BIO Integration

Original Article

Renal Effects of Graphite Oxide Sheets in Albino Rats (*Rattus norvegicus*): A Preliminary Study

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Abstract

Although diamond-like carbon-coated joint replacements are widely recommended for full bone replacements in humans, their clinical application is substantially limited by contamination with wear particles, specifically graphite nanoparticles, which are cytotoxic. This study was aimed at assessing the adverse effects of graphite oxide (GO) sheets on the blood and renal tissues of adult male albino rats. A total of 25 albino rats were procured from the Government College University Faisalabad, Punjab, Pakistan, and acclimated for 7 days in well-ventilated enclosures, after approval from the ethical committee at the University of Sialkot. The rats were randomly assigned to the following groups: a control group receiving no treatment, a vehicle control group receiving normal saline, and three treatment groups (G1, G2, and G3, administered GO nanosheets at doses of 5, 6, or 7 mg/kg body weight, respectively). Treatments were delivered through intraperitoneal injection on alternating days over 28 days. Animal mortality, hematological parameters, and kidney histology were assessed. The control and vehicle control groups showed normal findings, whereas the groups exposed to GO exhibited highly significant pathological changes in renal function tests ($p<0.05$). Histological alterations were more severe in the moderate- and high-dose treatment groups than the low-dose group, which displayed typical histological features. The treatment groups exhibited various histological changes, including vacuolation, renal fibrosis, inflammation, and tubular damage, which were significantly more pronounced in the G2 and G3 groups than the G1 group. Thus, exposure to GO sheets resulted in detrimental effects on renal tissues in albino rats. The findings suggested that the investigated doses of GO have detrimental effects on the health of living organisms.

Keywords

Graphite oxide sheet, histopathology, kidney, medicine, *Rattus norvegicus*.

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Introduction

Graphite oxide (GO) is a constituent of graphene oxide, a derivative of graphene. Graphene is a two-dimensional material consisting of a single layer of carbon atoms arranged in a hexagonal lattice [1]. GO is composed of stacked layers of graphene oxide sheets with oxygen-containing functional groups such as hydroxyl, epoxy, and carboxyl groups attached to the surface [2, 3]. GO sheets can induce inflammation and cell death in rats. However, owing to their high biocompatibility and low toxicity, carbon nanoparticles have substantial potential as a biomaterial for both diagnostic and therapeutic applications [4, 5].

GO sheets are biocompatible and hence suitable for use in biomedical applications such as drug delivery, imaging, and tissue engineering [6, 7]. Extensive research has been conducted both in vitro and in vivo to explore the properties and potential applications of GO. In vitro studies involve testing the effects of GO on cells and tissues outside a living organism [8, 9]. GO sheets have broad potential applications in fields including drug delivery, biosensors, and tissue engineering [10]. For instance, GO has been used as a drug carrier for cancer therapy, thus effectively delivering drugs to cancer cells while minimizing harm to healthy cells [11, 12].

The inflammation induced by graphene oxide in cells contributes to tissue damage and impaired kidney function. Although prior studies have not specifically addressed the effects of GO on the kidneys, they have suggested that graphene-based materials, including GO, might potentially cause kidney toxicity [5, 13]. A study published in the journal Nanotoxicology has investigated the potential toxicity of graphene oxide sheets in the kidneys in rats [14]. High doses of

graphene oxide sheets have been found to result in significant kidney tissue damage, including inflammation [15–18].

Methods

Experimental setup

A total of 25 post-weaning albino rats, all of similar weight, were obtained from the animal facility at Government College University, Faisalabad, Punjab, Pakistan. Ethical approval was granted by the Ethics Committee of the University of Sialkot, Punjab, Pakistan (Certificate No. USKT/EIRB/02). The rats were divided into five groups of five animals each. The rats were housed in steel cages under standard lighting conditions and were given free access to water and food. Before the start of the experiment, the rats were acclimated for 7 days. During that period, they were maintained at a temperature of 25±2° C, with a humidity level of 35±5% and a 12:12 day-night cycle. The sampling procedure in albino rats is shown in a flowchart (**Figure 1**).

