International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2024; 9(2): 542-549

www.veterinarypaper.com
Received: 15-01-2024
Accepted: 26-02-2024

Abdulkadir A Makwana
Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat, India

Dhaval T Fefar
Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat, India

Rajkumar S Delvadiya
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat, India

Khevana A Thakore
Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat, India

Swati S Patel
Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat, India

Urvesh D Patel
Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat, India

Amit R Bhadaniya
Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat, India

Kapil G Gajera
Department of Veterinary Surgery and Radiology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, Gujarat, India

Corresponding Author:
Abdulkadir A Makwana
Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat, India

Hepato-toxic effects of aluminium oxide nanoparticles following repeated dose 28-day oral exposure in Wistar rats

Abdulkadir A Makwana, Dhaval T Fefar, Rajkumar S Delvadiya, Khevana A Thakore, Swati S Patel, Urvesh D Patel, Amit R Bhadaniya and Kapil G Gajera

DOI: https://dx.doi.org/10.22271/veterinary.2024.v9.i2h.1257

Abstract
The significance of Aluminium oxide nanoparticles (Al_2O_3NPs) has grown due to their versatile applications across industrial and biomedical sectors. Despite their promising attributes, concerns about their potential toxicity have arisen. Hence, this study was conducted to investigate and address these concerns. Total four groups of Wistar rats (each group having 6 male and 6 female Wistar rats) were used, control group and other three treated groups (T_1, T_2 and T_3) which were orally treated with Al_2O_3NPs at following concentrations of 50 mg/kg b.wt., 100 mg/kg b.wt. and 200 mg/kg b.wt. respectively for 28 days. Exposure to Al_2O_3 NPs initiate the ROS formation and alter the antioxidant defence mechanism of liver which leads to increase in the activity of catalase and super oxide dismutase enzymes and elicit the level of malondialdehyde in treatment groups of animals. In haematological findings the effects of Al_2O_3NPs exposure alter the Haematocrit %, TEC and increase WBCs indicative for inflammatory response and Al_2O_3NPs exposure also leads to significant increase in hepatic leakage enzymes (ALT, AST), hepatic induced enzymes (ALP), total bilirubin, indirect bilirubin level in treatment groups. In histopathological examination treatment related changes were noted.

Keywords: Aluminium oxide nanoparticles, Oxidative stress, Hepato-toxicity, Sub-acute, Haematological, Biochemical, Histological

1. Introduction
Nanoparticles (NPs) are ultra-small particles, typically measuring between 1 and 100 nm, which can occur naturally or be synthesized through engineering. They exhibit unique and advantageous physical and chemical characteristics, showcasing enhanced catalytic, magnetic, electrical, mechanical, optical, biological and chemical properties when compared to their bulk counterparts (Dikshit *et al*., 2021) [10]. Surface area, shape, size, aspect ratio, crystallinity, surface coating, dissolution and agglomeration are key factors influencing the toxicity of nanoparticles (Egbuna *et al*., 2021) [13]. With widespread utilization in medicine, molecular biology, engineering, food, cosmetics and various industries, nanoparticles (NPs) have notably enhanced their effectiveness and significance in human life. However, as their use expands and their forms and applications become more widespread, the potential negative effects of these different NPs on human and animal health have also come under discussion (Roco, 2005; Shim *et al*., 2014; Amde *et al*., 2017) [26, 28, 1].
Aluminium (Al) ranks as the third most abundant metal on earth and is recognized as a prominent environmental neurotoxin. Prolonged exposure to excessive aluminium has been associated with several neurodegenerative conditions in humans, such as Alzheimer's disease and Parkinsonism-dementia (Savory *et al*., 2006) [27]. Aluminium oxide (alumina) nanoparticles (Al_2O_3NPs) represent one of the most extensively manufactured types of nanoparticles, constituting roughly 20% of the global nanoparticle market (Rittner *et al*., 2002) [25]. In 2010, global Al_2O_3 NPs production amounted to 18,500 tonnes per year. With a continuous increase in output, Al_2O_3NPs production is projected to exceed 100,000 tonnes by 2020. The remarkable dielectric properties, high melting point, thermal stability, wear

resistance, mechanical strength, electrical insulation, corrosion resistance and other attributes of alumina nanoparticles contribute significantly to their value. Dissolution and aggregation stand out as pivotal factors affecting the bioavailability of alumina nanoparticles in both aquatic and terrestrial environments (Asztemborska, 2018) [2]. In 2006, the Organization for Economic Co-operation and Development (OECD) initiated a program aimed at evaluating the hazards, exposure, and risks linked with nanoparticles. By 2007, they identified 14 groups of manufactured nanomaterials as high-priority, with Al_2O_3NPs being among them (www.oecd.org). Al_2O_3 NPs find widespread application in the chemical sector, notably in the production of paints, coatings, catalyst synthesis, electronics and optics (Asztemborska, 2018) [2]. Personal care items, antacids, buffered aspirins, drug delivery systems and various medical products also serve as sources for both occupational and nonoccupational exposure to Al_2O_3NPs.

Ecotoxicological research concerning Al_2O_3NPs primarily focuses on acute exposure assessments (Wagner *et al*., 2007) [29]. Al_2ONPs exhibit various adverse effects, including mitophagy (Huang *et al*., 2021) [16], genetic damage (Balasubramanyam *et al*., 2009) [5], inflammatory response (Oesterling *et al*., 2008) [20], carcinogenicity (Dey *et al*., 2008) [9], as well as the generation of reactive oxygen species (ROS) and cytotoxicity (Chen *et al*., 2008) [7]. Nanoparticles (NPs) possess the ability to traverse diverse cellular barriers and access highly sensitive organs such as the brain, liver, kidneys and lungs, where they interact with a wide array of cellular components ranging from DNA and various proteins to mitochondria. This interaction may lead to the generation of reactive oxygen species (ROS) and disruption of numerous cellular processes. NPs interactions can induce alterations in proteins, accumulation of NPs in the Golgi apparatus, DNA damage, lysosomal enzyme activity, mitochondrial dysfunction, apoptosis, damage to the cell membrane, cytoplasmic disturbances, depletion of ATP levels, ultimately resulting in the impairment of vital organ functions (Attarilar *et al*., 2020) [3].

