including mammals.

SCHOLARONE™
Manuscripts

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

The corrected items are marked with the bright green colour

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Studies on fate and toxicity of nanoalumina in male albino rats: 2. Oxidative stress in the brain, liver and kidney

Abstract

The present work aimed to evaluate the oxidative stress of nanoalumina (aluminium oxide nanoparticles, Al2O3-NPs) with a diameter <13nm (9.83±1.61 nm) as assessed by the perturbations in the enzymatic and non-enzymatic antioxidant as well as lipid peroxidation (LPO) in the brain, liver and kidney of male albino rats, after two days of injection of a single-acute dose (3.9g or 6.4g or 8.5g /kg), and a sub-lethal dose of 1.3g /kg/2day, over a periods of 28 days. According to two-ways ANOVA, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities as well as the levels of glutathione (GSH) and LPO were significantly affected by the injected-doses, organs and their interactions. On the other hand, in sub-lethal experiments, these parameters were affected by the experimental periods, organs and their interactions. Regression analysis confirmed that the activities of SOD, CAT, GPx and GSH levels in the brain, liver and kidney were inversely proportional with the acute doses, the experimental periods, and aluminium accumulated in these tissues, whereas levels of LPO exhibited a positive relationship. Correlation coefficient indicated that oxidative stress mainly depends on aluminium accumulated in the studied organs, followed by injected doses and the experimental periods. In comparison with the corresponding controls, the acute and sub-lethal doses of Al2O3-NPs caused significant inhibition of the brain, hepatic and renal SOD, CAT, GPx activities and a severe marked reduction in the concentrations of GSH that were associated with a significant elevation in the levels of malondialdehyde MDA (LPO). In conclusion, our data indicated that rats-injected with nanoalumina suffered from the oxidative stresses that were dose and time dependent. In addition, Al2O3-NPs released into the biospheres could be potentiating a risk to the environment and causing hazard effects on living organisms, including mammals.

Keywords
Nanoalumina; rats; oxidative stress; acute; sub-lethal

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Introduction

Nanoparticles (NPs) may be defined as materials that have at least one dimension less than 100nm (Balasubramanyam et al., 2009). Because of their unique chemical, mechanical, and biological properties, they are desirable for industrial and healthcare applications (Oberdörster et al., 2005). However, the increased surface area of NPs may exhibit greater biological activity, such as an increased generation of reactive oxygen species (ROS) when compared with the large particles at equivalent mass (Liu et al., 2010). Usually cells respond to these ROS by stimulating their antioxidant defence system in order to protect themselves (Prabhakar et al., 2012). Nevertheless, if the defence system fails to neutralize the oxidative burden, the ROS will oxidize cellular proteins, DNA and lipids, and inactivate specific enzymes by oxidation of cofactors, leading to a state called oxidative stress (Patlolla et al., 2011).

Nanoparticles universally, aluminium oxide nanoparticle (Al_2O_3-NPs) is one of the most important nanomaterial products because of its promising technological applications (Sadiq et al., 2009). Aluminium (Al) and Al2O3-NPs are widely used in drug delivery systems to increase solubility (Maquieira et al, 2012). They are also used in explosives, ammunition, artillery surface coatings, lithium batteries, resistant coatings on propeller shafts, fuels in boosters, missiles and rockets, gelled fuels, the ceramic industry, scratch- and abrasive-resistant coatings on sunglasses, car finishing and flooring, and orthopaedic implants (Monteiro-Riviere et al., 2010). Further, it was reported that Al2O3-NPs enhanced the anticancer effects of immunotherapy (Sun et al., 2010). Unfortunately, few studies have demonstrated that the administration of Al2O3-NPs may lead to adverse effects, such as genotoxicity (Balasubramanyam et al., 2009), inflammatory response (Oesterling et al., 2008), carcinogenicity (Dey et al., 2008), cytotoxicity (Di Virgilio et al., 2010) and mitochondrial dysfunction (Chen et al., 2008). Generally, the brain was reported to be especially vulnerable to oxidative stress because of its high metabolic rate and poor antioxidant defence system (Ma et al., 2010). The liver is the main site for detoxification of most chemicals resulting in free radical production that leads to lipid peroxidation and hepatotoxicity (Patlolla et al., 2011). Moreover, a very active oxidative metabolism in the kidneys results in generation of ROS that may cause major damage in cellular components (Mahieu et al., 2009).

Since the information about the toxicity of nanoalumina in tissues of mammals is limited, the present study aimed to evaluate the ability of Al2O3-NPs to induce oxidative stress in the

For Peer Review

brain, liver and kidney of male albino rats as mammalian model, during acute and sub-lethal administration.

Materials and Methods

Experimental animals

Healthy adult male albino rats, weighing 115±5g, were used as experimental model for the present work. Rats were purchased from the animal house of National Research Centre (NRC), Giza, Egypt. Rats were acclimatized to the laboratory conditions for two weeks prior to experiments, and housed in polyethylene cages, in air conditioned animal house (temperature 23±1°C, relative humidity 20.37%, and cyclic day light on 12h/day). The experimental animals had access *ad libitum* 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.

Chemicals

Aluminium oxide nanoparticles (Al2O3-NPs) was purchased from Sigma-Aldrich (Ward Hill, MA; 99.98% purity, Product number 718475, CAS number 1344-28-1, pH 9.4-10.1, boiling point 2.980°C, melting point 2.040°C and density 4.0g/cm^3). Nanoalumina was used in the ultrasonicated form, with diameter <13nm (9.83±1.61 nm), and their identification and characterization was done according to method described by Ali (2013).

Experimental Design

For acute experiments, twenty rats were divided randomly into four groups, each with five rats. Rats of the group I (control) were intraperitoneally-injected with saline, whereas those of the groups II, III and IV were injected with a single acute dose of 3.9g (30% of LD50), 6.4g (50% of LD50) and 8.5g (65% of LD50) of Al2O3-NPs/kg b. wt., respectively. For sub-lethal experiments, fifty rats were divided into two groups V and VI, each with 25 rats. Rats of group V (controls) were intraperitoneally-injected with saline, whereas those of group VI were injected with a sub-lethal dose of 1.3 g of Al2O3-NPs (10% of LD50), every two days, over a period of 28 days. The median lethal dose, for male albino rats, at 48 h (LD50=12.88 g/kg b. wt.) was measured by Ali (2013). The full design, for acute and sub-lethal experiments, is summarized in table 1.

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 1: The experimental design to evaluate the oxidative stresses of ultrasonicated Al_2O_3-NPs, in male albino rats, during the acute and sub-lethal experiments

Experimental Conditions	Acute groups				Sub-lethal groups	
	I	II	III	IV	V	VI
Saline	+++	---	---	---	+++	---
Al_2O_3-NPs (g /kg b. wt.)	---	3.9^{A}	6.4^{B}	8.5^{C}	---	1.3^{D}
Dosing	Single				Day after day	
Sample size (n) /group	Five rats				25 rats	
Sampling (after last dose)	2 days				1, 3, 7, 14, 28 days	

^{A,B,C,D}: equivalent to 30%, 50%, 65% and 10% of LD50, at 48 h

Rats were fasted 12 h prior to the sampling, with free access to water. At the sampling time, the experimental animals were sacrificed after being anesthetized with chloroform, and then dissected quickly to obtain the brain, liver and kidneys that were rinsed in ice cold physiological saline. In order to prepare the tissue homogenate, a considerable weight of the organ was homogenized in 5 ml potassium phosphate buffer solution (PH 7.4) and centrifuged at 10000 rpm for 15 min. in ice cool centrifuge (-4°C). The supernatant was drawn by Pasteur pipette and stored at -40°C for further antioxidants and lipid peroxidation assays.

