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Pathological Investigation of Alumunium Oxide Nanoparticles Toxicity and Protective Effect of Melatonine on Their Toxicity

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Abstract: The aim of this study was to evaluate the hazardous effect of aluminum oxide nanoparticles (AL_2o_3-NPs) and the protective role of melatonine. Twenty-eight adult male albino rats, was divided into 4 groups. Groups 1 received distilled water. Group 2 was received , Al_2O_3-NPs, (100 mg/kg b.wt) daily. Group 3 received Al_2O_3-NPs (100 mg/kg b.wt) + melatonine Mel) (10mg/kg b.wt)daily and group 4 received melatonine (Mel) (10 mg/kg b.wt) daily for 3 weeks. Blood and serum were collected for hematological, biochemical and antioxidant examinations. Group 2 revealed significant increase in RBCs count and PCV %. and hepatic enzymes including ALT, AST and ALP and urea levels. Renal enzymes urea, creatinine and uric acid were detected. Moreover, significant increase in MDA activity with significant decrease in SOD enzyme compared with control. Other treated groups exhibited an improvement in such altered parameters including RBCs, WBCs, AST, ALT, ALP, urea, uric acid, MDA and SOD when compared with group 2. It was concluded that aluminium nanoparticles induced oxidative damage associated with hepatic and renal dysfunction. Administration of Mel could alleviate the oxidative damage induced by aluminium nanoparticles.

Key words: Alumunim Oxide Nanoparticles · Hepato-Renal Dysfunction · Melatonin · Alleviate

INTRODUCTION

Aluminum is one of the most plentiful elements in the earths crust and is covered by an oxide film that generally inhibits corrosion. Acute and chronic exposure to Al has been shown to be toxic to animals and humans [1]. The rising concern for nanoparticles emerges because of their unique chemistry, extremely small size, large reactive surfaces and non-biodegradability. They rapidly get dispersed throughout the environment with unknown consequences [2].

Metallic NPs are closely related to generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) due to redox-cycling reactions. Currently, ROS and oxidative stress induced by NPs have been one of the best-developed paradigms for nanoparticle toxicity [3, 4]. There is evidence that exposure to aluminum may lead to inflammatory events and/or the breakdown of the blood- brain barrier [5]. The change in physicochemical and structural properties of NPs may be responsible for the interactions that could lead to toxicological effects [6]. These changes could lead to specific surface groups (e.g. thiol groups, carboxyl groups, carbonyl groups, SDS, DMSO, surfactants) to be reactive sites [7]. These reactive sites act as electron-donors or electron-acceptors and work with oxygen to induce superoxide radical (O_2^-), which can then generate reactive oxygen species (ROS) by dismutation or Fenton reaction [8].

However indiscriminate use of nanoaluminium may lead to release of the oxidized form of nano AL_2O_3 into the environment and may produce adverse effects, such as genetic damage, carcinogenicity, cytotoxicity etc [9]. Their small size facilitatedcellular uptake and transcytosis across epithelial and endothelial cells reaching into systemic circulation and affecting potentially sensitive target sites such as brain, bone marrow, lymph nodes, spleen and heart [10].

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Nephrotoxic action of aluminium arises from its accumulation in tissues, resultant in abnormal brush borders in proximal tubules of kidneys, fibrosis in the liver diffuse in periportal hepatocytes and parenchymal cells [11].

Aim of the work to investigate adverse effect of aluminium oxide nanoparticles toxicity and determine the protective role of melatonine

MATERIALS AND METHODS

Materials

Experimental Animals: Twenty-eight adult male albino rats weighing 100 gm were obtained from the Faculty of Medicine, Assiut University, Egypt. Prior to the experiment, the animals were kept in plastic cages with wire mesh covers under normal environmental conditions of temperature and humidity for a period 2 weeks. A standard commercial diet and water were supplied ad libitum throughout the experiment period. The rats were manipulated according to the experimental animal ethics approved by South Valley University, Qena, Egypt.

