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Hepatic and renal impairment and degenerative changes caused by carbon black nanoparticles in mice

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Carbon-based nanoparticles (CBNPs) have shown a notable increase in demand and are progressively encountering human exposure as a result of their extensive utilization across diverse industries and applications making it imperative to conduct comprehensive investigations of their potential impacts on human health. This study represents the inaugural investigation into the toxicity of CBNPs when administered orally (gavage) to mice over 30 days, dosing 5mg/kg, 10mg/kg, and 20mg/kg of the mice's body weight. The study depicted hyperactivity, social withdrawal, rolling behavior, the appearance of yellowish spots on the tail, alopecia, and a darkening of eye pigmentation. ALP and catalase levels decreased, ALT, AST, and glutathione levels increased, indicating liver and kidney physiological changes. High urea and creatinine levels indicated renal physiology disruption, whereas high bilirubin levels indicated hepatic physiology disturbance. Inflammation, necrotic foci, and binucleated cells were seen in kidney and liver tissue. The findings of the study suggested that the adverse effects resulting from exposure to CBNPs can be attributed to their tendency to aggregate, slow clearance rate, and excessive formation of reactive oxygen species (ROS), which in turn impair enzyme activities. Therefore, it may be deduced that exposure to CBNPs may induce a disruption of physiological processes, culminating in the development of severe and perhaps fatal illnesses.

Keywords: Gavage, Histology, Kidney, Liver, Morphology

Nanoparticles (NPs) exist naturally as well as a consequence of human activities. The exposure of particulate matter to humans has been seen from the early stages of human evolution. However, advancements in technology, such as combustion engines and nanotechnology, have introduced novel sorts of particles into the environment. Carbon Black Nanoparticles (CBNPs) are a specific subset of CNPs that exhibit a distinctive physical appearance. They manifest as finely divided powder with black colouration and possess a unique aciniform morphology[1]. CBNPs are very adaptable and widely utilized as the major reinforcing filler in various industries. In addition to its prominent role in the rubber sector, CBNPs are also extensively employed in the production of plastics, electronics, inks, coatings, and green technology[2]. Notably, the annual production of CBNPs is estimated to be approximately 10 million tonnes[3]. The growing presence of CBNPs in both human populations and the environment may be ascribed to several sources, such as paint spills, indirect ingestion of inks, leaching from plastic food packaging, inappropriate disposal of electrostatic discharge chemicals, and more[4]. Additionally, CBNPs has been used as a culinary colouring agent and a treatment for an extended period[2]. CBNPs exhibit high reactivity and have a low rate of clearance[5,6]. According to Ianni[6], it has been proposed that these elements could potentially contribute to heightened and protracted inflammation, hence elevating the likelihood of disease development.

Numerous research investigations have been conducted to investigate the toxicity of CBNPs. Research findings indicate that CBNP toxicity manifests in various ways across different organisms. CBNPs have been shown to hinder the growth and development of embryos, induce cell death, and trigger inflammation in lung tissues. Exposure to fine carbon black and Printex 90 can cause oxidative stress in A549 cells, similar to human primary bronchial epithelial cells which led to the proliferation of airway epithelium[7]. Studies have also shown mutations, epithelial hyperplasia, granulomas, and per-toxic lesions in lung and alveolar lining cells[8]. Printex 90

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can also cause inflammation in female offspring and decreased sperm production[9].

A study by Onoda found that exposure to Printex 90 particles caused granule enlargement, decreased macrophage numbers, and brain astrocyte phenotype changes[10]. The study also found an upregulation of collagen type VIII in juvenile mice's renal tissue and an increase in thymocytes and lymphocytes in offspring. Additionally, the study found partial inhibition of immune system maturation in progeny and reduced T cell populations in offspring[4]. Studies have shown that inhaling CBNPs, particularly Printex 90, can lead to lung inflammation, alveolar toxicity, type II epithelial hyperplasia, elevated catalase activity, and alterations in hepatocyte structural organization. Long-term exposure to CBNPs can also increase the ability to generate a pro-inflammatory response in rats' lungs, as demonstrated by Ianni[6]. Research also compared age, body mass index, smoking and drinking behaviours, exposure length, and work shift status between a control group and CBNPs exposure group wherein personal and fixed air samplers were used to detect CBNPs levels. While stationary samplers recorded a mean CBNPs concentration of 1.63 mg/m³ at the workplace, personal samplers from 15 volunteers reported a much higher mean concentration of 14.90 mg/m³, 4.26 times the Threshold Limit Value (TLV) of 3.5 mg/m³[1]. This data was considered to deduce the doses of CBNPs for the current study. The SPECT/CT imaging tests of a study also showed that 8.3-18.7% and 23.3-50.6% of the particles breathed for a brief duration were excreted and accumulated in the stomach of rats and mice[2]. CBNPs exposure primarily occurs during production, collection, and handling processes, with gastrointestinal system exposure being a significant route. While pulmonary exposure pathways are extensively studied, there is a lack of attention to other pathways, such as the gastrointestinal route, as they have not received as much attention despite their significance following pulmonary exposure. Understanding various exposure pathways is essential as the intensity of damage caused to the organs differ depending on the exposure route. For instance, in pulmonary exposure, the lungs are the main organ affected, while gastrointestinal exposure primarily affects the gastrointestinal tract. Furthermore, acute exposure to nanoparticles generally does not cause substantial harm to organs, as the majority of nanoparticles are eliminated from the body quickly. With prolonged exposure, nanoparticles have the opportunity to cause harm to the organs before they are eliminated from the body. Therefore, it is essential to examine the toxicity of NPs on organisms when they come into contact with the gastrointestinal system to better understand its toxicity.

