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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, where the rats were divided into six groups, with 8 rats in each group. The second group with a concentration of 30 mg/kg, the third group with a concentration of 40 mg/kg, the fourth group with a concentration of 50 mg/kg, and the fifth group with a concentration of 60 mg/kg of nano graphene oxide. The sixth group is 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 when comparing between the control group and the treated groups, and 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. 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.

***Keywords*---**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 (Noveselov et al., 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 Hexagonal materials give graphene unique properties that distinguish it from other forms of carbon (Novoselov et al., 2009). 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 (Lijima et al., 1999). 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 to It can act as a transporter that combines genes, drugs and some molecules to improve bone regeneration due to functional oxygen groups (Liu et al., 2014). 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 the possible negative effects on human health (Yang et al., 2013). Laboratory rats.

Materials and Methods

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

Study design

The animals were divided into six groups with 8 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, and the sixth group is a control group. Animals were dosed orally with nano graphene oxide solution using special dosing needles with a pointed tip to prevent animal wounding, using a medical syringe used for diabetic patients, at an amount of 0.1 ml for each animal, once a day for a period of time. 30 days . The levels of ALT, AST, ALK and GGT enzymes were measured using the measuring kit supplied by the French company Biolabo. Microstrips were prepared based on the method (Luna, 1968; Bancroft and Gamble 2008, to

find out the changes in electron microscopy, as well as the electron microscope. at the cellular level.

Statistical analysis

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 the analysis of variance for one factor Anova One-way, and the smallest significant difference was used L.S.P.

Results

Phenotypic and behavioral results

The current study showed a set of phenotypic and behavioral changes in the group animals treated with nano graphene oxide compared to the control group, where 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 color of the stool, which became dark black compared to the control group animals.

Effect of graphene oxide nanoparticles on average body weight

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. The average body weight is (170.88 ± 1.43, 170.14 ± 2.30, 170.77 ± 1.72, 170.83 ±0.96, 170.76 ± 1.38 g) respectively. The results of the statistical analysis also showed a significant decrease (P≤0.05) in the average body weight after the last day of the experiment when comparing the control group with the groups treated with graphene oxide, where the average body weight in the control group was (178.37±3.77 grams), while the average body weight was In the treated groups, it was (172.34 ± 4.13, 171.67 ±3.81, 169.56 ± 2.98, 167.78 ± 2.50, 166.72 ± 1.80 g) respectively, and the results of the current study showed a significant decrease (P≤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, where the average difference in body weight in the control group was (7.98 ± 2.96 grams), 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, 4.05 ± 2.04 g) respectively as shown in Table (1).

Table 1
Shows the effect of nano-graphene oxide on average body weight of rats in different groups

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

- The numbers in the table represent (Mean ± S.D) and L.S.D values.
- The different letters indicate a significant difference at the level of probability (P≤0.05) when comparing the totals vertically.
- Similar letters indicate that there is no significant difference when comparing the totals vertically.

Effect of graphene oxide nanoparticles on the average weight and length of the liver

The results of the statistical analysis of the current study showed a significant decrease (P≤0.05) in the average liver weight when comparing between the control group and the treated groups, where the average liver weight in the control group was (5.25 ± 0.21 g), 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, 2.93 ± 0.49 g) respectively, and the results of the current study showed a significant decrease in the mean liver length when comparing between the control group and the groups treated with concentrations 20 30, 40 and 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, 3.56 ± 0.20 cm), respectively, as shown in Table (2).

Table 2
Shows the effect of graphene oxide nanoparticles on average weight and length of liver in rats of different groups

Groups	Liver Weight	Liver length
Control	5.25±0.21 a	4.53±0.18 a
Treatment 20 mg/kg	3.77±0.52 b	4.35±0.15 b
Treatment 30	3.40±0.21 b c	4.35±0.10 b

mg/kg		
Treatment 40 mg/kg	3.18±0.37 c	3.98±0.18 c
Treatment 50 mg/kg	3.15±0.66 c	3.76±0.19 c
Treatment 60 mg/kg	2.93±0.49 c	3.56±0.20 c
LSD	0.44	0.17

- The numbers in the table represent (Mean ± S.D) and L.S.D values.
- The different letters indicate a significant difference at the level of probability (P≤0.05) when comparing the totals vertically.
- Similar letters indicate that there is no significant difference when comparing the totals vertically.

