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Relationship between Bioaccumulation of Aluminum Oxide Nanoparticles and some Elements in Male Rats at Acute Experiments

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ABSTRACT

The current work aims to study the bioaccumulation of aluminum (Al) ions in the tissues of male rats, as well as to shed light on the relationship of that accumulation to the ionic content of some elements in these tissues. To achieve this goal, the rats were divided into two groups, the first group (control) was intranasal instilled with deionized water, whereas the second was given a single acute dose of nanoalumina (LD_{25} at 96h that's equivalent to 1.66 g /kg b. wt.). After 24 hours, compared to the control group, the bioaccumulation of Al ions in the liver, kidneys, spleen, lungs, and brain had a significant increase. The bioaccumulation of Al ions in all tissues was associated with a significant decrease in the ionic content of Fe, Zn, and Cu. On the contrary, the ionic content of Ca was increased. The Al accumulated in most tissues has exhibited an inverse significant relationship with the levels of Fe, Zn, and Cu but the Ca ions showed a positive relationship. In conclusion, the bioaccumulation of Al ions caused a significant effect on the most tissues ionic content of Fe, Zn, Cu, and Ca in male rats.

Keywords

Al_2O_3NPs, Iron, Zinc, Copper, Calcium

1. INTRODUCTION

Recently, the rise of nanotechnology has become an essential component of everyday human life and our environment [1]. Nanotechnology is the design, production and application of structures, materials in nanometer scale. It is established that nanoparticles (NPs) are molecules having a critical dimension of less than 100 nanometer (nm). Due to their size, wide surface area and volume to mass ratio, NPs have specific optical, mechanical, electrical, chemical, and magnetic characteristics that make them more reactive compared to their bulk materials [2]. The materials in the

nanoparticle form are usually very different in their properties compared to those of the large material [3].

Nanoparticles can provide many applications in mechanical industries as in coating, lubricants and adhesive applications. Silver nanoparticles (AgNPs) have been widely used to facilitate numerous antimicrobial activities, which can inhibit the growth of Gram negative and Gram positive bacteria as well as yeasts [4]. Beside many industrial and medical applications, there are certain toxicities which are associated with nanoparticles [5]. Nanotoxicology defined as the study of the toxicity of nanomaterials [6]. Nanoparticles represent possible dangers, both medically and environmentally [7] [8]. Most of these dangers are due to the high surface to volume ratio, which can make the particles very reactive or catalytic [9]. Copper nanomaterials have been documented to possess toxic effects on the liver and kidney. Nano-copper has resulted severe impairment in liver, kidney, and spleen in experimental animals after oral administration and interacting with gastric juice [10]. Silver NPs have been detected in various organs, including lungs, spleen, kidney, liver, and brain after exposing the rats to silver nanoparticles either via inhalation or by subcutaneous injection [11]. Cytotoxicity, cell membrane damage, and increased oxidative stress have been developed in various mammalian cell lines as the most common toxic effect of zinc nanoparticles [12].

Nanoparticles can be administrated to human body through inhalation exposure; this is the most common route of exposure to airborne particles in the workplace [13]. Some studies suggested that nanomaterials could enter the body through intact skin during occupational exposure also they can enter the body through wounds [14]. Ingestion can occur from unintentional hand-to-mouth of materials; it also could happen during handling of nanomaterials [15].

One of the widely studied and commonly used nanoparticles is aluminum oxide nanoparticles (Al_2O_3-NPs). Aluminum micro particles and aluminum-containing nanomaterials have been applied by industry, including in food products [16]. Also, they are used as food additives (anticaking agents, neutralizing agents, texturizers) and in food contact materials, such as cooking tools and food packaging [17] [18]. This extensive usage leads to significant releases of aluminum oxide NPs into the environment. These NPs are likely to present greater toxic potency than the same micro sized materials [19] [20]. In humans, the main target of aluminum toxicity is the brain, where it has been associated with dementia, osteomalacia, Alzheimer's disease, and Parkinson's disease [21]. Few studies have demonstrated that the administration of Al_2O_3-NPs may lead to adverse effects, such as genotoxicity [22], inflammatory response [23], carcinogenicity [24], and mitochondrial dysfunction [25]. Reports on neurotoxicity of Nano-alumina mainly focus on the damages to hippocampus, cortex, and cognitive function [26].

The recent database showed that there is a scarcity and/or absence of studies on the relationship of aluminum ions accumulated in tissues with the ionic content of some essential elements in those tissues, following administration of aluminum oxide nanoparticles. Therefore, the present work was designed to assess the effect of aluminum ions accumulated in the tissues of the liver, kidneys, spleen, lungs, and brain on their ionic contents of iron, zinc, copper, and calcium as well as its relationship with the ionic content of those organs, following intranasal instillation with an acute dose of nanoalumina.