Materials and Method

Experimental setup

A total of 24 adult male Swiss albino mice aged 6-8 weeks were procured from the National Institute of Biosciences, Pune, India. The mice were acclimatized to the laboratory environment for a week, kept in polycarbonate cages with standard bedding, and subjected to a light/dark cycle of 12 h each. They were provided with commercial food and tap water unrestrictedly. The study received ethical approval from the Institutional Animal Ethics Committee (1091/GO/Bt/D/07/CPCSEA) and humane treatment in strict accordance with CPCSEA rules. The mice were divided into four groups, with three groups gavaged with CBNPs (5, 10, and 20 mg/kg body weight respectively) suspended in saline solution and the fourth group (control) received only saline solution[2]. Animals were regularly checked for behavioral changes, morphological alterations, and other noticeable changes during the exposure period. After 30 days of continuous oral exposure (gavage), all animals were sacrificed by cervical dislocation.

Histology

After sacrificing, the mice were dissected open and the liver and kidney tissues were isolated, aseptically, washed in mammalian saline thoroughly to remove all the traces of blood, weighed, and immediately stored in 10% formalin. They were processed via embedding in paraffin, sectioned at 0.1 μm, and stained with hematoxylin and eosin. The slides were analyzed under an Olympus BX51 microscope, equipped with a ProgRes® Capture Pro 2.8.8 JENOPTIK Camera.

Biochemical Analysis

Post mice dissection, the liver and kidney specimens were isolated and subjected to a meticulous cleansing process using chilled 0.9% mammalian saline solution to eliminate any residual blood. Subsequently, the specimens were frozen at a temperature of -20°C until they were ready for use. In addition, blood samples were obtained through cardiac puncture for serum and plasma collection. Furthermore, urine samples were collected following the urination protocol outlined by Chew[11]. The tissue homogenates were prepared in different

buffers to estimate various metabolites and enzymes using standard protocols *viz*., total carbohydrates[12], free sugars[12], total proteins[12], albumins and globulins[12], total cholesterol[12], triglycerides[12], reduced glutathione (GSH)[13], bilirubin[14], Catalase[13], Alanine aminotransferase (ALT, Product no. HTBC009, Himedia), Aspartate aminotransferase (AST, Product no. HTBC008, Himedia), Alkaline phosphatase (ALP, Product No. CCK035, Himedia), Urea (Product no. MAK006-1KT, Sigma Aldrich) and Creatinine (Product No. MAK080-1KT, Sigma Aldrich)

Statistical Analysis

Statistical analyses were done using GraphPad Prism 9 software. Results were expressed as mean±standard deviation. Student *t-test* and One-way Analysis of variance (One-way ANOVA) were carried out by considering the statistical significance range as $P \leq 0.05$ (significant), $P \leq 0.001$ (highly significant), and $P \leq 0.0001$ (very highly significant).

Results

Behavior

The mice exhibited incremental differences concerning several behavioral changes throughout 30 days of exposure to CBNPs, as depicted in Table 1. At the initiation of the exposure period, the occurrence of tail rolling was noted, and throughout the subsequent 14 day period, this behavior exhibited a steady escalation in the experimental groups. In comparison, Group 3 had the greatest degree of tail rolling after 25 days of treatment. The animals in the Control group exhibited typical behavior throughout the trial, but the groups displayed hyperactivity that progressively intensified in the third group throughout the exposure time. The mice in the Control and Group 1 exhibited normal feeding behavior, however, a steady decline in feeding was noted in Group 2 and 3. The control group mice did not exhibit isolation behavior, but the groups demonstrated a gradual rise in isolation behavior, particularly in groups 2 and 3. In the fourth week, an abrupt separation of all the animals belonging to group 3 was noted.

Morphology

The animals exhibited incremental differences concerning several morphological changes across the duration of exposure to CBNPs, as depicted in Fig. 1 and Table 2. The mean weight of animals across all groups ranged from approximately 22 to 28 g. An