Chemicals:

- Aluminum Oxide nanoparticles, it was obtained from Nanotechnology Unit, Faculty of Science, South Valley University, Qena, Egypt.
- Melatonin: It is white semi-hard tablet. It was purchased from NATROL, Inc. Company USA.
- Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) kits were estimated using technique delineated by Reitman and Frankel [12]. Alkaline phosphatase (ALP) was assessed via method of Belfield and Goldberg [13]. Catalogue number 260 001, 264 001 and 216 001, respectively. (Spectrum company).
- Serum urea, creatinine and uric acid were estimated by methods of Fawcett and Scott [14], Bartels *et al.* [15] and Barham and Trinder [16] respectively. Catalogue number 321 001, 235 001 and 323 001, respectively. (Spectrum company)
- Superoxide dismutase (SOD) was evaluated by the techniques of Nishikimi *et al.* [17]. Malondialdehyde (MDA) concentration was measured according to Ohkawa *et al.* [18].Catalogue number SD 25 21 and MD 25 29 respectively.(Biodiagnostic company).

Methods

Experiment Design: Animals were divided equally into seven groups, with each group containing seven rats.

Group 1: (Control) received distilled water.
Group 2: Received orally aluminium oxide nanoparticles (Al_2O_3NPs) at dose 100 mg/kg b.wt.
Group 3: Received oraly aluminum oxide nanoparticles (Al_2O_3NPs) + melatonine (mel) at dose 10 mg/kg b.wt..
Group 4 received oraly melatonine (mel) at dose 10 mg/kg b w.t.

The rats were dosed daily for a period 3 weeks. The clinical signs and mortality rate of the animals were daily recorded. All rats were anaesthetized by halothane. Blood and serum samples were collected for hematology and biochemical examinations.

Statistical Analysis: The data were analyzed using one-way analysis of variance followed by post hoc analysis (Dunnett's test) using SPSS (Statistical package for Social Sciences) version 17. A difference of $P<0.05$ was considered statistically significant.

RESULTS

Hematological Findings: Table (1) showed significant increase was recorded ($P<0.05$ %) in RBCs count and PCV % of groups 2 & 3 in comparsion with control. Hemoglobin concentration expressed non-signifcat changes in all exposed groups when comapred with control and group 2. WBCs count displayed significat increase ($P<0.05$ %) in group 2 when compared with control, while groups 3 & 4 showed significat decrease ($P<0.05$ %) in comparsion with group 2. Platelets counts were significatly increased ($P<0.05$ %) in groups 2 & 3in comparsion with control. Group 4 expressed significat decrease ($P<0.05$ %) in platelets when compared with group 2.

Table (2) revealed significant increase ($P<0.05$ %) was detected in lymphocytes % of groups 2 & 3 when compared with control. While, in comparison with group 2; there was significant decrease ($P<0.05$ %) in lymphocytes % of groups 3& 4. Neutrophil % significantly decreased in groups 2 & 3 when compared with control, group 4 showed significant increase ($P<0.05$ %) when compared with group 2. Also, monocytes % displayed significant decrease ($P<0.05$ %) in group 2 in comparison with control. On the contrast, monocytes % of group 4 showed significant increase ($P<0.05$ %) when compared with group 2. Both eosniophil and basophil % showed significant changes only in group 3 when compared with control.

Table 1: The mean and standard error of RBCs, hemoglobin concentration (Hb), packed cell volume (PCV %), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), WBCs and platelets count of group (1), group (2), group (3) and group (4)

Parameters	RBC's ($x10^6$)	Hb. (gm/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC%	WBCs ($x10^3$)	Platelets ($x10^9$)
Group (1)	5.9±0.2	12.4±0.17	35.8±0.3	58.1 ±4.1	20.0 ±1.3	34.6±0.4	10.5±0.6	348.3±16.3
Group (2)	7.5±0.3 [a]	14.2±0.7	41.7±2.1 [a]	55.2 ±5.3	18.7±0.7	33.9±0.2	17.0±0.8 [a]	400.3±8.6 [a]
Group (3)	7.6±0.2 [a]	14.9±0.5	43.5±1.5 [a]	53.2±1.6	19.3±0.9	34.1±0.2	13.3±0.5 [b]	415.0±6.3 [a]
Group (4)	6.3±0.4	12.1±0.3	34.5±0.6	54.8±4.0	19.3±1.4	35.2±0.4	11.2±0.9 [b]	341.0±11.8 [b]

a → significant difference when compared with control (G.1) when P<0.05 %.

b → significant difference when compared with group 2 when P<0.05 %.