Toxicity assessment

For assessment of toxicity, the rats were divided into five groups of five rats each (**Table 1**). The first group was the untreated control group. The second group received intraperitoneal injections of 1 ml normal saline (vehicle control). The remaining three groups, G1, G2, and G3, received intraperitoneal injections of GO nanoparticles (GOs) at doses of 5, 6, or 7 mg/kg body weight, respectively, every other day over a period of 28 days. Exposure to GO has been predicted to have cytotoxic effects [19–22]. The flowchart in **Figure 2** shows the process of investigation of toxicity induced by GO sheets.

Table 1 Doses in Experimental Groups

Group	Graphene oxide dose (mg/kg, intraperitoneal)
Control	0.00
Vehicle	0.00
G1	5.00
G2	6.00
G3	7.00

Blood sampling and sacrifice

Blood samples were collected from all animals at the start of the trial and again after 28 days of treatment. These samples were used for various hematological analyses including CBC and RFT. On the 29th day, the rats were euthanized, and their kidneys were obtained for histological analysis with hematoxylin-eosin staining. The method of dissection for blood sampling is shown in **Figure 3**.

Sample collection

Blood samples for hematological examination were collected at the beginning of the study and after 28 days of exposure. On the 28th day, the animals were fasted overnight, and blood samples were collected from the caudal vein of individual

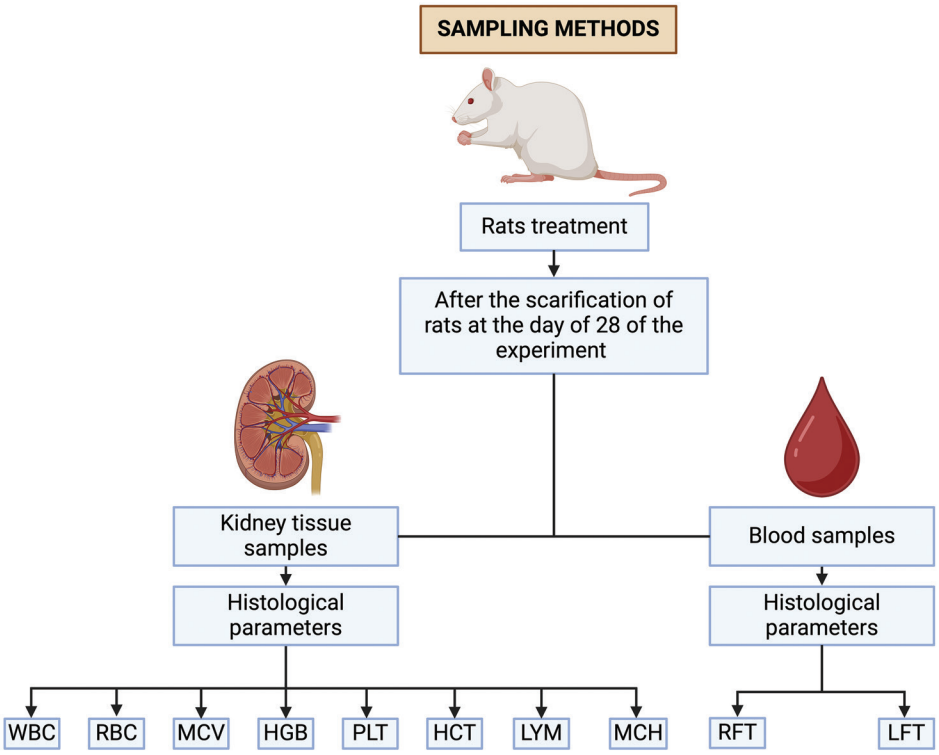

Figure 1 Flowchart of sampling methods.

