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# Histopathological Changes Resulting from the Effect of Nano-graphene Oxide on the Liver in Laboratory Rats

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**ABSTRACT**

The current study included the use of 48 white male laboratory rats which were divided into six groups, with eight rats in each group. These groups were treated with a concentration of 20, 30, 40, 50 and 60 mg/kg nano graphene oxide. The sixth group was the control group. The results of the statistical analysis of the study showed a significant decrease in the average body weight. On the last day of the experiment, the results showed a significant decrease in the average liver weight when comparing between the control group and the treated groups, central vein, hepatic sinusoidal dilatation, hepatocyte nucleus replication, hepatocyte rupture and nucleolysis as well as thickening of the nuclei in some hepatocytes, infiltration of inflammatory cells, hemorrhage and dissolution and necrosis of hepatocytes. In conclusion, the results of the over-study by transmission electron microscope also showed changes at the cellular level, represented by the rupture of the nucleus and cytoplasm, in addition to the dissolution of the nucleus due to exposure to nano-graphene oxide.

**Key words :** Histopathological changes, nano-graphene oxide, liver, laboratory rats

## INTRODUCTION

Graphene is an allotrope of carbon and is the first two-dimensional material discovered in 2004. It is a one-atom-thick sheet of atoms arranged in a two-dimensional hexagonal crystal structure resembling a slice of a honeycomb, and this structure gives graphene unique properties that distinguish it from other forms of carbon (Wang *et al.*, 2017). Graphene is also known to be one of the strongest materials known to man and was found to be 200 times stronger than steel (Nezakati *et al.*, 2018). Graphene oxide is a graphene sheet covalently decorated with oxygen functional groups (Wang *et al.*, 2018). Graphene oxide nanoparticles have been widely used in biomedical fields due to their physical and chemical properties making them a useful material for drug delivery and tissue engineering applications, and bio-imaging, in biosensors, as an antimicrobial and in the treatment of tumors (Mahajan *et al.*, 2019). According to several studies, it has been reported that graphene oxide enhances cell adhesion, proliferation and differentiation due to its aromatic composition (Raucci *et al.*, 2017). In addition, it can act as a transporter that combines genes, drugs and some molecules to improve bone regeneration due to functional oxygen groups (Priyadarsini *et al.*, 2018). In addition to the unique chemical and physical properties of graphene-based nanomaterials, there are concerns arising from the toxic effects resulting from the use of these substances in the field of biomedical applications where there has been a growing debate in recent times about the potential toxicity of these materials in the field. In addition, the increasing use of graphene and its derivatives means that there is an urgent need to understand possible negative effects on human health or laboratory rats (Tonelli *et al.*, 2015; Lotfi *et al.*, 2017).

## MATERIALS AND METHODS

The current study included the use of 48 male laboratory rats, their ages ranged between 10 to 12 weeks, average weight about 170 g, and they were in good health condition. All animals were placed under standard laboratory conditions of temperature, humidity, ventilation and lighting for five days, while continuously providing water and food. Use the graphene oxide nanopowder, supplied from Sky Spring Nanomaterials, Inc. USA, had the following specifications (which was in the form of black powder) purity 97%, thickness 2 nm and average diameter 3-10 nm. Different

concentrations of it were prepared by dissolving graphene oxide nanopowder in normal saline.

The animals were divided into six groups with eight rats in each group. The first group was treated with a concentration of 20 mg/kg, the second group was treated with a concentration of 30 mg/kg, the third group was treated with a concentration of 40 mg/kg, the fourth group was treated with a concentration of 50 mg/kg, and the fifth group was treated with a concentration of 60 mg/kg of nano-graphene oxide solution. The sixth group was a control group. Animals were dosed orally with nano-graphene oxide solution using special dosing needles with a pointed tip to prevent animal wounding. Medical syringe was used for diabetic patients with an amount of 0.1 ml for each animal once a day for a period of 30 days. The levels of ALT, AST, ALK and GGT enzymes were measured using the measuring kit supplied by the French company Biolabo, to find out the changes in electron microscopy, as well as the electron microscope at the cellular level. The weights of the liver in the different groups were measured using a sensitive scale, and the lengths of these organs were also measured using the ruler, and then the statistical analysis was performed using one-way the analysis of variance, and the least significant difference (LSD) was used.