While Al_2O_3NPs offer significant industrial benefits, their accumulation in the environment poses risks to both human and animal health. To effectively employ nanomaterials in biomedicine, it's crucial to comprehend their fate and potential toxicity *In vivo*. This study, involving subacute oral gavage exposure of Al_2O_3NPs in Wistar rats, aims to shed light on their toxicity potential in vital organs like the liver. The data generated from this research will serve as valuable insights for future investigations.

2. Materials and Methods

2.1 Experimental animals and environment

Adult male and female Wistar Albino rats (*Rattus norvegicus*), aged 7-8 weeks, were procured from Cadila Pharmaceuticals Limited in Dholka, Ahmedabad, Gujarat, India. They were acclimatized for one week before the study initiation. The rats were housed under standard environmental conditions following the guidelines outlined in the Committee for Control and Supervision of Experiments on Animal (CCSEA, 2003) publication. They were provided with standard rodent pellet diet and water *ad libitum*. The experimental protocol received approval from the Institutional Animal Ethics Committee (IAEC) at the College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat (Protocol no.: KU/JVC/IAEC/SA/91/22; Dated: 08/07/2022).

2.2 Chemicals, Preparation of Al_2O_3NPs and Experimental design

Al_2O_3NPs (Gamma alumina nanopowder) were procured from SRL (Sisco Research Laboratories Pvt. Ltd) (CAS No.: 1344-28-1), while all other chemicals utilized in the experiments were of either molecular or analytical grade.

The Al_2O_3NPs underwent ultrasonication to prepare the metal oxide nanoparticles for characterization and subsequent administration to the experimental rats. A considerable quantity of Al_2O_3NPs was ultrasonicated in Milli-Q water using the Sonics ultrasonic homogenizer (Model: 1-800-745-1105, USA). This process involved vibration at 20 kHz with a continuous pulse of 40% of the total pulse power, resulting in a power output of 40 W. Ultrasonication was carried out for 5 minutes with an elapsed time of 30 seconds before administration, following the method described by Morsy, *et al*., 2016a [19].

Rats were divided into 4 groups, each of which includes 6 male and 6 female adult Wistar rats, such as control group and other three treated groups (T_1, T_2 and T_3) which were orally treated with Al_2O_3NPs at 50 mg/kg b.wt., 100 mg/kg b.wt and 200 mg/kg b.wt. respectively. Rats were orally administered their respective dose daily for 28 days. The design of experimental groups was depicted in Table 1.

2.3 Sample collection

At the conclusion of the experiment, all rats were euthanized in a humane manner using the CO_2 method, adhering to the guidelines provided by the National Institute of Health American Research Advisory Committee (NIH-ARAC). Subsequently, during necropsy, liver samples were collected and preserved in 10% neutral buffered formalin for histopathological analysis. For oxidative stress analysis liver tissue samples were collected in their respective buffer based on measurements of oxidative stress marker *viz.*, for catalase (CAT) activity in phosphate buffered saline (PBS; pH:7.5, for super oxide dismutase (SOD) activity in Tris-EDTA buffer (pH: 8.2-8.5) and for MDA level in butylated hydroxyl toluene (BHT) buffer were collected.

2.4 Evaluation of oxidative stress markers in liver

SOD activity was assessed following established protocols (Marklund and Marklund, 1974), while CAT activity was measured according to the procedure outlined by Beers and Sizer (1952) [6]. The level of MDA was determined by the formation of the MDA-TBA complex using thiobarbituric acid (TBA) (Lykkesfeldt, 2001) [18].

2.5 Hemato-Biochemical analysis

Haematological were estimated by an automated haematology analyser (Abacus Junior Vet 5, Diatron, Hungary) and Serum biochemical parameters were estimated from serum samples using standard kits on automated biochemistry analyzer (Dia-chem 240 plus, Diatek, China).

2.6 Histopathology

Sampling and trimming of liver samples for histopathological examination were carried out as per the method described in RITA and NACAD guidelines. Subsequently, the livers from all groups were fixed in 10% neutral buffered formalin and underwent standard histological processing. Following paraffin embedding, 4 µm sections were cut using a semi-automated rotary microtome (Leica Biosystems, Germany) and stained with haematoxylin and eosin (H & E). Microscopic examinations of the stained slides were

conducted using an optical microscope (Zeiss primo star) equipped with a camera (ZEISS Axiocam ERC 5), and microscopic images were captured using Carl Zeiss ZEN 2 (Blue edition 3.4) software.

2.7 Statistical analysis
All data underwent statistical analyses utilizing GraphPad Prism 9.4.1 software. The normality of the data was assessed using the Kolmogorov-Smirnov test and the equality of variances was confirmed using Bartlett's test. As the data did not exhibit both normal distribution and equal variances, the Kruskal-Wallis test followed by Dunn's test was employed for analysis. A significance threshold of $p<0.05$ (*) denoted statistical significance, while $p<0.01$ (**), $p<0.005$ (***), and $p<0.001$ (****) were considered highly statistically significant.

3. Results
All the rats were closely observed for the development of clinical and behavioural symptoms throughout the experimental period. Noticeable clinical signs were not observed in rats of any experimental groups except mild diarrhoea, dull and depression occurred during 4th week of experimental period in T_3 group. Additionally, no any mortality in any of treatment groups were recorded during the experimental study.

3.1 Hematology
The mean ± SE values of hematology parameters in male and female rats of all groups are presented in Table-2 and table Table-3.

