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TOXICS COMPARISON OF GRAPHENE NANO SHEETS AND CARBON NANOTUBES EXPLORING HEPATO-INTESTINAL AND HEMATOLOGICAL DISPARITIES IN CIRRHINUS MRIGALA

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Abstract

This study was conducted to explore the comparative toxicity exploration of graphenenano sheets and carbon nanotubes in fish Cirrhinusmrigala. The experiment was performed in Department of Zoology, University of Education, Lahore, Jauharabad campus. The experimental fish were obtained from chashma fish hatchary and transported to experimental site. The experiment was started after the division of fish in seven different groups (n=70) named as C, GNS-I, GNS-II, GNS-III, CNT-I, CNT-II and CNT-III. Group C was control group while group GNS-I, GNS-II and GNS-III were treated waterborne with 40.45, 60.58 and 121.37 mg/L of graphenenano tubes. Group CNT-I, CNT-II and CNT-III were treated orally with 10.92, 16.38 and 32.76 mg/kg of carbon nano tubes. The hematological results reveal that hemoglobin, erythrocytes count, pack cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and lymphocytes were significantly ($p<0.05$) decreased while mean corpuscular volume, leucocyte count, neutrophils and monocytes were significantly increased in both graphenenanosheets and carbon nanotubes exposed fish.The liver was reported with histological abnormalities such as Pyknotic nuclei, Atrophy, Acute cellular swelling, melanomacrophage centers, hepatic vacuolation, sinusoid dilation, hydropic degeneration, necrosis and perivascular degeneration. In the intestine intestinal damage, collapsed epithelial layer, intestinal interior architecture damage, atrophy, bigger intercellular gaps, and degenerated goblet cells were reported. This study concludes that graphenenano sheets and carbon nano tubes are toxic at different doses in correlation to the route of exposure.

Keywords: Graphenenano sheets, Carbon nanotubes, Fish Cirrhinusmrigala, Toxicity exploration, Hematological results

Introduction

Graphene is made up of carbon atoms that are arranged in a hexagonal lattice (Obodo et al., 2019). It has been emerged as a material with extraordinary properties, driving it into exploration of technologicala dvancements (Dhinakaran et al., 2020). Graphene has extraordinary strength, electrical conductivity, and thermal conductivity with a wide range of applications that garnered significant attention exploring its diverse uses in various fields (Kong et al., 2023).Graphenenano sheets have been extensively investigated, leading to a deeper understanding of its potential applications. Particle size affect distribution of graphenenano sheets across different domains(Amaro-Gahete et al., 2019).Graphenenano sheets are use as electromagnetic interference (EMI) shielding(Wei et al., 2020), energy storage (Wei et al., 2019),environmental applications such as soluble graphenenano sheets for sunlight-induced photo degradation of dye mixtures, emphasizing the potential for real-life applications in photocatalysis and environmental remediation (Gunture et al., 2019), electro chemical sensors, (Dou et al., 2019) such as electro chemical detection with potential clinical applications, enhancement of thermal conductivity (Yang et al., 2019), In the realm of therapeutical applications, graphene-based nanomaterials are making significant strides such as their use in in tissue engineering and regenerative medicine(Orsu and Koyyada2020). In the flow of these wide range applications graphene-based materials continue to captivate researchers(Tiwari et al., 2020). According to above applications graphene is a gateway to scientific community due to its dynamic and evolving nature.

Carbon nanoparticles have emerged as a class of materials with diverse applications. Now a days Carbon Nano Tubes (CNTs) are synthesized through chemical, physical, and green Methods. CNTs are tubular elongated structure with diameters ranging from 1 to 2 nm, underscores their unique characteristics (Rathinavel et al., 2021). Synthesis and classification of carbon nanotubes highlights their diverse applications, setting the stage for a deeper exploration (Ijaz et al., 2020). The wider applications of carbon nano tubes includes construction materials (Mohajerani et al., 2019) showcasing the potential for enhanced performance and functionality, Supercapacitor (Hussain et al., 2020), catalytic applications that opens avenues for the utilization of carbon nanoparticles in catalysis (Narayan et al., 2019), electrochemical sensing (Asadian et al., 2019) to develop advanced sensing devices for various applications, thermal energy storage and cooling applications (Wong-Pinto et al., 2020), agriculture, addressing gaps in the use of nanotechnology for crop improvement and pest management (Singh et al., 2021), fabrication of metal nanoparticles through environmentally friendly approaches (Siddiqi and Husen 2020). These nanomaterials hold significant promise in shaping the future of various industries.