## RESULTS AND DISCUSSION

The current study showed a set of phenotypic and behavioural changes in the groups of animals treated with nano-graphene oxide compared to the control group. These changes were represented by an increase in aggressiveness among the animals, especially after dosing with graphene oxide. The results showed other changes such as lethargy, swelling and slow movement, especially in the last days of the treatment period. The results also showed that there was congestion in the eyelids of the rats treated with graphene oxide, in addition to a change in the colour of the stool, which became dark black as compared to the control group animals.

The results of the statistical analysis of the current study showed that there was no significant difference in the average body weight on the first day of the experiment when comparing the control group with the other treatment groups (Table 1). The average body weight was 170.88±1.43, 170.14±2.30, 170.77±1.72, 170.83±0.96 and 170.76±1.38 g, respectively. The results of the statistical analysis also showed a significant decrease ($P \leq 0.05$) in the average body weight on the last day of the experiment when comparing the control group with the groups treated with graphene oxide. The average body weight in the control group was 178.37±3.77 g, while the average body weight in the treated groups was 172.34±4.13, 171.67±3.81, 169.56±2.98, 167.78±2.50 and 166.72±1.80 g, respectively. The results of the current study also showed a significant decrease ($P \leq 0.05$) in the mean difference in body weight between the days. The first and last of the experiment when comparing between the control group and the treated groups, the average difference in body weight in the control group was 7.98±2.96 g, while the difference in the mean body weight in the treated groups was 3.58±1.44, 3.67±2.59, 2.04±2.17, 3.45±2.48 and 4.05±2.04 g, respectively.

The results of the statistical analysis of the current study showed a significant decrease ($P \leq 0.05$) in the average liver weight when comparing between the control group and the treated groups (Table 2). The average liver weight in the control group was 5.25±0.21 g,

**Table 1.** The effect of nano-graphene oxide on average body weight (mean±SD) of rats in different groups

| Groups | Weight before treatment | Weight after treatment | Weight difference |
|---|---|---|---|
| Control | 170.33±1.34 | 178.37±3.77a | 7.98±2.96a |
| Treatment 20 mg/kg | 170.88±1.43 | 172.37±4.13b | 3.58±1.44b |
| Treatment 30 mg/kg | 170.14±2.30 | 171.67±3.81b | 3.67±2.59b |
| Treatment 40 mg/kg | 170.77±1.72 | 169.56±2.98c | 2.04±2.17b |
| Treatment 50 mg/kg | 170.83±0.96 | 167.78±2.50c | 3.45±2.48b |
| Treatment 60 mg/kg | 170.76±1.38 | 166.72±1.80c | 4.05±2.04b |
| LSD | NS | 3.88 | 3.93 |

Figures followed by different letters indicate a significant difference at the level of probability ($P \leq 0.05$).
NS–Not Significant.

while the average liver weight in the treated groups was 3.77±0.52, 3.40±0.21, 3.18±0.37, 3.15±0.66 and 2.93±0.49 g, respectively. The results of the current study also showed a significant decrease in the mean liver length when comparing between the control group and the groups treated with concentrations 20, 30, 40, 50 and 60 mg/kg, where the average liver length in the control group was 4.53±0.18 cm, while the average liver length in the treated groups was 4.35±0.10, 4.35±0.15, 3.98±0.18, 3.76±0.19 and 3.56±0.20 cm, respectively.

**Table 2.** The effect of graphene oxide nanoparticles on average weight and length of liver (mean±S.D.) in rats of different groups

| Groups | Liver weight | Liver length |
|---|---|---|
| Control | 5.25±0.21a | 4.53±0.18a |
| Treatment 20 mg/kg | 3.77±0.52b | 4.35±0.15b |
| Treatment 30 mg/kg | 3.40±0.21bc | 4.35±0.10b |
| Treatment 40 mg/kg | 3.18±0.37c | 3.98±0.18c |
| Treatment 50 mg/kg | 3.15±0.66c | 3.76±0.19c |
| Treatment 60 mg/kg | 2.93±0.49c | 3.56±0.20c |
| LSD | 0.44 | 0.17 |

The figures followed by different letters indicate a significant difference at the level of probability (P≤0.05).