Effect of graphene oxide nanoparticles on the rate of liver enzymes

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, where it was 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, 43.25 ± 4.46) respectively, as shown in the table. (3) 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 where the rate of 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, 38.23±1.92), respectively, as shown in Table. (3)

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 where the rate of 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, 30.56±2.78), respectively, as shown in Table. (3) The results of the current study 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 (22.82±3.31), while the rate of this enzyme in the treated groups was (22.16±3) 5.19, 22.44±6.03, 21.34±4.84, 25.67±2.67, 25.90±2.26) respectively as shown in Table (3).

Table 3
Shows the effect of graphene oxide nanoparticles on the rate of liver enzymes ALT, AST, ALk and GGT in rats of different groups

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

- The numbers in the table represent (Mean ± S.D) and L.S.D values.
- The different letters indicate a significant difference at the level of probability (P≤0.05) when comparing the totals vertically.
- Similar letters indicate that there is no significant difference when comparing the totals vertically.

Histological study
Effect of graphene oxide on the liver

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 as in Figure.(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. Inflammatory cells, hepatocyte nuclei doubling, nucleolysis, in addition to hepatocyte sporulation, as shown in Figures (5,4,3,2), respectively.

The histological sections of the liver in the rats treated with a concentration of 40 mg/kg showed hepatocyte nucleolysis, nucleoli doubling, and rupture of hepatocytes in addition to the congestion of the central vein and the expansion of the hepatic sinusoids as well as the infiltration of inflammatory cells. Severe central vein, thickening of hepatocyte nuclei, infiltration of inflammatory cells in addition to bleeding, hepatocyte rupture and hepatocyte degeneration, in addition to the expansion of hepatic sinusoids and necrosis of hepatocytes as shown in Figures (9,8,7,6) respectively, as shown by the histological sections of the liver. In the rats of the group treated with a concentration of 60 mg/kg central vein congestion, hepatocyte nuclei doubling, hepatocyte necrosis, inflammatory cell

infiltration, hepatic sinusoidal dilatation, hepatocyte rupture in addition to hemorrhage and hepatocyte nucleus thickening as shown in Figures (11,10) respectively.

The results of the meta-study

Transmission electron microscopy images of the liver in the control group rats showed the structure of the hepatocyte containing two nuclei, where the nucleus and nucleolus can be observed in addition to the mitochondria and the plasma network as shown in Figure (12), and the TEM images of the liver in the rats of the treated group at a concentration of 20 mg/kg blunt nucleus showed the addition of The TEM images of the liver in the rats treated with a concentration of 30 mg/kg showed the nucleolysis as in the figure (14), and the TEM images of the liver in the rats of the treated group at a concentration of 40 mg/kg showed the nucleolus free. The nucleoli in addition to the rupture and decomposition of the cytoplasm as shown in Figure (15), and the TEM images of the liver in the rats of the treated group at a concentration of 50 mg/kg showed the nucleolysis as well as the rupture of the cytoplasm as in the figure (16), and the liver images of the rats of the treated group at a concentration of 60 mg/kg showed. kg of nucleus without a nucleus as in Figure (17).

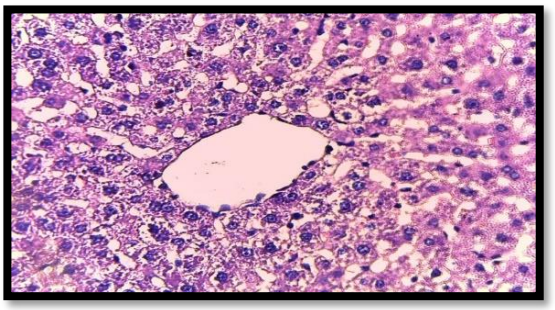

Figure 1. A section of the liver tissue of the control group showing A central vein B hepatocytes C hepatic sinusoids D Kupffer cells (400X) (H&E)