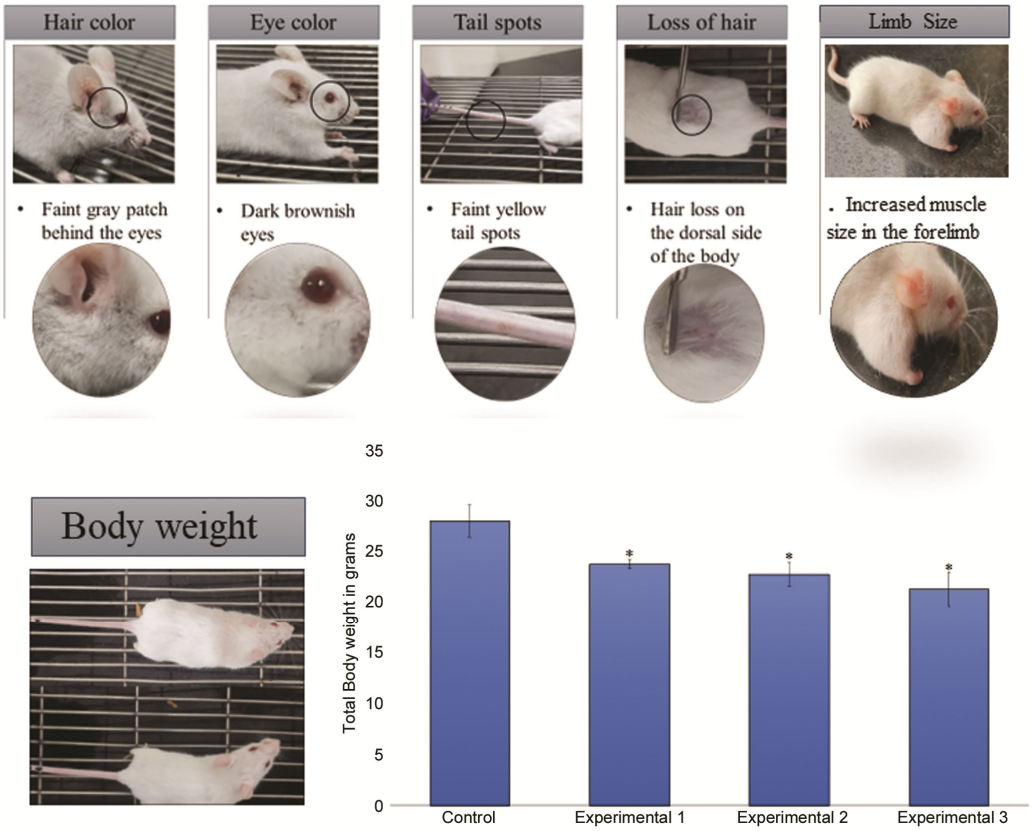

Fig. 1 — CBNPs effect on morphology of mice

ongoing upward trend in the weight of the Control group was noted, reaching a maximum of 35 g. Nevertheless, it was observed that the experimental groups saw a significant reduction in body weight, amounting to 60%. Notably, Groups 2 and 3 exhibited a particularly pronounced decline in body weight during the final week of the study. The animals exhibited a uniform presence of pure white hair throughout the experiment, except Group 3. In this group, a slight gray colouration was observed behind the eyes of the mice, while no changes in hair texture were recorded. All mice had ocular pigmentation characterized by red coloured eyes. However, a progressive transition from red to a shade of reddish brown was noted in animals belonging to Groups 2 and 3. At the onset of the exposure period, the animals exhibited typical hair consistency. This characteristic remained unaltered in the Control group and Group 1. Conversely, Groups 2 and 3 displayed a progressive loss of hair over time. The regions that exhibited the most pronounced hair loss were the dorsal side, neck region, and belly region. Notably, Group 3 displayed conspicuous hair loss in patchy patterns. An anomalous alteration in the muscular dimensions of the forelimbs was noted in the mice belonging to Groups 2 and 3, wherein a progressive augmentation in inflammation was noticed throughout the exposure.

Histology

The study examined the liver and kidney structures of mice. The control group had a typical liver structure with intact cords and sinusoids. Group 1 had hepatic steatosis, characterized by acute inflammation and increased vesicles. Group 2 showed alterations in sinusoidal morphology, inflammatory cell aggregation, and hepatocyte structural modifications. Group 3 showed multiple necrotic foci, hemorrhage, edema, bi-nucleated cells, activated kupffer cells vesicles, distorted hepatocytes, and inflammatory cell aggregates (Fig. 2). Kidney tissue showed normal glomerulus and renal tubules, while Group 1 had distorted glomeruli and damaged tubular epithelium. Group 2 also showed inflammatory cells surrounding distorted glomeruli and necrotic foci supplementing damaged tubular epithelium. Group 3 also showed distorted glomeruli surrounded by inflammatory cells and distorted tubular epithelium (Fig. 3).

Biochemical analysis of liver

The study found that CBNPs exposure significantly increased the concentration of total carbohydrates

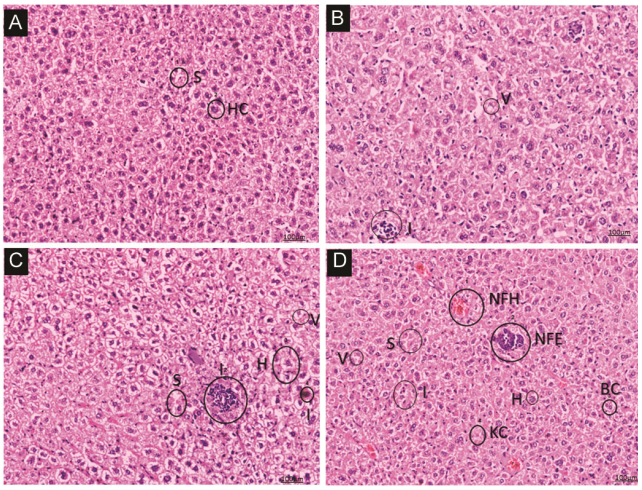

Fig. 2 — CBNPs effect on histopathology of the Liver (40×) A-Control mouse liver section showing normal hepatic architecture with normal (HC) hepatic cords and (S) sinusoids. B-Exp. 1 characterized by steatosis with (I) acute inflammation and (V) an increase in vesicles. C- Exp. 2 indicates (S) sinusoid changes (I) some aggregation of inflammatory cells (H) hepatocyte structural changes, (V) an increase in vesicles, and (I) inflammation. D- Exp. 3 depicts (NFH) multi necrotic foci filled with hemorrhage, (NFE) necrotic foci filled with edema and surrounded by inflammatory cells, (BC) bi-nucleated cells (KC) activated kupffer cells along with (S) sinusoid changes (I) aggregation of inflammatory cells (H) hepatocyte structural changes, (V) an increase in vesicles and (I) inflammation.

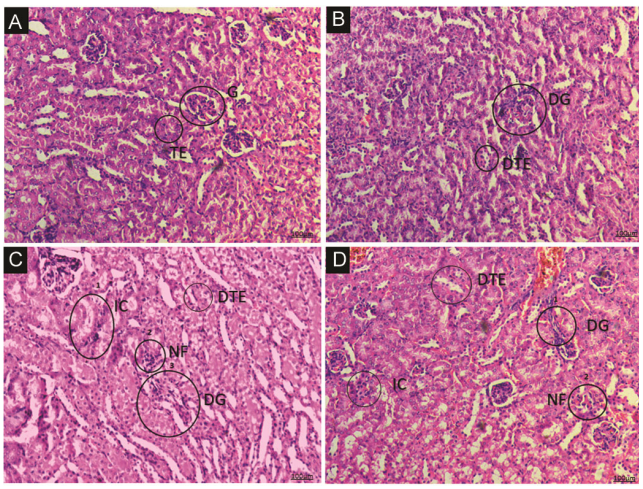

Fig. 3 — CBNPs effect on histopathology of the Kidney (40×) A- Control mouse kidney section showing (G) normal glomerulus and (TE) renal tubules with intact epithelium. B-Exp. characterized by (DG) distorted glomeruli and (DTE) damaged tubular epithelium. C- Exp. 2 shows (IC) inflammatory cells surrounding distorted glomeruli, (NF) necrotic foci in addition to (DG) distorted glomeruli, and (DTE) damaged tubular epithelium. D- Exp. 3 depicts (IC) excessive aggregation of inflammatory cells, (DG) distorted glomeruli surrounded by inflammatory cells along with (DTE) damaged tubular epithelium and (NF) necrotic foci.