2. MATERIALS AND METHODS

2.1. Experimental Animals and Chemicals

Healthy adult male albino rat with average weight 180±5 g was used as an experimental mammalian model. Rats were purchased from the animal house of the National Research Centre, Dokki, Giza, Egypt. The experimental animals were acclimatized to the laboratory conditions for two weeks prior the experiments. Male rats were housed in polyethylene cages in the air-conditioned

animal house at temperature of 25±1°C, relative humidity 20-35% and cyclic daylight on 12 h/day, with a full free access to drinking water and balanced commercial pelleted diet. Every day, the food debris and wastes were removed from cages and were cleaned continually to keep cages dry and suitable for the normal environmental conditions through the course of acute experimentation.

Aluminum oxide nanoparticles (Al_2O_3NPs) with a diameter ≤13nm, were purchased from Sigma-Aldrich (St. Louis, Missouri, USA; 99.98% purity, Product number 718475, CAS number 1344-28-1) and the pure concentrated nitric acid (HNO_3) were purchased from Al-Goumhoria company.

2.2. Ultra-Sonication and Characterization of Nanoparticles

Nanoalumina was ultra-sonicated to be ready for the processes of characterization and administration (intranasal instillation) to the experimental rats during acute experiment. The ultrasonication of aluminum oxide nanoparticles was done by the aid of the biologics ultrasonic homogenizer, Model 150VT. A considerable weight of non-sonicated aluminum oxide nanoparticles was ultra-sonicated in deionized water medium, and immediately vortexes prior to the process of intra-nasal instillation, following the vibration at 20 kHz, with continuous pulse of 40% resulting in a power output of 40 Watts, for five min pre-instillation.

In order to identify the characterization of aluminum oxide nanoparticles (the shape, size and aggregation in the deionized water) were established by the aid of Transmission Electron Microscopy (TEM), according to the technique described by Balasubramanyam [27], where a beam of electrons is transmitted through (Al_2O_3NPs) specimen to form an image. The TEM technique measurements were carried out in the laboratory of the Faculty of Agriculture Research Park, Cairo University, Giza, Egypt, by using JEOL TEM, Model JEM-1400. A drop, of ultra-sonicated suspension of nano-alumina was placed on a sheet of parafilm, and the electron microscope copper grids were made directly off on the specimens. Each grid was placed in a drop of 2% filtered stain of phosphor-tungstic acid (PTA), at pH 7.0, and then incubated in a petri - dish with specimen-side up, until examined by TEM. The output images of aluminum oxide nanoparticles were formed from the interaction of the emitted electrons with the Al_2O_3NPs sample in deionized water medium as the beam is transmitted through the sample. The output pictures of ultra-sonicated aluminum oxide nanoparticles were captured by the Charged Coupled Device (CCD), Optronics camera; model AMT, with a resolution of 1632x1632 pixels. The diameters of Al_2O_3NPs were measured in a random view field, Al_2O_3NPs were amorphous shape with a diameter range of 5.60 nm to 13.51 nm with average mean ± standard error of mean 8.26 ± 1.987 (**Plate 1**).

2.3. Experimental Design

The present work was designed and based on input stage of the acute experimentation. The required total sample sizes of the experimental rats, for acute experiment, were computed by the aid of G-power software (version 3.9.1). The input parameters were: 1- Effect size: 0.75, 2- Alpha level (α): 0.05, 3- Number of groups: 2.0, 4- The response variables: 3.0. The output total sample size was ten animals (N=10) with the actual power was 0.8.

Ten male rats were divided into two groups, each with five (n=5). Rats of the group I (control) were intranasal instilled with deionized water, whereas those of the group II were intranasal instilled with a single acute dose of ultrasonicated Al_2O_3NPs that required to kill 25% of rat's population after 96 hours (LD_{25} of Al_2O_3NPs at 96 h=1.66 g /kg b. wt.). The dose was selected according to the results of the lethality percentiles doses that were measured according to Master Thesis, under supervision (Morsy et al., 2021, in publication). The sampling of specimens was performed after 24h of intranasal instillation with Al_2O_3NPs. Rats were euthanized and dissected

quickly to get the desired tissues (liver, kidney, spleen, Lung, and brain). Then after, the tissues were stored at -20°C for further measurements of bioaccumulation of aluminum ions and the concentrations of iron, zinc, copper, and calcium.