As observed in table 2 & 3, the T_2 group exhibited a significant reduction in hematocrit (%) and Total Erythrocyte Count (10^6/µl) (TEC) compared to the C group. Conversely, both Total Leukocyte count (10^3/µl) (TLC) and absolute lymphocyte counts (10^3/µl) increased significantly in the T_2 and T_3 groups relative to the T_1 group among male rats. In female rats, there was a significant elevation in TLC and absolute lymphocyte count (10^3/µl) in the T_3 group compared to the T_1 group by the end of the experiment.

3.2 Serum-Biochemical parameters
The mean ± SE values of serum-biochemical parameters in male and female rats of all groups are presented in Table-4 and Table-5.

In the present study, exposure to Al_2O_3NPs in male rats led to a significant increase in hepatocellular leakage enzymes (ALT) and hepatic induced enzyme (ALP) as well as total bilirubin and indirect bilirubin levels in the T_3 group compared to the control, T_1 and T_2 groups. Conversely, there was a significant decrease in total protein concentration in the T_3 group compared to the control, T_1 and T_2 groups. Similarly, in female rats, exposure to Al_2O_3NPs resulted in a significant increase in hepatocellular leakage enzymes (ALT, AST), total bilirubin level, indirect bilirubin level, total protein level, globulin level and a non-significant increase in the level of hepatic induced enzyme (ALP) in treated groups compared to the control group.

3.3 Oxidative Stress Parameters of Liver
The mean ± SE values of SOD and catalase activity and levels of MDA in liver tissue of male and female rats of all groups are depicted graphically in Figure-1.

Exposure to Al_2O_3NPs in both male and female rats led to a marked, dose-dependent reduction in SOD activity across the T_1, T_2 and T_3 groups compared to the control group and within the treatment groups. Similarly, a significant decline in catalase activity was observed in the liver of the T_3 group relative to the control, T_1 and T_2 groups in both sexes of animals. Furthermore, there was a notable increase in MDA levels in the liver of the T_3 group compared to the control and T_1 groups in both sexes.

3.4 Histopathology
Microscopic examination of the liver (figure-2) revealed normal hepatic parenchyma, portal triad and central vein in control group (figure-A). Whereas, marked dilatation and congestion of central vein and portal vein (figure-B, D & F) were observed in all treatment groups. Periportal inflammatory cells infiltration (figure-F), hepatocyte swelling, anisokaryosis, karyomegaly, karyolitic nuclei distorted hepatic parenchyma with vacuolated degeneration and necrosis (figure-F) were also detected in T_2 and T_3 groups of animals.

4. Discussions
The alterations observed in hematological parameters, specifically in HCT and TEC due to exposure to Al_2O_3NPs, suggest the presence of anemia. These findings align with previous research by Yousef *et al.* (2019a) [31], who documented a decrease in HCT and TEC values compared to the control group following oral exposure to alumina nanoparticles at a dose of 70 mg/kg for 75 days in Wistar rats. In our study, the changes in HCT and TEC may be linked to liver and kidney damage, which are known to play crucial roles in heme synthesis and extramedullary erythropoiesis, respectively.

Similarly, consistent with our results, Park *et al.* (2015) [22] reported an increase in TLC and lymphocyte values in mice treated with Al_2O_3NPs at a dose rate of 6 mg/kg over a 13-week repeated dose study. Furthermore, several earlier studies by researchers such as Morsy *et al.* (2016) [19], Yousef *et al.* (2019a) [31] have also highlighted an increase in TLC and lymphocyte values in higher Al_2O_3NPs treated groups compared to control groups.

The elevation in TLC and lymphocyte values corresponds to the body's inflammatory reaction to Al_2O_3NPs, a correlation supported by numerous studies conducted by various researchers over time. These studies have consistently shown that the impact of Al_2ONPs on different tissues leads to a dose-dependent increase in inflammatory cytokines such as IL-1β, IL-2 and IL-6. It is proposed that these inflammatory cytokines contribute to the inflammatory response, potentially responsible for the rise in the number of WBCs circulating in the blood (Dong *et al.*, 2019) [12].

The impairment of liver enzymes such as ALT and AST signals hepatocellular injury, while elevated ALP levels indicate a disruption in the normal flow of bile. Changes in total protein levels and bilirubin parameters further suggest hepatocellular dysfunction. In male rats treated with higher doses of Al_2O_3NPs, increased levels of ALT, AST, ALP, total bilirubin and decreased protein concentration indicate compromised liver function. Conversely, in female rats, elevated total protein levels may be attributed to mild dehydration. The findings of present study are also supported by various researcher *viz.* Park *et al.* (2011) [21], Li *et al.* (2012), Park *et al.* (2015) [22], El-Hussainy *et al.* (2016) [14], Morsy *et al.* (2016) [19], Yousef *et al.*, (2019a) [31] found that repeated oral exposure to Al_2O_3NPs and non-nano aluminum oxide in SD rats at a dose of 50 mg/kg body weight every two

days for 60 days resulted in a significant increase in ALT, AST and ALP activity in the Al_2O_3NPs treated group compared to the non-nano aluminum oxide-treated group and the control group.

Similarly, in a study conducted by Yousef *et al*. (2019a) [31], oral sub-chronic exposure to Al_2O_3NPs, zinc oxide nanoparticles (ZnONPs), and their combination at a dose of 70 mg/kg body weight for 75 days in Wistar rats led to elevated levels of ALT, AST, ALP, TB and LDH in all three treatment groups compared to the control group. They also observed a significant decrease in levels of total protein, albumin and globulin in all treated groups relative to the control group. These alterations in the activities of liver biomarkers (ALT, AST, ALP, total bilirubin and direct bilirubin) in the serum indicate the impact of Al_2O_3NPs on the liver.