(F=0.4046, *P*>0.01), free sugars (F=3,477, *P*<0.05), and protein (F=4.383, *P*<0.05) in Group 2 and 3 mice, with progressive depletion in albumin levels (F=4.7993, *P*<0.05) hence the globulins levels were calculated to elevate by 11%±2 (Fig. 4). A pronounced increase was also evident in total cholesterol (F=5.675, *P*<0.05), triglycerides (F=4.122, *P*<0.05), reduced glutathione (F= 3.071, *P*<0.05), total and conjugated bilirubin concentrations (F=17.87, *P*<0.0001), with conjugated bilirubin showing the highest increase in Group 3 (Fig. 4). Simultaneously, total bilirubin also showed a significantly increased level but its concentration was less in comparison to conjugated bilirubin (F=3.448, *P*<0.05) (Fig. 4). Remarkable changes were also observed in catalase (F=3.486, *P*<0.05), ALP (F=2.329, *P*>0.1), AST (F= 3.971, *P*<0.05), and ALT (F=5.924, *P*<0.01) activity in mice exposed to CBNPs, with a decrease in CBNPs exposed mice, and an increase in AST and ALT activity (Fig. 4). On the other hand, AST and ALT activity also increased in the serum (F= 1.1, *P*>0.05; F=5.924, *P*<0.0) (Fig. 4)

Biochemical analysis of kidney

CBNPs exposure led to significant decreases in total carbohydrates (F=3.305, *P*<0.05) in the kidneys, increased free sugar concentrations (F=3.299, *P*< 0.05), elevated total proteins levels (F=1.333, *P*>0.05), and restrained declines in albumin (F=0.8296, *P*>0.05) in the experimental group mice (Fig. 5). Cholesterol levels increased significantly in all groups (F=4.691, *P*<0.05), with triglyceride concentrations up to twofold in the 2nd and 3rd Groups (F=4.553, *P*<0.05), and reduced glutathione levels were increased dose-dependently (F=8.956, *P*<0.001) (Fig. 5). Total and conjugated bilirubin concentrations altered significantly with conjugated bilirubin showing the most significant increase (F=7.403, *P*<0.001; F=3.343, *P*<0.05) (Fig. 5). Furthermore, significant decrease in catalase activity in mice's kidneys (F=4.973, *P*<0.05), declines in ALP activity (F=4.929, *P*<0.05), and increased levels of AST (F=3.786, *P*<0.05) and ALT in mice exposed to CBNPs were seen, with the 3rd Group showing twofold more significant differences (F=4.924, *P*<0.05) (Fig. 5). Significant increases were also seen in urea concentration in CBNPs exposed mice, while serum and urine creatinine levels varied significantly, with serum creatinine concentrations notably higher than urine (F=5.262, *P*<0.01; F=9.514, *P*<0.001) (Fig. 5).

Discussion

CBNPs increase the susceptibility of disorders due to their unique property of forming agglomerates attributing to high interaction between the particles[4].

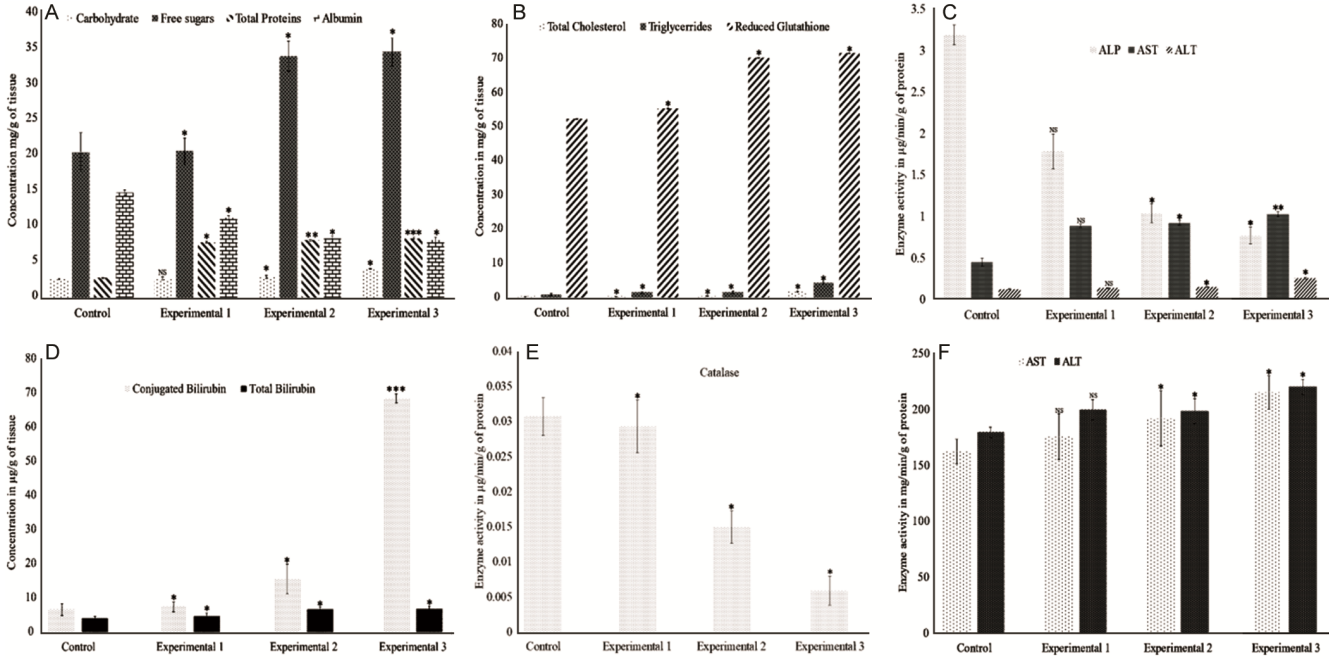

Fig. 4 — Effect of CBNPs on A-Carbohydrates, Free sugars, Total Protein and Albumin, B-Total cholesterol, Triglycerides and Reduced Glutathione, C-Conjugated Bilirubin and Total Bilirubin, D-ALP, AST and ALT, E- Catalase content of liver and F-AST and ALT of serum. * ($P \leq 0.05$); ** ($P \leq 0.01$); *** ($P \leq 0.001$)