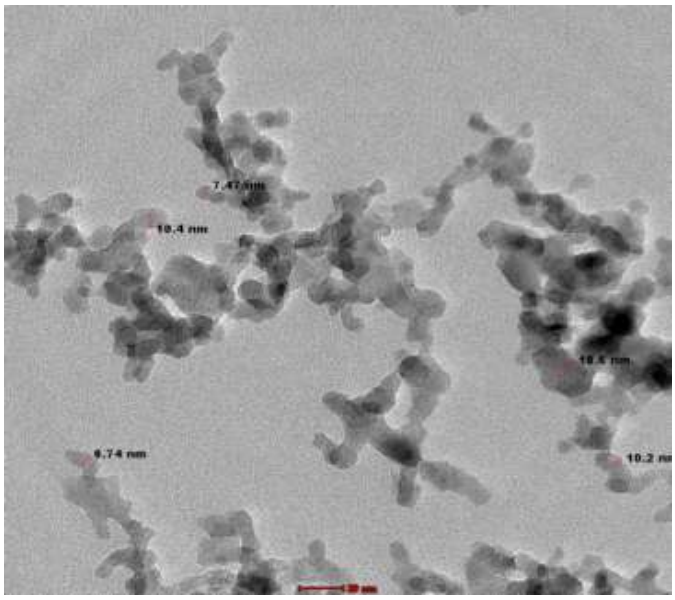

Plate 1. Diameter Measurments of Aluminum Oxide Nanoparticles

2.4. Metals Assay

The concentrations of Al, Fe, Zn, Cu, and Ca ions were measured in the liver, kidney, spleen, lung, and brain of rats was performed according to the method described by Morsy [28]. The measured ions in the tissues were analyzed two times against standards in a linear range by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The concentrations were expressed as microgram per gram dry weight (μg /g dry wt.).

2.5. Statistical Analysis

All data of the present work were normally distributed as affirmed by Shapiro-Wilk and Kolmogorov-Smirnov distribution analyses, and therefore the parametric statistical analyses were applied. Two-ways analysis of variance (ANOVA), for acute experimentation, was applied to test the effects of organs (liver, kidney, spleen, Lung, and brain), dosage of ultrasonicated Al_2O_3NPs (0.00 and LD_{25} at 96h), and their interactions on the bioaccumulation of Al ions and the concentrations of the iron, zinc, copper, and calcium ions in these organs. The analysis of variances was followed by the post hoc Tukey (For homogenous data) and Games-Howell's (For heterogenous data) tests to compare between each two of the desired experimental groups. Additionally, the regression analyses and the Pearson's correlation were applied to expect and to fit the negative or positive relationship and correlation coefficients between the desired independent (factors) or/and dependent (metallic ions) variables. The present data were represented as a mean of five rats ± standard error of mean (SEM). The significant levels were computed at $P<0.05$. All the statistical analyses were done with the aid of the IBM Statistical Package for the Social Sciences, SPSS version 26.

3. RESULTS

Two-way analysis of variances (ANOVA) affirmed that the bioaccumulation of Al ions in the studied organs was significantly affected by the intra-nasal dosage (0.00 and $LD_{25\ @\ 96hr}$ =1.66 g/kg b.

wt.: $F_{(1,4),0.0001}$=1882, P<0.0001), the types of organs (liver, kidney, spleen, lung, and the brain: $F_{(4,4),0.0001}$=83, P<0.0001), and their interaction ($F_{(4,4),0.0001}$=63, P<0.0001) after 24h of instillation with Al_2O_3NPs. In rats of the group I, according to the post hoc Tukey's and Games-Howell's test, the hepatic and the renal Al contents didn't differ but each of them was significantly greater than its content in the spleen and the lungs (Table 1). In addition, the average levels of Al ions in the kidneys of the group I were markedly lesser than its concentration in the brain, and this Al content was significantly greater than its level in the spleen and lungs (Table 1). In a descending order, the brain Al content was significantly > the hepatic Al content > the Al renal content > the splenic > Al level in the lungs (Table 1).

According to the statistical analysis of the current results by Student's test or Welch's t-test, the Al ions accumulated in all the studied tissues of the liver, kidneys, brain, spleen, and lungs of the second group rats that intranasal instilled with a single acute dose of nanoalumina (Al_2O_3NPs), increased significantly when compared with their counterparts in the rats of the first group (Table 1). In addition to the above, it was also evident from the results that the significant bioaccumulation of aluminum in the rats of the second group was accompanied by a sharp and significant decrease in the ionic content of iron, zinc, and copper in all the tissues of the studied organs, when compared with their counterparts in the first group, except the spleen copper content didn't differ (Table 1). Contrary to what we previously observed, we noticed that the Al accumulations in all organs of the second group, except the Ca content of the spleen, were associated with a strong significant increase in calcium ions when compared with their counterparts in the first group (Table 1).

As shown in figures 1, 2, and 3 according to regression analysis and correlation coefficients, it has been proven that there are an inverse strong relationship between aluminum accumulated in the liver, kidneys, brain, lungs, and spleen with the ion content of iron, zinc, and copper except for the concentration of copper ions in the spleen. The observed significant relationships were associated with a negative significant correlation coefficient (Figure 1, 2, and 3).

In contrast to the above, it was observed that the ionic content of calcium in the all organs, except the spleen, showed a strong direct significant relationship with the aluminum ions accumulated in these organs, and these relations were associated with a positive strong significant correlation coefficient (Figure 1, 2, and 3).