The results of the current study showed a non-significant (P≤0.05) increase in the rate of ALT enzyme when comparing between the control group and the treated group at a concentration of 20 mg/kg, while there was a significant increase in the rate of ALT enzyme when comparing between the control group and the other treated groups (Table 3). The rate of this enzyme in the control group was 22.41±1.95, while the rate of this enzyme in the treated groups was 24.55±4.20, 30.70±2.02, 32.97±4.92, 39.73±3.35 and 43.25 ± 4.46, respectively.

Similarly, the results of the current study showed that there was a non-significant (P≤0.05) increase in the rate of AST enzyme when comparing between the control group and the treated group at a concentration of 20 mg/kg, while there was a significant increase in the rate of this enzyme when comparing between the control group and the other treated groups (Table 3). This enzyme in the control group was 18.98±3.14, while the rate of this enzyme in the treated groups was 21.31±4.01, 25.74±3.20, 27.34±4.53, 36.49±4.07 and 38.23±1.92, respectively.

The results of the current study showed that there was a non-significant (P≤0.05) increase in the rate of ALK enzyme when comparing between the control group and the treated group at a concentration of 20 mg/kg, while there was a significant increase in the rate of ALK enzyme when comparing between the control group and the other treated groups. This enzyme in the control group was 22.41±3.72, while the rate of this enzyme in the treated groups was 23.46±0.79, 30.04±3.63, 27.75±0.92, 28.76±3.69 and 30.56±2.78, respectively.

The results of the current study also showed that there was no significant difference (P≤0.05) in the rate of GGT enzyme when comparing between the control group and the treated groups, where the rate of this enzyme in the control group was 21.34±4.84, while the rate of this enzyme in the treated groups was 22.16±5.19, 22.44±6.03, 22.82±3.31, 25.67±2.67 and 25.90±2.26, respectively (Table 3).

The histological sections of the liver in the control group rats showed the normal structure of the liver, where the regular hepatocytes around the central vein can be observed radially, as well as the hepatic sinusoids separating the hepatocytes, in addition to the Kupffer cells (Fig. 1).

The histological sections of the liver in the rats of the group treated with a concentration of 20 mg/kg showed the presence of blood congestion in the central vein in addition to the expansion of the hepatic sinusoids and the infiltration of inflammatory cells and the doubling of the nuclei of hepatocytes.

**Table 3.** The effect of graphene oxide nanoparticles on the rate of liver enzymes ALT, AST, ALK and GGT in rats

| Groups | ALT | AST | ALK | GGT |
|---|---|---|---|---|
| Control | 22.41±1.95a | 18.98±3.14a | 22.41±3.72a | 21.34±4.84 |
| Treatment 20 mg/kg | 24.55±4.20a | 21.31±4.01ab | 23.46±0.79a | 22.16±5.19 |
| Treatment 30 mg/kg | 30.70±2.02b | 25.74±3.20b | 27.75±0.92b | 22.44±6.03 |
| Treatment 40 mg/kg | 32.97±4.92b | 27.34±4.53bc | 28.76±3.69b | 22.82±3.31 |
| Treatment 50 mg/kg | 39.73±3.35c | 36.49±4.07be | 30.04±3.63b | 25.67±2.67 |
| Treatment 60 mg/kg | 43.25±4.46c | 38.23±1.92be | 30.56±2.78b | 25.90±2.26 |
| LSD | 6.15 | 6.02 | 4.28 | NS |

The figures followed by different letters indicate a significant difference at the level of probability (P≤0.05).