The liver is particularly vulnerable to free radical injury due to its role in metabolizing the majority of toxic substances, which can lead to lipid peroxidation and consequent hepatic damage. Nano-alumina's capacity to induce the production of free radicals consequently induces oxidative stress within cells (Zhang *et al*., 2011) [32]. The toxicity of Al_2O_3NPs may arise from their direct interaction with cell organelles, leading to the formation of chemical compounds with DNA, RNA, proteins and other molecules. This accumulation within cells, tissues and organs can result in oxidative damage to these vital structures (Bai *et al*., 2010) [4].

The findings of the current study align with those of Prabhakar *et al*. (2012) [24], who observed that rats administered acute doses of Al_2O_3NPs (30 and 40 nm) exhibited alterations in liver oxidative stress parameters, including increased liver MDA levels and inhibition of SOD activity in the nano-alumina-treated groups. Similarly, the results of the present study are consistent with those of Yousef *et al*. (2019a) [31], who reported that oral sub-chronic exposure to Al_2O_3NPs in Wistar rats led to a significant decrease in the levels of all antioxidant parameters, including glutathione (GSH), SOD, CAT, GPx and Total Antioxidant Capacity (TAC), along with a significantly higher MDA level. In the histopathological examination, the observed hepatic necrosis may be attributed to the overproduction of reactive oxygen species (ROS) induced by exposure to Al_2O_3NPs. Al_2O_3NPs can impair the endogenous antioxidant system, leading to increased lipid peroxidation (LPO) and subsequent cellular damage. The congestion of blood sinusoids observed could be linked to Al_2O_3NPs ability to induce the expression of inflammatory molecules, such as intercellular adhesion molecule-1, interleukin-8, monocyte chemotactic protein-1 and various adhesion molecules, resulting in endothelial dysfunction and sinusoid congestion (Gojova *et al*., 2007) [15].

The histopathological findings of the liver are consistent with serum biomarkers of liver function, i.e, ALT, AST and ALP, as well as oxidative stress parameters, indicating the cytotoxic effects of Al_2O_3NPs on liver tissue. These findings are supported by various previous studies. For instance, Yousef *et al*. (2019a) [31] gave oral sub-chronic exposure to Al_2O_3NPs, zinc oxide nanoparticles (ZnONPs) and their combination at a dose of 70 mg/kg body weight for 75 days in Wistar rats. They observed degenerative hydropic changes, cellular infiltration in numerous hepatocytes, lytic necrosis, piecemeal necrosis and sinusoidal blood vessel congestion in the Al_2O_3NPs treated group during histopathological examination of the liver.

Table 1: Group of animals and different treatments

Groups	No. of Animals		Dose (Oral gavage for 28 days)
	Male	Female	
Control (C)	6	6	Milli-Q Water
Treatment 1 (T_1)	6	6	50 mg/kg b.w./day Al_2O_3 NPs in Milli-Q water
Treatment 2 (T_2)	6	6	100 mg/kg b.w./day Al_2O_3 NPs in Milli-Q water
Treatment 3 (T_3)	6	6	200 mg/kg b.w./day Al_2O_3 NPs in Milli-Q water
Total	24	24	

Table 2: Comparison of mean values (Mean ± SE; n=6) of different haematological parameters in male rats following Al_2O_3 NPs exposure for 28 days

Parameters	Treatment groups (Mean ± SE)			
	Control	T_1 (50 mg/kg)	T_2 (100 mg/kg)	T_3 (200 mg/kg)
Hb (g/dL)	16.68 ± 0.32^{a}	16.42 ± 0.71^{a}	14.7 ± 0.46^{a}	14.95 ± 0.54^{a}
HCT (%)	51.23 ± 0.61^{a}	48.47 ± 1.47^{ab}	45.9 ± 1.11^{b}	47.31 ± 1.47^{ab}
TEC (10^6/µL)	10.33 ± 0.16^{a}	9.96 ± 0.33^{ab}	9.07 ± 0.24^{b}	9.63 ± 0.30^{ab}
MCV (fl)	49.5 ± 0.67^{a}	48.67 ± 0.56^{a}	50.5 ± 0.72^{a}	49 ± 0.44^{a}
MCH (pg)	16.17 ± 0.26^{a}	16.5 ± 0.34^{a}	16.2 ± 0.35^{a}	15.53 ± 0.28^{a}
MCHC (g/dL)	32.55 ± 0.44^{a}	33.87 ± 0.85^{a}	31.95 ± 0.53^{a}	31.6 ± 0.54^{a}
Platelets (10^3/µL)	670.33 ± 65.00^{a}	781.67 ± 132.62^{a}	724 ± 62.48^{a}	874.5 ± 119.96^{a}
TLC (10^3/µL)	10.66 ± 1.09^{ab}	9.50 ± 1.43^{b}	14.14 ± 0.92^{a}	14.91 ± 1.07^{a}
Lymphocytes (%)	81.8 ± 1.73^{a}	77.06 ± 4.03^{a}	84.1 ± 0.71^{a}	84.00 ± 3.12^{a}
Abs. Lymphocytes (10^3/µL)	8.78 ± 0.98^{ab}	7.03 ± 0.99^{b}	11.90 ± 0.80^{a}	12.61 ± 1.17^{a}
Neutrophils (%)	11.63 ± 1.23^{a}	11.06 ± 1.16^{a}	8.53 ± 0.92^{a}	8.62 ± 0.89^{a}
Abs. Neutrophils (10^3/µL)	1.20 ± 0.13^{a}	1.00 ± 0.13^{a}	1.19 ± 0.12^{a}	1.27 ± 0.13^{a}
Monocytes (%)	5.29 ± 0.99^{a}	10.2 ± 3.47^{a}	5.78 ± 0.98^{a}	4.99 ± 1.25^{a}
Abs. Monocytes (10^3/µL)	0.53 ± 0.08^{a}	1.09 ± 0.49^{a}	0.83 ± 0.17^{a}	0.71 ± 0.15^{a}
Eosinophils (%)	1.28 ± 0.29^{a}	1.67 ± 1.01^{a}	1.58 ± 0.35^{a}	2.38 ± 1.06^{a}
Abs. Eosinophils (10^3/µL)	0.13 ± 0.04^{a}	0.20 ± 0.14^{a}	0.22 ± 0.05^{a}	0.31 ± 0.13^{a}

