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Acute and Chronic Effects of Aluminum Smelter Dust on Hematology, Metal Bioaccumulation and Oxidant-antioxidant Status in Rat

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ABSTRACT

Background: Aluminum smelting industry is implicated with documented health risks. This study examined toxic effects of aluminum smelting dust as a real-life chemical hazard instead of single aluminum compounds, which seldom exist in real life.

Methods: Adult male rats were gavaged acute (3 consecutive days) and chronic (6 weeks) doses of two dust concentrations (10 and 20 mg kg^{-1}). Experimental investigation included toxic metal accumulation and biochemical analysis of blood and liver.

Result: Aluminum and iron were highest in dust and respectively accumulated in brain, liver and kidneys. Anemia, inflammation, liver and kidney damage and oxidative stress were established in view of thrombocytopenia (35%), leukocytosis (41%), lymphocytosis (55%) and alterations in aminotransferases, creatinine, malondialdehyde, superoxide dismutase and catalase. Aluminum facilitation of iron-mediated lipid peroxidation is suggested. These findings drew attention to the magnitude (dose-dependent) and persistence (time-dependent) of aluminum dust as health compromising and are of particular significance to workers in aluminum smelting industries.

Key words: Aluminum dust, Bioaccumulation, Hemogram, Oxidative stress, Serum biochemistry.

INTRODUCTION

Dust in industrial settings is a biological hazard that can pose health threats, particularly to those involved in the mining or smelting industries. Aluminum smelting scrap is usually contaminated with numerous foreign metals such as iron, nickel, chromium, cadmium, lead, manganese and other metals (Reuter *et al.,* 2005). Heavy metals are transported into body cells and tissues by binding to proteins and nucleic acids, destroying these macromolecules and disrupting their cellular functions (Engwa *et al.,* 2019). That's why metal accumulation in tissues is normally attended with metal toxicity.

Hematological techniques are the most common methods to detect stress and pollutant induced physiological changes. Animal studies have shown that aluminum is implicated as a contributing factor and an etiologic agent in anemia (Adham *et al*., 2011; Geyikoglu *et al.,* 2013; Muayad *et al*., 2018; Argente *et al*., 2019). Effects of aluminum on liver and kidney functions in rat were evidenced by the increased serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine (Geyikoglu *et al.,* 2013; Balgoon, 2019).

Aluminum compounds have been shown to generate free radicals, induce oxidative stress, increase the levels of reactive oxygen species and modify the peroxidation of lipids and the activities of anti-oxidatant enzymes (Abubakar *et al.,* 2003). Malondialdehyde (MDA) is the end product of lipid peroxidation and is an index of oxidative stress (Kumar *et al.*, 2018). Superoxide dismutase (SOD) and catalase (CAT) are antioxidant enzymes that are responsible for eliminating free radicals like superoxide and hydrogen peroxide (Newairy *et al.*, 2009; Wu *et al.*, 2012; Makwana *et al*., 2019). In certain conditions, an increase in oxidant levels and a decrease in antioxidant system elements may occur, causing oxidative stress due to changes in the oxidative/antioxidative balance (Canli and Canli, 2017).

This study followed the novel concept of real-life risk simulation (RLRS) (Margina *et al*., 2019) by examining the toxic effects of aluminum smelting dust as a real-life chemical hazard instead of a single aluminum compound, which seldom exists in real life. This study is an attempt to create reliable predictions and estimates of hematotoxic, nephrotoxic, hepatotoxic and oxidative risks imposed upon human accidently exposed to aluminum dust.