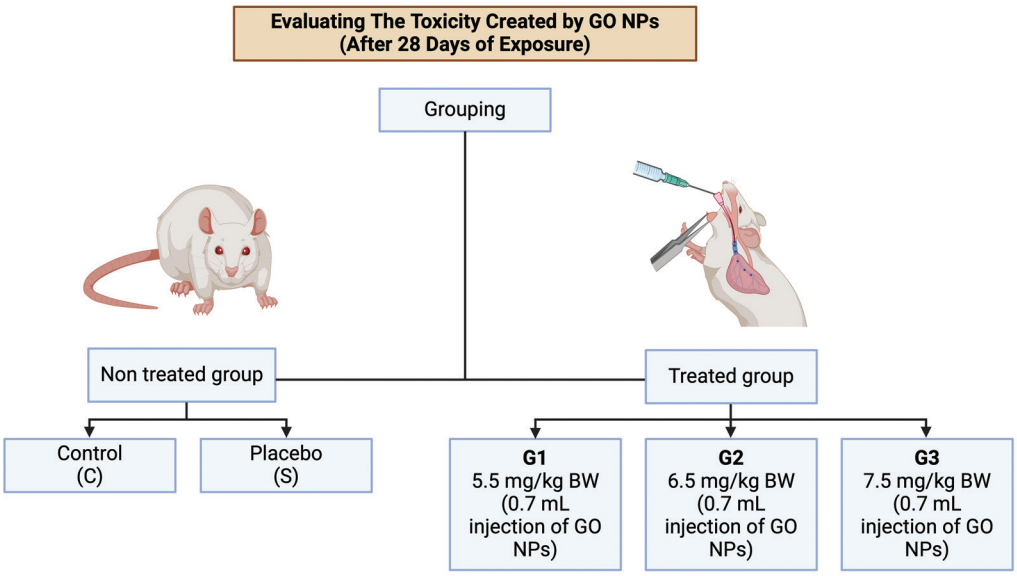

Figure 2 Flow chart of toxicity induced by GO sheets.

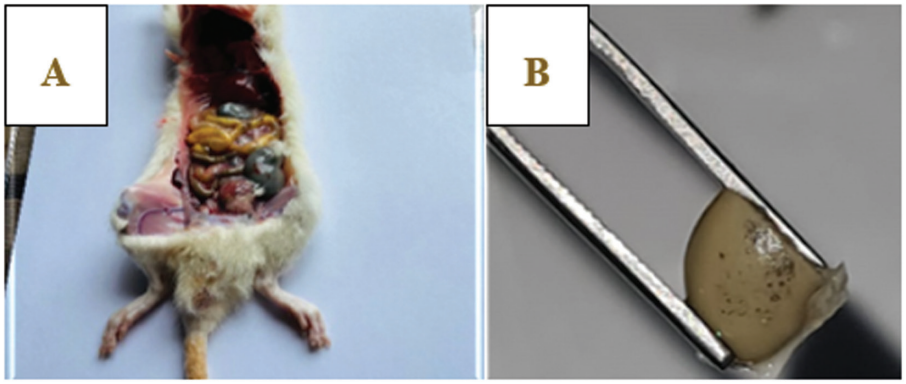

Figure 3 Dissection of albino rats: (A) rat dissection, (B) kidney view.

rats into EDTA anticoagulant tubes. These samples were analyzed with a hematology autoanalyzer (Shanghaic Drawell Intelligent Instrument Co., Ltd, DW-TEK5000 robotized blood hematology analyzer) to assess hematological variables. The rats were then anesthetized with ketamine hydrochloride (30 mg/kg BW) and sacrificed. Their renal tissues were weighed with a Sartorius balance and fixed for histological processing [23, 24].

Hematological analysis of blood

Blood samples were collected from the animals' hearts and transferred into two tubes containing calcium EDTA for subsequent CBC analysis. Hematology parameters, including RBC, HGB, WBC, LYM, MCH, MCHC, and PLT, were immediately analyzed with a hematology auto-analyzer.

Tissue preparation

For tissue fixation, small tissue portions were submerged in a solution of formaldehyde (30–35 ml), 100% alcohol (55–60 ml), and glacial acetic acid (10–15 ml). After fixation, the tissues were dehydrated with various grades of ethanol, then immersed in cedarwood oil until they became transparent. Subsequently, the tissues were embedded in benzene and paraplast, to form blocks for sectioning [25].