Bioassay of enzymatic and non-enzymatic antioxidants

The enzymatic (superoxide dismutase, SOD; catalase, CAT; glutathione peroxidase, GPx) and non-enzymatic (glutathione, GSH) antioxidants were estimated in the homogenate of the brain, liver and kidney of controls male albino rats and those administered a single acute or repeated sub-lethal doses of nanoalumina. Activities of SOD, CAT and GPx were assayed by Biodiagnostic kits, according to the methods described by Nishikimi *et al.* (1972), Aebi (1984) and Paglia and Valentine (1967) respectively. Activities of antioxidant enzymes were expressed as units of SOD per gram of tissue (U/g).

The concentrations of GSH in tissue homogenates were estimated as described by Beutler *et al.* (1963), and expressed as milligrams GSH per gram of tissue (mg/g).

Lipid Peroxidation

The lipid peroxidation was measured according to the method described by Ohkawa *et al.* (1979), by using Biodiagnostic kits. This method was based on estimation of the released malondialdehyde (MDA) molecules, as a result of oxidative damage of cell membranes. The

For Peer Review

concentrations of MDA were expressed as nanomole of MDA per gram of tissue (nmol/g tissue).

Statistical Analysis

The present data were statistically analysed by aid of Statistical Package for the Social Sciences (SPSS) version 20 package software. Two-ways ANOVA was used to test the effect of doses (3.9, 6.4, 8.5 g Al_2O_3-NPs), types of organs and their interactions (in acute experiments), and the experimental periods, organs and their interaction (in sub-lethal experiments) on the studied parameters in tissues. Regression analyses and correlation coefficient were applied to fit the relationships between the different studied variables. Least significant difference (LSD) was used to compare between the various studied variables. Data were represented as mean ± standard error of mean (SEM). N.B., all the computed relationships between various variables were executed on the raw data.

Results

Two-ways ANOVA revealed that the activities of SOD, CAT, GPx as well as the levels of GSH and MDA in the brain, liver and kidneys of groups II, III and IV were significantly affected by the injected-acute doses of Al_2O_3-NPs (3.9, 6.4, 8.5 g), types of organs (brain, liver, kidney) and their interaction together, except GPx activity didn't affect by doses-organs interaction (Table 2). In table 3, the fitting equations derived from the regression analyses, revealed that the injected-acute doses of nanoalumina were inversely proportional with either the brain, hepatic and renal SOD, CAT, GPx activities, and GSH contents with negative correlation coefficients, whereas the levels of MDA exhibited a direct positive relation with positive correlation coefficients. This indicated that the activities of SOD, CAT, GPx, concentrations of GSH in the brain, liver and kidney decreased significantly with increasing the values of injected doses, whereas the levels of MDA were markedly increased. According to LSD, after two days of injection of a single acute dose of Al_2O_3-NPs, the brain, hepatic and renal SOD, CAT, GPx activities and the GSH contents of group II, III and IV were significantly decreased, except for the brain's GSH content, in comparison with the corresponding controls (group I), whereas the concentrations of MDA were elevated, (Table 3). The highest percentage of changes, in relation to controls, for activities of SOD (-89.7%) and GPx (-73.4%) were recorded in kidney and CAT (-80.7%) in the brain of group IV (Table 3). In addition, the highest percentage changes, in comparison with group I, for the levels of GSH (-84.5%) and MDA (+142.7%) were recorded in the kidney and liver of group IV, respectively (Table 3).

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 4 revealed that in rats of group V (controls), during sub-lethal experiments, the concentrations of GSH, MDA and activities of SOD, CAT and GPx, in all studied tissues, were significantly affected by the types of organs but neither affected significantly by the experimental periods or their interactions with the type of organs, whereas those of group VI were markedly affected by the experimental periods (time), types of organs, and their interactions together (Table 4). In rats of group VI, The experimental periods exhibited negative relationship with the activities of SOD, CAT, GPx (Table 5), levels of GSH in the brain, liver and kidney with negative correlation coefficients (Table 6), whereas, MDA content showed a positive correlations (Table 6). The brain, hepatic and renal activities of SOD, CAT and GPx were significantly decreased and became severely less than the corresponding controls (group V), at all the experimental periods, except GPx, in all tissues, after one day (Table 5). In comparison with the corresponding controls, the concentrations of GSH in the brain, liver and kidney, of group VI, were significantly decreased, at all experimental periods, but after one day of injection the brain's GSH content was not significantly differed (Table 6). On the contrary, the concentrations of MDA, in all the studied tissues of group VI, were significantly higher than the corresponding controls, at most experimental periods excluding after one day, in the brain and liver, as well as after three days in the brain only (Table 6). In descending order, the percentage of change for the hepatic, brain and renal GSH contents, in relation to controls, were -88.97%, -79.96 , -75.75%, and the MDA levels in the kidney, liver and the brain, after 28 days, were +680%, 302.09% and 95.44% respectively (Table 6). In addition, the highest percentage of changes, in relation to corresponding controls, for SOD, CAT and GPx activities were recorded after 28 days in the liver, -84.8; the brain, -95.5%; and the kidneys, -75.4% respectively (Table 5).

Regression analysis and correlation coefficients (Table 7), in both acute and sub-lethal experiments, revealed that aluminium accumulated in the brain, hepatic and renal tissues were inversely proportional with either the activities of SOD, CAT, GPx or the concentrations of GSH in these tissues, whereas, the levels of MDA exhibited a positive correlation. This indicated that the activities of SOD, CAT, GPx, concentration of GSH decreased significantly with increasing the concentrations of aluminium accumulated in the brain, hepatic and renal tissues, whereas, the levels of MDA markedly increased.

Discussion

The present data, in the acute and sub-lethal experiments, demonstrated that the brain, hepatic and renal GPx, CAT and SOD are potential targets for Al2O3-NPs toxicity, leading to

http://mc.manuscriptcentral.com/TIH

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

significant reduction in the activities of these antioxidant enzymes as a result of Al accumulated in these tissues. In addition, the significant effects of injected doses of nanoalumina, types of organs, experimental periods and their interaction together indicated that their activities were doses, organs, and time dependants. This lead to suggest that the recorded inhibition of SOD, GPx and CAT activities may be attributed to: 1)- the depletion in the levels of total protein necessary for synthesis of antioxidant enzymes, 2)- the alteration in gene expression related to these enzymes, 3)- the reduction of essential elements such as Se, Cu, Mn and Zn, that act as cofactors for antioxidant enzymes, 4)- the damage of the brain, hepatic and renal tissues as a result of over-production of ROS.

Proteins are necessary for enzyme synthesis, and any factor blocks the process of protein synthesis will be in turn reduces the synthesis of enzymes, including antioxidant enzymes, and consequently lead to the inhibition of these enzymes (Albendea et al., 2007). Ali (2013) reported significant depletion in serum total protein content of rats injected with Al2O3-NPs, and was negatively correlated with administered doses as demonstrated in acute experiments and with the experimental time as shown in sub-lethal experiments. The depletion of protein synthesis, necessary for the synthesis of antioxidant enzymes, was attributed to nanoalumina that might enhance the generation of ROS that damaged mitochondria and endoplasmic reticulum necessary for energy production and protein synthesis, respectively (Ali, 2013). Accordingly, the reduction of protein synthesis and excessive generation of free radicals reduced the production of antioxidant enzymes and consequently their activities (Newairy et al., 2009).