Table 2: The mean and standard error of the differential leukocytic counts of group (1), group (2), group (3) and group (4)

Parameters	Lymphocytes	Neutrophil	Monocytes	Eosinophil	Basophil
Group (1)	5.4±0.3	0.5±0.1	10.5±0.6	10.5±0.6	10.5±0.6
Group (2)	5.9±0.4	0.5±0.1	17.0±0.8[a]	17.0±0.8[a]	17.0±0.8[a]
Group (3)	4.3±1.2	0.5±0.1	13.3±0.5 [b]	13.3±0.5 [b]	13.3±0.5 [b]
Group (4)	1.65.4	0.1±0.7	0.7±1.5	0.7±1.5	0.7±1.5

a → significant difference when compared with control (G.1) when P<0.05 %.

b → significant difference when compared with group 2 when P<0.05 %.

Table 3: The mean and standard error of serum aspartate-aminotranferase (AST), alanine-aminotransferase (ALT) and alkaline phosphatase (ALP) of group (1), group (2), group (3) and group (4)

Parameters	AST (IU/l)	ALT (IU/l)	ALP (IU/l)
Group (1)	197.3±4.6	38.3±1.4	175±2.8
Group (2)	287±3.0 [a]	79.0±2.6 [a]	207.7±3.8 [a]
Group (3)	277.7±2.9 [ab]	58.6±2.3 [ab]	182.7±4.8 [ab]
Group (4)	188.7±6.1 [b]	40.0±3.5 [b]	164.7±5.2 [b]

a → significant difference when compared with control (G.1) when P<0.05 %.

b → significant difference when compared with group 2 when P<0.05 %.

Table 4: The mean and standard error of serum urea, creatinine and uric acid of group (1), group (2), group (3) and group (4).

Parameters	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Group (1)	34.0 ±2.1	0.9±0.05	3.8±0.5
Group (2)	45.3±2.6 [a]	1.0±0.08	4.3±0.2
Group (3)	35.3±1.2 [b]	0.9±0.3	3.3±0.2 [b]
Group (4)	31.3±1.3 [b]	0.8±0.03	3.2±0.1 [b]

a → significant difference when compared with control (G.1) when P<0.05 %.

b → significant difference when compared with group 2 when P<0.05 %.

Table 5: Mean and standard error of oxidative stress marker (MDA) and antioxidant enzyme (SOD) (IU/l) of group (1), group (2), group (3) and group (4).

Parameters	MDA (µmol/l)	SOD (IU/l)
Group (1)	11.1±0.4	392.7±3.8
Group (2)	20.2±1.2 [a]	254.0±5.0 [a]
Group (3)	15.2±0.6 [ab]	352.1±4.5 [ab]
Group (4)	11.7±0.9 [b]	346.6±4.0 [ab]

a → significant difference when compared with control (G.1) when P<0.05 %.

b → significant difference when compared with group 2 when P<0.05 %.

Table (3) showed significant elevation (P<0.05 %) in the enzymatic activities of liver function tests involving AST, ALT and ALP in groups 2 & 3 in comparison with control. Significant decreases (P<0.05 %) were detected in the same enzymes of groups 3 & 4 in comparison with group 2.

Table (4) exhibited significant increase (P<0.05 %) in serum urea of group 2 when compared with control. Groups 3 &, 4 expressed an improvement in urea level indicated by significant decrease (P<0.05 %) in comparison with group 2. Creatinine level was insignificantly changes in all exposed groups when

compared with control. Uric acid showed significant decrease (P<0.05 %) in groups 3 & 4in comparison with group 2.

