BPB Reports

Report

Aluminum Oxide Nanoparticles Induce Nephrotoxicity and Acute Kidney Injury Following Coadministration with Cisplatin or 5-Aminosalicylic Acid

Azusa Araki, Funa Kasai, Tatsuya Asano, Kotaro Takasaki, and Katsuhiro Isoda*

Laboratory of Medical Pharmacy Education, Faculty of Pharmaceutical Sciences, Teikyo Heisei University, Nakano-ku, Tokyo 164-8530, Japan

Received October 8, 2025; Accepted November 16, 2025

The use of nanoparticles in various industrial fields is increasing. Aluminum oxide nanoparticles (nAlO) exhibit excellent functionality and are thus widely used for industrial purposes. However, the potential biological hazards of nAlO have not been addressed. Therefore, we investigated the *in vivo* effects and drug interactions of nAlO. Administration of nAlO to mice via the tail vein induced acute kidney injury but not liver injury. nAlO induced kidney injury when co-administered with cisplatin or 5-aminosalicylic acid. Thus, our results indicate that nAlO exhibits potential nephrotoxicity either alone or through drug interactions.

Key words aluminum oxide nanoparticles, acute kidney injury, cisplatin, 5-aminosalicylic acid

INTRODUCTION

Nanomaterials have attracted considerable attention as functional materials due to their high potential for providing unprecedented superior performance.[1,2] Nanoparticles are already being used in a wide range of fields, from practical engineering applications such as information and communications technology, biotechnology, energy engineering, and medical engineering to basic sciences such as physics, chemistry, biology, and medicine.[3,4] Indeed, nanomaterials are utilized in products such as cosmetics as a skin-whitening agent and UV-absorbing material.[5] However, studies have reported that fullerenes and carbon nanotubes, which are non-toxic at the macro level, become toxic when they are reduced to nanosize due to a dramatic increase in their reactivity at the electrochemical level.[6] For example, exposure to industrially used carbon nanotubes via inhalation reportedly damages lung tissue. Nanofullerene, a UV-absorbing material, migrates to the brain via the blood circulation after skin application, leading to damage to brain tissue.[7,8] At the very least, nanofullerene has the potential to penetrate biological barriers such as the skin and blood-brain barrier.

Aluminum is present in a wide range of products used in daily life because of its excellent strength and workability.[9] Aluminum oxide (alumina), which can be artificially produced, is used as an industrial material due to its high strength and heat resistance.[10] Aluminum oxide is also used as a fine ceramic material in industrial products, medical products, and art objects.[11] Nano-sized aluminum oxide particles have a large surface area to volume ratio that results in improved functionality of industrial products such as thin electrical insulating films and purification filters. In addition, aluminum oxide nanoparticles (nAlO) exhibit improved light transmittance, and as this property leads to reduced power consumption, the application of nAlO in electrical appliances is highly anticipated.[11,12] However, reports describing the safety of nAlO are lacking, even though they are likely to be present in a wide range of products used in daily life in the future.

Nanotoxicology studies have revealed that silica nanoparticles are cytotoxic, hepatotoxic, and cause placental damage.[13,14] Another study reported that carbon nanotubes can induce pulmonary mesothelioma.[15] However, the pharmacologic effects resulting from interactions between nanoparticles and drugs are generally unknown. In this study, we investigated the effects of nAlO in mice and further investigated whether they synergistically exacerbate the toxicity of certain pharmaceuticals, such as cisplatin, a widely used antitumor drug,[16, 17] and 5-aminosalicylic acid (5-ASA), a common anti-inflammatory drug. This study investigated the safety of nAlO in mammals and the safety and interactions when co-administered with drugs.