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MATERIALS AND METHODS

Dust sampling and analysis

Over 5 consecutive days, 5 different samples of dust were collected from electro filters of a dust collector in an aluminum smelter in Ras Al Kair Industrial City located on the eastern coast of Saudi Arabia, 60 km north of Jubail. Dust and airborne contaminants trapped in the filters were analyzed to determine metals included. Dust was digested using 65% nitric acid and 40% hydrogen fluoride (HF). An amount of 200 mg of sample was placed into 60 mL capacity DAP-60+ pressure vessels (Berghof/America, 3773 NW 126th Ave, Building 1 Coral Springs, FL 33065). Six mL nitric acid and three mL HF were added in each vessel. The mixture was shaken carefully with a glass bar before being heated in the microwave oven until a clear solution is obtained. Metal concentrations were detected in digested dust using iCAP Q ICP-MS (Thermo Fisher Scientific, Instrument, USA). Metal concentrations were detected in digested tissues using iCAP Q ICP-MS (Thermo Fisher Scientific Instrument, USA). The approach relies on the use of soluble hard base anion ligands for coordination of hard acid cation metals, and soft base anion ligands for coordination of soft acid cation metals (Mark and Workman, 2010).

Experimental animal and group allocation

A total number of 60 adult male Wistar rat, *Rattus norvegicus*, weighing 180-220 g, was obtained from the animal house facility, King Saud University, Riyadh, Saudi Arabia. In the lab, animals were housed in stainless steel rat cages under standard laboratory conditions (temperature, 24±3°C; humidity, 40-60%) with a 12-h light-dark cycle. Throughout the experimental duration (18 months starting December 2017), rats were given *ad libitum* access to tap water and commercially available rodent pelleted chow. After one week of acclimation, rats were randomly assigned into six groups (n=10), according to the specified dosage. Daily oral gavages were given over 3 consecutive days (acute) or 6 weeks (chronic). Each of the two acute and two chronic groups received water suspension of aluminum dust in a dosage of either 10 mg kg^{-1} or 20 mg kg^{-1}. Dose selection was based on an earlier study (Balgoon, 2019) that found that 10 and 20 mg kg^{-1} application of $AlCl_3$ over 8 weeks induced kidney failure based on an increase in serum creatinine and histopathological assessment. Two parallel acute and chronic control groups received distilled water for the specified duration. Animal experiments were conducted in accordance with the guidelines of the National Committee for Medical and Bio-ethics, PO Box 6086, 11442, Riyadh, Saudi Arabia and the policy and standard guidelines for ethical conduct in the care and use of nonhuman animals in research (412-383-2008) (IACUC, 2008).

Sampling and preparing blood and tissue for analysis

At the end of experimental period animals were sacrificed by decapitation and trunk blood was collected. One aliquot was placed immediately in EDTA tubes (no clotting) for blood picture analysis. The other aliquot was collected in non-heparinized tubes and left undisturbed to clot for 30 min at 25°C then centrifuged at 2000 ×g for 15 minutes. Top serum layer was pipetted carefully and stored in clean Eppendorf tubes and stored at -80°C until later used in liver and kidney function tests. Scarified animals were dissected and liver, kidneys and brain were excised and stored at -80°C until later used in metal accumulation analysis, lipid peroxidation and antioxidant enzyme analysis.

Metal determination in dust and animal tissue

All tissue samples were dried at 75°C for 24 hours. The dried samples were ground and homogenized, using an agate pestle and mortar and stored in polyethylene bags, until used in acid digestion. Before digestion all the plastic, glassware and vessels were washed with 10% nitric acid and then thoroughly rinsed with double deionized water (Milli-Q Millipore 18.2 µS cm^{-1} conductivity). Digestion of liver, kidneys and brain was performed by microwave-assisted pressure digestion system (Topwave Analytik Jena microwave digestion system, UK) using 65% nitric acid. About 200 mg of sample was put into the DAP-60+ pressure vessels (60 ml capacity) then 10 mL of nitric acid was added and heated in the microwave oven until reaching the clear solution (Tüzen, 2003). Metal concentrations were detected in digested tissues using iCAP Q ICP-MS (Thermo Fisher scientific Instrument, USA).

Complete blood count analysis

Hematologic indices were determined according to the manufacturer's instructions, using COULTER® Ac·T™ 5diff CP (Cap Pierce) (Beckman Coulter, USA) as an experimental device. Tests included counts of red blood cells RBCs (10^6 μL^{-1}), white blood cells WBCs (10^3 μL^{-1}), packed cell volume (PCV/hematocrit) (10^3 μL^{-1}), mean corpuscular volume MCV (femtoliters, or 10^{-15}L) and platelets PLT (10^3 μL^{-1}), differential counts (10^3 μL^{-1}) of neutrophils, lymphocytes, monocytes and eosinophils; and the concentrations of hemoglobin Hb (g dL^{-1}) and mean corpuscular hemoglobin concentration MCHC (g dL^{-1}).