Nanoscale materials find their way into diverse ecosystems, concerns have arisen regarding their potential environmental impact, particularly on aquatic organisms (Farré et al., 2009). fish, being integral components of aquatic food chains, are of particular interest in understanding the toxicity of graphenenano sheets (Dasmahapatra et al., 2019).The toxicity studies have encompassed various fish species, with a focus on notable models such as zebrafish (Daniorerio) and blackfish (Capoetafusca) (Malhotra et al., 2020; Sayadi et al., 2021). Nanomaterials impact on fish at the cellular level (Siqueira et al., 2022). Grapheneoxide induces lipid peroxidation at the mitochondrial level and redox modulation in freshwater fish (Anabas testudineus) (Paital et al., 2019). Among the organisms vulnerable to carbon nanotubes toxicity, fish have emerged as crucial subjects of study due to their ecological significance and susceptibility to environmental changes (Chowdhry et al., 2019). Researchers have explore the toxicity of carbon nanotubes in fish through diverse experimental approaches and model systems (Silva et al., 2021).Cirrhinus mrigala, commonly known as Mrigal, has emerged as a prominent model organism in the study of fish toxicity due to its relevance in assessing the impact of various environmental stressors (Ramesh et al., 2021)

Materials and Methods

Experimental Site:

This study was conducted at the Department of Zoology, University of Education, Lahore, Jauharabad campus.The Cirrhinusmrigala, an experimental fish sourced from Chashma Fish Hatchery and transported to the experimental site.

Grouping:

The fish were divided into seven different groups (n=70)

Groups	Doses
Group C:	Control group
Groups GNS-I, GNS-II, and GNS-III:	Treated waterborne with 40.45, 60.58, and 121.37 mg/L of graphenenano sheets, respectively.
Groups CNT-I, CNT-II, and CNT-III:	Treated orally with 10.92, 16.38, and 32.76 mg/kg of carbon nanotubes, respectively.

Toxicant Exposure:

Groups GNS-I, GNS-II, and GNS-III were subjected to different concentrations of graphenenano sheets in their aquatic environment.

Groups CNT-I, CNT-II, and CNT-III were orally administered different concentrations of carbon nanotubes.

Hematological Analysis:

Hematological parameters, including hemoglobin, erythrocyte count, pack cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, leucocyte count, lymphocytes, neutrophils, and monocytes, were assessed. Statistical significance ($p<0.05$) was determined.

Ethical Approval

This experiment was approved from ethical committee on animal experimentation in the Department of Zoology, UE (Jauharabad campus)

Histology

The fish were captured from the experiment tanks with the help of small hand net. Fish were dissected after capture and the necessary organs were separated for histology. Organs were temporarily preserved in 10% formalin. Histology was done according to the standard method following the steps of washing, dehydration, clearing, infiltration, embedding, and microtomy. The sections were again processed for dehydration, clearing, staining, and mounting. The slides were examined through a computer-assisted microscope and sections were observed with magnification power set at 400x.

Results and Discussion

In the current study graphenenano sheets and carbon nanotubes induce hematological changes in fish CirrhinusmrigalaThe hematological results reveal that hemoglobin, erythrocytes count, pack cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and lymphocytes were significantly ($p<0.05$) decreased while mean corpuscular volume, leucocyte count, neutrophils and monocytes were significantly increased. In the previous study conducted by Aguado-Henche et al., 2022 involved intraperitoneal injection of graphene and graphene oxide nanosheets in rats to assess potential changes in blood after 15 and 30 days. Hematological changes involved alterations to the red blood cells, such as microcytosis or higher mean hemoglobin concentrations, changes in prothrombin and thromboplastin leading to longer coagulation times.Sayadiet al., 2023investigated the hematological changes in male mice exposed to graphene oxide nanoparticles (GO NPs). The study involved 48 mice divided into six groups. The results of this study showed that GO NPs at both concentrations (30 and 60 mg/kg/bw) decreased the number of red blood cells (RBC) compared to the control group. Morphological changes in RBC induced by GO NPs were also observed. The studies of Aguado-Henche et al., Sayadi et al., 2023 supports the current study findings related to hematological abnormalities induced by graphenenano sheets. The histological alterations in intestine in current study are intestinal damage, collapsed epithelial layer, intestinal interior architecture damage, atrophy, bigger intercellular gaps, and degenerated goblet cells. Aprevious study by Zheng et al. (2019) reported thatgraphene-family materials (GR, GO, rGO) induce alterations in gut bacterial communities, GFMs altered intestinal morphology, inducing more vacuolation and generating more goblet cells. Another study by Sayadi et al., 2022 explored that multi-layer graphenes (MLGs) in blackfish led to increased acute toxicity and histopathological changes in gills and intestines. The findings of Duo et al., 2022 described that GO exposure led to serious skin and intestinal damage, inhibited earthworm growth, and decreased reproductive capacity. A previous findings of Liu et al., 2021 reported that oral administion of graphene oxide (GO) during gestational days 7–16 in mice led to altered gut microbiome, placenta barrier dysfunction, and adverse pregnancy outcomes. Studies by Sayadi et al., 2021 and Manjunatha et al., 2021 reported that graphenenanosheets (GNs) showed tissue-dependent bioaccumulation and elimination patterns in Blackfish, graphene oxide (GO) induced multi-organ defects in developing zebrafish larvae, affecting the brain, heart, hepatocytes, and mitochondria. These studies are evidences to support the current study about the histological changes in intestine induced by graphenenano sheets.