4. DISCUSSION

The present data affirmed that the bioaccumulation of Al ions in the brain and kidneys of rats that given Al_2O_3NPs (Group II) did not differ statistically, but that accumulation was significantly greater than in the liver, the spleen, then the lungs, but these ions in both the liver and the spleen did not show a significant difference. Because the experimental rats were given Al_2O_3NPs via the nostril, and accordingly did not get absorbed through the intestine and nor the liver directly, but passed to the blood network directly to the lungs to enter the systemic circulatory blood and then into the heart that pumps the blood loaded with these nanoparticles to all organs [29]. Therefore, the Al_2O_3NPs were not subjected to the first-pass biotransformation by the gastrointestinal tract and/or the liver at all, meaning that there was no systematic pre-elimination of these nanoparticles, and thus, the bioavailability of Al_2O_3NPs was very high leading to their accumulation in descending order in all tissues of the kidneys, brain, liver, spleen, and lungs. The bioaccumulation of these nanoparticles in the brain can be attributed to several reasons such as their high bioavailability in the bloodstream, as mentioned above, their nano-size, and their lipophilicity. The high bioavailability of nanoparticles gives them an impulsive force to surround the brain at high concentrations and due to its large surface area stimulates its outer surface to auto-ionization and then generate some reactive oxygen species (ROS) that in turn destroy and penetrate the blood brain barrier (BBB) and accumulate at high percentage in the brain tissue as observed in the current data [30]. In addition, Al_2O_3NPs are highly

lipid-soluble and this allows them to easily cross nerve cell membranes and accumulate within them at high levels [30].

The kidneys, liver, and spleen, of rats given Al_2O_3NPs, accumulated high levels of Al ions. The average diameter of Al_2O_3NPs, in the present results, was 10 nm and this represents a great obstacle to getting rid of these particles through the urine, as the glomerular pores are about 5.5 nm, and this leads to continuous ionic trapping of these nanoparticles in the kidneys, which led to a decrease of that ions elimination via the urine leading to a significant increase in its levels in the renal tissues [31]. The high accumulation of Al ions in the liver reveals its role as a vital organ involved in the processes of biotransformation to eliminate Al_2O_3NPs outside the body via urine [32]. This might be attributed to the histological architecture of the liver that has a wide discontinuous endothelial layer that permits the passage of Al_2O_3NPs freely to the liver [33]. In addition, the reticular-endothelial structure of the spleen plays a vital function in trapping, fixing, and eliminating Al_2O_3NPs by opsonization and phagocytosis by the aid of the spleen [34].

The influence of the bulk aluminum on the essential elements in tissues of mammals was previously studied [35] whereas, according to the recent database, there is no enough information or studies about the effect and/or the relationship of Al_2O_3NPs on/and with these metallic ionic contents absolutely. The results of the current work have found that the bioaccumulation of Al in the liver, kidneys, spleen, lungs, and brain was attended by a significant decrease in the ionic content of Fe, Zn, and Cu, whereas the ionic content of Ca has increased substantially when compared to the control rats of the group I. In addition, there was an inverse significant relationship between the Al accumulated in most tissues with the ionic content of all elements in these tissues except the Ca ions that exhibited a direct relationship with a strong significant positive correlation coefficient. These results can be attributed to each of the physicochemical properties of Al_2O_3NPs as well as its effect on their metabolic pathways as a direct and/or indirect response to their interaction with them in the studied tissues.

Chemically and according to the electrochemical series of the reactivity, the Al ions are more reactive than Zn, Fe, and Cu but lesser than that of the Ca ions [36]. Accordingly, the Al ions had the reactivity required to display the Fe, Zn, and Cu ions in most tissues, leading to a significant depletion of them, as observed in the present results. On the contrary, the reactivity of the Ca ionic contents greater than that of the Al and therefore Al isn't able to display the Ca ionic in the tissues. Kinetically, it is known that hypercalcemia is usually associated with a marked decrease or/and inhibition of the absorption of either Zn and Cu ions that in turn caused a significant depletion of these ions in the blood circulation and consequently decreased their levels in the tissues [37], as shown in the current data. Additionally, as a response to the toxicity of Al ions accumulated in the pancreas, the protein zinc-binding factor interacts with the Zn ions to form an inactive hydrophilic complex causing the reduction of Zn absorption that will be stay bounded in the liver with the metallothionines by the -SH group leading to lower its levels in the blood and in turn various tissues as well as facilitate the process of Zn elimination and excretion from the blood and tissues [38].

Physiologically, the significant depletion of the ionic Fe, Zn, Cu as well as the increased Ca contents can be attributed to the direct and/or indirect disturbances and defects in the metabolism of these ionic contents as a response to the accumulated Al ions in the tissues, which has reached the level of Al overload.