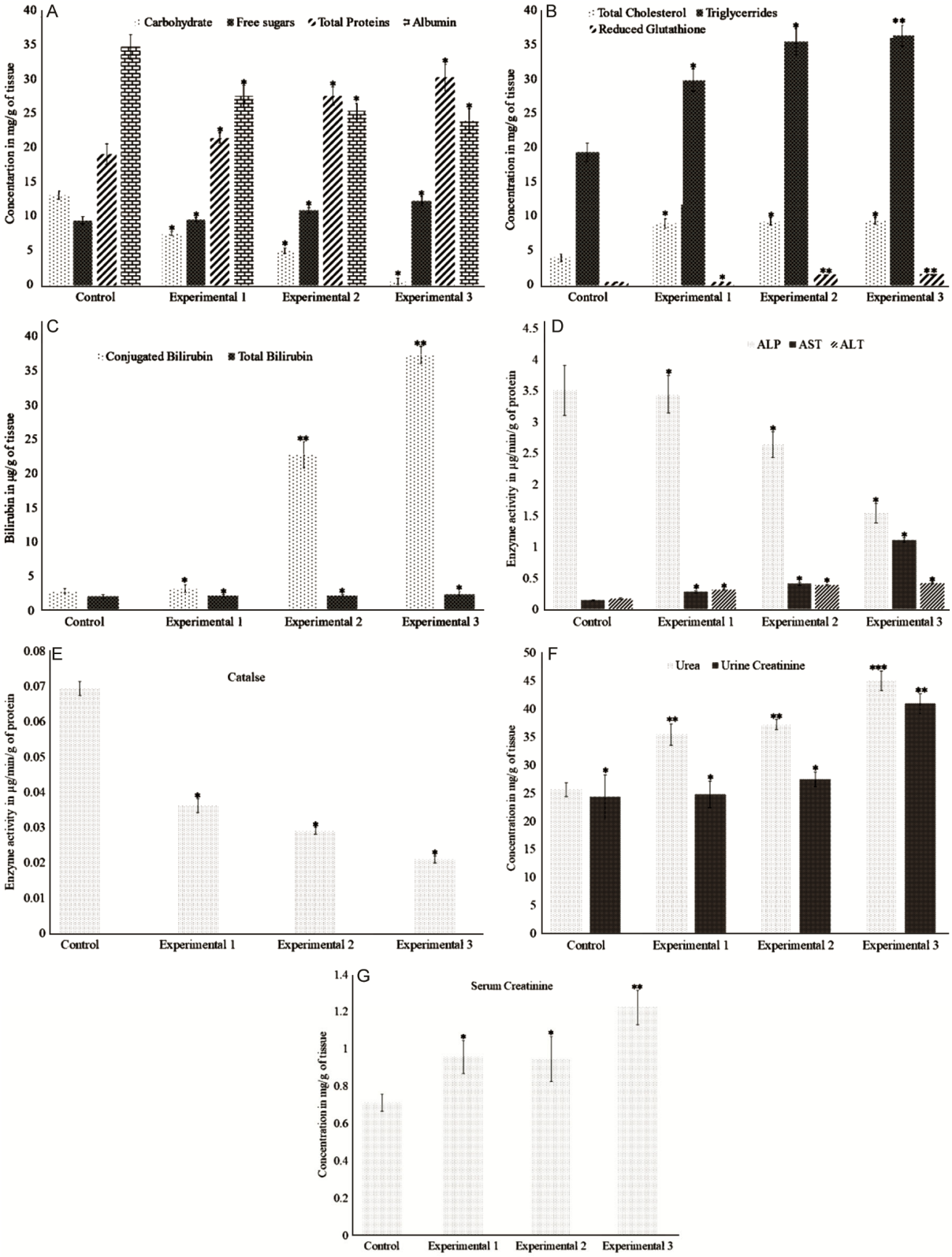

Fig. 5 — Effect of CBNPs on A-Carbohydrates, Free sugars, Total Protein and Albumin, B-Total cholesterol, Triglycerides and Reduced Glutathione, C-Conjugated Bilirubin and Total Bilirubin, D-ALP, AST, and ALT, E-Catalase, F-Urea, Urine Creatinine, and G-Serum Creatinine content of mice kidney. * ($P \leq 0.05$); ** ($P \leq 0.01$); *** ($P \leq 0.001$)

The results of the present study investigated the toxicity of CBNPs on different parameters associated with morphology, behavior, anatomy, and hepatic and renal functioning. This study is the first to reveal the detrimental effect of chronically exposed CBNPs through oral routes, highlighting the potential interactions with animals and the potential consequences for humans, as most studies focused on acute periods.

The study revealed gradual behavioral changes in mice during exposure to CBNPs, including increased tail rolling, hyperactivity, isolation, and decreased feeding frequency. These changes may be linked to hormonal changes and potential CNS toxicity[15]. Subsequently, hyperactivity was evident through biting around the tail region, which could be due to the effect of CBNPs on neuropeptides modulating stress response[16]. Isolation behavior of mice was seen which may be a result of increased expression of inflammatory markers due to CBNPs, keeping in par with the behavioral studies in mice due to prolonged silver nanoparticle exposure[17].

Morphology gives a primary outlook on health since change is visible to the naked eye determining the underlying variations in the body. The study found that mice exposed to CBNPs experienced weight loss, reduced food intake, and may be linked to chronic diseases like cancer also the highest dose resulted in faint grayish patches, possibly due to CBNPs drainage to the epidermis similar to the results explained by Saweres[18]. Eye colour underwent a significant change from red to dark brown. The eye colour variations were evident with increased eye redness and irritation which can be attributed to cellular toxicity caused by CBNPs affecting either the lens, retina, or even optic nerves[19]. The mice's tail, clear with blood vessels, showed faint yellowish spots post-CBNPs exposure, indicating potential blood infection symptoms that might have led to tail lesions[20]. Hair is one of the most important parts of the body derived from the ectoderm and acts as a protective entity. The exposure studies of CBNPs signified mice undergoing hair loss that can be due to inflammation in dermal cells leading to wounds, furthermore similar results were also seen in oral toxicity caused by acute exposures to drugs and metals like mercury[21] whereas hair texture was noted to be smooth without any variations.