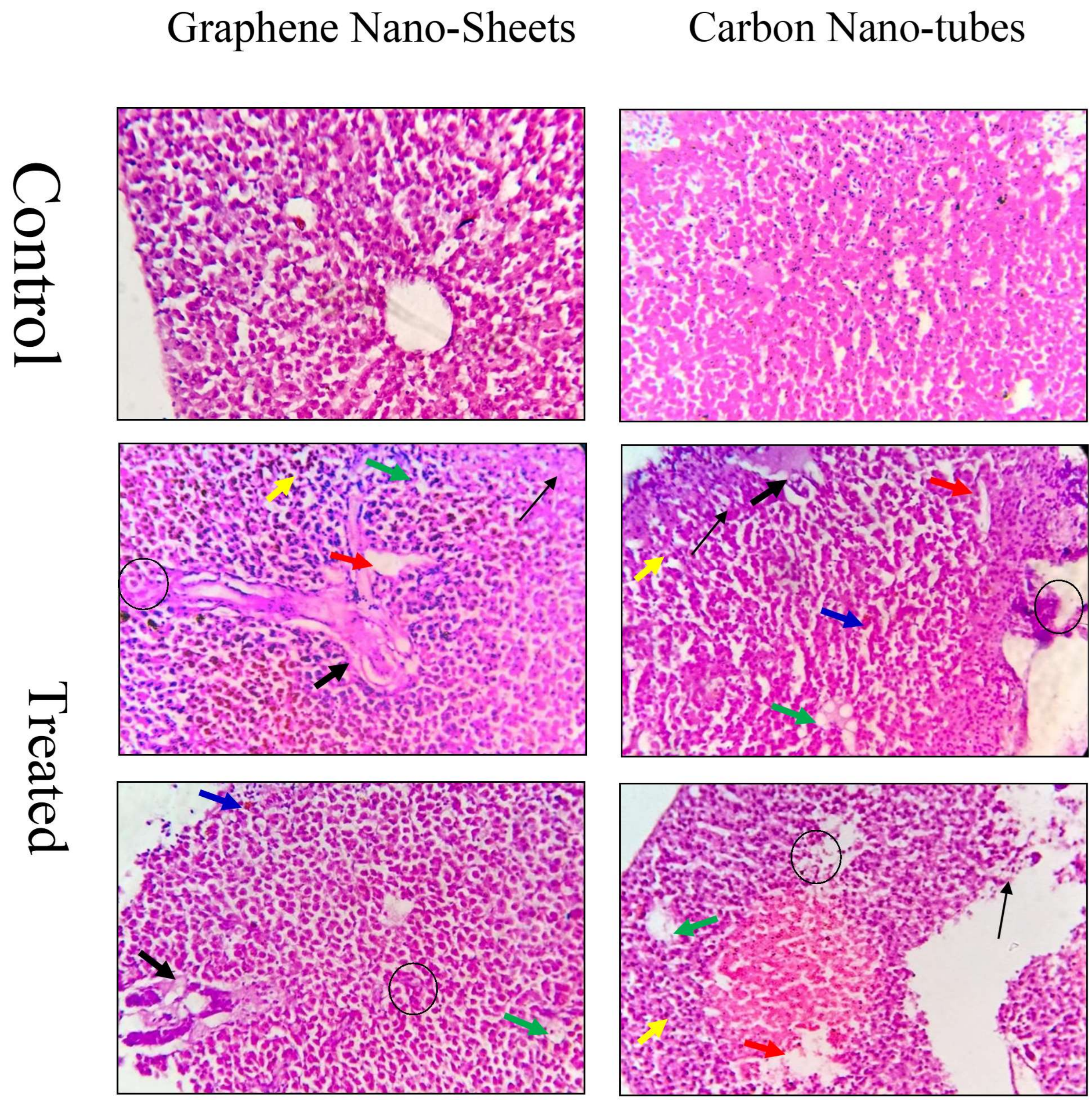
The liver of C. mrigala exposed to graphenenanosheets reported with histological abnormalities such as Pyknotic nuclei, Atrophy, Acute cellular swelling, melanomacrophage centers, hepatic vacuolation, sinusoid dilation, hydropic degeneration, necrosis and perivascular degeneration. A previous study of Nirmal et al., 2021 reported that hepatotoxicity was induced by graphene oxide (GO) in Wistar rats after intraperitoneal administration resulted in dose-dependent toxicity that was marked with histopathological changes in the liver included inflammation, vacuolations, hepatocytic injury, and abnormal hepatic sinusoids. Another study by Rhazouani et al., 2023 found that GO at different doses (2 mg/kg and 5 mg/kg) for five days in male mice reported with liver inflammation.