The chemical resemblances of Al and Fe let the Al, which has no vital and/or physiological role in mammals, to interfere and disturb the process of iron metabolism [39]. Accordingly, we can say that the significant decrease in the tissue iron content may be attributed to two reasons. First, as we know hepcidin is a hormone that produced by the liver, and play an important regulatory role of iron

metabolism, i.e., it is a key regulator of the entry of iron into the circulation in mammals [40]. Hepcidin blocks and inhibits iron transport by binding the iron exporter ferroprotein (FPN) of the duodenal enterocytes, the inhibited FPN letting the iron out of the intestinal cells [41] [42]. In the current work, most of the tissues, including the liver, have accumulated aluminum ions to a degree that exceeded the usual and reached the overload limit, causing an excessive increase of hepcidin hormone secretion, which in turn led to preventing and/or decreases the passage of ferrous ions (F^{+2}) through the intestinal cells to the outside, and this, of course, led to a great decrease of ionic ferrous content in the blood which is the main distributor of ions to the hard and soft tissues, and this, in turn, led to a marked depletion of iron in tissues. Secondly, the transferrin is a glycoprotein which bind to and consequently mediate the transport of ferric ions (Fe^{+3}) through blood plasma, from the ileum to bone marrow for the process of erythropoiesis [43]. The Al ions accumulated in tissues, in the present work, maybe led to a significant disturbance in the metabolism of iron. It was found that iron absorption by transferrin decreased by 60% in a mammalian model that intake an acute dose of the bulk Al [44]. Mostaghi and Skylin concluded that Al ions frighteningly compete with iron to bind with transferrin, which leads to an imbalance and disruption of iron metabolism by interfering with cell absorption of iron [45], which leads to a reduction and/or inhibition of heme synthesis, which causes a significant decrease in iron and hemoglobin levels, and this was accompanied by a significant noticeable elevation in the levels of zinc protoporphyrin (ZPP) [46]. In addition, as a result of the large surface area of Al_2O_3NPs accumulated in the tissues, their outer layer surfaces began a process of auto-ionization, which led to the release of high concentrations of Al ions [47]. The liberated Al ions expel the Fe ions from tissues in the form of transferrin [48] and inhibit the ceruloplasmin that is responsible to enhancement and acceleration the incorporation of the ferric ion into transferrin [49] leading to a marked decrease of absorption Fe and consequently lead to a significant iron depletion in the various tissues [28].

The Cu ions was significantly decreased and negatively correlated with the Al ions accumulated in the most tissues. This may be attributed to the kinetics, metabolic disturbance of Cu, and the histopathological changes as a direct or indirect Al toxicity. Because the high levels of Al ions in the bloodstream, it will be reach and accumulate in the stomach, intestine, and the liver with high concentrations [28] without first-pass metabolism. In the stomach and intestine the accumulated Al ions interact with the copper-transporting ATPase ATP7A protein, localized in trans-Golgi Network (TGN) and the cytosolic vesicles, causing the block and/or inhibit the bounding of Cu ions with the albumin to form the Cu-albumin complex that is responsible to transfer the Cu into the liver leading to a decrease in its absorption by the stomach and the intestine and facilitate the Cu excretion via the abnormal pathway of urine as well as reduce its availability as a substrate to the copper-transporting ATPase ATP7B protein produced by the hepatocytes [50]. In the hepatic cells, the Al ions attack the cell organelles and destroying them, the TGN and the cytosolic vesicles by the liberated reactive oxygen species leading to liberate high levels of the coded ATP7B protein [51]. Accordingly, this coded protein migrates from the TGN and cytosolic vesicles to the hepatocytic membranes and transfer the Cu ions into the bile that in turn collected in the gall bladder and then accelerate the excretion of the Cu-protein by the exocytosis into the intestine and then outside the body with the feces [52] leading to decrease the absorption and bioavailability of Cu in the tissues.

According to the toxicokinetic of Al_2O_3NPs and its route of administration, these particles accumulated in the lungs and then poured directly into the systemic circulatory blood that transfers them to the various organs including the thyroid and parathyroid glands in which these nanoparticles accumulated and caused histopathological and physiological disorders [28]. The Al ions compete Fe to bind with the Fe regulatory protein to interrupt Fe metabolism and liberating the redox-active ferrous and ferric ions, lead to redox cycling resulting in excessive production of reactive oxygen species

(ROS) of HO, ROO, and H_2O_2 causing oxidative stress [53] [54]. It has been found that the high accumulation of Al ions increases the possibility of its interaction with $O_2^{\bullet}$ to produce Al-superoxide ($AlO_2^{\bullet 2+}$) that is more powerful than $O_2^{\bullet -}$ in attacking the lipoproteins of the cell and organelle membranes, leading to increase the process of the lipid peroxidation and causing some physiological disturbances in the thyroid and/or parathyroid glands [55] [56]. Therefore, the parathyroid glands could be inflamed and secreted excessive amounts of parathyroid hormone (PTH) and calcitriol into the blood circulation causing hyperparathyroidism and hypervitaminosis of vitamin D (calcitriol). The excessive secretion of uncontrolled PTH induced the bone to release its stores of Ca ions into the systemic blood circulation and in turn in tissues causing hypercalcemia. Additionally, the hypervitaminosis of vitamin D (calcitriol) potentates the reabsorption of Ca ions from the distal convoluted tubules to the blood stream causing hypercalcemia.

Table 1. The concentrations of Al, Fe, Zn, Cu, and Ca ions in the liver, kidneys, brain, spleen, and lungs of male rats intranasal instilled with deionized water (Group I) and those intranasal instilled with Al_2O_3NPs (Group II), after 24h of instillation.