Table (5) revealed significant increase (P<0.05 %) in MDA level was noticed in groups 2 & 3 when compared with control. On the other hand, groups 3 & 4 revealed significant reduction (P<0.05 %) in MDA values when compared with group 2. Group 4 showed significant decrease in the MDA level when compared with group 2. In case of SOD activity, there were significant decreases (P<0.05 %) in group 2 in comparison with control, while exposed other groups (G. 3&4) showed significant reduction (P<0.05 %) in activity of SOD when compared with group 2.

DISCUSSION

Aluminium is widely distributed in the environment and extensively used in daily life, which causes its easy exposure to human beings. It gets access to human and animal's body via gastrointestinal and respiratory tracts [19]. It is widely accepted that nervous system is the most sensitive target of aluminum toxicity and it may induce cognitive deficiency and dementia when it enters brain. Moreover, cardiotoxic, nephrotoxic and hepatotoxic effects have also been provoked by aluminium [20]. Today, many drugs have been found to have antioxidant activity, each of these nutrients has specific activities, it have been suggested as adjunct to metals antidotes and often work synergistically to enhance the overall antioxidant capacity of body.

Hematological findings displayed signifcat increase in RBCs count and PCV % of groups 2 & 3 in comparsion with control. WBCs count displayed significat increase in group 2 when compared with control, while groups 3 & 4 showed significat decrease in comparsion with group 2. Platelets counts were siginifcatly increased in groups 2& 3 in comparsion with control. Group 4 expressed significat decrease in platelets when compared with group 2. Previous studies mentioned a significant decrease in RBCs count and PCV % according to Abdel Aziz and Zabut [21] and Manisha *et al.* [22] who noticed that aluminum-induced anemia after long term exposure. The significant increase in white blood cell levels of aluminium-treated rats might indicate activation of the immune system, a normal cell-mediated immune response [23].

Biochemical assay showed increased level of serum AST, ALT and ALP after long term administration of Al. These results are in accordance with Wilhelm *et al.* [24] who found an increased release of the enzymes AST and ALT into the hepatic perfusate due to high dose of Al, this suggest that chronic Al exposure induce hapato-toxicity manifested by elevation of liver function enzymes [23]. Significant increase of serum urea and creatinine concentration in animals receiving aluminium chloride is of interest. The increase of serum urea and creatinine concentration can be a consequence of critical accumulation of this metal in kidneys and following renal failure development. Aluminium is excreted mainly by kidneys. Many studies have reported elevated concentrations of Al_3^+ in kidney, that may lead to renal damage [25, 26]. Serum urea and creatinine are the most sensitive biochemical markers for diagnosis of renal damage because they are excreted through kidney. An increase in serum level of these markers is an indication of impairment of renal function of treated groups. Other workers also arrived at similar conclusion in alumunium exposed animals [27, 28]. A significant increase in MDA levels accompanied by marked decrease in SOD activity following oral administration Al_2O_3-NPs. [29] demonstrated that Al_2O_3-NPs might induce free radical generation that further initiated the process of lipid peroxidation and damaging cellular components.

In the current study, administration of melatonin before alumuinum exposure revealed an improvement in hemato-biochemical parameters, since all altered hematological and biochemical started to return near to the normal level [30]. Displayed that melatonin inhibits lipid peroxidation by scavenging free radicals which is the main cause of hepato, nephro and neuro toxicity. The inhibitory action on lipid peroxidation was reflected by the decrease in level of TBARS in liver, kidney and brain tissues in groups through improvement the biochemical indices that treated with melatonin prior to alumunium Also it alleviate toxicity induced injury to liver, kidney and brain which was associated with a decline in all abnormal changes arise by aluminium toxicity through induces significant increase could be se in synthesis of tissue metallothionein (MT) [28].

It could be concluded that aluminium nanoparticles induced oxidative damage associated with hepatic and renal dysfunction. Administration of Mel could alleviate the oxidative damage induced by aluminium nanoparticles.

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