The present study is reported with severe hematological changes induced by CNTs. Smith et al. (2007) reported thatin Rainbow trout (Oncorhynchus mykiss) carbon nanotubes (CNTs) showed no major disturbances in red and white blood cell counts, hematocrits, whole blood hemoglobin, or plasma Na+ and K+.The study of Tavabe et al. (2020) showed that Common carp (Cyprinuscarpio) was reported with decreased weight gain, specific growth rate, and survival rate with increasing MWCNT concentrations butIncrease in cortisol secretion, blood glucose level, and anti-oxidative enzyme activities with higher MWCNT concentrations. Ma et al. (2017) studied that CNT materials provoke interleukin-6 (IL-6) production and stimulate hepcidin induction, leading to disordered iron homeostasis. The investigations of Bhardwaj and Saxena (2015) in which intravenous administration of AF-SWCNTs results in transient anemia, preferentially affecting older erythrocytes in mice.The accumulation of old erythrocytes in circulation, indicating selective elimination of younger erythrocytes produces toxic effect on older subpopulation of erythrocytes. Park et al., (2011) studied significant changes in blood parameters, such as altered cytokine secretion. Fibrotic histopathological changes observed, indicating an inflammatory response. Another study bySachar and Saxena (2011) reported AF-SWCNTs induce lysis of erythrocytes. In vivo, intravenous administration results in transient anemia, predominantly affecting older erythrocytes. Xie et al. (2017) found that in mice significant changes in hematology and serum biochemistry were reported. Slight oxidative stress observed in RES organs at high dosages at day 7 post-exposure.According to previous studies, current study is in line for the abnormalities produced in blood and blood associated parameters.