Ions	Organs	Liver	Kidneys	Brain	Spleen	Lungs
Al	Group I	10.8±0.217	10.1±0.263	11.3±0.269	6.3±0.272	2.8±0.068
	Group II	112.6±5.804*	159.4±4.580*	158.5±6.188*	96.3±5.550*	47.6±5.198*
	% change[■]	+952%	+1475%	+1294%	+1425%	+1578%
Fe	Group I	132±3.674	84.60±1.990	21.87±0.971	723.20±38.85	132.0±3.674
	Group II	105.0±5.282*	51.60±3.600*	13.80±0.860*	528.80±44.535*	60.80±5.142*
	% change[■]	-21%	-39%	-37%	-27%	-54%
Zn	Group I	15.6±0.50	21.80±0.735	11.00±0.451	18.00±0.707	15.6±0.510
	Group II	10.60±0.51*	13.40±0.748*	6.80±0.374*	13.00±0.894*	12.80±0.860*
	% change[■]	-32%	-39%	-38%	-28%	-18%
Cu	Group I	4.54±0.25	3.33±0.047	1.68±0.088	0.932±0.023	182.6±7.019
	Group II	3.16±0.12*	2.52±0.206*	1.25±0.046*	0.914±0.043	150.80±5.704*
	% change[■]	-30%	-24%	-26%	-2%	-17%
Ca	Group I	2.48±0.06	3.54±0.194	32.40±2.064	4.60±0.201	3.96±0.267
	Group II	3.38±0.25*	5.18±0.514*	51.20±2.746*	5.59±0.533	5.48±0.219*
	% change[■]	+36%	+46%	+58%	+21%	+39%

Data are represented as a mean of five rats ± SEM.

*: Significant difference in comparison with the corresponding control (Group I) at α=0.05 (P<0.05).

% change[❶]: Percentage of change in relation to the corresponding control (Group I).

5. CONCLUSION

It is evident from the present results that the bioaccumulation of aluminum ions that follows the intranasal instillation of an acute dose of aluminum nanoparticles completely depended on the dose (0.00 and 1.66 mg/kg b. wt.) and the type of organs (the liver, kidneys, brain, lungs, and spleen). The accumulation of aluminum ions in the liver, kidneys, and brain led to a significant decrease in the ionic content of iron, zinc, and copper, with a marked increase in the ionic content of calcium in these tissues. In addition to the above, the bioaccumulation of aluminum was inversely

proportional to iron, zinc, and copper ions but was positively proportional to the ionic content of calcium.