Table 3: Comparison of mean values (Mean ± SE; n=6) of different haematological parameters in female rats following Al_2O_3 NPs exposure for 28 days

Parameters	Treatment groups (Mean ± SE)			
	Control	T_1 (50 mg/kg)	T_2 (100 mg/kg)	T_3 (200 mg/kg)
Hb (g/dL)	13.25 ± 1.00^a	14.18 ± 1.54^a	13.45 ± 0.61^a	14.43 ± 0.51^a
HCT (%)	41.36 ± 2.94^a	46.57 ± 4.04^a	44.27 ± 1.10^a	46.40 ± 1.36^a
TEC (10^6/µL)	8.23 ± 0.61^a	8.81 ± 0.82^a	8.55 ± 0.27^a	8.73 ± 0.20^a
MCV (fl)	50.33 ± 1.14^a	53 ± 0.73^a	51.66 ± 0.84^a	53.16 ± 0.60^a
MCH (pg)	16.17 ± 0.53^a	15.92 ± 0.30^a	15.75 ± 0.63^a	16.57 ± 0.23^a
MCHC (g/dL)	33.02 ± 2.04^a	30.13 ± 0.77^a	30.35 ± 0.85^a	31.18 ± 0.24^a
Platelets (10^3/µL)	825.83 ± 99.44^a	862.17 ± 196.9^a	1044.83 ± 49.34^a	922.66 ± 27.70^a
TLC (10^3/µL)	8.30 ± 1.47^{ab}	5.92 ± 0.0.93^b	6.68 ± 0.64^{ab}	10.35 ± 0.59^a
Lymphocytes (%)	81.78 ± 1.97^a	75.83 ± 3.73^a	80.57 ± 1.18^a	81.88 ± 2.52^a
Abs. Lymphocytes (10^3/µL)	7.24 ± 1.53^{ab}	4.46 ± 0.94^b	5.37 ± 0.50^{ab}	8.53 ± 0.71^a
Neutrophils (%)	11.64 ± 1.04^a	13.61 ± 3.19^a	12.55 ± 1.52^a	9.06 ± 1.33^a
Abs. Neutrophils (10^3/µL)	0.81 ± 0.07^{ab}	0.67 ± 0.05^b	0.84 ± 0.14^{ab}	1.02 ± 0.06^a
Monocytes (%)	4.71 ± 0.94^a	8.45 ± 0.62^a	5.66 ± 1.54^a	6.42 ± 1.54^a
Abs. Monocytes (10^3/µL)	0.37 ± 0.02^a	0.64 ± 0.12^a	0.39 ± 0.11^a	0.63 ± 0.13^a
Eosinophils (%)	1.87 ± 0.60^a	2.10 ± 0.93^a	1.22 ± 0.32^a	1.64 ± 0.54^a
Abs. Eosinophils (10^3/µL)	0.14 ± 0.03^a	0.15 ± 0.08^a	0.08 ± 0.02^a	0.16 ± 0.05^a

Table 4: Comparison of mean values (Mean ± SE; n=6) of serum-biochemical parameters in male rats following Al_2O_3 NPs exposure for 28 days

Parameters	Treatment groups (Mean ± SE)			
	Control	T_1 (50 mg/kg)	T_2 (100 mg/kg)	T_3 (200 mg/kg)
ALT (IU/L)	69.46 ± 3.07^{ab}	64.17 ± 2.22^b	61.47 ± 2.69^b	104.92 ± 9.4^a
AST (IU/L)	228.2 ± 16.9^a	193.6 ± 24.3^a	171.6 ± 24.3^a	205.8 ± 30.4^a
Total bilirubin (mg/dL)	0.14 ± 0.01^b	0.16 ± 0.01^a	0.23 ± 0.01^a	0.20 ± 0.02^a
Direct bilirubin (mg/dL)	0.003 ± 0.003^a	0.01 ± 0.007^a	0.008 ± 0.008^a	0.012 ± 0.007^a
Indirect bilirubin (mg/dL)	0.14 ± 0.014^b	0.15 ± 0.02^b	0.22 ± 0.02^a	0.2 ± 0.02^{ab}
Total protein (g/dL)	7.43 ± 0.17^{ab}	7.33 ± 0.27^{ab}	7.57 ± 0.16^a	6.71 ± 0.24^b
Albumin (g/dL)	3.92 ± 0.09^a	3.99 ± 0.05^a	4.06 ± 0.08^a	3.53 ± 0.24^a
Globulin (g/dL)	3.51 ± 0.17^a	3.34 ± 0.23^a	3.51 ± 0.14^a	3.18 ± 0.19^a
ALP (IU/L)	172.92 ± 13.03^b	225.55 ± 12.39^{ab}	259.55 ± 23.00^a	251.52 ± 7.92^a
LDH (IU/L)	2314.05 ± 529.4^a	2312.81 ± 434.9^a	2172.51 ± 481.1^a	2366.27 ± 257.8^a

Note: Values with different superscripts in a row were significantly different ($p<0.05$)

Table 5: Comparison of mean values (Mean ± SE; n=6) of serum-biochemical parameters in female rats of following Al_2O_3 NPs exposure for 28 days