Hematoxylin and eosin staining

A hematoxylin stain was prepared by dissolving 2 g hematoxylin in 100 ml ethanol. An eosin stain was prepared by dissolving 1 g eosin in 100 ml 70% ethanol. The staining process involved hydration, deparaffinization, staining, and mounting.

Light microscopy

Tissue sections 5 μm thick were examined under a light microscope at 40× and 100× magnification. Slides from the control and treatment groups were thoroughly examined and documented.

Histological examination

After the animals were sacrificed, fresh kidney tissues were fixed, dehydrated, and embedded in benzene and paraplast. The tissues were cut into 5 µm sections with a microtome and mounted on clean albumenized glass slides. Hematoxylin and eosin staining were performed, and images were captured for histological examination [24, 26].

Statistical analysis

To assess the effects of GO sheets on various hematology parameters, we conducted statistical analysis (ANOVA with a linear model) in Minitab 17 software. A Tukey test was performed to compare the mean values of different groups, with a significance threshold set at $p < 0.05$ [27].

Results

The study was conducted at the Exploration Research Center, Division of Zoology, Government College University Faisalabad, Punjab, Pakistan. The sublethal dose of GOs was determined on the basis of the histology, hematology, and mortality.

General health observations

Rats were carefully observed daily throughout the experiment. The rats in the control and GO sheet-treatment groups displayed good health and exhibited energetic behavior. Rats treated with a dose of 7 mg/kg body weight showed signs of irritability and diminished activity, but no mortality within 7 days. Rats treated with 6 mg/kg remained healthy and showed normal behavior. Weekly weight measurements over 4 weeks revealed significant weight loss in the treated rats by the end of the study. After 28 days, the control rats were thriving, whereas the GO sheet-treated rats exhibited symptoms such as dry skin, decreased coelomic fluid, shrunken body cavities, and organ fat depletion, thus indicating toxicity at a GO sheet dose of 7 mg/kg (**Table 2**).

Table 2 Descriptions of Experimental Treatments

Group	Treatment
Control	No treatment
Vehicle	Normal saline (intraperitoneally injected)
G1	GO_S 5 mg/kg BW (intraperitoneally injected)
G2	GO_S 6 mg/kg BW (intraperitoneally injected)
G3	GO_S 7 mg/kg BW (intraperitoneally injected)

Changes in body weight across groups

The animals in the vehicle and control groups behaved normally and typically gained weight during the study. During the first 2 weeks of exposure, no significant changes were observed in body weight in any groups (control and treatment) ($p>0.05$). However, during the third and fourth weeks of exposure, we observed significantly ($p<0.05$) lower body weight in the treatment groups receiving moderate (6 mg/kg BW) or high doses (7 mg/kg BW) than in the control, vehicle control, and low dose treatment groups (5 mg/kg BW) (**Table 3**).

Exposure to GO caused dose-dependent toxicity to the kidney cells, including inflammation, and disruption of cellular processes. GO nanosheets were administered to the rats, and their effects on kidney function and histopathology were evaluated through social tests including open-field tests, maze tests, Morris water maze tests, and novel item acknowledgment tests (**Table 4**).

CRE, UA, and BUN were significantly lower ($p>0.05$) in the G2 and G3 groups (treated with moderate and high GO NP doses, respectively) than in the vehicle control group. The degree of urea and TBIL were fundamentally expanded in treated bunches in a portion subordinate way ($p<0.05$) (**Table 5**).

ALT, AST, LDH, and ALP were significantly higher ($p<0.05$) in the G2 and G3 treated groups than in the control, vehicle control, and low dose treatment groups (**Table 6**).