MATERIALS AND METHODS

Materials nAlO with a diameter of 60 nm were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). The size distribution of the nAlO was 67.1 ± 23.7 nm. The particles were spherical and nonporous, and they were stored as a 0.8 mg/mL aqueous suspension. Prior to use in experiments, the suspension was thoroughly dispersed using sonication, and the nan-

*To whom correspondence should be addressed. e-mail: k.isoda@thu.ac.jp

oparticles were diluted with water. An equal volume of suspension was injected for each treatment. CDDP, and 5-ASA (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) were dissolved in saline and stored at −20°C before use. The CDDP concentration was adjusted to 80 μmol/kg, and 5-ASA to 500 mg/kg. All reagents used were of research grade.

Animals Eight-week-old BALB/c male mice were purchased from Funabashi Farm Co., Ltd. (Chiba, Japan). Mice were maintained in a controlled environment (temperature: 23 ± 1.5°C; 12-h light/dark cycle) with free access to standard rodent chow and water. The mice were given 1 week to adapt before commencing the experiments. The experimental protocols conformed to the ethical guidelines of the Graduate School of Pharmaceutical Sciences, Teikyo Heisei University, Japan.

Biochemical Analyses Serum ALT and AST levels were measured using commercially available kits (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) according to the manufacturer's protocols. Briefly, collected serum (10 μL) was combined with 1 mL of color A reagent and incubated at 37°C for 15 min. Following the addition of 1 mL of color B reagent, the sample was incubated at 37°C for 10 min. Absorbance was measured at a wavelength of 570 nm. BUN levels were measured using a commercially available kit (Arbor Assays, Inc., Michigan, USA) according to the manufacturer's protocol. Absorbance was measured at a wavelength of 450 nm. Cre levels were measured using a commercially available kit (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) according to the manufacturer's protocol. Absorbance was measured at a wavelength of 520 nm.

Statistical Analyses Statistical analyses were performed using Statcel 3 add-in software forms for Excel (EMS Publication Co., Ltd., Saitama, Japan). All data are presented as the mean ± standard error of the mean (SEM). Significant differences between the control and experimental groups were determined using the Dunnett test; a P value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Initially, the acute toxicity of nAlO was investigated by injecting mice via the tail vein at doses of 100, 200, and 300 μg/kg. All mice that received nAlO at a dose of 300 μg/kg died, whereas all mice that received nAlO at doses of 100 and 200 μg/kg survived. Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as indicators of liver damage were not increased in the 100 and 200 μg/kg nAlO groups compared with the control group (Fig. 1A). Levels of blood urea nitrogen (BUN) and creatinine (Cre) as indicators of kidney injury were significantly elevated in the 200 μg/kg nAlO group compared with the control group (Fig. 1B, C), suggesting that nAlO induce acute kidney injury.

Next, we investigated the interaction between cisplatin (CDDP) and nAlO. Administration of CDDP can lead to significant adverse effects such as kidney and hepatic failure.[18] Co-administration of CDDP (80 μmol/kg) and nAlO did not significantly increase serum ALT or AST levels (Fig. 2A). However, synergistic increases in the BUN level from 26.5 to 60.2 mg/dL and serum Cre level from 0.42 to 0.91 mg/dL were observed (Fig. 2B, C).

We also investigated the interaction between 5-ASA and nAlO. Administration of 5-ASA can lead to significant adverse effects such as nephritis and liver failure.[19] Co-administration of 5-ASA (500 mg/kg) and nAlO did not induce an increase in serum ALT or AST levels (Fig. 3A). However, synergistic increases in the BUN level from 19.4 to 43.3 mg/dL and serum Cre level from 0.17 to 0.52 mg/dL were observed in mice co-administered nAlO and 5-ASA (Fig. 3B, C). The interaction between nAlO and 5-ASA resulted in acute kidney injury. These results demonstrate that co-administration of nAlO with either CDDP or 5-ASA induces acute kidney injury but not liver injury.