Histological examinations identify cytoarchitecture in tissues and organs, detecting changes caused by toxicants. The liver is crucial for substance accumulation due to its role in toxin breakdown and hepatocyte oxidation, making it vulnerable to nanoparticle damage and inflammation. The liver's histopathology revealed structural changes, inflammatory cell aggregation, and necrotic foci. These changes were primarily due to NPs interacting with hepatic tissue proteins and enzymes, generating reactive oxygen species and disrupting antioxidant defense, leading to hepatocyte apoptosis or necrosis[22]. The study supports the findings that liver damage is a result of CBNPs toxicity, as evidenced by similar histopathological findings in mice livers exposed to ZnO nanoparticles[22].

The kidney, responsible for excretion and metabolism, is damaged by CBNPs exposed mice, leading to pathological distortions such as inflammatory cells and minor epithelium changes. These can be due to membrane damage, oxidative stress, mitochondrial injury, cytoskeletal changes, apoptosis, and necrosis[22]. These results are also supported by studies of mice exposed to ZnO NPs[22].

The liver function test findings suggest that the principal consequences of CBNPs toxicity are likely in our present investigation. The total carbohydrate was raised, indicating changes in carbohydrate metabolism, which may be attributed to altered lipogenesis[23] or enhanced metabolism owing to reported hyperactivity in the mice. The study by Sim[24] found a negative link between lipogenesis and triglyceride accumulation. This correlation supports the idea that the failure of adipose tissue to metabolize glucose conversion leads to an increase in free sugar concentrations. This may also result from enhanced glucose absorption in the liver to synchronize with the stress induced by the inflammatory alterations caused by CBNPs.

The liver is the central hub for the production of albumin and a primary contributor to the synthesis of most proteins. The elevated concentrations of total protein exhibited a positive correlation with the heightened levels of globulin, leading to a subsequent reduction in albumin levels. This phenomenon might be attributed to the suppression of enzymes that facilitate the breakdown of globulins, and the limited excretion of hormones that regulate protein metabolism, which is caused by the impact of CBNPs[25]. Several studies have shown a correlation between liver dysfunction and an increase in protein content, namely in the liver[26].

The liver has a crucial function in the process of cholesterol metabolism. The increase in total

cholesterol was statistically significant, consistent with the findings of Aberare[27], who observed elevated total cholesterol levels in rats exposed to petrol and kerosene fumes. This variation demonstrates a distinct correlation between the administration of CBNPs by oral gavaging and its impact on lipid metabolism. The occurrence of cholesterol buildup in liver hepatocytes may be attributed to oxidative stress and endoplasmic reticulum stress induced by CBNPs. The existence of Kupffer cells effectively eliminates the possibility of cholesterol buildup in liver tissue[28].

Glutathione (GSH) is an antioxidant that reduces peroxides in enzymatic processes. Exposure to CBNPs leads to a rise in GSH concentration, potentially causing tumor growth in liver cells due to enhanced antioxidant capacity and resistance to oxidative stress, which may also indicate the onset of tumor growth[29]. The study also found a correlation between increased levels of total and conjugated bilirubin, highlighting the effects of CBNPs on liver functioning. Disruption of sinusoids impairs bilirubin excretion, increasing cholesterol and intrahepatic cholestasis risk[30].

Four enzymatic estimates, namely catalase, ALP, AST, and ALT, were conducted to assess liver functioning. The study found a significant decrease in catalase activity, a biomarker of liver oxidation-reduction equilibrium, due to the interaction of catalase molecules with CBNPs, leading to the overproduction of radicles which in turn has inhibitory effects on the catalase enzyme and its antioxidant properties, thus stating its relation with tissue damage and inflammation and hence also obliging to the results on Ultrafine Carbon Black (UCB) explained by Zhang[31]. High doses of CBNPs negatively affected liver function, leading to a significant decrease in ALP activity due to cell damage and inflammation thus promoting its loss[32].

AST and ALT are the markers of hepatocellular injury. The significant increase in AST and ALT enzyme activity in liver tissue and serum, suggests a potential risk of liver cirrhosis or chronic hepatitis due to inflammation and cell necrosis caused by increased cell permeability or damage *via* CBNPs[33].

The kidney is regarded as the secondary organ that is most affected by the toxicity of NPs. Since the kidney is responsible for excretion, it is anticipated that NPs would have a negative impact on the renal system. The renal function tests revealed a correlation with the previously mentioned liver functions. The CBNPs treated animals exhibited a large drop in total carbohydrate content, but free sugars showed a substantial rise. This indicates a correlation between experimental groups and a change in their energy-yielding mechanism, resulting in weight loss. This finding is also supported by Mieczkowska[34] suggesting that the inflammation generated by CBNPs may also contribute to the development of chronic renal disorders. The correlation between total proteins and albumin levels was negative, likely as a result of renal injury induced by CBNPs. These particles raised glomerular pressure and impacted the tubular epithelium, leading to a drop in albumin levels owing to increased excretion of albumin-derived substances impairing kidney function[35]

CBNPs markedly elevated cholesterol and triglyceride levels in renal tissue, resulting in renal impairment and inflammation. The buildup of CBNPs in the liver tissue is associated with the development of chronic renal disorders[36].

A significant rise in GSH may be attributed to cellular damage caused by CBNPs. This damage might lead to an increase in glutathione monoethyl ester, a protective agent against cellular damage. When combined with GSH, this can result in renal failure[37]. As a result, there was a notable rise in both total and conjugated bilirubin levels in the mice that were administered CBNPs orally. Wen[38] observed similar results in rats that were treated with silver NPs. The accumulation of CBNPs in tissue may directly impact renal functioning, while the liver's inability to metabolize bilirubin results in its outflow into the kidney.