Physiological changes

The body loads of the rodents were verified weekly for 28 days. Toward the end of the trial, the treated rodents in bunches 6, 7, and 8 exhibited a significant decrease in body weight. The decrease in body weight in the albino rats was associated with the kidney tissue histology. GO nanosheet

Table 3 Weekly Body Weight (g) and Renal Somatic Index of Rats in Control and Treatment Groups (Mean±SE)

Parameter	Group				
	Control	G1	G2	G3	Vehicle
0 week	121.80 ± 0.80^{a}	122.00 ± 1.87^{a}	121.60 ± 1.51^{a}	121.40 ± 0.89^{a}	121.6 ± 2.40^{a}
1st week	132.40 ± 1.10^{a}	133.40 ± 1.14^{a}	132.40 ± 1.14^{a}	133.40 ± 1.14^{a}	132.2 ± 1.00^{a}
2nd week	143.40 ± 0.80^{a}	143.20 ± 1.30^{a}	143.00 ± 1.58^{a}	143.00 ± 1.87^{a}	142.8 ± 1.00^{a}
3rd week	153.60 ± 1.10^{a}	150.40 ± 0.54^{b}	146.20 ± 0.83^{c}	$141.80\pm2.28^{d}\downarrow$	153.4 ± 1.10^{a}
4th week	163.60 ± 0.50^{a}	159.80 ± 1.09^{b}	144.80 ± 1.64^{c}	$136.20\pm2.59^{d}\downarrow$	164.2 ± 0.80^{a}
R/S index	1.73^{b}	1.73^{b}	1.77^{b}	2.33^{a}	2.34^{a}

^{a,b,c,d}Level of significant increase or decrease in body weight of treated and non-treated groups.

Table 4 Effects of GO Nanosheets on Hematological Parameters in Albino Rats

Parameter	Group				
	Control	G1	G2	G3	Vehicle
RBC	6.71±0.15[a]	5.64±0.11[b]	4.27±0.05[c]	3.2±0.24[d] ↓	6.67±0.21[a]
WBC	13.36±0.43[c]	13.80±0.51[c]	17.53±0.37[b]	22.25±0.56[a] ↑	13.82±0.62[c]
LYM%	48.59±0.56[d]	51.38±1.61[c]	66.03±0.74[b]	96.76±0.49[a] ↑	47.87±0.62[d]
MCH	17.38±0.45[cd]	17.99±0.38[c]	20.75±0.61[b]	24.60±0.40[a] ↑	17.05±0.48[d]
MCHC	30.83±0.43[d]	33.20±0.58[c]	41.56±0.14[b]	43.28±0.44[a] ↑	31.04±0.41[d]
MCV	52.43±0.42[d]	55.03±0.57[c]	59.71±0.19[b]	63.69±0.52[a] ↑	51.94±0.67[d]
HCV	42.02±0.72[d]	54.29±0.54[c]	59.09±0.59[b]	62.38±0.46[a] ↑	42.58±0.32[d]
PLT	682.40±16.70[a]	640.40±15.39[b]	582.40±21.63[c]	416.6±19.90[d] ↓	686.60±22.23[a]
GRA	0.42±0.25[a]	0.52±0.35[a]	0.46±0.96[a]	0.21±0.16[a]	0.54±0.34[a]
GRA%	3.90±1.15[a]	4.72±0.22[a]	5.16±0.83[a]	5.45±0.41[a]	4.41±0.81[a]
HGB	13.68±0.20[a]	12.79±0.16[b]	6.62±0.38[c]	4.75±0.24[d] ↓	13.55±0.13[a]
RDW%	14.17±0.65[a]	14.29±0.59[a]	11.19±0.61[b]	9.27±0.49[c] ↓	14.29±0.60[a]
MPV	6.30±0.19[b]	6.05±0.14[b]	6.05±0.20[b]	6.96±0.52[a] ↑	6.31±0.39[b]
PDW%	43.91±0.77[a]	31.26±0.99[b]	19.98±0.58[c]	6.52±0.23[d] ↓	44.87±3.24[a]
PCT	0.24±0.01[d]	0.39±0.01[c]	0.59±0.02[b]	0.97±0.03[a] ↑	0.25±0.01[d]
LPCR	6.21±0.46[b]	6.14±0.29[b]	5.76±0.46[b]	11.12±2.32[a] ↑	6.00±0.53[b]
MID	0.54±0.22[ab]	0.61±0.06[ab]	0.63±0.05[a]	0.35±0.19[b]	0.63±0.04[a]
MID%	8.49±1.54[a]	7.24±.060[a]	8.33±0.82[a]	6.58±4.30[a]	8.29±1.14[a]
Eosin	3.82±0.20[b]	1.80±0.44[c]	1.80±0.44[c]	6.00±0.70[a] ↑	1.80±0.44[c]
Mocyt	0.90±0.37[b]	2.60±0.54[b]	4.10±1.81[b]	8.40±0.54[a] ↑	2.60±0.54[b]
Nepils%	69.60±6.11[a]	32.60±4.39[c]	43.00±8.69[c]	27.60±1.67[c] ↓	32.60±4.39[c]