BUN and Cre levels could not be determined in the mice in the group that received 300 μg/kg nAlO because all of the animals died, but in mice that received 200 μg/kg nAlO, BUN and Cre levels increased significantly relative to the control group (Fig. 1B, C). Based on these results, we investigated potential drug interactions with nAlO at a dose of 100 μg/kg. As shown in Fig. 1, the increases in BUN and Cre levels indicated that administration of nAlO induces acute kidney injury. Further detailed analysis of the acute toxicity of nAlO in mice following administration via the tail vein is thus required. A previous study reported that repeated oral administration of nAlO is nephrotoxic, hepatotoxic, and accumulative.[20] Our results showed that administration of nAlO to mice via the tail vein induces acute kidney injury but not acute liver injury. In this study, all mice survived at a dose of 200 μg/kg of aluminum oxide nanoparticles, whereas all died at 300 μg/kg. This sharp change in outcome suggests that the toxicity of the nanoparticles may exhibit a threshold-like behavior related to their biodistribution and organ accumulation. The kidney is a major excretory organ for nanoparticles, and previous studies have reported renal toxicity of nAlO, including oxidative stress and tubular damage.[20] It is therefore likely that at 300 μg/kg, the renal excretory capacity was exceeded, leading to a rapid progression of organ damage. Taken together, these findings indicate that a threshold dose for toxicity exists between 200 and 300 μg/kg.

The dose of nAlO used in this study (100–300 μg/kg) is a high experimental dose that far exceeds the general environmental or occupational exposure levels reported to date. Therefore, this test does not directly reflect the actual exposure situation in humans, but was set with the aim of exploring safety margins and toxicity thresholds. Future work will be required to evaluate the long-term effects at concentrations at real-world environmental levels.

As shown in Fig. 2, the BUN and Cre levels in mice co-administered nAlO and CDDP were significantly elevated relative to control mice, indicating that acute kidney injury was induced. One of the more serious adverse effects of CDDP is kidney damage, which results from the active uptake of organic cations into cells via organic cation transporters present in the basement membrane of the proximal tubules, resulting in cytotoxicity and proximal tubule necrosis.[21] Our results suggest that nAlO enhances the absorption of CDDP via organic cation transporters, resulting in kidney injury.

As shown in Fig. 3, BUN and Cre levels in mice co-administered nAlO and 5-ASA were also significantly elevated relative to control mice, indicating that acute kidney injury was induced. 5-ASA is metabolized to a stable acetyl form primarily by *N*-acetyltransferase in the liver and digestive tract.[22] The acetylated form of 5-ASA is then excreted via the urine, and the unchanged form is not detected.[23] As administration of nAlO alone is sufficient to induce acute kidney inju-