The inhibition of enzymes may also be referred to the effect of Al that alters and/or disturbs the expression of mRNA of endogenous antioxidants (Gonzalez et al., 2007). Hamad (2012) reported that titanium oxide nanoparticles caused a significant severe damage to DNA in the brain, liver and kidney, leading to the production of abnormal strands of mRNA that control the synthesis of antioxidant enzymes in cells, and consequently reduce their production. Ali (2013) reported that Al accumulated in the brain, hepatic and renal cells, after injection of acute and sub-lethal doses of Al2O3-NPs, were directly correlated with comet parameters (tail intensity, tail moment and olive tail moment). Thus, in the present work, the disturbance in DNA molecules, under the effect of Al2O3-NPs may alter and disturbed the expression of mRNA causing a significant depletion in the synthesis of the intracellular proteins, leading to the shortage in protein precursor necessary for antioxidant synthesis. Moreover, the inhibition of antioxidant enzymes, in the studied tissues may be also related to the depletion of some essential elements such as Fe, Zn, Cu and Mn that function as cofactor to the antioxidant

For Peer Review

enzymes, especially SOD, and consequently inhibit these enzymes (Flora et al., 2008). As lead metal, Al is known to inhibit heme synthesis by liberating its ionic core of ferritin, and since CAT is a heme-containing enzyme, it causes CAT activity to decrease (Mylroie et al., 1984). Copper ions appear to have a functional role in the reaction by undergoing alternate oxidation whereas zinc ions seem to stabilize the enzyme in the cytosol (Halliwell and Gutteridge, 1989).

Biochemically, acute and sub-lethal doses, of Al2O3-NPs, caused renal failure that confirmed by the histological and physiological alterations (Ali, 2013). As previously confirmed by recent work, the renal failure is usually associated with significant increase in the rate of essential element, cofactors for antioxidant enzyme, elimination from the body, leading to decrease their levels in tissues and serum, and consequently inhibit Cu/Zn-SOD in cytoplasm and Mn-SOD in mitochondria (El-Khawaga and El-Sayed, 2012). Tripathi et al. (2009) reported that Al accumulated in the tissues of rats was accompanied with a significant decrease in the levels of Se, Cu and Mn. In general, the inhibition of SOD activity could be due to the high flux of superoxide radicals resulting in H2O2 production in cells (Li et al., 2010). The H2O2 in cells is further responsible for the changes observed in the activities of antioxidant enzymes. Particularly high levels of H2O2 up regulate CAT activity and down-regulate the activity of SOD (Nehru and Anand, 2005).

Prabhakar et al. (2012) demonstrated a significant increase in MDA levels and CAT activity accompanied by marked decrease in SOD activity with no marked change in GPx activity in the brain, liver, kidneys and heart of rats, after 14 days following oral administration of 0.5, 1.0 and 2.0 g Al2O3-NPs/kg b. wt.. They concluded that Al2O3-NPs might induce free radical generation that further initiated the process of lipid peroxidation and damaging cellular components. In addition, CAT and GPx share the same substrate, H2O2, but with different affinities, since GPx is more effective at low levels of H2O2 whereas CAT is more effective at high levels of H2O2, and the reduction of SOD activity may be related to the high generated levels of H2O2 (Powers and Jackson 2008). However, it may be suggested that the increased production of H2O2 may lead to increased utilization of CAT and GPx and naturally their compensation will be very slowly due to the shortage of protein (Albendea et al., 2007) and some essential elements (El-Khawaga and El-Sayed, 2012), as a result of intracellular accumulation of Al, causing a reduction in their synthesis, concentrations and consequently inhibit these enzymes activities, as demonstrated in the present work.

The current data revealed that the concentrations of GSH in all the studied tissues were significantly reduced except in the brain of rats of group II, III and IV, after 48 h and those of

For Peer Review

group VI, at 24 h post-injection. Several authors have demonstrated that GSH is decreased in the brain, liver, kidney of rats exposed to micro-sized Pb, Al, Cisplatin and Cd (Shrivastava, 2011; Afifi, 2010; Al-Hashem et al., 2009). Glutathione reductase (GR) is a flavoprotein which reduces glutathione disulphide (GSSG) to GSH, thereby supporting the antioxidant defence system (Franco et al., 2008). GR has a disulphide bond in its active site, and Al may interfere and react with the disulphide bond, inhibit the GR activity (Newairy et al., 2009), and inhibit the conversion of GSSG to GSH, making cells more susceptible to oxidative damage (Othman and El Missiry, 1998), leading to marked depletion in the levels of GSH. In addition, GSH is known to protect cells against oxidative stress and any alteration in GSH levels (either a decrease or an increase) indicates a disturbed oxidant status, and when cells are oxidatively challenged, GSH synthesis increases (Li et al., 2010). As oxidative stress continues and the tissue protein contents significantly depleted, as a result of the total protein oxidation by the Al in these tissues, GSH synthesis cannot efficiently supply the demand; therefore, GSH depletion occurs (Khan et al., 2012). Several studies reported GSH depletion in tissues, after administration of micro-sized Cd & Al and the titanium oxide nano-powder, TiO2 (Karmakar et al., 1998; Shrivastava, 2011, Hamad, 2012). Orihuela et al. (2005) reported that high doses of Al induced free radicals and resulted in reduced GSH synthesis by decreasing glutathione synthase activity. From the present data, it was observed that Al2O3-NP toxicity was mainly mediated through the altered antioxidant status of the cells.

The estimated significant increases in the levels of MDA in the brain, liver and kidney of rats injected with acute or sub-lethal doses of Al2O3-NPs, were the results of lipid peroxidation (LPO) which is the main manifestation of oxidative damage (Abdel Wahab, 2012 and El-Demerdash, 2004). The concentrations of MDA, in all studied tissues, were dose- (during acute experiments), time- (in sub-lethal experiments) and organs- dependent, suggesting that the injected nanoalumina might have induced generation of active free radicals that further initiated lipid peroxidation, signifying that the experimental rats suffered severe oxidative stress condition (Hamad, 2012). The toxicity of nanoalumina can be attributed to the physical (nano-diameter, 13<nm; high surface tension) and chemical (hyperactivity of surface) properties of the injected Al2O3-NPs, that facilitate the process of penetration to intracellular organelles across the cell membrane and emission of free Al ions from the hyperactive surface of nanoalumina (Chang et al., 2012), respectively. Thus, nanoalumina may exerts its toxicity by: (1)- the direct interaction with cell organelles, formation of chemical compounds with DNA, RNA, proteins, etc. and (2)- its accumulation in cells, tissues and organs, leading to oxidative damage of organs. Specifically, we thought