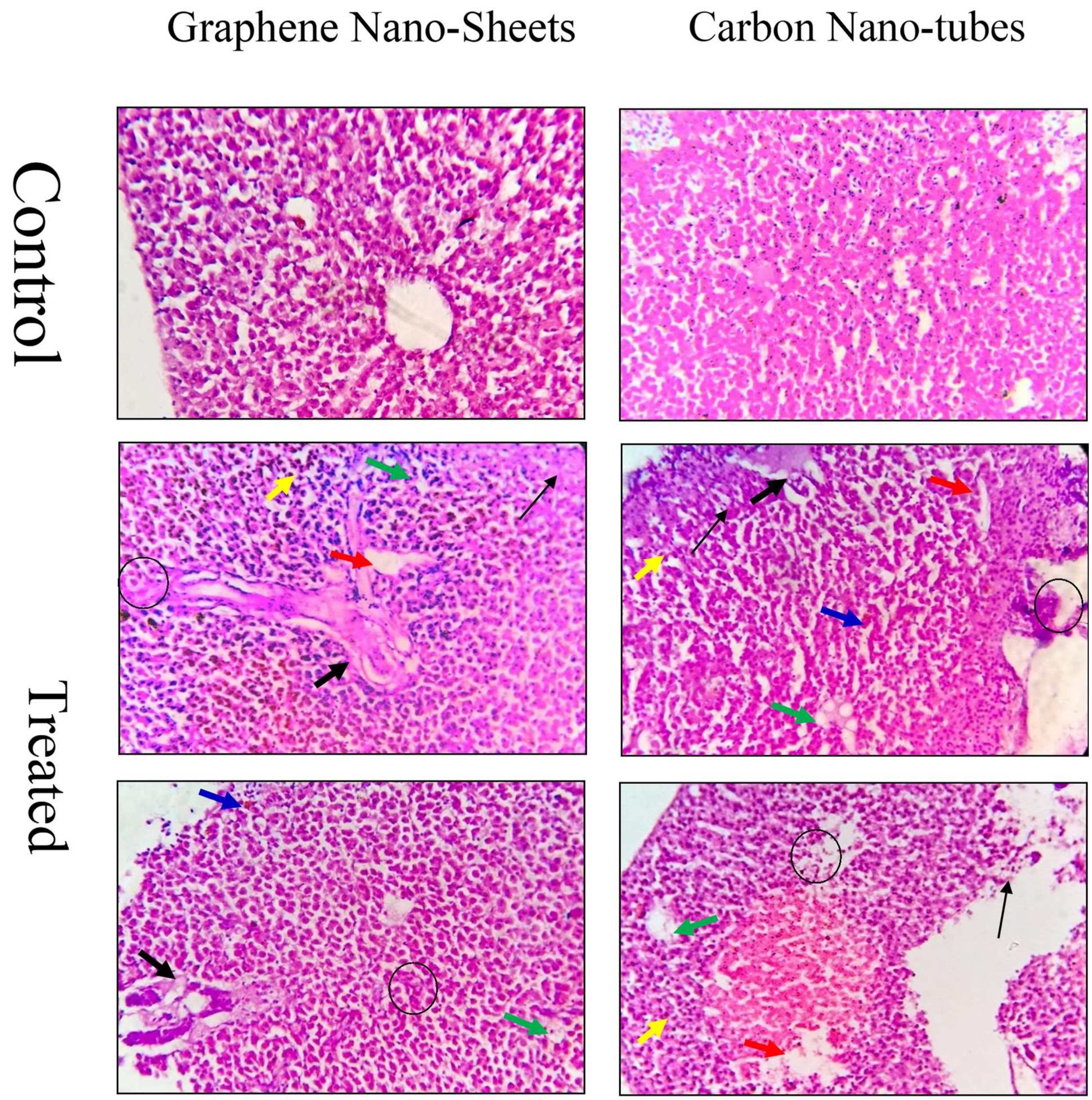
Liver damage in previous studies was reported and showed multiple abnormalities in hepatic-histology. Tavabe et al. (2020) reported liver damages, including apoptosis, sinusoidal spaces, fibrosis, hepatocyte degeneration, and necrosis. Smith et al. (2007) reported liver pathologies, including condensed nuclear bodies (apoptotic bodies) and abnormal nuclear division.Ahmadi et al., 2017 reported histopathological observations indicated some injuries in the liver, despite no significant changes in oxidative stress and biochemical markers (75 and 150 μg/mouse)Patlolla et al., 2011 study showed significant morphological alterations in the liver tissue compared to controls. Swiss–Webster mice exposed to purified/functionalized single-walled carbon nanotubes (SWCNT) revealsimpairment or damage to the central vein, presence of vacuoles within hepatocytes, Necrosis, Pyknotic cells. Awasthi et al., 2013 reported that in swiss albino mice Single doses of 60 and 100 mg/kg body weight ,showed histopathological changes in liver, Injury to macrophages, cellular swelling, unspecific inflammation, spot necrosis, blood coagulation.Tavabe et al., 2020 Investigate the toxicity effects of multi-walled carbon nanotubes (MWCNTs) on common carp (Cyprinuscarpio), showed apoptosis, sinusoidal spaces, fibrosis, hepatocyte degeneration, and necrosis.Smith et al. (2007) reportedintestinal pathology due to fish ingesting SWCNT-containing water, resulting in gut lumen precipitation. The current study is in accordance with all the above mentioned studies related to liver and intestine abnormalities induced by carbon nano tubes.