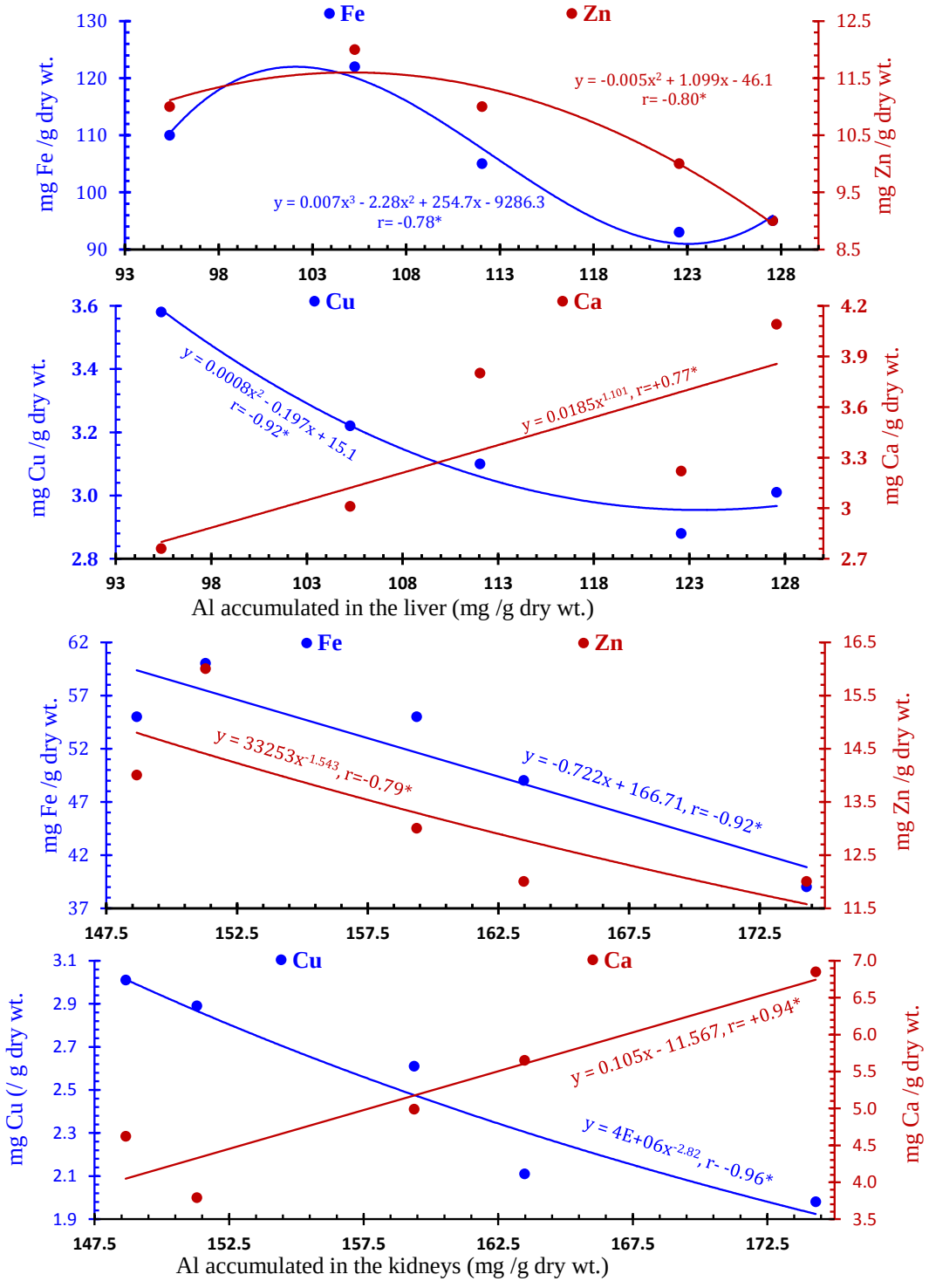

Figure 1. The relationship of the Al ions accumulated in the liver and the kidneys with their ionic content of iron, zinc, copper, and calcium in the second group rats, after 24h of intranasal instillation with a single acute dose of 1.66 g/kg b. wt. x: Al ions accumulated in the tissues. y: the Fe, Zn, Cu, and Ca ions contents. r*: significant correlation coefficient.