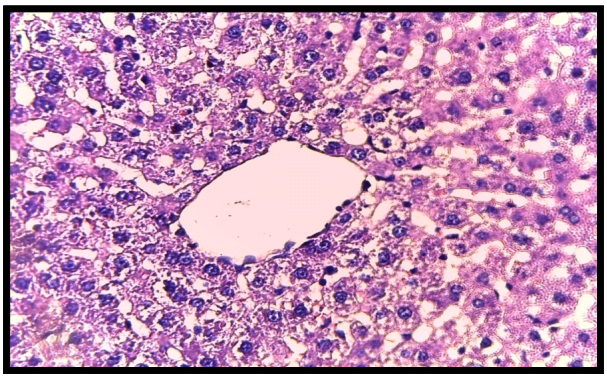

Fig. 1. A section of the liver tissue of the control group showing A central vein, B hepatocytes, C hepatic sinusoids and D Kupffer cells (400X) (H&E).

Inflammatory cells, hepatocyte nuclei doubling, nucleolysis and hepatocyte sporulation are shown in Figs. 2, 3, 4, 5, 6, 7, 8a and 8b, respectively.

The results of the current study showed a set of phenotypic and behavioural changes in animals treated with graphene oxide,

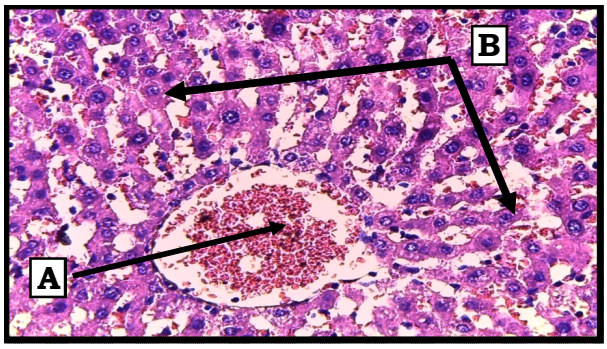


Fig. 2. A section of the liver tissue of rats treated with a concentration of 20 mg/kg showing A central venous congestion and B dilation of hepatic sinusoids (400X) (H&E).

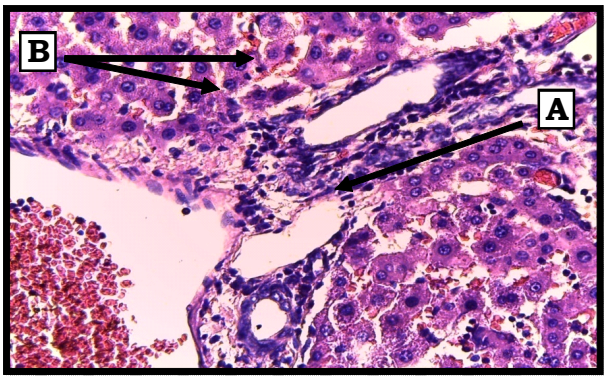


Fig. 3. A section of the liver tissue of rats treated with a concentration of 20 mg/kg showing A the infiltration of inflammatory and B cells that doubled the nuclei of hepatocytes (400X) (H&E).

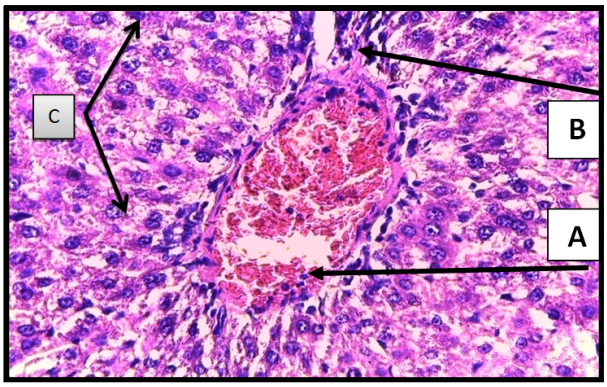


Fig. 4. A section of the liver tissue of rats treated with a concentration of 30 mg/kg showing A central vein congestion, B infiltration of inflammatory cells and C doubling the nuclei of hepatocytes (400X) (H&E).