Enzyme studies carried out in the liver were also undertaken for the kidney. Interestingly, the findings from both organs showed a correlation with each other. The results of our research indicate a decrease in catalase activity, which is significantly influenced by the toxicity of CBNPs. This toxicity reduces the ability of CBNPs to effectively remove hydrogen peroxide. This phenomenon may arise from the excessive synthesis and buildup of hydrogen peroxide caused by CBNPs, leading to stressful situations and directly impacting renal processes. These findings align with the research conducted by Balas[39]. Similarly, the activity of both AST and ALT exhibited a substantial rise, with AST demonstrating a more pronounced difference in comparison. The elevated levels indicate renal impairment caused by the impact of CBNPs. This might be attributed to altered

metabolism resulting from renal damage induced by inflammation. Research also elucidates the fundamental correlation between the diminished activity of these enzymes and chronic renal disorders[40].

Urea and creatinine play a vital role in the functioning of the kidneys since they are responsible for eliminating 80-90% of non-protein nitrogenous waste. CBNPs can elevate urea levels, which may have impacted the metabolism causing a buildup of proteins. Glomerular damage leads to a reduction in filtration rate, which in turn affects the filtration of creatinine. Evidence from toxicity studies indicates that elevated levels of urea and creatinine are indicative of renal function deterioration. Previous research has documented comparable investigations conducted on mice that were administered ZnO NPs[22].

The outcomes of this research avenues the damaging effects of CBNPs which are exposed daily to humans *via* newspapers, inks, plastic wraps, plastic pipes, industrial bags, tires, ink tattoos, *etc*. The current study demonstrated how CBNPs cause toxicity in the mice physiology specifically in liver and kidney tissue along with effects on their behavior, morphology, and anatomy. Thus, this is the first report that serves as supplementary knowledge in relating the CBNPs toxicity in humans not only *via* oral route but also *via* intravenous, subcutaneous, intraperitoneal, and intratracheal exposure. Hence the exposure of these nanoparticles *via* different processes during the manufacturing, handling, and disposal of CBNPs products can induce toxic effects in humans leading to detrimental diseases and in the bargain also having the potential to cause bioaccumulation on their entry into the waterbodies. In due course, this research will be an eye-opener for all the people who often encounter CBNPs but are unaware of the possible hazardous effects associated with them.

Conclusion

CBNPs can remarkably alter the behavior, morphology, and anatomy of the mice. These NPs can alter the biochemical parameters changing the enzyme activities and metabolite concentrations in turn affecting normal renal and kidney functioning. This study has eventually facilitated the correlation of its impact on human physiology which fosters the desire to implement the necessary preventative measures that will decrease the direct and indirect exposure to CBNPs to people by also maintaining control over the quantitative indirect exposure via various goods.

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Conflict of interest

The authors declare that there is no conflict of interest.

References

1 Zhang R, Dai Y, Zhang X, Niu Y, Meng T, Li Y, Reduced pulmonary function and increased pro-inflammatory cytokines in nanoscale carbon black-exposed workers. *Part Fibre Toxicol*, 11 (2014) 73.
2 Zhang R, Zhang X, Gao S, Liu R, Assessing the in vitro and in vivo toxicity of ultrafine carbon black to mouse liver. *Sci Total Environ*, 655 (2019) 1334.
3 Tang Q, Tu B, Jiang X, Zhang J, Bai L, Meng P, Exposure to carbon black nanoparticles during pregnancy aggravates lipopolysaccharide-induced lung injury in offspring: an intergenerational effect. *Am J Physiol Lung Cell Mol Physiol*, 321 (2021) 900.
4 Chaudhuri I, Fruijtier-Pölloth C, Ngiewih Y, Levy L, Evaluating the evidence on genotoxicity and reproductive toxicity of carbon black: a critical review. Critical Reviews in Toxicology. *Taylor and Francis Ltd*, 48 (2018) 143.
5 Teng C, Jiang C, Gao S, Liu X, Zhai S, Fetotoxicity of Nanoparticles: Causes and Mechanisms. *Nanomaterials*, 11 (2021) 791.
6 Di Ianni E, Erdem JS, Møller P, Sahlgren NM, Poulsen SS, Knudsen KB, In vitro-in vivo correlations of pulmonary inflammogenicity and genotoxicity of MWCNT. *Part Fibre Toxicol*, 18 (2021) 25.
7 Wei X, Liu N, Feng Y, Wang H, Han W, Zhuang M, Competitive-like binding between carbon black and CTNNB1 to ΔNp63 interpreting the abnormal respiratory epithelial repair after injury. *Sci Total Environ*, 929 (2024) 172652.
8 Pei Z, Ning J, Zhang N, Zhang X, Zhang H, Zhang R, Genetic instability of lung induced by carbon black nanoparticles is related with Plk1 signals changes. *NanoImpact*, 26 (2022) 100400.
9 Akarsu SA, Ömür AD, Nanoparticles as Food Additives and their Possible Effects on Male Reproductive Systems in *Nanotechnology in Reproduction* (Özgür Publications, Şehitkamil/Gaziantep), 2023, 13.
10 Onoda A, Umezawa M, Takeda K, Ihara T, Sugamata M, Effects of Maternal Exposure to Ultrafine Carbon Black on Brain Perivascular Macrophages and Surrounding Astrocytes in Offspring Mice. *PLoS One*, 9 (2014) 94336.
11 Ortiz SR, Field MS, Sucrose Intake Elevates Erythritol in Plasma and Urine in Male Mice. *J Nutr*, 175 (2023) 1889.
12 Pandey S, Goswami SK, Jain BP, Carbohydrates in *Protocols in Biochemistry and Clinical Biochemistry* (Academic Press, India), 2020, 5.
13 Kondaparthi P, Deore M, Naqvi S, Flora SJS, Dose-dependent hepatic toxicity and oxidative stress on exposure to nano and bulk selenium in mice. *Environ Sci Pollut Res*, 28 (2021) 53034.
14 Gogoi N, Gogoi A, Neog B, Singh KD, Hepatoprotective effect of Garcinia xanthochymus and Garcinia lanceifolia