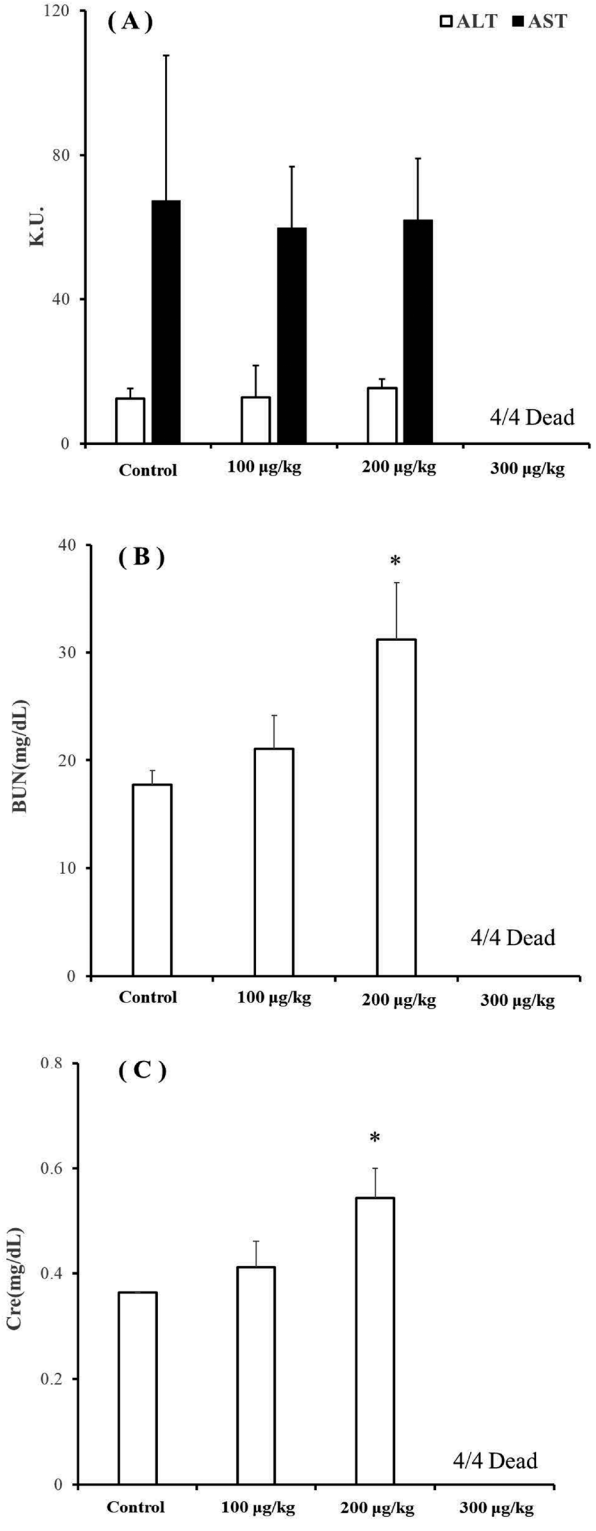

Fig. 1. Dose Dependence of nAlO-Induced Liver Injury and Kidney Injury

Serum levels of the liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (A), blood urea nitrogen (BUN) (B), and creatinine (Cre) (C) were determined using commercially available kits (see 'Biochemical analyses' section) 24 h after intravenous administration of aluminum oxide nanoparticles (nAlO) at the indicated doses. Data are representative of three independent experiments and shown as the mean ± SEM (n = 4). *Significant difference compared with vehicle group (*$P < 0.05$).