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

that Al ions liberated from outer surface of nanoalumina, chemically interacted with the iron in different tissues liberating the redox-active free iron (Gutteridge et al., 1985). Mahieu et al. (2000) reported that Al uptake could be facilitated as iron binds to transferrin and compete with the ferric ions, leading to the utilization of the Fe uptake route for entry into erythrocytes, and this explains the significant increase in zinc protoporphyrin (ZPP), indicating mild anaemia (Flora et al., 2003). Thus, there is a probability that aluminium may interfere with iron metabolism in the event of aluminium overload and the elevation of ZPP could be attributed to the lower iron requirement as a result of reduced heme synthesis after the competition with Al (Mahieu et al., 2000). Oshiro et al. (2000) reported that aluminium binds directly with iron regulatory protein to disrupt iron metabolism in rat cortical cells. Accordingly, in the present work, the liberation of the redox-active ferrous and ferric ions, leads to redox cycling resulting in excessive production of ROS ($HO^{\bullet}$, $ROO^{\bullet}$ & H2O2), causing oxidative stress (Flora et al., 2003). In addition, Al has a modulatory role on the non-iron induced lipid peroxidation (Verstraeten and Oteiza, 2000); non-iron-mediated oxidation of NADH (Kong et al., 1992); and non-iron-mediated formation of the hydroxyl radical (Méndez-Álvarez et al., 2002). The released ferrous and ferric ions are able to activate the molecular oxygen and other compound to produce reactive oxygen radicals.
A significant increase in whole brain thiobarbituric acid reactive substances, a marker of LPO, after stimulation by aluminium salts was observed by Julka and Gill (1996). The finding that Fe^{2+} caused a significant increase in the MDA content of the brain agreed with earlier report where Fe^{2+} was shown to be a potent initiator of lipid peroxidation in the brain (Oboh et al., 2007). The increased lipid peroxidation in the presence of Fe^{2+} could be attributed to the fact that Fe^{2+} can catalyse one electron transfer actions that generates ROS, such as the reactive $OH^{\bullet}$, which is formed from H2O2 through the Fenton's reaction. Iron also decomposes lipid peroxides, thus generating peroxyl and alkoxyl radicals, which favours the propagation of lipid oxidation (Zago et al., 2000). The ionic radii of Al^{3+} most closely resemble those of Fe^{3+}; therefore, the appearance of Al^{3+} in Fe^{3+} sites is probable, and Al is known to be bound by the Fe^{3+} carrying protein transferrin thus reducing the binding of Fe^{3+} (Nehru and Anand, 2005). The increase in free intracellular Fe^{3+} causes the peroxidation of membrane lipids and thus causes membrane damage. Moreover, Fleming and Joshi (1987) have reported that the amount of aluminium found in ferritin extracted from Alzheimer's disease affected brains was 5.6 times higher than in ferritin from matched control samples. This increase may be due to a general increase in the availability of aluminium to the brain of patients with Alzheimer's disease, and raised the possibility that aluminium releases iron as

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Fe^{3+}. Inside the living cells, the increased concentrations of Al could increase the chance of interaction between Al and superoxide anion leading to formation of Al superoxide ($AlO_2^{\bullet 2+}$) that is more potent oxidant than $O_2^{\bullet -}$ in attacking lipids, causing increased LPO (El-Demerdash et al., 2004; Sánchez-Iglesias et al., 2009). This was confirmed, in present study, by the positive relationships between the bioaccumulation of Al and the concentrations of MDA in the studied organs.

The mitochondrial respiration as the major source of ROS is promoted by lipid peroxidation and therefore enhances oxidative stress induced by metal toxicity (Ayres et al., 2008; Hamad, 2012). Throughout our acute experiments, the insignificant decrease in GSH content of the brain could be explained on basis of reduced GPx activity in the brain leading to less utilization of GSH in detoxification of free radicals and consequently accumulation of this antioxidant (Sánchez-Iglesias et al., 2009). This is in agreement with Abubakar et al. (2004) who reported, after 21 days of intra-peritoneal injection of 5 mg Al/kg b. wt./day, no significant change in the concentrations of GSH in the brain of rats were recorded. They suggested that at low concentrations of Al, it may act as antioxidant. Flora et al. (2003) observed a significant increase in TBARS accompanied with no change in GSH level, following exposure to Al nitrate.

Our data of sub-lethal experiments showed that, after one day, the lowest recorded concentration of Al was associated with insignificant change in the levels of MDA and GSH, in the brain of rats of group VI. This was followed by significant reduction in the levels of GSH without any marked elevation in MDA, at the third day post-injection. However, on the seventh day, significant decrease in the GSH content was recorded in association with marked elevation in MDA in the brain of group VI. This could be attributed to the very low concentrations of Al accumulated in the brain, after one day, which weren't enough to induce lipid peroxidation or elicit the anti-oxidative response of GSH. On the other hand, on the third day post-injection, the concentrations of Al in the brain were significantly higher than the first day to the degree that disrupt the synthesis of GSH, by reducing the proteins required for its production (Gonzalez et al., 2007), and this was confirmed by the depletion of serum total protein content (Ali, 2013), at the third day post-injection, in our present study. However, from the seventh day till the end of experiment, LPO was potentiated as a response to the significant accumulation of Al in the brain, leading to more reduction in the brain GSH content that consumed as antioxidant (El-Demerdash, 2004). This was supported by negative relationships between the concentrations of MDA and GSH, in the studied organs of group

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

VI, in the present study. These data is in accordance with several other studies using different nanoparticles (NPs) that have reported the ability of NPs to induce oxidative stress in vivo and in vitro. Nano-titanium dioxide ($TiO2$-NPs), injected in the abdominal cavity of mice, caused significant increased LPO, decreased GSH levels and altered antioxidant enzyme activity in a dose-dependent way (Hamad, 2012; Ma et al., 2010). Similarly, an in vitro study on primary mouse embryo fibroblast cells treated with carbon nanotubes, carbon black, silicon dioxide and zinc oxide showed significant increase in the LPO and decrease in GSH content as well as SOD activity, in a dose-dependent manner (Yang et al., 2009). Zhu et al. (2008) showed that nano and submicron-sized Fe2O3 intra-tracheal instillation produced oxidative stress whereas nano-Fe2O3 increased cell damage in the lung epithelium and affected blood coagulation parameters more significantly than submicron-sized Fe2O3. Our results are also in agreement with Al-bulk administered in the form of AlCl3 through oral gavage, which showed significant induction in LPO in the tissues as compared with normal controls (Bhalla and Dhawan, 2009).

For Peer Review

Conclusion

The present results show that Al2O3-NPs have exerted the following effects:

1). It induced a marked increase in ROS level, as indicated by increasing process of lipid peroxidation (expressed in the levels of MDA), thereby causing oxidative stress in the brain, hepatic and renal tissues.

2). It produced oxidative imbalance in the brain, liver and kidney, probably because these organs are more sensitive to Al2O3-NPs.

3). Aluminium accumulated in the studied tissues was positively correlated with the oxidative stress that proved by inversely relationships between activities of SOD, CAT, GPx, levels of GSH and the Al accumulated in the same tissues, whereas MDA exhibited positive correlation.

4). Oxidative stresses induced by nano-alumina were dose, time and organs dependent, and the first factor that induced tissues damage is the levels of aluminium accumulated in tissues, followed by the injected doses.

References

Abdel Wahab WM (2012): $AlCl_3$-Induced toxicity and oxidative stress in liver of male rats: protection by melatonin. Life Sci. J., 9(4): 1173-1182.

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

Abubakar MG, Taylor A, and Ferns GA (2004): The effects of aluminium and selenium supplementation on brain and liver antioxidant status in the rat. Afr. J. Biotechnol., 3(1): 88-93.

Aebi H (1984): Catalase *in vitro*. Methods enzymol. 105, 121-126.

Afifi ME (2010): Effect of camel's milk on cisplatin-induced nephrotoxicity in Swiss Albino Mice. Am. J. Biochem. Biotechnol., 6: 141-147.

Albendea CD, Trullen EM, Broto LE, Miana-Mena FG, Plano SM, Gonzales MC, Balların EM, Garcia JJ (2007): Melatonin reduces lipid and protein oxidative damage in synaptosomes due to aluminium. J. Trace Elem. Med. Biol., 21: 261–268.

Al-Hashem F, Dallak M, Bashir N, Abbas M, Elessa R, Khalil M, Al-Khateeb M (2009): Camel's milk protects against cadmium chloride induced toxicity in white albino rats. Am. J. Pharmacol. Toxicol., 4: 107-117.

Ali AA (2013): Studies on fate and toxicity of nanoalumina in male albino rats. PhD Thesis, University of Cairo, Faculty of Science, Zoology Department

Ayres JG, Cassee FR, Castranova V et al. (2008): Evaluating the toxicity of airborne particulate matter and nanoparticles by measuring oxidative stress potential- a workshop report and consensus statement. Inhal. Toxicol., 20: 75-99.

Balasubramanyam A, Sailaja N, Mahboob M, Rahman MF, Hussain SM, Grover P. (2009): *In vivo* genotoxicity assessment of aluminium oxide nanomaterials in rat peripheral blood cells using the comet assay and micronucleus test. Mutagensis, 24(3): 245–251.