[a,b,c,d]Level of significant increase or decrease in body weight of treated and non-treated groups.

Table 5 Effects of GO Nanosheets on Hematological Parameters in Albino Rats

Parameter	Group				
	Control	G1	G2	G3	Vehicle
CRE	1.22±0.07[a]	1.07±0.03[b]	0.85±0.03[c]	0.37±0.04[d]	1.20±0.07[a]
UA	4.66±0.07[a]	4.31±0.05[b]	3.85±0.02[c]	3.42±0.18[d]	4.64±0.07[a]
Urea	49.52±0.26[c]	53.71±0.51[b]	72.41±0.47[a]	72.41±0.47[a]	49.50±0.26[c]
TBIL	0.46±0.05[c]	0.60±0.10[c]	1.90±0.09[b]	2.54±0.30[a] ↓	0.44±0.05[c]
BUN	21.42±0.45[a]	15.86±0.66[b]	9.64±0.18[c]	6.89±0.60[d] ↓	21.40±0.45[a]

[a,b,c,d]Level of significant increase or decrease in body weight of treated and non-treated groups.

Table 6 Significantly Increased Parameters (ALT, AST, LDH, and ALP) after Exposure to GO Nanosheets ($p<0.05$)

Parameter	Group				
	Control	G1	G2	G3	Vehicle
ALT	13.64±0.06[d]	47.08±0.90[c]	123.11±0.51[b]	144.18±0.53[a] ↑	13.62±0.06[d]
AST	40.63±9.30[b]	48.90±0.66[ab]	53.71±0.39[a]	59.35±0.45[a] ↑	40.60±9.30[b]
ALP	84.50±1.19[d]	859.00±15.41[c]	975.40±12.62[b]	1172.20±17.90[a] ↑	85.52±0.72[d]
LDH	1337.98±0.09[d]	1551.15±7.92[c]	2246.80±17.46[b]	2769.20±24.00[a] ↑	1337.96±0.09[d]

[a,b,c,d]Level of significant increase or decrease in body weight of treated and non-treated groups.

treatment significantly increased ALT, ASP, ALP, and LDH, and significantly decreased CRE, UA, and BUN, thus leading to physiological changes in kidney structure.

Histopathological analysis

The histology analysis revealed histopathological changes in kidney tissue. Exposure of albino rats to GO sheets led to glomerulosclerosis, endothelial cell injury, and thickening of the glomerular basement membrane (**Figure 4**).

Discussion

GO sheets are economically important because of their widespread industrial use [28–30]. Assessment of their potential toxicity is crucial, because nanosheets can come into direct or indirect contact with the human body through various routes of exposure, such as cutaneous penetration, inhalation, oral consumption, or injection [31]. A recent study has investigated the toxicity of anatase GO Ns on histology and blood parameters in albino rats (liver function and CBC tests). For 28 days, rats were given GO N