Parameters	Treatment groups (Mean ± SE)			
	Control	T_1 (50 mg/kg)	T_2 (100 mg/kg)	T_3 (200 mg/kg)
ALT (IU/L)	60.3 ± 4.56^b	78.7 ± 7.36^{ab}	73.9 ± 6.50^{ab}	90.9 ± 8.54^a
AST (IU/L)	167.33 ± 6.74^b	204.04 ± 19.56^{ab}	190.70 ± 14.88^{ab}	239.88 ± 12.0^a
Total bilirubin (mg/dL)	0.15 ± 0.032^b	0.18 ± 0.025^{ab}	0.23 ± 0.031^{ab}	0.29 ± 0.027^a
Direct bilirubin (mg/dL)	0.005 ± 0.005^a	0.003 ± 0.003^a	0.007 ± 0.007^a	0.01 ± 0.008^a
Indirect bilirubin (mg/dL)	0.15 ± 0.03^b	0.18 ± 0.02^a	0.22 ± 0.03^a	0.28 ± 0.02^a
Total protein (g/dL)	6.80 ± 0.19^{ab}	6.86 ± 0.34^{ab}	6.35 ± 0.24^b	7.62 ± 0.21^a
Albumin (g/dL)	3.88 ± 0.12^a	3.94 ± 0.14^a	3.72 ± 0.12^a	4.16 ± 0.25^a
Globulin (g/dL)	2.92 ± 0.25^{ab}	2.92 ± 0.21^{ab}	2.63 ± 0.14^b	3.46 ± 0.16^a
ALP (IU/L)	142.54 ± 28.55^a	105.72 ± 9.12^a	127.22 ± 9.58^a	156.37 ± 19.18^a
LDH (IU/L)	2437.55 ± 374.37^a	2334.53 ± 197.48^a	2329.5 ± 163.77^a	2014.02 ± 291.74^a

Note: Values with different superscripts in a row were significantly different ($p<0.05$)

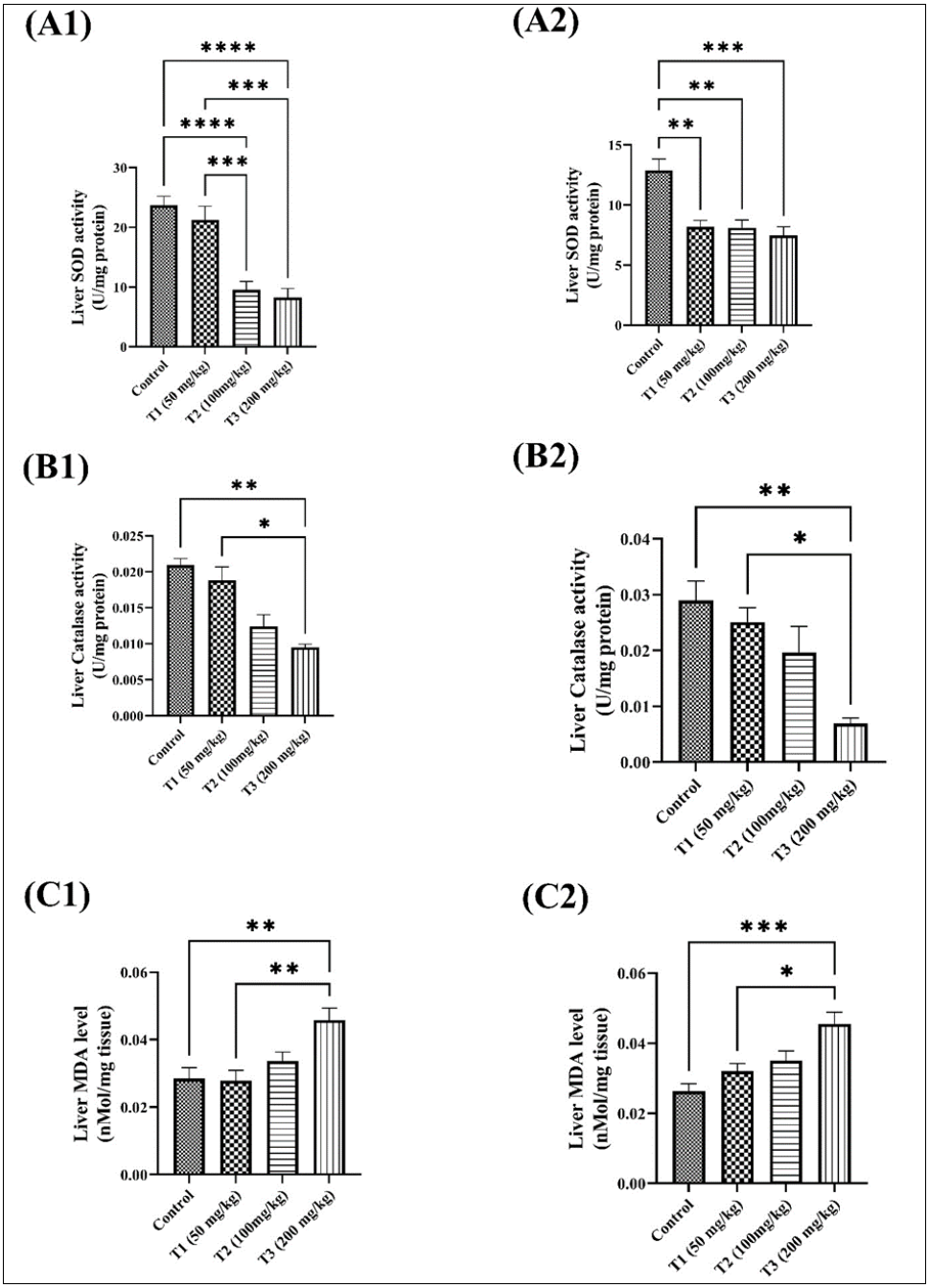

Fig 1: Comparison of oxidative stress parameters in the liver of male (A1, B1, C1) and female (A2, B2, C2) rats following Al_2O_3 NPs exposure for 28 days. Where *indicates $p<0.05$, **indicates p<0.01, *** indicates p<0.005, **** indicates p<0.001.