Beutler E, Duron O and Kelly BM (1963): Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61: 882-888.

Bhalla P, Dhawan DK (2009): Protective role of lithium in ameliorating the aluminium-induced oxidative stress and histological changes in rat brain. Cell. Mol. Neurobiol., 29: 513–521.

Chang YN, Zhang M, Xia L, Zhang J, Xing G (2012): The Toxic Effects and Mechanisms of CuO and ZnO Nanoparticles. Materials, (5): 2850-2871.

Chen L, Yokel RA, Hennig B, Toborek M (2008): Manufactured Aluminum oxide nanoparticles decrease expression of tight junction proteins in brain vasculature. J. Neuroimmune Pharmacol., 3(4): 286–295.

Dey S, Bakthavatchalu V, Tseng MT, Wu P, Florence RL, et al. (2008): Interactions between SIRT1 and AP-1 reveal a mechanistic insight into the growth promoting properties of alumina (Al_2O_3) nanoparticles in mouse skin epithelial cells. Carcinogenesis, 29: 1920–1929.

Di Virgilio AL, Reigosa M, de Mele MF (2010): Response of UMR 106 cells exposed to titanium oxide and aluminium oxide nanoparticles. J. Biomed. Mater. Res. A, 92: 80–86.

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

El-Demerdash FM (2004): Antioxidant effect of vitamin E and selenium on lipid peroxidation, enzyme activities and biochemical parameters in rats exposed to aluminium. J. Trace Elem. Med. Biol. 18, 113–121.

El-Demerdash FM, Yousef MI, Kedwany FS, Baghdadi HH (2004): Role of α-tocopherol and β-carotene in ameliorating the fenvalerate-induced changes in oxidative stress, haematobiochemical parameters and semen quality of male rats. J. Environ. Sci. Health Bull., 39: 443-459.

El-Khawaga OY, El-Sayed IH (2012): Evaluation of trace elements and antioxidants in pre and post hemodialysis of chronic renal failure patients. Int. J. Sci. Nat., 3(3): 617-620.

Fleming J, Josh JG (1987): Ferritin isolation of aluminium–ferritin complex from brain. *Proceedings of the National Academy of Sciences of the United* 84:7866–7870.

Flora SJ, Mehtaa A, Satsangib K, Kannana GM, Gupta M (2003): Aluminium-induced oxidative stress in rat brain: response to combined administration of citric acid and HEDTA. Comp Biochem Physiol C Toxicol Pharmacol., 134(3): 319-328.

Flora SJ, Mittal M and Mehta A. (2008): Heavy metal induced oxidative stress & its possible reversal by chelation therapy. Indian J. Med. Res., 128: 501-523

Franco JL, Posser T, Brocardo PS, Trevisan R, et al. (2008): Involvement of glutathione, ERK1/2 phosphorylation and BDNF expression in the antidepressant-like effect of zinc in rats. Behav. Brain Res., 188: 316–323.

Gonzalez MA, Alvarez ML and Pisani GB (2007): Involvement of oxidative stress in the impairment in biliary secretory function induced by intraperitoneal administration of aluminium to rats. Biol. Trace Elem. Res., 116: 329-348.

Gutteridge JMC, Quinlan GJ, Clark I, Halliwell B (1985): Aluminium salts accelerate peroxidation of membrane lipids stimulated by iron salts. Biochem. Biophys. Acta, 835: 441–447.

Halliwell B, Gutteridge JMC (1989): Eds. Free Radicals in Biology and Medicine. 2nd edition. Oxford: Clarendon Press.

Hamad HR (2012): Modulation of the genotoxic effect induced by titanium dioxide nanoparticles in mice (*Mus musculus*). Ph.D. Thesis, Cairo. University, Faculty of Science, Zoology Department.

Julka D, Gill KD (1996): Effect of aluminium on regional brain antioxidant defense status in Wistar rats. Res. Exp. Med. 1996:187–194.

Karmakar R, Banik S, Bandyopadhyay S, Chatteqee M (1998): Cadmium-induced alterations of hepatic lipid peroxidation, glutathione S-transferase activity and reduced glutathione level

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

and their possible correlation with chromosomal aberration in mice: a time course study. Mut. Res., 397: 183-190.

Khan H, Khan MF, Khan BA, Razaque G, Haque N, Akhter B, Zareen B (2012): Evaluation of the interaction of aluminium metal with glutathione in human blood components. Biomed. Res., 23(2): 237-240

Kong S, Liochev S, Fridovich I (1992): Aluminium (III) facilitates the oxidation of NADH by the superoxide anion. Free Radic. Biol. Med., 13: 79–81.

Li ZH, Li P, Randak T (2010): Effect of a human pharmaceutical carbamazepine on antioxidant responses in brain of a model teleost in vitro: an efficient approach to biomonitoring. J. Appl. Toxicol., 30: 644–648.

Liu S, Xu L, Zhang T, Ren G, Yang Z (2010): Oxidative stress and apoptosis induced by nanosized titanium dioxide in PC12 cells. Toxicology, 267: 172–177.

Ma L, Liu J, Li N, Wang J, Duan Y, et al. (2010): Oxidative stress in the brain of mice caused by translocated nanoparticulate TiO_2 delivered to the abdominal cavity. Biomaterials, 31: 99–105.

Mahieu S, Contini MC, González M, Millen N, Elias MM (2000): Aluminium toxicity. Haematological effects. Toxicol. Lett., 111: 235–242.

Mahieu S, Contini MC, González M, Millen N (2009): Melatonin reduces oxidative damage induced by aluminium in rat kidney. Toxicol. Lett., 190(1): 9-15.

Maquieira Á, Brun EM, Garcés-García M, Puchades R (2012): Aluminium oxide nanoparticles as carriers and adjuvants for eliciting antibodies from non-immunogenic haptens. Anal Chem., 84(21): 9340-9348.

Méndez-Álvarez E, Soto-Otero R, Hermida-Ameijeiras A, López-Real AM, Labandeira-García JL (2002): Effects of aluminium and zinc on the oxidative stress caused by 6-hydroxydopamine autoxidation: relevance for the pathogenesis of Parkinson's disease. Biochim. Biophys. Acta, 1586: 155–168.

Monteiro-Riviere NA, Oldenburg SJ, Inman AO (2010): Interactions of aluminium nanoparticles with human epidermal keratinocytes. J. Appl. Toxicol., 30: 276–285.

Mylroie AA, Umbles C and Kyle J (1984): Effects of dietary copper supplementation on erythrocyte superoxide dismutase activity, ceruloplasmin and related parameters in rats ingesting lead acetate. In: Trace Substances in Environmental Health. Hemphill Ed.; Univ. of Missouri Press, Columbia, 18: 497-504.

Nehru B, Anand P (2005): Oxidative damage following chronic Aluminium exposure in adult and pup rat brains. J. Trace Elem. Med. Biol., 19: 203- 208.

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Newairy AS, Salama AF, Hussien HM, Yousef MI (2009): Propolis alleviates aluminium-induced lipid peroxidation and biochemical parameters in male rats. Food Chem. Toxicol., 47: 1093-1098.

Nishikimi M, Roa NA and Yogi K (1972): Measurement of superoxide dismutase. Biochem. Biophys. Res. Common., 46: 849-854.

Oberdörster G, Oberdörster E and Oberdörster J (2005): Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. Environ. Health Perspect., 113: 823–839.

Oboh G, Puntel RL and Rocha JB (2007): Hot pepper (Capsicum annuum, tepinand Capsicum Chinese, Habanero) prevents Fe^{2+}-induced lipid peroxidation in brain– *in vitro*. Food Chem., 102: 178–185.