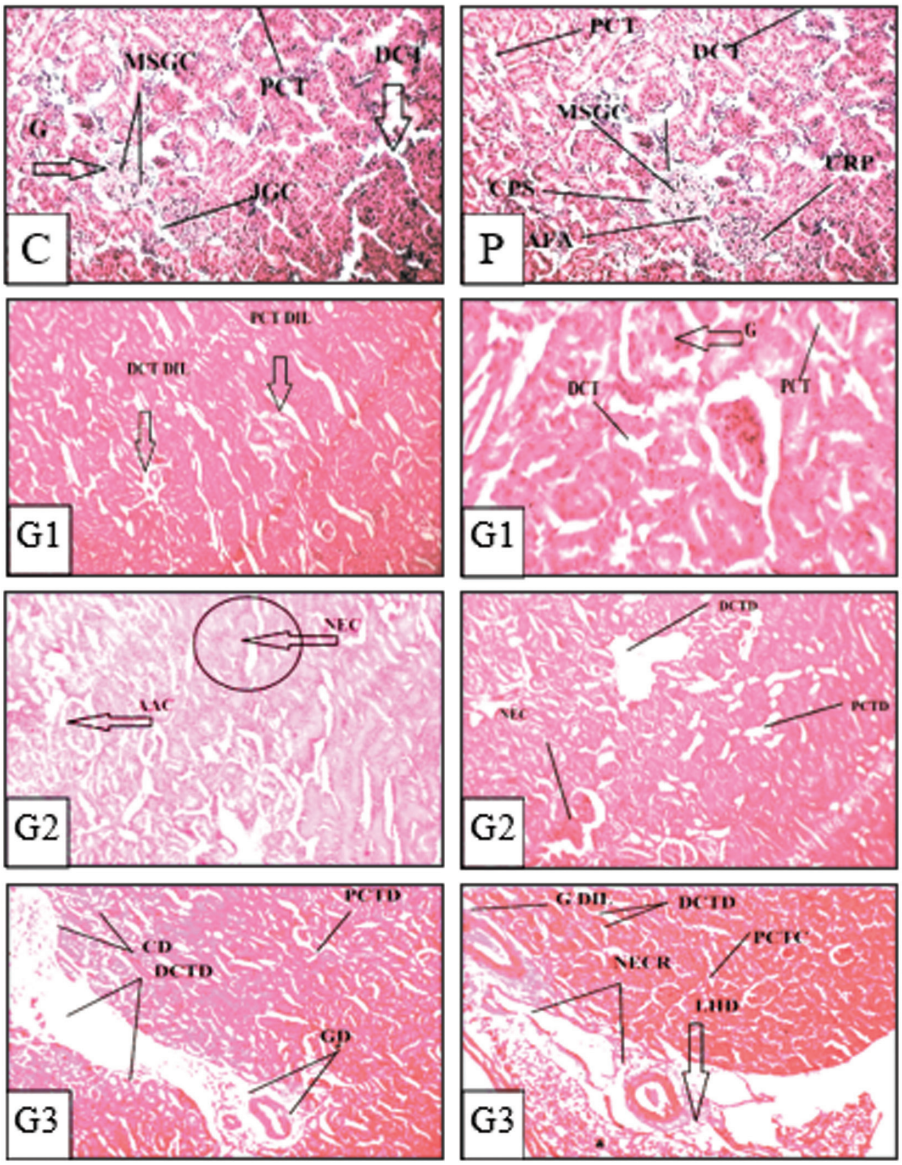

Figure 4 Histopathological analysis in albino rats.

doses of 5 mg/kg, 6 mg/kg, or 7 mg/kg every other day. The rats in all groups showed no mortality but exhibited behavioral alterations. Sacrifice, appetite reduction, and violent behavior were observed in the G3 (7 mg/kg) and G2 (6 mg/kg) groups, but not in the G1 (5 mg/kg) group. Similar behavioral changes were observed by Gurunathan in 2012, in which animals treated with high doses of GO showed low appetites and diminished physical activity. Herein, we investigated the pathophysiological and physiological alterations caused by GO Ns in rats. For 28 days, rats were administered GO Ns at doses of 5 mg/kg, 6 mg/kg, and 7 mg/kg on alternating days. The rats in all groups died at the same rate. Although sacrifice, appetite reduction, and violent behavior were observed in the G2 (6 mg/kg) and G3 (7 mg/kg) groups, the G1 (5 mg/kg) group maintained normal behavior. Exposure of GO causes adverse impact on brain cells due to which they become unable to perform their activities properly [26, 32]. Physiological effects also occurred in the treated rodents, particularly in bunches 6, 7, and 8, which exhibited the body weight were significantly decrease the current result of research are not in accordance with [33, 34].