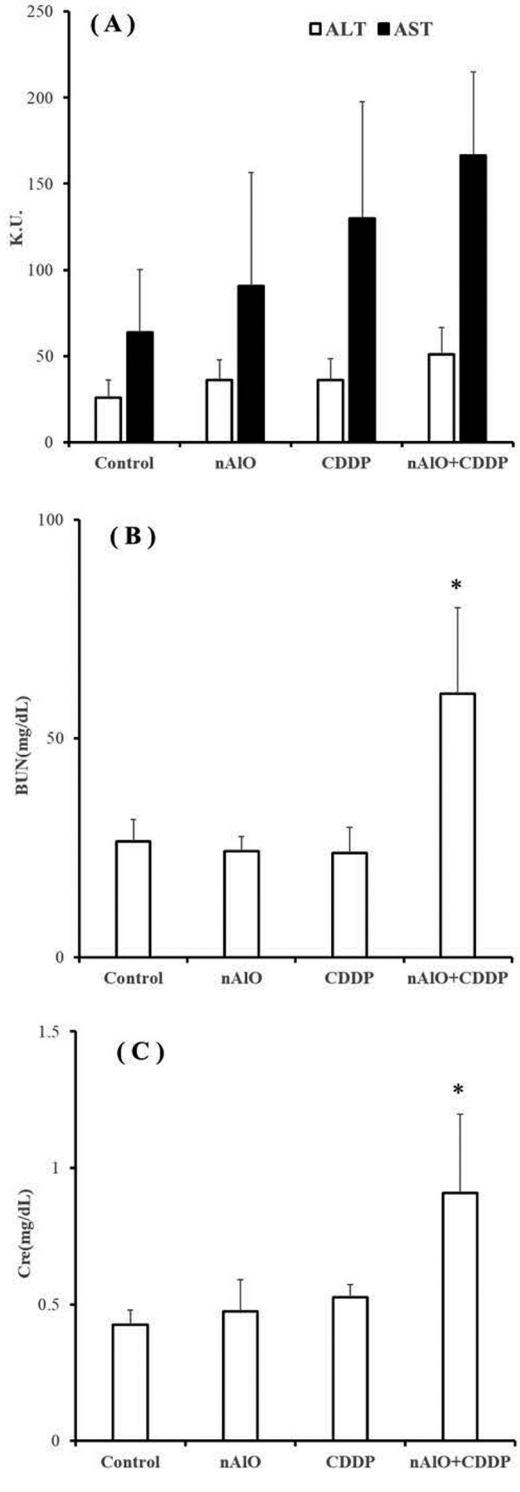

Fig. 2. Effect of nAlO on Cisplatin (CDDP) Changes

Mice were injected intraperitoneally with CDDP at 80 μmol/kg together with nAlO injected intravenously at a dose of 100 μg/kg. ALT (A), AST (A), BUN (B), and Cre (C) levels were assayed as described in the Experimental section. Data are shown as the mean ± SEM (n = 4). Significant difference between vehicle and CDDP-treated group (*$P < 0.05$).