Oesterling E, Chopra N, Gavalas V, et al. (2008): Alumina nanoparticles induce expression of endothelial cell adhesion molecules. Toxicol. Lett., 178: 160–166.

Ohkawa H, Ohishi N and Yagi K (1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95(2): 351-358.

Orihuela D, Meichtry V, Pregi N, Pizarro M (2005): Short-term oral exposure to aluminium decreases glutathione intestinal levels and changes enzyme activities involved in its metabolism. J. Inorg. Biochem., 99: 1871-1878.

Oshiro S, Kawahara M, Kuroda Y, Zhang C, Cai Y, Kitajima S, Shirao M (2000): Glial cells contribute more to iron and aluminium accumulation but are more resistant to oxidative stress than neuronal cells. Biochim. Biophys. Acta, 1502: 405–414.

Othman AI, El Missiry MA (1998): Role of selenium against lead toxicity in male rats. J. Biochem. Mol. Toxicol., 12: 345-349.

Paglia DE, Valentine WN (1967): Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med., 70 (1): 158-169.

Patlolla AK, Berry A and Tchounwou PB (2011): Study of hepatotoxicity and oxidative stress in male Swiss-Webster mice exposed to functionalized multi-walled carbon nanotubes. Mol. Cell Biochem., 358(1-2): 189-199.

Powers SK, Jackson MJ (2008): Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. Physiol. Rev., 88: 1243–1276.

Prabhakar PV, Reddy UA, Singh SP, Balasubramanyam A, et al. (2012): Oxidative stress induced by aluminium oxide nanomaterials after acute oral treatment in Wistar rats. J. Appl. Toxicol., 32(6): 436-445.

Sadiq IM, Chowdhury B, Chandrasekaran N and Mukherjee A (2009): Antimicrobial sensitivity of *Escherichia coli* to alumina nanoparticles. Nanomedicine, 5: 282–286.

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Sánchez-Iglesias S, Méndez-Alvarez E, Iglesias-González J, Muñoz-Patiño A, Sánchez-Sellero I, Labandeira-García JL, Soto-Otero R (2009): Brain oxidative stress and selective behaviour of aluminium in specific areas of rat brain: potential effects in a 6-OHDA-induced model of Parkinson's disease. J. Neurochem., 109(3): 879-888.

Shrivastava S (2011): S-Allyl-cysteines reduce amelioration of aluminium induced toxicity in rats. Am. J. Biochem. Biotechnol., 7(2): 74-83.

Sun Z, Wang W, Wang R, et al. (2010): Aluminium nanoparticles enhance anticancer immune response induced by tumor cell vaccine. Cancer Nano., 1: 63–69.

Tripathi S, Mahdi AA, Nawab A, Chander R, et al. (2009): Influence of age on aluminium induced lipid peroxidation and neurolipofuscin in frontal cortex of rat brain: A behavioural, biochemical and ultrastructural study. Brain Res., 1253: 107–116.

Verstraeten SV, Oteiza PI (2000): Effects of Al^{3+} and related metals on membrane phase state and hydration: correlation with lipid oxidation. Arch. Biochem. Biophys., 375: 340–346.

Yang H, Liu C, Yang D, Zhang H, Xi Z (2009): Comparative study of cytotoxicity, oxidative stress and genotoxicity induced by four typical nanomaterials: the role of particle size, shape and composition. J. Appl. Toxicol., 29: 69–78.

Zago MP, Verstraeten SV and Oteiza PI (2000): Zinc in the prevention of Fe^{2+} initiated lipid and protein oxidation. Biol. Res., 33(2): 143–150.

Zhu MT, Feng WY, Wang B, Wang TC, Gu YQ, Wang M, et al. (2008): Comparative study of pulmonary responses to nano- and submicron-sized ferric oxide in rats. Toxicology, 247: 102–111.

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 2. Two-ways ANOVA to analyse the effects of the injected acute-doses, types of organs and their interaction (int.) on activities of SOD, CAT, GPx, the concentrations of GSH and MDA in the brain, liver and kidneys of group II, III and IV, during acute experiments. df: degree of freedom.

Source	Sum of squares	df	Mean squares	$F_{calculated}$	P-value
Doses:					
SOD	5887292.90	2	2943646.45	1135.41	<0.0001
CAT	6.94	2	3.47	360.87	<0.0001
GPx	383.95	2	191.98	208.49	<0.0001
GSH	287.97	2	143.98	40.66	<0.0001
MDA	555.24	2	277.62	174.05	<0.0001
Organs					
SOD	3305961.94	2	1652980.97	637.58	<0.0001
CAT	9.77	2	4.89	507.83	<0.0001
GPx	985.46	2	492.73	535.11	<0.0001
GSH	2262.06	2	1131.03	319.38	<0.0001
MDA	1394.33	2	697.17	437.09	<0.0001
Doses-Organ Int.					
SOD	2477623.97	4	619405.99	238.92	<0.0001
CAT	8.93	4	2.23	232.12	<0.0001
GPx	3.15	4	0.79	0.86	>0.05
GSH	264.07	4	66.02	18.64	<0.0001
MDA	30.35	4	7.59	4.76	<0.01
Error					
SOD	93332.85	36	2592.58		
CAT	0.35	36	0.01		
GPx	33.15	36	0.92		
GSH	127.49	36	3.54		
MDA	57.42	36	1.60		

P>0.05: insignificant effect; P<0.01 and P<0.0001: significant effect at α=0.01 and 0.0001, respectively.

For Peer Review

Table 3. Activities of SOD (x10^3), CAT, GPx (U/g), the concentrations of GSH (mg/g) and MDA (nmol/g) in the brain, liver and kidneys of controls male albino rats (groups I) and those injected with a single low (group II), medium group III) or high (group IV) acute doses, of nanoalumina, after two days of injection . Each value is a mean of five rats ± SEM. % change: percentage of change with the corresponding controls.

Parameters	and organs	Group I	Group II	% change	Group III	% change	Group IV	% change	Regression analysis [1] (Fitting equations)	(r)
SOD	Brain	2.01±0.05	1.70±0.02*	-15.3%	0.92±0.03*	-54.2%	0.56±0.05*	-71.9%	$y= -1471\ln(x)+3686.4$	-0.98
	Liver	0.48±0.01	0.45±0.01•	-6.8%	0.43±0.01♦	-10.5%	0.35±0.002*	-26.6%	$y=560.2e^{-0.051x}$	-0.82
	Kidney	2.06±0.04	1.61±0.02*	-21.9%	0.74±0.01*	-64.3%	0.21±0.002*	-89.7 %	$y= -1787\ln(x)+4042.8$	-0.99
CAT	Brain	2.96±0.15	2.87±0.01	-3.0 %	2.56±0.03♦	-13.5%	0.57±0.05*	-80.7%	$y=-0.488x+5.054$	-0.90
	Liver	1.62±0.14	0.91±0.04*	-43.8%	0.88±0.03*	-45.7%	0.84±0.03*	-48.2%	$y=-0.016x+0.975$	-0.38
	Kidney	3.23±0.17	1.80±0.06*	-44.3%	1.61±0.07*	-50.2%	1.45±0.04*	-55.1%	$y=-0.077x+2.102$	-0.78
GPx	Brain	33.16±1.07	22.51±1.01*	-32.1%	19.35±0.36*	-41.7%	14.77±0.38*	-55.5%	$y=32.6e^{-0.09x}$	-0.91
	Liver	12.25±0.09	11.11±0.34♦	-9.3%	9.29±0.35*	-24.2%	4.37±0.19*	-64.3%	$y=-1.441x+17.29$	-0.93
	Kidney	23.37±0.36	12.89±0.24*	-44.8%	10.39±0.18*	-55.5%	6.21±0.09*	-73.4%	$y=-1.441x+18.86$	-0.97
GSH	Brain	15.38±0.24	14.96±1.21	-2.7%	14.59±0.22	-5.2%	14.01±0.56	-8.9%	$y=-0.205x+15.80$	-0.24
	Liver	14.83±0.48	8.74±0.40*	-41.1%	6.52±0.54*	-56.0%	5.62±0.28*	-62.1%	$y= -4.05\ln(x)+14.19$	-0.83
	Kidney	116.24±1.93	32.22±1.48*	-72.3%	22.58±1.33*	-80.6%	18.04±0.25*	-84.5%	$y=87.68x^{-0.737}$	-0.92
MDA	Brain	15.99±0.79	19.09±0.12•	+19.4%	24.73±1.03*	+54.7%	29.02±0.84*	+81.5%	$y=9.229x^{0.533}$	+0.93
	Liver	5.93±0.56	7.41±0.11•	+25.0%	10.50±0.45*	+77.1%	14.39±0.14*	+142.7%	$y=4.202e^{0.144x}$	+0.98
	Kidney	9.54±0.04	12.49±0.32*	+30.9%	14.20±0.68*	+48.9%	21.25±0.54*	+122.8%	$y=7.619e^{0.114x}$	+0.89