Determination of serum aminotransferases and creatinine

Serum activities (U L^{-1}) of AST and ALT and concentrations (mg dL^{-1}) of creatinine were determined using diagnostic strips (Reflotron®, Roche, Basel, Switzerland) and were read using Reflotron® Plus instrument (Roche, Basel, Switzerland).

Determination of hepatic lipid peroxidation and antioxidant enzymes

Following dissection, liver tissue was rinsed with a phosphate buffered saline (PBS) solution (pH 7.4) to remove any red blood cells and clots. The tissues were homogenized on ice in 5 mL of cold buffer (*i.e.*, 50 mM potassium phosphate, pH 7.0, containing 1 mM EDTA)

per 0.5 g tissue. The homogenate was centrifuged at 10,000 xg for 15 minutes at 4°C. The supernatant was removed for assay and stored at -80°C. Commercial ELISA kits (Cayman Chemical Company, Ann Arbor, Michigan 48108, USA) were used to quantify MDA, SOD and CAT in liver homogenate. All measurements were done according to the manufacturer's instructions, using Zenyth 340R ELISA reader (Anthos, Krefeld, Germany) as an experimental device.

Statistical analysis

Results were expressed as mean ± standard error (SE). Comparisons of data of experimental groups with the control groups were performed using one-way analysis of variance (ANOVA) using GraphPad Prism software (Graph Pad Software, Inc.). Differences at P≤0.05, P≤0.01 and P≤0.001 were considered significant, highly significant and very highly significant, respectively.

RESULTS AND DISCUSSION

Metal content in dust and animal tissue

As expected, pilot tests of dust showed aluminum as the metal with the highest concentration among other metals analyzed. There are several guidelines of heavy metals to compare with (Table 1). Based on the most relevant guidelines to the present results, Pb and Cd content in aluminum dust fall within permissible levels of road dust guidelines (CCME, 2007) and thus were excluded. Being the second highest and based on the assumptions that aluminum could induce iron accumulation (Wu *et al*., 2012), iron was measured, in addition to aluminum, in animal tissue.

Tissue metal accumulation (Table 2) showed a significant increase in the concentrations of both aluminum (P≤ 0.001) and iron (P≤ 0.01- ≤ 0.001) in all experimental groups. Aluminum accumulation (p ≤ 0.01- p ≤ 0.001) was highest in the brain (55-79%) followed by the liver (62-71%) and then kidneys (42-64%). Iron concentration was highest in the brain tissue (up to 171%) followed by the kidneys (up to 49%) and then the liver (up to 36%). Aluminum accumulation in tissues is rationally anticipated following aluminum dust exposure. Yet the marked iron accumulation in rat tissue could be due to either its relative high concentration in the dust or to the hypothesis that iron accumulation is a feedback of aluminum accumulation itself. Aluminum treatment might result in the cell sensing a lack of Fe and that Fe accumulation was probably a feedback or compensating result of this deficiency (Wu *et al*., 2012).

The hemogram

Red and white blood cell indices fluctuated in a dose and time-dependent manner (Fig 1-6 and Fig 7-11). Anemia was implied in the general decline in red blood cell indices and the marked thrombo cytopenia (35%). Thrombo cytopenia with the overall increase in leukogram indices, particularly

Table 1: Mean ± SE metal concentrations (µg g^{-1}) of 5 samples of aluminum dust collected over 5 consecutive days from an aluminum smelter in Ras Al Kair Industrial City. Permissible values of different guidelines are indicated.