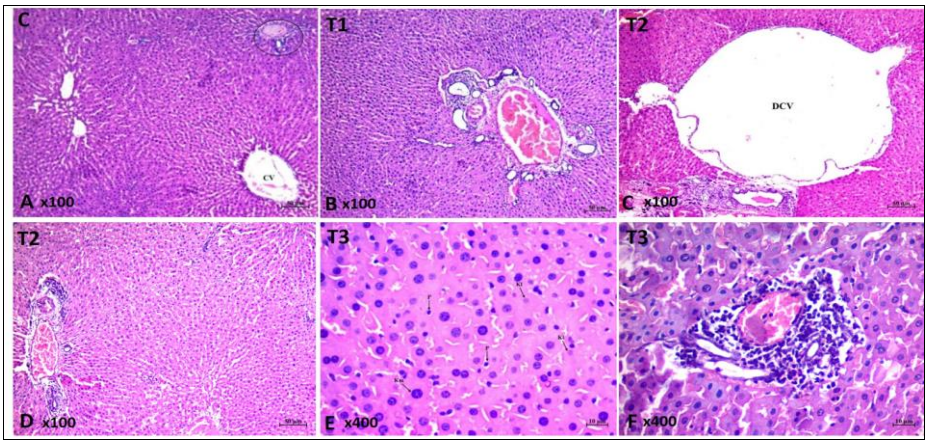

Fig 2: Histopathological changes in the liver of Wistar rats from different groups. A: Normal hepatic architecture from control group B: Microscopic view of liver from T_1 group showing congestion of portal vein C: Liver from T_1 group showing dilatation of central vein D: Liver from T_2 group showing marked portal vein congestion along with mild distorted hepatic parenchyma E: Liver from T_3 group showing pyknotic nuclei (P), karyolytic nuclei (Kl) and karyomegaly (Km) F: liver from T3 group showing periportal inflammatory cells infiltration (H & E)

5. Conclusion

The current investigation revealed that Al_2O_3NPs induced hepatotoxicity by generating ROS and oxidative stress. These effects are linked to compromised antioxidant defense systems and changes in hemato-biochemical parameters. In conclusion, although Al_2O_3NPs hold promise for diverse applications, their potential toxicity poses considerable concerns for human health and the environment. Further research endeavors are necessary to uncover the underlying mechanisms of toxicity, establish dependable risk assessment approaches and devise suitable mitigation strategies to ensure the safe utilization of these nanoparticles across industrial and biomedical domains.

6. Acknowledgements

The authors express their sincere gratitude to Dr. H. B. Patel, Assistant Professor in the Department of Veterinary Pharmacology and Toxicology at the College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, India, for his invaluable assistance during the course of this study. Additionally, appreciation is extended to Dr. B. B. Javia, Associate Professor and Head of the Department of Veterinary Microbiology at the same institution, for generously providing the necessary facilities for conducting this research.

7. Funding: The study was carried out using the funds provided to the Department by the Institute/University.

8. Ethical approval: Ethics approval and consent to participate Experimental procedure were approved by the Institutional Animal Ethics Committee of the college (Experimental Protocol no.: KU-JVC-IAEC-SA-91-22; Dated: 08/07/2022).

9. Consent for publication: All the authors approved the manuscript for publication.

10 References

1. Amde M, Liu JF, Tan ZQ, Bekana D. Transformation and bioavailability of metal oxide nanoparticles in aquatic and terrestrial environments. A review. Environmental Pollution. 2017;230:250–267. https://doi.org/10.1016/j.envpol.2017.06.064
2. Asztemborska M. Alumina nanoparticles and plants: environmental transformation, bioaccumulation, and phytotoxicity. In: Phytotoxicity of Nanoparticles. Springer, Cham. 2018:335-345.
3. Attarilar S, Yang J, Ebrahimi M, *et al*. The Toxicity Phenomenon and the Related Occurrence in Metal and Metal Oxide Nanoparticles: A Brief Review from the Biomedical Perspective. Frontiers in Bioengineering and Biotechnology. 2020;8:822. https://doi.org/10.3389/fbioe.2020.00822
4. Bai Y, Zhang Y, Zhang J, *et al*. Repeated administrations of carbon nanotubes in male mice cause reversible testis damage without affecting fertility. Nature Nanotechnology. 2010;5(9):683–689. https://doi.org/10.1038/nnano.2010.153
5. Balasubramanyam A, Sailaja N, Mahboob M, *et al*. *In vivo* genotoxicity assessment of aluminum oxide nanomaterials in rat peripheral blood cells using the comet assay and micronucleus test. Mutagenesis. 2009;24(3):245–251. https://doi.org/10.1093/mutage/gep003
6. Beers RF, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. Journal of Biological Chemistry. 1952;195(1):133-140. https://doi.org/10.1016/S0021-9258(19)50881-X
7. Chen L, Yokel RA, Hennig B, *et al*. Manufactured aluminum oxide nanoparticles decrease expression of tight junction proteins in brain vasculature. Journal of Neuroimmune Pharmacology: The official Journal of the Society on NeuroImmune Pharmacology. 2008;3(4):286–295. https://doi.org/10.1007/s11481-008-9131-5
8. De A, Ghosh S, Chakrabarti M, *et al*. Effect of low-dose exposure of aluminium oxide nanoparticles in Swiss albino mice: Histopathological changes and oxidative damage. Toxicology and Industrial Health. 2020;36(8):567–579. https://doi.org/10.1177/0748233720936828
9. Dey S, Bakthavatchalu V, Tseng MT, *et al*. Interactions between $SIRT_1$ and AP-1 reveal a mechanistic insight into the growth promoting properties of alumina (Al2O3) nanoparticles in mouse skin epithelial cells. Carcinogenesis. 2008;29(10):1920–1929. https://doi.org/10.1093/carcin/bgn175
10. Dikshit PK, Kumar J, Das AK, *et al*. Green synthesis of metallic nanoparticles: Applications and Limitations. Catalysts. 2021;11(8):902.
11. Dong E, Wang Y, Yang ST, *et al*. Toxicity of nano gamma alumina to neural stem cells. Journal of Nanoscience and Nanotechnology. 2011;11(9):7848–7856. https://doi.org/10.1166/jnn.2011.4748
12. Dong L, Tang S, Deng F, *et al*. Shape-dependent toxicity of alumina nanoparticles in rat astrocytes. The Science of The Total Environment. 2019;690:158–166. https://doi.org/10.1016/j.scitotenv.2019.06.532
13. Egbuna C, Parmar VK, Jeevanandam J, *et al*. Toxicity of Nanoparticles in Biomedical Application: Nanotoxicology. Journal of Toxicology. 2021;2021:9954443. https://doi.org/10.1155/2021/9954443
14. El-Hussainy el-HM, Hussein AM, Abdel-Aziz A, *et al*. Effects of aluminum oxide (Al2O3) nanoparticles on ECG, myocardial inflammatory cytokines, redox state, and connexin 43 and lipid profile in rats: possible cardioprotective effect of gallic acid. Canadian Journal of Physiology and Pharmacology. 2016;94(8):868–878. https://doi.org/10.1139/cjpp-2015-0446
15. Gojova A, Guo B, Kota RS, *et al*. Induction of inflammation in vascular endothelial cells by metal oxide nanoparticles: effect of particle composition. Environmental Health Perspectives. 2007;115(3):403–409. https://doi.org/10.1289/ehp.8497
16. Huang T, Guo W, Wang Y, *et al*. Involvement of Mitophagy in Aluminum Oxide Nanoparticle-Induced Impairment of Learning and Memory in Mice. Neurotoxicity Research. 2021;39(2):378–391. https://doi.org/10.1007/s12640-020-00283-0
17. Li XB, Zheng H, Liu R, *et al*. Cell activation and function alteration in rat livers induced by repeated aluminum oxide nanoparticles exposure. Advanced Materials Research. 2012;486:75-79. https://doi.org/10.4028/www.scientific.net/AMR.486.75
18. Lykkesfeldt J. Determination of malondialdehyde as dithiobarbituric acid adduct in biological samples by HPLC with fluorescence detection, comparison with ultraviolet-visible spectrophotometry. Clinical Chemistry.