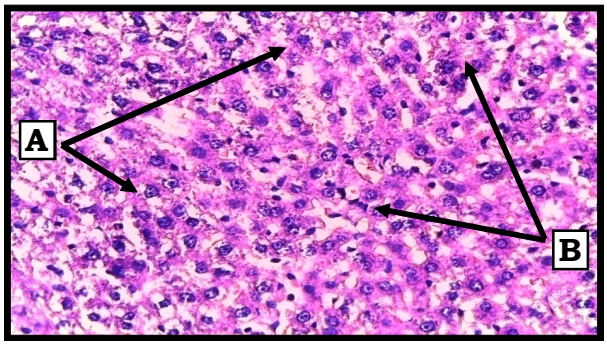


Fig. 5. A section of the liver tissue of rats treated with a concentration of 30 mg/kg showing A nucleolysis of hepatocytes and B cells (400X) (H&E).

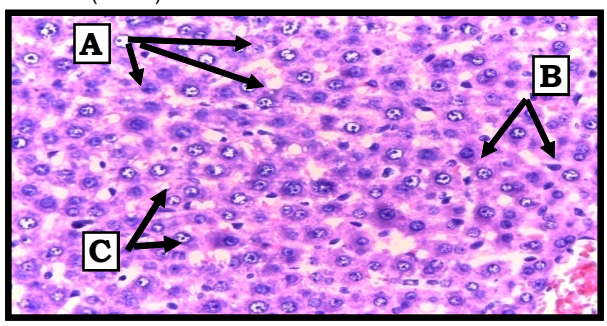


Fig. 6. A section of the liver tissue of rats treated with a concentration of 40 mg/kg showing A hepatocyte nucleolysis, B nuclei doubling and C sporulation of hepatocytes (400X) (H&E).

represented by an increase in aggression among animals, especially in the first days of treatment, in addition to other changes such as lethargy, swelling and slow movement, especially in the last days of treatment, as well as congestion in the eyelids, in addition to the changed stool colour as it became dark black compared to the control group animals, and this

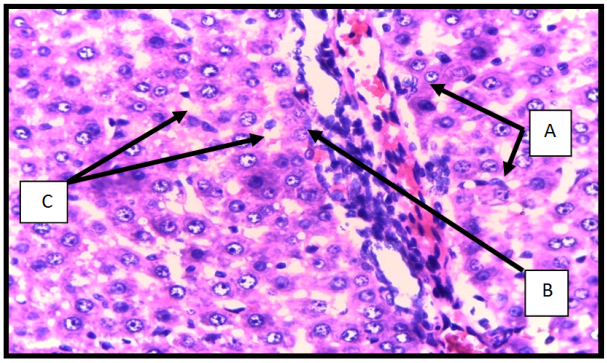


Fig. 7. A section of the liver tissue of rats treated with a concentration of 40 mg/kg showing A hepatocyte doubling, B infiltration of inflammatory cells and C swelling of hepatocytes (400X) (H&E).

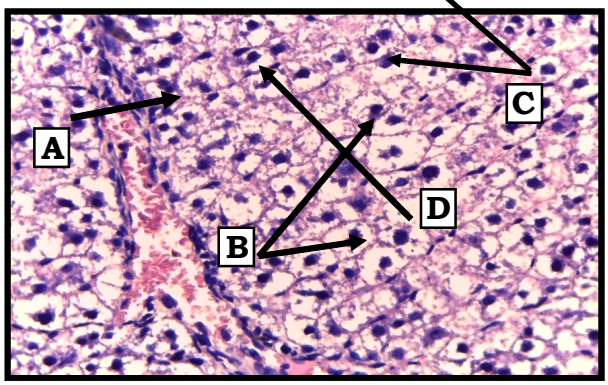


Fig. 8a. Section of the liver tissue of rats treated with a concentration of 50 mg/kg showing A hemorrhage, B hepatocyte exudation, C thickening of hepatocyte nuclei and D hepatocyte hemolysis (400X) (H&E).