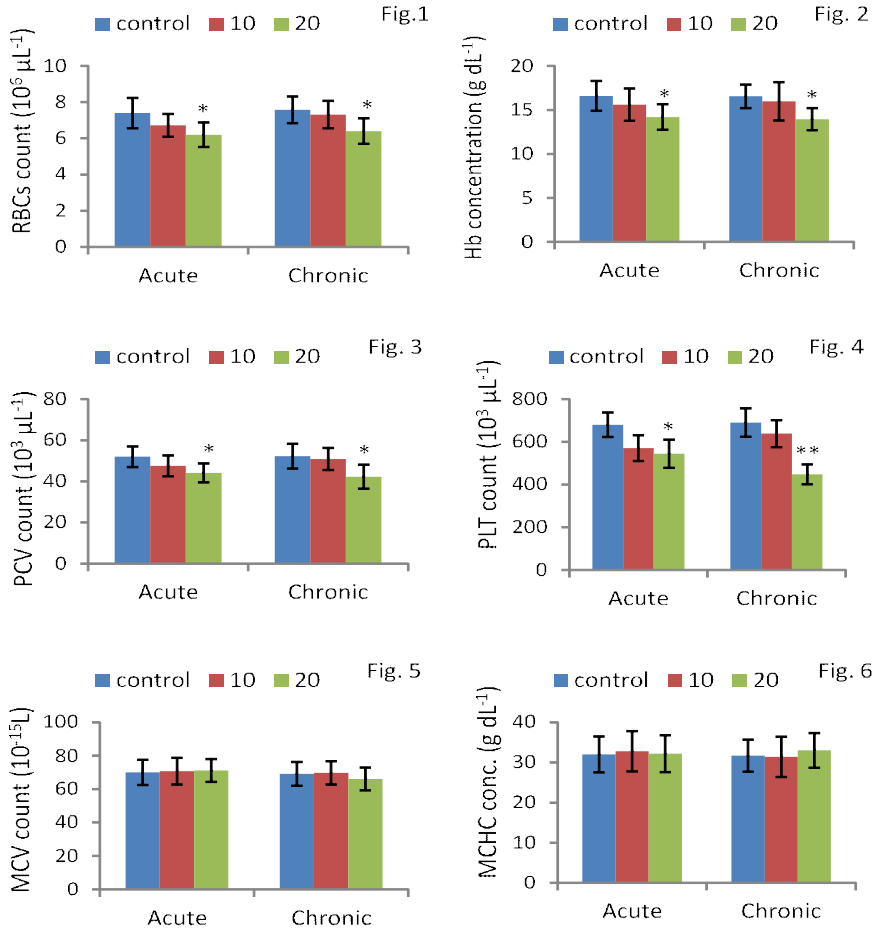
Metal	Concentrations in aluminum smelter dust	Maximum allowable limits in soils	Permissible limits in road dust	Ingestion reference dose (mg/70 kg/day)
Al	1820 ± 257.4	NE	NE	NE
Fe	686.7± 111.6	150	NE	0.49
Pb	108.4±2.783	10-70	140	0.245
Mn	99.35±1.954	NE	NE	NE
Cd	90.55± 0.903	NE	10	0.07
Guideline/ reference	Current study	FAO/WHO (1996, 2000)	CCME (2007)	Nkpaa *et al*., 2016

Ingestion Reference Dose is given on the basis of a 70 Kg person; NE, not identified.
All values are given as µg g^{-1}.

Table 2: Mean ± SE aluminum and iron concentrations (µg g^{-1}) in liver, kidneys and brain of rats acutely and chronically exposed to two dust doses versus control.

Qrgan	Dose	Aluminum		Iron	
		Aute	Chronic	Acute	Chronic
liver	control	53.4±4.9	43.9±6.9	1166.2±130.9	1249.3±102.8
	10 mg kg^{-1}	164.8±17.7***	115.4±10.4***	1724.5±180.0**	1960.5±189.0**
	20 mg kg^{-1}	162.6±18.8***	151.0±15.5***	1748.1±198.2**	1700±168.1**
kidneys	control	73.0±6.8	75.8±5.4	527.4±55.1	541.2±51.3
	10 mg kg^{-1}	137.1±13.2**	129.8±7.2**	889.6±110.4**	832.9±84.6**
	20 mg kg^{-1}	145.2±11.5**	209.9±9.1***	975.2±138.5**	1050.7±97.74**
brain	control	9.51±1.1	12.55±0.5	61.9±7.2	68.2±7.2
	10 mg kg^{-1}	21.1±0.6**	32.76±1.8 ***	100.2±10.4**	125.5±12.1**
	20 mg kg^{-1}	46.2±3.2***	53.93±5.9***	137.1±14.7***	184.8±20.3***