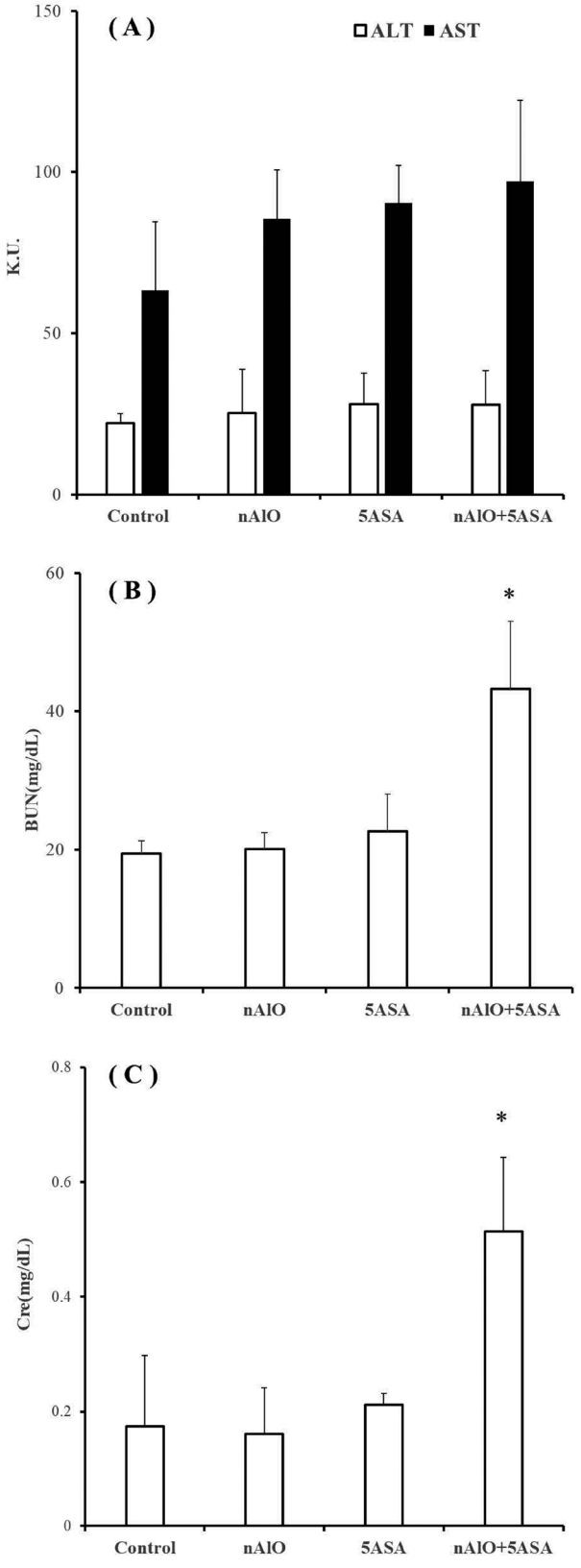

Fig. 3. Effect of nAlO on 5-Aminosalicylic Acid (5-ASA) Changes

Mice were injected with 5-ASA at 500 mg/kg together with nAlO injected intravenously at a dose of 100 μg/kg. ALT (A), AST (A), BUN (B), and Cre (C) levels were assayed as described in the Experimental section. Data are shown as the mean ± SEM (n = 4). Significant difference between vehicle and 5-ASA-treated group (*$P < 0.05$).

ry, our results suggest that 5-ASA may have exacerbated the acute kidney injury–inducing effect of nAlO. Also, 5-ASA has been reported to induce mitochondrial dysfunction and ROS production in renal tubular cells under certain conditions, and nAlO itself may also promote ROS production and inflammation.[24,25] The combination of these may synergistically enhance oxidative and inflammatory injury in the kidney, thereby exacerbating renal dysfunction.

The above results indicate that nAlO induces acute kidney injury not only when administered alone but also when administered in combination with CDDP or 5-ASA. The mechanism by which nAlO induces acute kidney injury could not be elucidated in this study, however. In the future, it will thus be necessary to investigate the pharmacokinetics and mechanism of action of nAlO *in vivo*.

Acknowledgments The authors wish to thank all members of the laboratory for their useful comments and discussions.

Conflict of interest The authors declare no conflict of interest.

REFERENCES

1) Kiseleva AP, Kiselev GO, Nikolaeva VO, Seisenbaeva G, Kessler V, Krivoshapkin PV, Krivoshapkina EF. Hybrid Spider Silk with Inorganic Nanomaterials. *Nanomaterials (Basel)*, **10**, 1853 (2020).
2) Diez-Pascual AM, Rahdar A. Functional Nanomaterials in Biomedicine: Current Uses and Potential Applications. *ChemMedChem*, **17**, e202200142 (2022).
3) Li Y, Liao Q, Hou W, Qin L. Silver-Based Surface Plasmon Sensors: fabrication and Applications. *Int. J. Mol. Sci.*, **24**, 4142 (2023).
4) Awashra M, Młynarz P. The toxicity of nanoparticles and their interaction with cells: an *in vitro* metabolomic perspective. *Nanoscale Adv.*, **5**, 2674–2723 (2023).
5) Egambaram OP, Kesavan Pillai S, Ray SS. Materials Science Challenges in Skin UV Protection: A Review. *Photochem. Photobiol.*, **96**, 779–797 (2020).
6) Migliore L, Uboldi C, Di Bucchianico S, Coppedè F. Nanomaterials and neurodegeneration. *Environ. Mol. Mutagen.*, **56**, 149–170 (2015).
7) Dong J. Signaling Pathways Implicated in Carbon Nanotube-Induced Lung Inflammation. *Front. Immunol.*, **11**, 552613 (2020).
8) Som C, Wick P, Krug H, Nowack B. Environmental and health effects of nanomaterials in nanotextiles and façade coatings. *Environ. Int.*, **37**, 1131–1142 (2011).
9) Zheng J, Pang Q, Hu Z, Sun Q. Recent Progress on Regulating Strategies for the Strengthening and Toughening of High-Strength Aluminum Alloys. *Materials (Basel, Switzerland),* **15**, (2022).
10) Koech AK, Mwandila G, Mulolani F. A review of improvements on electric vehicle battery. *Heliyon*, **10**, e34806 (2024).
11) Lawson NC, Burgess JO: Dental ceramics: a current review. *Compendium of continuing education in dentistry (Jamesburg, N.J.: 1995),* **35**, 161-166; quiz 168 (2014).
12) Ku CA, Yu CY, Hung CW, Chung CK. Advances in the Fabrication of Nanoporous Anodic Aluminum Oxide and Its Applications to Sensors: A Review. *Nanomaterials (Basel)*, **13**, 2853 (2023).
13) Nishimori H, Kondoh M, Isoda K, Tsunoda S, Tsutsumi Y, Yagi K: Silica nanoparticles as hepatotoxicants. *European journal of pharmaceutics and biopharmaceutics: official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V,* **72**, 496-501 (2009).
14) Yamashita K, Yoshioka Y, Higashisaka K, Mimura K, Morishita Y, Nozaki M, Yoshida T, Ogura T, Nabeshi H, Nagano K, Abe Y, Kamada H, Monobe Y, Imazawa T, Aoshima H, Shishido K, Kawai Y, Mayumi T, Tsunoda S, Itoh N, Yoshikawa T, Yanagihara I, Saito S, Tsutsumi Y. Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. *Nat. Nanotechnol.*, **6**, 321–328 (2011).