•,♦,*: significant difference in comparison with the corresponding controls (group I), at $\alpha= 0.05$ ($P<0.05$), 0.01 ($P<0.01$) , 0.0001 ($P<0.0001$), respectively. [1]**:** Relationship between the administered acute doses (3.9, 6.4, 8.5 g) and the corresponding parameter in each organ, with correlation coefficient (r).

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

Table 4. Two-ways ANOVA to analyse the effects of the experimental time, organs (brain, liver, kidney) and their interaction (int.) on the activity of SOD, CAT, GPx, the concentrations of GSH and MDA in male albino rats of groups V and VI. df: degree of freedom.

Source	Group V (controls)					Group VI (injected with Al2O3-NPs)				
	Sum squares	df	Mean squres	$F_{calculated}$	P-value	Sum squares	df	Mean squres	$F_{calculated}$	P-value
Time:										
SOD	9282.485	4	2320.621	0.319	> 0.05	4584717.83	4	1146179.46	189.387	< 0.0001
CAT	0.348	4	0.087	1.189	> 0.05	9.48	4	2.371	79.587	< 0.0001
GPx	3.393	4	0.848	0.672	> 0.05	1985.31	4	496.33	553.392	< 0.0001
GSH	9.851	4	2.463	0.799	> 0.05	2244.08	4	561.02	410.062	< 0.0001
MDA	1.105	4	0.276	0.474	> 0.05	11374.60	4	2843.65	2105.665	< 0.0001
Organs:										
SOD	43150385.98	2	21575192.99	2964.71	< 0.0001	6006698.60	2	3003349.30	496.254	< 0.0001
CAT	38.98	2	19.492	266.07	< 0.0001	10.61	2	5.306	178.096	< 0.0001
GPx	5340.10	2	2670.048	2115.13	< 0.0001	1951.83	2	975.92	1088.124	< 0.0001
GSH	164707.58	2	82353.79	26707.00	< 0.0001	19858.41	2	9929.20	7257.471	< 0.0001
MDA	1653.89	2	826.95	1418.61	< 0.0001	13344.69	2	6672.34	4940.732	< 0.0001
Time-Organ int.:										
SOD	7234.817	8	904.352	0.124	> 0.05	2685839.91	8	335729.99	55.474	< 0.0001
CAT	0.730	8	0.091	1.245	> 0.05	3.390	8	0.424	14.225	< 0.0001
GPx	13.355	8	1.669	1.322	> 0.05	435.38	8	54.423	60.680	< 0.0001
GSH	10.984	8	1.373	0.445	> 0.05	616.57	8	77.072	56.333	< 0.0001
MDA	2.652	8	0.332	0.569	> 0.05	5183.22	8	647.902	479.758	< 0.0001
Error:										
SOD	436640.999	60	7277.350			363122.657	60	6052.04		
CAT	4.396	60	0.073			1.788	60	0.030		
GPx	75.741	60	1.262			53.813	60	0.897		
GSH	185.016	60	3.084			82.088	60	1.368		
MDA	34.976	60	0.583			81.029	60	1.350		

P>0.05: insignificant effect; P<0.0001: significant effect at α=0.0001

For Peer Review

Table 5. Activities of SOD (x10^3), CAT, GPx (U/g), in the brain, liver and kidneys of control male albino rats (group V) and those administered 1.3 g Al_2O_3-NPs/kg (group VI), after 1, 3, 7, 14 and 28 days post-injection. Each value is a mean of five rats ± SEM. ►% change: percentage of change with the corresponding controls.

Parameters and organs			Experimental Periods (days)					Regression analysis [(1)] (Fitting equations)	*r*
			1	3	7	14	28		
SOD (U/g)	Brain	Group V	2.05 ± 0.006	2.05 ± 0.077	2.04 ± 0.023	2.07 ± 0.063	2.08 ± 0.012	-----	-----
		Group VI	1.85 ± 0.109*	0.97 ± 0.008*	0.70 ± 0.026*	0.62 ± 0.005*	0.51 ± 0.007*	y= 1.642x$^{-0.377}$	-0.71
		► (%Change)	(-9.79 %)	(-52.89 %)	(-65.56 %)	(-70.24 %)	(-75.69 %)		
	Liver	Group V	0.46 ± 22.64	0.48± 0.012	0.47 ± 0.015	0.45 ± 0.010	0.49 ± 0.006	-----	-----
		Group VI	0.35 ± 0.012*	0.33 ± 0.016*	0.33 ± 0.007*	0.27 ± 0.008*	0.07 ± 0.001*	y= -0.010+0.38	-0.98
		► (%Change)	(-25.05 %)	(-30.24 %)	(-29.41 %)	(-43.06 %)	(-84.77 %)		
	Kidney	Group V	2.11 ± 0.019	2.08 ± 0.038	2.08 ± 0.039	2.11 ± 0.080	2.11 ± 0.025	-----	-----
		Group VI	1.06 ± 0.062*	0.84 ± 0.005*	0.82 ± 0.004*	0.79 ± 0.030*	0.41 ± 0.009*	y= 1.042e$^{-0.03x}$	-0.95
		► (%Change)	(-49.64 %)	(-59.36 %)	(-60.76 %)	(-62.38 %)	(-80.41 %)		
CAT (U/g)	Brain	Group V	2.97 ± 0.15	2.89 ± 0.19	2.93 ± 0.15	2.97 ± 0.03	3.13 ± 0.09	-----	-----
		Group VI	1.33 ± 0.24*	1.09 ± 0.03*	0.92 ± 0.02*	0.72 ± 0.04*	0.14 ± 0.01*	y= -0.041x+1.27	-0.99
		(%Change)	(-55.22%)	(-62.28%)	(-68.60%)	(-75.76%)	(-95.53%)		
	Liver	Group V	1.66 ± 0.05	1.62 ± 0.14	1.41 ± 0.14	1.72 ± 0.06	1.56 ± 0.12	-----	-----
		Group VI	0.96 ± 0.06*	0.73 ± 0.04*	0.64 ± 0.01*	0.62 ± 0.01*	0.62 ± 0.05*	y= 0.905x$^{-0.126}$	-0.67
		► (%Change)	(-42.17%)	(-54.94%)	(-54.60%)	(-63.95%)	(-60.26%)		
	Kidney	Group V	3.46 ± 0.09	3.39 ± 0.11	3.13 ± 0.15	3.08 ± 0.11	3.10 ± 0.11	-----	-----
		Group VI	2.64 ± 0.09*	1.58 ± 0.01*	1.39 ± 0.08*	1.31 ± 0.08*	0.92 ± 0.22*	y= 2.459x$^{-0.283}$	-0.78
		► (%Change)	(-23.70%)	(-53.40%)	(-55.59%)	(-57.47%)	(-70.32%)		
GPx (U/g)	Brain	Group V	31.81 ± 0.49	33.35 ± 0.79	33.14 ± 0.45	32.37 ± 1.24	33.91 ± 0.77	-----	-----
		Group VI	30.02 ± 1.08	24.60 ± 0.52*	16.85 ± 0.49*	14.43 ± 0.68*	13.75 ± 0.24*	y= 30.179x$^{-0.257}$	-0.80
		► (%Change)	(-5.63%)	(-26.24%)	(-49.16%)	(-55.42%)	(-59.45%)		
	Liver	Group V	12.37 ± 0.09	12.16 ± 0.35	12.11 ± 0.09	12.63 ± 0.18	12.21 ± 0.04	-----	-----
		Group VI	12.12 ± 0.20	8.25 ± 0.39*	7.79 ± 0.21*	6.55 ± 0.05*	4.52 ± 0.43*	y= -2.082ln(x)+11.6	-0.87
		(%Change)	(-2.02%)	(-32.15%)	(-35.67%)	(-48.14%)	(-62.98%)		
	Kidney	Group V	24.20 ± 0.21	24.19 ± 0.23	23.52 ± 0.23	23.57 ± 0.48	23.86 ± 0.08	-----	-----
		Group VI	25.08 ± 0.08	9.59 ± 0.07*	8.26 ± 0.03*	6.81 ± 0.02*	5.87 ± 0.24*	y= 20.009x$^{-0.41}$	-0.63
		► (%Change)	(+3.64%)	(-60.36%)	(-64.88%)	(-71.11%)	(-75.40%)		