Asterisks denote highly (**, p ≤ 0.01) and very highly (***, p ≤ 0.001) significant differences from control.



Figs 1-6: Red blood cell indices. Mean ± SE of red blood cell count (10^6 µL^{-1}), hemoglobin concentration (g dL^{-1}), PCV/hematocrit count (10^3 µL^{-1}), platelets PLT count (10^3 µL^{-1}), MCV count (femtoliters, or 10^{-15}L) and MCHC concentration (g dL^{-1}) in rats acutely and chronically exposed to two dust doses. 10 and 20 refer to 10 and 20 mg kg^{-1} doses, respectively. Asterisks denote significant (*, $p \leq 0.05$) and highly significant (**, $p \leq 0.01$) differences from parallel control values.

leukocytes (41%) and lymphocytes (55%) suggested an inflammatory type of leukogram, which is characterized by a rapid transient thrombocytopenia (Baskett *et al.,* 1997) and leukocyte production (Abdelhamid *et al.,* 2020). Earlier studies emphasized the damaging potential of aluminum (Chmielnicka *et al.,* 1994) to the structure of RBCs by binding to proteins and enzymes that alter their activity and cause hemolysis (Engwa *et al.,* 2019). Potential causes of anemia include oxidative damage of erythroid cells and the concomitant failure of erythropoiesis and short survival of erythrocytes in the circulation (Fibach and Rachmilewitz, 2008).