Conclusion

In conclusion, this study conducted a comprehensive investigation into the comparative toxicity of graphenenano sheets and carbon nanotubes in the fish species Cirrhinusmrigala. The experiment, carried out at the Department of Zoology, University of Education, Lahore, Jauharabad campus, involved the exposure of fish to varying doses of these nanomaterials through different routes. The hematological analysis demonstrated significant alterations in various blood parameters, indicating adverse effects on the fish's physiological well-being. Histological examinations of the liver and intestine revealed a range of abnormalities, highlighting the impact of both graphenenano sheets and carbon nanotubes on these vital organs.



Liver Micrographs (400x) Black Arrow: Central vein injury Blue arrow: Melanomacrophage center (MMC), Yellow Arrow: Pyknotic nuclei; Red Arrow: Dilation of sinusoids; Green Arrow: Vacuolation; Thin Black Arrow: Necrosis of hepatocytesBlack Circle: Congestion



Intestine Micrographs (400x) Black Arrow: Necrosis in the villi of the intestine; Yellow Arrow: Destroyed intestinal integrity ; Red Arrow: Inflammation in the intestine; Green Arrow: Disintegration of the epithelial layer; Blue Arrow: Vacoulation ; Thin Black Arrow: Degenerated Goblet cell.

Hematological alteration induced by waterborne exposure of graphenenanosheets and oral exposure of carbon nano tubes in Cirrhinus mrigala

Parameters	Groups						
	C	GNS-I	GNS-II	GNS-III	CNT-I	CNT-II	CNT-III
Hemoglobin (g/dl)	12.56 ± 0.04^{A}	12.47 ± 0.02^{A}	9.65 ± 0.03^{B}	7.32 ± 0.02^{C}	12.58 ± 0.03^{A}	9.67 ± 0.03^{B}	7.29 ± 0.02^{C}
Erythrocyte (106/mm3)	1.93 ± 0.02^{A}	1.93 ± 0.01^{A}	1.75 ± 0.02^{B}	1.47 ± 0.01^{C}	1.93 ± 0.02^{A}	1.75 ± 0.01^{B}	1.34 ± 0.02^{D}
Pack cell volume (%)	26.46 ± 0.01^{A}	26.46 ± 0.01^{A}	23.6 ± 0.03^{B}	21.63 ± 0.04^{C}	26.46 ± 0.01^{A}	23.6 ± 0.03^{B}	20.32 ± 0.05^{D}
Mean corpuscular volume (fl)	158.91 ± 0.18^{D}	158.86 ± 0.2^{D}	174.23 ± 0.15^{C}	180.1 ± 0.18^{B}	159.32 ± 0.2^{D}	173.93 ± 0.21^{C}	182.61 ± 0.11^{A}
Mean corpuscular Hemoglobin (pg)	34.5 ± 0.05^{A}	34.45 ± 0.03^{A}	32.84 ± 0.07^{B}	31.3 ± 0.02^{C}	34.43 ± 0.03^{A}	32.91 ± 0.06^{B}	30.41 ± 0.02^{D}
Mean corpuscular hemoglobin concentration (g/dl)	32.9 ± 0.07^{A}	32.75 ± 0.07^{A}	28.42 ± 0.05^{B}	27.62 ± 0.04^{C}	32.81 ± 0.07^{A}	28.4 ± 0.04^{B}	26.59 ± 0.06^{D}
Leukocyte counts (103/mm3)	15.3 ± 0.05^{D}	15.32 ± 0.07^{D}	17.44 ± 0.12^{C}	20.49 ± 0.11^{B}	15.27 ± 0.05^{D}	17.61 ± 0.1^{C}	21.58 ± 0.09^{A}
Neutrophil (%)	25.48 ± 0.08^{D}	25.48 ± 0.1^{D}	27.23 ± 0.07^{C}	29.62 ± 0.11^{B}	25.48 ± 0.1^{D}	27.44 ± 0.1^{C}	31.52 ± 0.1^{A}
Lymphocyte (%)	15.46 ± 0.09^{A}	15.49 ± 0.1^{A}	13.61 ± 0.1^{B}	12.41 ± 0.08^{C}	15.56 ± 0.09^{A}	13.53 ± 0.09^{B}	10.53 ± 0.09^{D}
Monocyte (%)	2.26 ± 0.06^{D}	2.21 ± 0.04^{D}	2.62 ± 0.03^{C}	3.3 ± 0.04^{B}	2.21 ± 0.04^{D}	2.63 ± 0.02^{C}	4.18 ± 0.02^{A}

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