In liver function tests, a dose-dependent significant improvement in ALT, AST, and ALP enzymes was observed. Studies have shown that urea, creatinine, and uric acid levels increase in the presence of GO nanoparticles [35, 36]. GO Ns were administered to rats intravenously as a single injection (5 mg/kg BW) of GO N suspension in saline. The tissue content of GO Ns was measured after 1, 14, and 28 days. The kidneys showed a high GO nanosheet content. Liver function tests revealed dose-dependent increases in ALT, AST, and ALP enzymes—findings similar to those reported by Abbasi-Oshaghi *et al.* in 2019. The impacts of GO sheets on the kidney, GO sheet were found in renal cells, and glomeruli infection can produce pathological changes and nephron-like toxicity [37, 38]. Furthermore, as compared with the control group, 25 mg/kg GO N treatment markedly increases serum urea levels [39–42].

Renal toxicity induced by GO nanoparticles in albino rats can lead to various physiological changes within the kidneys and the overall organism; frequently observed physiological changes include renal function impairment, inflammation, tubular damage, distal tubular damage, histopathological changes, renal fibrosis, functional impairment, and other factors influencing renal toxicity. Research examining the effects of GO on kidney function impairment has indicated disruption of normal function [43], manifesting as changes in the glomerular filtration rate, impaired tubular reabsorption and secretion, and altered electrolyte and fluid balance [44–47]. Impaired kidney function may lead to disturbances in urine production and composition, and to inflammation in the body, particularly in the kidneys. The exposure of albino rats to GO can trigger an inflammatory response in the kidneys [48–51]. This response involves the release of pro-inflammatory cytokines and chemokines, recruitment of immune cells, and activation of inflammatory pathways. Sustained inflammation can exacerbate renal injury and contribute to further tissue damage [48, 51].

GO has been shown to accumulate in the renal tubules in albino rats, particularly the proximal tubules. This accumulation can result in tubular damage, including tubular dilation, vacuolization, and epithelial cell necrosis, thus leading to glomerular damage [52–54]. These changes can impair the filtration function of the glomeruli and lead to proteinuria, the presence of excessive protein in the urine [55]. Despite glomerular damage, GO exposure in the distal tubules can impair renal function, and contribute to electrolyte abnormalities and metabolic acidosis [56]. Renal fibrosis also occurred in albino rats after excessively long exposure to GO. Prolonged exposure to GO nanoparticles can lead to the development of renal fibrosis, characterized by aggregation of extracellular lattice components within renal tissue [57]. Renal fibrosis further impairs kidney function and contributes to the progression of renal damage [58, 59]. Furthermore, renal toxicity induced by GO leads to significant functional impairments in albino rats. The glomerular filtration rate is frequently affected, thus decreasing filtration efficiency [60–63]. Several factors influence the severity and extent of renal toxicity induced by GOs, including GO dose and concentration, exposure duration, administration route, and individual susceptibility. Higher doses and prolonged exposure to GOs tend to result in more severe renal damage [64, 65]. Exposure of albino rats to GO sheets resulted in observable kidney histopathological changes, observed through microscopic examination of kidney tissue. Glomerular damage is characterized by glomerulosclerosis, endothelial cell injury, and thickening of the glomerular basement membrane [66, 67].

Conclusion

GO sheets were observed to cause dose-dependent renal toxicity in albino rats. GOs decreased physical activity while increasing hematological and histological changes. The harmful effects of GOs on the blood and kidney tissues in male albino rats were investigated. Histological findings revealed necrosis and apoptosis in the GO sheet treatment groups, in contrast to the control and vehicle control groups. The observed histological alterations in the kidneys were caused by inflammation, tubular damage, renal fibrosis, and distal tubular damage. Our results indicated that GO sheet administration in rats had significant negative effects on blood parameters and kidney function.

Ethical approval

This study was approved by The University of Sialkot, Punjab, Pakistan.

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Competing interests

All authors declare that there are no conflicts of interest in any capacity, including competing or financial.

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