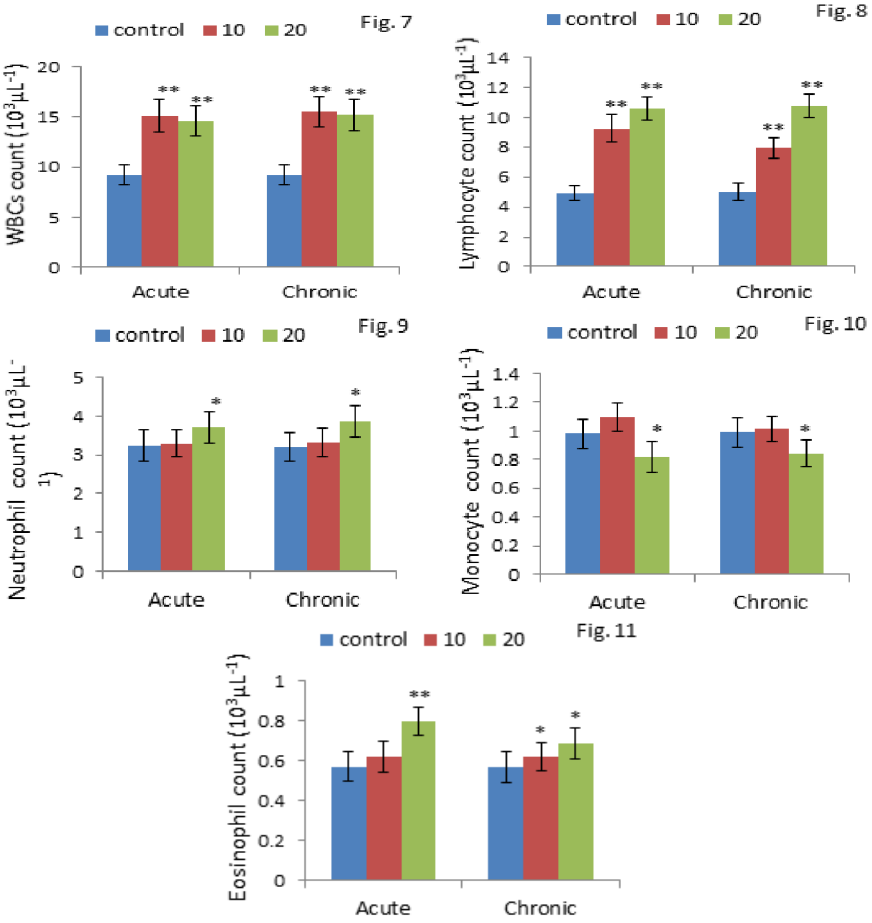
Liver and kidney function

The current rise of serum activities of ALT and AST (Fig 12 and 13) suggested aluminum-induced liver impairment, damage or dysfunction (Newairy *et al.,* 2009). Hepatocellular injury was found to be the trigger for the release of ALT and AST into the circulation (Lala *et al*., 2020) as they are intracellular enzymes and their presence in serum indicates damage to hepatocyte membranes. There is a correlation between creatinine levels and renal function as serum creatinine is a waste product of skeletal muscle metabolism and its elimination is exclusively renal (Levey *et al.,* 1988).

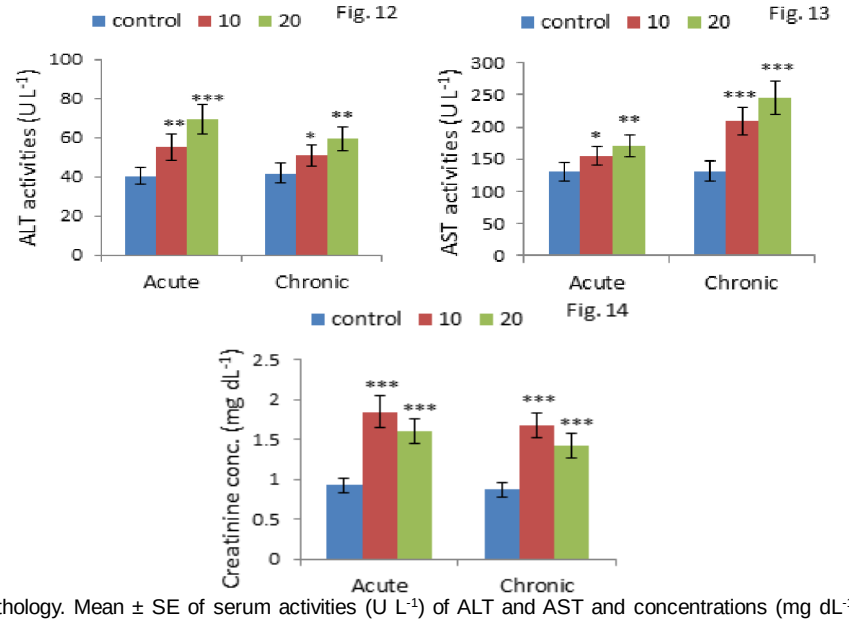
Oxidative stress and antioxidant defense

The current rise in hepatic MDA and SOD and decline in CAT (Fig 15-17) indicated the induction of oxidative stress and initiation of antioxidant activities (Nehru and Anand, 2005). As a main dust constituent, aluminum is not a transition metal and cannot initiate peroxidation (Newairy *et al.*, 2009). A correlation was suggested between aluminum accumulation and dramatic increase of iron accumulation (Wu *et al.*, 2012). The current induction of oxidative stress could be due to iron accumulation in animal tissue powered by iron high concentration in dust in addition to aluminum-induced iron accumulation in animal tissue.