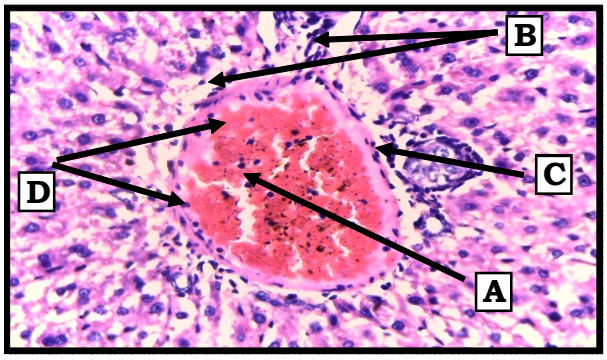


Fig. 8b. Section of the liver tissue of rats treated with a concentration of 50 mg/kg showing A hemorrhage, B hepatocyte exudation, C thickening of hepatocyte nuclei and D hepatocyte hemolysis (400X) (H&E).

may be caused by trauma or the increased load from dosing with nano-graphene oxide. In the treated animals, it led to a change in walking behaviour, lethargy, and an increase in aggression, and these changes were associated with levels of dose. Zheng *et al.* (2016) supported this result where he showed that nanoparticles with diameters of 10-200 nm could penetrate the blood-brain barrier BBB and could enter into cells and thus affected the central nervous system as gold nanoparticles could pass through the blood-brain barrier.

The results of the current study showed a decrease in the average body weight in the animals treated with graphene oxide compared to the animals of the control group and compared to their weight before the dose. Toxic to rats treated with graphene oxide and therefore this substance leads to a disturbance in the absorption process by affecting the lining of the alimentary canal. It is allowed to easily accumulate or adhere to the cell membrane, as mentioned (Xu *et al.,* 2016) that graphene oxide can interact with the cell membrane to induce cytotoxicity. It was shown that intraperitoneal injection of graphene nanoparticles GNPs in rats at concentrations of 5 and 15 mg/kg for 27 days led to a decrease in the average body weights of the animals.

The results of the current study showed a decrease in the average liver weight in the groups treated with graphene oxide compared with the control group. The control group consisted of congestion in the central vein, dilatation of the hepatic sinusoids, infiltration of inflammatory cells, multiplication of hepatocyte nuclei, in addition to rupture of hepatocytes and nucleolysis. This may be caused by damage to hepatocytes as a result of exposure to graphene oxide, which leads to an increase in the permeability of the hepatocyte membrane and thus the release of ALT and AST. It causes an increase in their concentration in the body, and these results are consistent with Patlolla *et al.* (2017), who showed that the liver was a vital organ responsible for many biochemical processes in biological systems, and that most toxic chemicals are metabolized in the liver, a condition that causes high risk of infection and leads to liver poisoning.

The reason for these changes in the liver tissue may be attributed to the fact that graphene oxide can travel through the blood and reach the liver, and, therefore, the contact between it and hepatocytes causes cytotoxic reactions such as the release of reactive oxygen species ROS and oxidative stress, and this is consistent with

what was mentioned by Li *et al.* (2021) who showed that indirect contact between nanomaterials and cells caused toxic reactions that led to the release of reactive oxygen species and oxidative stress that was followed by the release of cytokines and inflammation, which was primarily a response to reactive oxygen species. These results agree with what was mentioned by Nirmal *et al.* (2020) and Hafsan *et al.* (2022), who showed that treating rats with graphene oxide by intraperitoneal injection at different doses for 30 days led to changes in the levels of ALT, AST and ALP in serum, especially in animals treated with high doses. However, these changes were few in the groups treated with low and medium doses, as it was clear that the animals treated with medium and high doses of graphene oxide showed varying degrees of histopathological changes such as inflammation around the central vein, portal veins, diaphragm and hepatocyte injury, in addition to the presence of abnormal sinusoids. These results are supported by what was mentioned by Tang *et al.* (2022), who showed that the main mechanisms of graphene oxide toxicity were oxidative stress, physical destruction, inflammatory response, programmed cell death, necrosis and autophagy.

## CONCLUSION

In conclusion, the results of the study by transmission electron microscope showed changes at the cellular level, represented by the rupture of the nucleus and cytoplasm, in addition to the dissolution of the nucleus due to exposure to nano-graphene oxide.

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