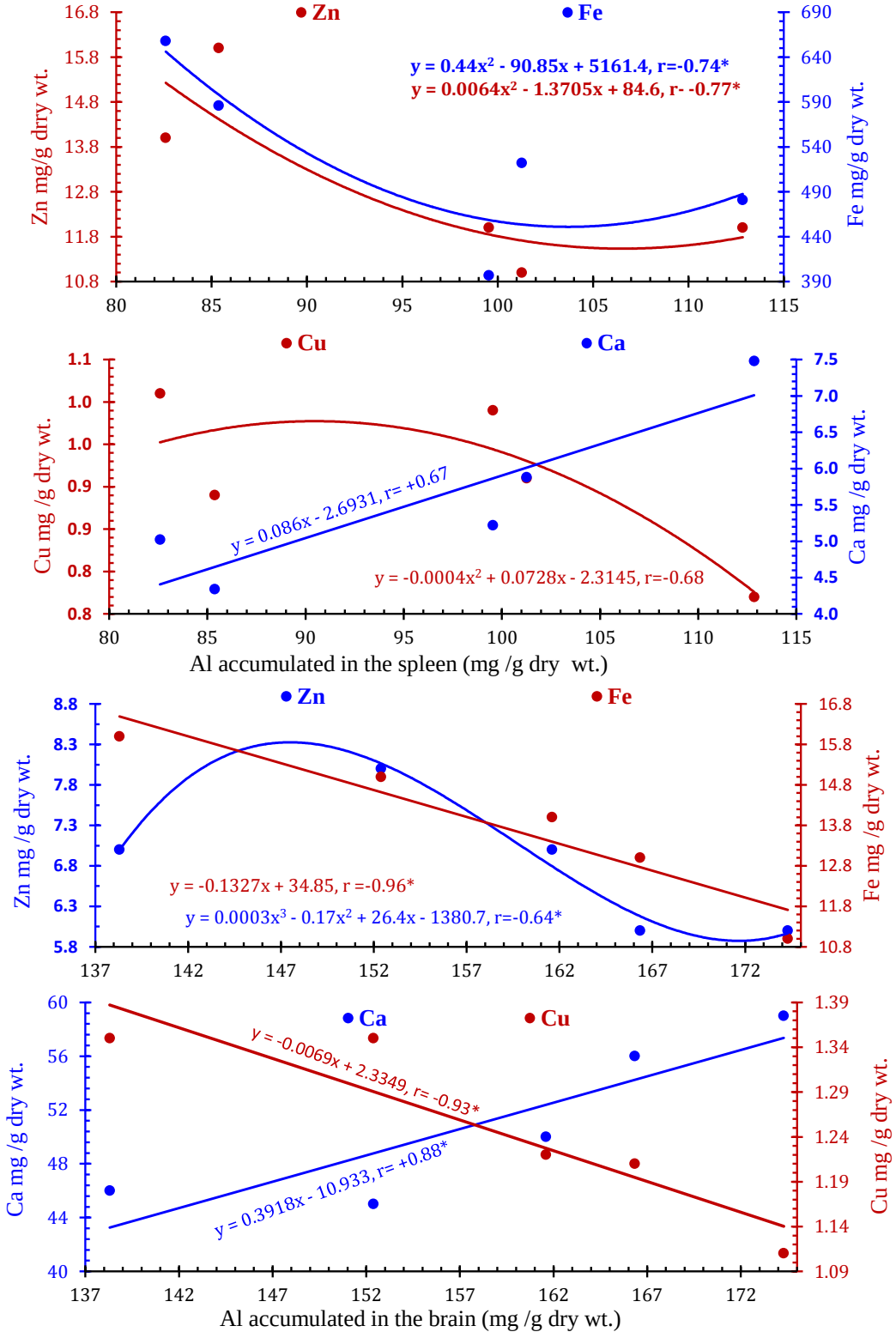

Figure 2. The relationship of the Al ions accumulated in the spleen and the brain with their ionic content of iron, zinc, copper, and calcium in the second group rats, after 24h of intranasal instillation with a single acute dose of 1.66 g/kg b. wt. x: Al ions accumulated in the tissues. y: the Fe, Zn, Cu, and Ca ions contents. r*: significant correlation coefficient.

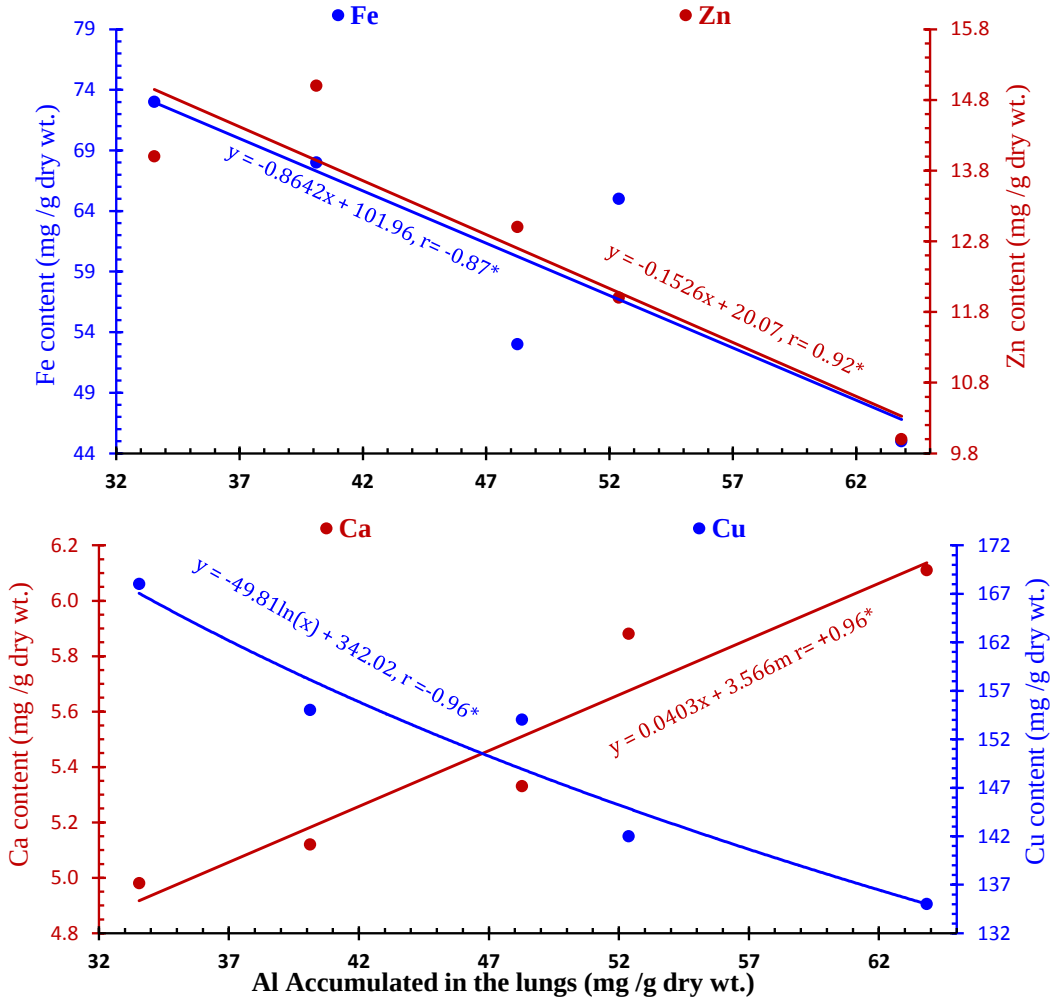

Figure 3. The relationship of the Al ions accumulated in the lungs with its ionic content of iron, zinc, copper, and calcium in the second group rats, after 24h of intranasal instillation with a single acute dose of 1.66 g/kg b. wt. x: Al ions accumulated in the tissues. y: the Fe, Zn, Cu, and Ca ions contents. r*: significant correlation coefficient.

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