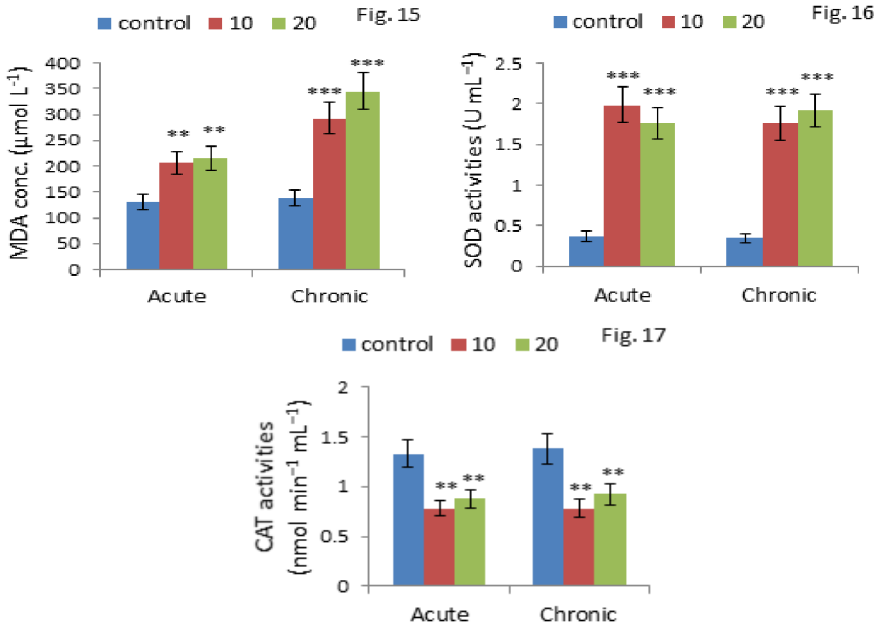
The drastic increase in SOD activities ($P \leq 0.001$) could be necessary to detoxify increased lipid peroxidation



Figs 7-11: The leukogram. Mean ± SE of white blood cell WBC and differential counts (10^3 μL^{-1}) of lymphocytes, neutrophils, monocytes and eosinophils in rats acutely and chronically exposed to two dust doses. 10 and 20 refer to 10 and 20 mg kg^{-1} doses, respectively. Asterisks denote significant (*, $p \leq 0.05$) and highly significant (**, $p \leq 0.01$) differences from parallel control values.



Figs 12-14: Serum pathology. Mean ± SE of serum activities ($U L^{-1}$) of ALT and AST and concentrations (mg dL^{-1}) of creatinine in rats acutely and chronically exposed to two dust doses. 10 and 20 refer to 10 and 20 mg kg^{-1} doses, respectively. Asterisks denote significant (*, $p \leq 0.05$), highly significant (**, $p \leq 0.01$) and very highly significant (***, $p \leq 0.001$) differences from parallel control values.



Figs 15-17: Oxidative stress and antioxidant defense. Mean ± SE concentrations of MDA (µmol L^{-1}) and activities of SOD (U mL^{-1}) and CAT (nmol min^{-1} mL^{-1}) in liver homogenate of rats acutely and chronically exposed to two dust doses. 10 and 20 refer to 10 and 20 mg kg^{-1} doses, respectively. Asterisks denote highly (**, $p \leq 0.01$) and very highly (***, $p \leq 0.001$) significant differences from parallel control values.

(Hundekari *et al.,* 2013) in rats constantly suffering from oxidative stress based on the consistent rise in MDA that was not inhibited by antioxidants (Adham *et al.,* 2014). SOD is the first enzyme that catalyzes the conversion of superoxide to H_2O_2 before CAT or GPx subsequently convert H_2O_2 to water and oxygen (Ighodaro and Akinloye, 2018). Contrarily, the physiological role of CAT would be limited to situations in which cellular concentration of the inorganic hydrogen peroxide reaches high value (Chance *et al.,* 1979). If oxidative stress outweighs CAT activities, this could probably lead to defense exhaust, when the cell cannot produce more protective enzymes under the free radical attack (Porokhovnik *et al*., 2015). This is typically attended by oxidative damage of CAT molecules (Trykova and Kostova, 2005) and can give grounds for the drop in CAT levels.

CONCLUSION

Exposure to aluminum polymetal dust is implicated in metal toxic accumulation in addition to hematotoxic, nephrotoxic, hepatotoxic and oxidative effects. These findings drew attention to the magnitude (dose-dependent) and persistence (time-dependent) of aluminum polymetal dust in compromising human health and are of particular significance to workers in aluminum smelting industries.

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