15) Dong J, Ma Q. Integration of inflammation, fibrosis, and cancer induced by carbon nanotubes. *Nanotoxicology*, **13**, 1244–1274 (2019).
16) Ozols RF, Young RC. Ovarian cancer: where to next? *Semin. Oncol.*, **18**, 307–310 (1991).
17) Witjes JA. Current recommendations for the management of bladder cancer. Drug therapy. *Drugs*, **53**, 404–414 (1997).
18) Oliveira CA, Mercês ÉAB, Portela FS, Malheiro LFL, Silva HBL, De Benedictis LM, De Benedictis JM, Silva CCDE, Santos ACL, Rosa DP, Velozo HS, de Jesus Soares T, de Brito Amaral LS. An integrated view of cisplatin-induced nephrotoxicity, hepatotoxicity, and cardiotoxicity: characteristics, common molecular mechanisms, and current clinical management. *Clin. Exp. Nephrol.*, **28**, 711–727 (2024).
19) Deltenre P, Berson A, Marcellin P, Degott C, Biour M, Pessayre D. Mesalazine (5-aminosalicylic acid) induced chronic hepatitis. *Gut*, **44**, 886–888 (1999).
20) Park EJ, Sim J, Kim Y, Han BS, Yoon C, Lee S, Cho MH, Lee BS, Kim JH. A 13-week repeated-dose oral toxicity and bioaccumulation of aluminum oxide nanoparticles in mice. *Arch. Toxicol.*, **89**, 371–379 (2015).
21) Manohar S, Leung N. Cisplatin nephrotoxicity: a review of the literature. *J. Nephrol.*, **31**, 15–25 (2018).
22) Ramírez-Alcántara V, Montrose MH. Acute murine colitis reduces colonic 5-aminosalicylic acid metabolism by regulation of N-acetyltransferase-2. *Am. J. Physiol. Gastrointest. Liver Physiol.*, **306**, G1002–G1010 (2014).
23) Tokui K, Asai Y, Arakawa T, Matsumoto T, Nabeshima T. Comparative absorption of 5-aminosalicylic acid (5-ASA) after administration of a 5-ASA enema and salazosulfapyridine (SASP) after an SASP suppository in Japanese volunteers. *Biol. Pharm. Bull.*, **25**, 264–267 (2002).
24) Salimi A, Bahreini F, Jamali Z, Pourahmad J. Mesalazine Induces Oxidative Stress and Cytochrome c Release in Isolated Rat Heart Mitochondria: An Analysis of Cardiotoxic Effects. *Int. J. Toxicol.*, **39**, 241–247 (2020).
25) Alghriany AAI, Omar HEM, Mahmoud AM, Atia MM: Assessment of the Toxicity of Aluminum Oxide and Its Nanoparticles in the Bone Marrow and Liver of Male Mice: Ameliorative Efficacy of Curcumin Nanoparticles. *ACS Omega*, **7**.13841-13852 (2022).