***:** significant difference in comparison with the corresponding group V, at $\alpha = 0.0001$ ($P<0.000$). [(1)]: Relationship between the experimental periods and the corresponding parameter in each organ of rats of group VI, with correlation coefficient (r) .

For Peer Review

Table 6. Concentrations of GSH and MDA in the brain, liver and kidney of control male albino rats (group V) and those administered 1.3g Al_2O_3-NPs/kg (group VI). Each value is a mean of five rats ± SEM. ►% change: percentage of change with the corresponding controls.

Parameters and organs			Experimental Periods (days)					Regression analysis (Fitting equations)	*r*
			1	**3**	**7**	**14**	**28**		
GSH (mg/g)	Brain	Group V	14.9±0.68	15.2±0.48	15.4±0.05	16.0±0.05	15.0±0.66	-----	-----
		Group VI	14.4±0.04	13.0±0.19*	11.2±0.03*	3.9±0.19*	3.0±0.21*	$y= 14.718e^{-0.063x}$	-0.92
		►(%Change)	(-3.48 %)	(-14.58 %)	(-27.30 %)	(-75.61 %)	(-79.96 %)		
	Liver	Group V	15.4±0.30	15.1± 0.28	14.6±0.62	15.2±0.36	14.6±0.32	-----	-----
		Group VI	9.2±0.16*	5.8±0.08*	5.1±0.24*	2.6±0.16*	1.6±0.14*	y= -2.252ln(x)+8.92	-0.87
		►(%Change)	(-40.00 %)	(-61.82 %)	(-64.96 %)	(-83.09 %)	(-88.97 %)		
	Kidney	Group V	114.0±1.11	115.7±1.63	113.6±1.72	115.1± 0.65	114.4±0.24	-----	-----
		Group VI	53.6±0.58*	48.35±0.44*	41.8±0.53*	35.0±1.09*	27.8±1.36*	y= -7.786ln(x)+55.4	-0.96
		►(%Change)	(-52.98 %)	(-58.21 %)	(-63.16 %)	(-69.60 %)	(-75.75 %)		
MDA (nmol/g)	Brain	Group V	17.4±0.18	17.8±0.22	17.7±0.51	17.53±0.57	17.8±0.25	-----	-----
		Group VI	17.4±0.21	17.6±0.34	21.0±0.03*	28.8 0.02*	34.8±0.68*	y= 0.685x+16.66	+0.98
		►(%Change)	(-0.34 %)	(-1.12 %)	(+19.21 %)	(+64.29 %)	(+95.44 %)		
	Liver	Group V	6.5±0.43	6.7±0.32	6.2±0.44	6.8±0.47	6.7±0.22	-----	-----
		Group VI	6.6±0.06	12.2±0.11*	14.2±0.03*	20.5±0.34*	26.9±0.43*	$y= 6.933x^{0.407}$	+0.97
		►(%Change)	(+1.24 %)	(+84.16 %)	(+129.89 %)	(+203.56 %)	(+302.09 %)		
	Kidney	Group V	9.3±0.31	9.4±0.30	9.8±0.17	9.0±0.19	9.7±0.13	-----	-----
		Group VI	19.0±0.24*	20.8±0.15*	52.0±1.40*	69.9±0.23*	75.5±1.00*	y= 19.45ln(x)+12.36	+0.88
		►(%Change)	(+104 %)	(+123 %)	(+432 %)	(+680 %)	(+680 %)		

***:** significant difference in comparison with the corresponding control, at $\alpha = 0.0001$ ($P<0.000$). (1): Relationship between the experimental periods and the corresponding parameter in each organ of rats of group VI, with correlation coefficient (r) .

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 7. The relationships between aluminium,[1] accumulated in the brain, liver and kidney of group II, III, IV(acute experiments) and group VI (sub-lethal experiments) with the activities of SOD, CAT, GPx, concentrations of GSH and MDA organs.

Parameters	Acute Experiments		Sub-lethal experiments	
	Fitting equation	*r*	Fitting equation	*r*
SOD (U/g)				
Brain	$y= -776.5\ln(x) + 131.5$	-0.93	$y= -586\ln(x) + 881.2$	-0.89
Liver	$y= 1005.9x +555.9$	-0.75	$y= -128.3x + 419.5$	-0.55
Kidney	$y= -1329.7x + 2356.3$	-0.92	$y= -310.8 + 1149.1$	-0.87
CAT (U/g)				
Brain	$Y=-4.61 + 3.58$	-0.94	$y= -0.61x + 1.55$	-0.79
Liver	$y= 0.242x^{-0.68}$	-0.57	$y= -0.16\ln(x) + 0.7$	-0.81
Kidney	$y= 1.68x-0.302$	-0.65	$y= -0.7\ln(x) + 1.51$	-0.65
GPx (U/g)				
Brain	$y= 12.13x^{-0.38x}$	-0.94	$y= -9.79x + 31.4$	-0.91
Liver	$y= -59.99x + 16.9$	-0.83	$y= -3.39x + 12.4$	-0.91
Kidney	$y= -12.4x + 24.49$	-0.89	$y= 9.07x^{-0.63}$	-0.51
GSH (mg/g)				
Brain	$y=-2.26 + 15.4$	-0.21	$y= -6.69x + 16.9$	-0.85
Liver	$y= -90.43 + 20.5$	-0.80	$y= -4.11x + 9.67$	-0.93
Kidney	$y= -39.6\ln(x) + 29.9$	-0.90	$y= -14.28x + 57.99$	-0.80
MDA (nmol/g)				
Brain	$y= 6.74 + \ln(x) + 32.3$	+0.93	$y= 14.61e^{0.39x}$	+0.83
Liver	$y= 3.31e7.83x$	+0.88	$y= 5.91e^{0.76x}$	+0.83
Kidney	$y= 8.59e^{0.52x}$	+0.80	$y= 34.91x + 6.65$	+0.74

[1]: Data from Ali (2013).

For Peer Review