against carbon tetrachloride-induced hepatic damage in rat. ||| *Bangladesh J Pharmacol*,18 (2023) 91.
15 Zarzoliani SR, Mohapatra SS, Sinha A, A review on toxicity induced by the insecticide indoxacarb. *Pharma Innovation*, 12 (2023) 2730.
16 Reichmann F, Holzer P, Neuropeptide Y: A stressful review. *Neuropeptides*, 55 (2016) 99.
17 Antsiferova A, Kopaeva M, Kashkarov P, Effects of Prolonged Silver Nanoparticle Exposure on the Contextual Cognition and Behavior of Mammals. *Materials*, 11 (2018) 558.
18 Saweres-Argüelles C, Ramírez-Novillo I, Vergara-Barberán M, Carrasco-Correa EJ, Lerma-García MJ, Simó-Alfonso EF, Skin absorption of inorganic nanoparticles and their toxicity: A review. *Eur J Pharm Biopharm*, 182 (2023) 128.
19 Zhu S, Gong L, Li Y, Xu H, Gu Z, Zhao Y, Safety Assessment of Nanomaterials to Eyes: An Important but Neglected Issue. *Adv Sci*, 6 (2019) 1802289.
20 National Research Council (US) Committee on Infectious Diseases of Mice and Rats, Skin and Joints in *Infectious Diseases of Mice and Rats* (National Academies Press, US), 1991, 8.
21 Xiao W, Wang X, Wang C, Wang M, Fei C, Zhang L, Acute and 30-day oral toxicity studies of a novel coccidiostat – ethanamizuril. *Toxicol Res Camb*, 8 (2019) 686.
22 Srivastav AK, Dhiman N, Tiwari R, Arjaria N, Prakash J, Jagdale P, Sub-acute oral exposure of zinc oxide nanoparticles causes alteration in iron homeostasis through acute phase response: A protective effect by surface modification. *J Trace Elem Med Biol*, 52 (2019) 270.
23 Zhou Y, Addai FP, Zhang X, Liu Y, Wang Y, Lin F, Heavy metal-induced lipogenic gene aberration, lipid dysregulation and obesogenic effect: a review. *Environ Chem Lett*, 20 (2022) 1611.
24 Sim WC, Lee W, Sim H, Lee KY, Jung SH, Choi YJ, Downregulation of PHGDH expression and hepatic serine level contribute to the development of fatty liver disease. *Metabolism*, 102 (2020) 154000.
25 Murray RK, Plasma Proteins & Immunoglobulins in *Harper's illustrated biochemistry* (McGraw-Hill, USA), 2003, 693.
26 Sagar K, Vidyasagar GM, Evaluation of acute and sub acute toxicities of leaf extract of Caesalpinia bonducella (L.) flem. *Int J Pharma Bio Sci*, 1 (2010) 1.
27 Aberare O, Okuonghae P, Mukoro N, Dirisu J, Osazuwa F, Odigie E, Triglycerides, total cholesterol, high density lipoprotein cholesterol and low density lipoprotein cholesterol in rats exposed to premium motor spirit fumes. *N Am J Med Sci*, 3 (2011) 277.
28 Aberare O, Okuonghae P, Mukoro N, Dirisu J, Osazuwa F, Odigie E, Triglycerides, total cholesterol, high density lipoprotein cholesterol and low density lipoprotein cholesterol in rats exposed to premium motor spirit fumes. *N Am J Med Sci*, 3 (2011) 277.
29 Ballatori N, Krance SM, Notenboom S, Shi S, Tieu K, Hammond CL, Glutathione dysregulation and the etiology and progression of human diseases. *Bchm*, 390 (2009) 191.
30 Thayer V, Gogolski S, Felten S, Hartmann K, Kennedy M, Olah GA, AAFP/EveryCat Feline Infectious Peritonitis Diagnosis Guidelines. *J Feline Med Surg*, 24 (2022) 905.
31 Zhang R, Wu Q, Liu R, Characterizing the binding interaction between ultrafine carbon black (UFCB) and catalase: electron microscopy and spectroscopic analysis. *RSC Adv*, 7 (2017) 42549.
32 Goltzman D, Miao D, Alkaline Phosphatase in *Encyclopedia of Endocrine Diseases* (Academic Press, US) 2004, 164.
33 Ozer J, Ratner M, Shaw M, Bailey W, Schomaker S, The current state of serum biomarkers of hepatotoxicity. *Toxicol*, 245 (2008) 194.
34 Mieczkowska A, Nguyen V, Smulikowska S, Effect of dietary fat on fatty acid composition of lipids from breast muscle and abdominal fat of broiler chickens. *J Anim Feed Sci*,10 (2001) 279.
35 Koltun M, Nikolovski J, Strong K, Nikolic-Paterson D, Comper WD, Mechanism of hypoalbuminemia in rodents. *Am J Physiol Heart Circ Physiol*, 288 (2005) 1604.
36 Honzumi S, Takeuchi M, Kurihara M, Fujiyoshi M, Uchida M, Watanabe K, The effect of cholesterol overload on mouse kidney and kidney-derived cells. *Ren Fail*, 40 (2018) 43.
37 Scaduto RC, Gattone VH, Grotyohann LW, Wertz J, Martin LF, Effect of an altered glutathione content on renal ischemic injury. *Am J Physiol Renal Physiol*, 255 (1988) 911.
38 Wen H, Dan M, Yang Y, Lyu J, Shao A, Cheng X, Acute toxicity and genotoxicity of silver nanoparticle in rats. *PLoS One*, 12 (2017) 185554.
39 Balas M, Popescu Din IM, Hermenean A, Cinteza LO, Dinischiotu A, Exposure to Iron Oxide Nanoparticles Coated with Phospholipid-Based Polymeric Micelles Induces Renal Transitory Biochemical and Histopathological Changes in Mice. *Materials*, 14 (2021) 2605.
40 Liberato I, Lopes E, Cavalcante M, Pinto T, Moura I, Loureiro-Jr L, Liver enzymes in patients with chronic kidney disease undergoing peritoneal dialysis and hemodialysis. *Clinics*, 67 (2012) 131.