2001;47(9):1725-1727. https://doi.org/10.1093/clinchem/47.9.1725
19. Morsy GM, Abou El-Ala KS, Ali AA. Studies on fate and toxicity of nanoalumina in male albino rats: Oxidative stress in the brain, liver and kidney. Toxicology and Industrial Health. 2016;32(2):200–214. https://doi.org/10.1177/0748233713498462
20. Oesterling E, Chopra N, Gavalas V, *et al*. Alumina nanoparticles induce expression of endothelial cell adhesion molecules. Toxicology Letters. 2008;178(3):160–166. https://doi.org/10.1016/j.toxlet.2008.03.011
21. Park EJ, Kim H, Kim Y, *et al*. Repeated-dose toxicity attributed to aluminum nanoparticles following 28-day oral administration, particularly on gene expression in mouse brain. Toxicological & Environmental Chemistry. 2011;93(1):120-133.
22. Park EJ, Sim J, Kim Y, *et al*. A 13-week repeated-dose oral toxicity and bioaccumulation of aluminum oxide nanoparticles in mice. Archives of Toxicology. 2015;89(3):371–379. https://doi.org/10.1007/s00204-014-1256-0
23. Patlolla A, McGinnis B, Tchounwou P. Biochemical and histopathological evaluation of functionalized single-walled carbon nanotubes in Swiss-Webster mice. Journal of Applied Toxicology: JAT. 2011;31(1):75–83. https://doi.org/10.1002/jat.1579
24. Prabhakar PV, Reddy UA, Singh SP, *et al*. Oxidative stress induced by aluminum oxide nanomaterials after acute oral treatment in Wistar rats. Journal of Applied Toxicology: JAT. 2012;32(6):436–445. https://doi.org/10.1002/jat.1775
25. Rittner MN. Market analysis of nanostructured materials. American Ceramic Society Bulletin. 2002;81(3):33-36.
26. Roco MC. International perspective on government nanotechnology funding in 2005. Journal of Nanoparticle Research. 2005;7:707-712.
27. Savory J, Herman MM, Ghribi O. Mechanisms of aluminum-induced neurodegeneration in animals: Implications for Alzheimer's disease. Journal of Alzheimer's Disease: JAD. 2006;10(2-3):135–144. https://doi.org/10.3233/jad-2006-102-302
28. Shim KH, Hulme J, Maeng EH, *et al*. Analysis of zinc oxide nanoparticles binding proteins in rat blood and brain homogenate. International Journal of Nanomedicine; c2014. p. 217–224. https://doi.org/10.2147/IJN.S58204
29. Wagner AJ, Bleckmann CA, Murdock RC, *et al*. Cellular interaction of different forms of aluminum nanoparticles in rat alveolar macrophages. The Journal of Physical Chemistry. 2007;111(25):7353–7359. https://doi.org/10.1021/jp068938n
30. www.oecd.org. Six years of OECD work on the safety of manufactured nanomaterials: Achievements and future opportunities; c2012.
31. Yousef MI, Mutar TF, Kamel MAEN. Hepato-renal toxicity of oral sub-chronic exposure to aluminum oxide and/or zinc oxide nanoparticles in rats. Toxicology Reports. 2019;6:336-346.
32. Zhang QL, Li MQ, Ji JW, *et al*. *In vivo* toxicity of nano-alumina on mice neurobehavioral profiles and the potential mechanisms. International Journal of Immunopathology and Pharmacology. 2011;24:23S–29S.