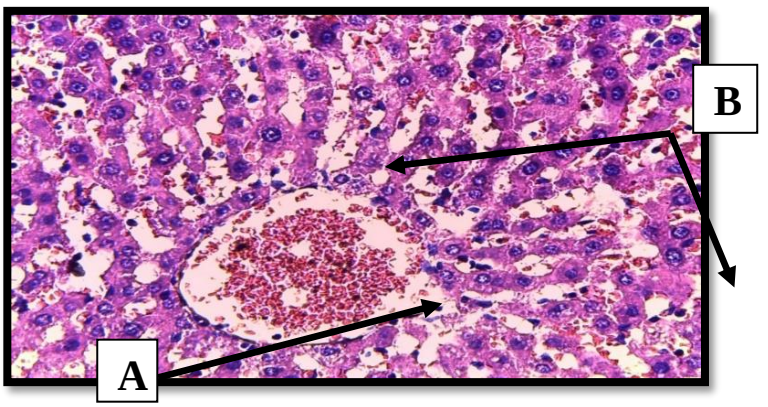

Figure 2. A section of the liver tissue of rats treated with a concentration of 20 mg / kg shows A central venous congestion B dilation of hepatic sinusoids (400X) (H&E)

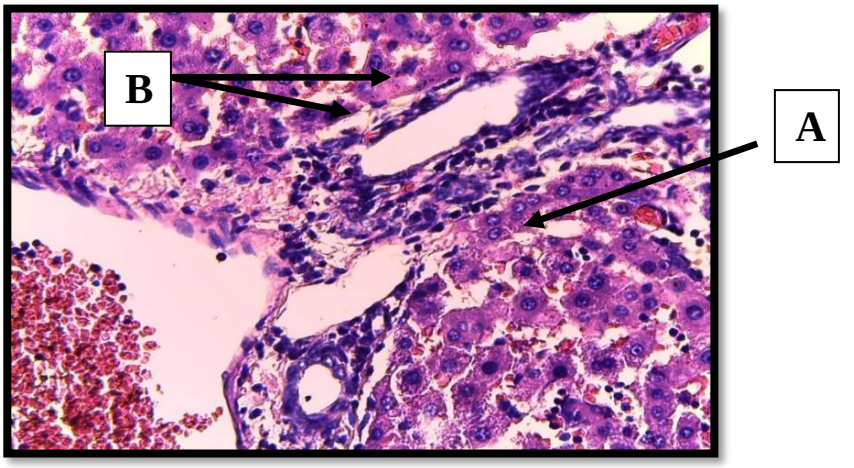

Figure 3. A section of the liver tissue of rats treated with a concentration of 20 mg/kg A shows the infiltration of inflammatory B cells that doubled the nuclei of hepatocytes (400X) (H&E

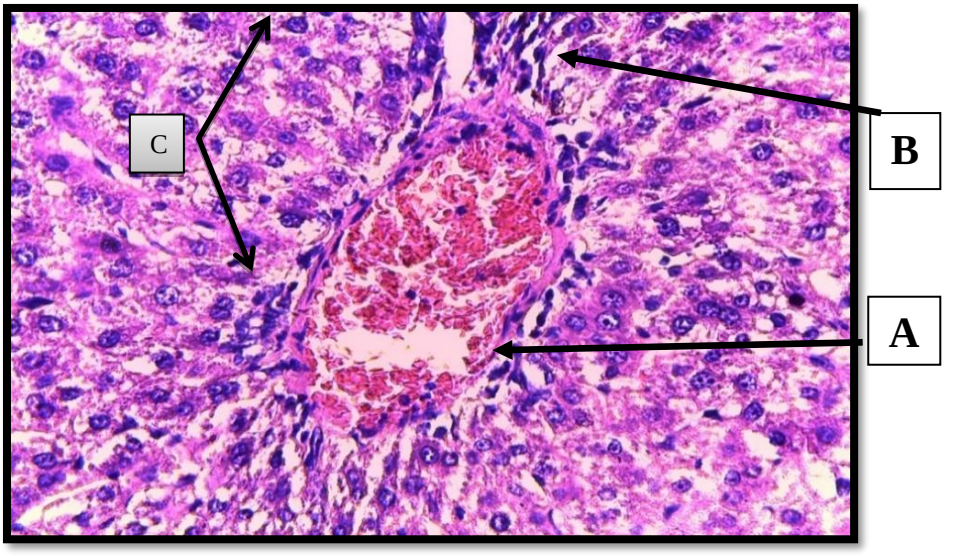

Figure 4. A section of the liver tissue of rats treated with a concentration of 30 mg/kg shows A central vein congestion B infiltration of inflammatory cells C doubling the nuclei of hepatocytes (400X) (H&E)

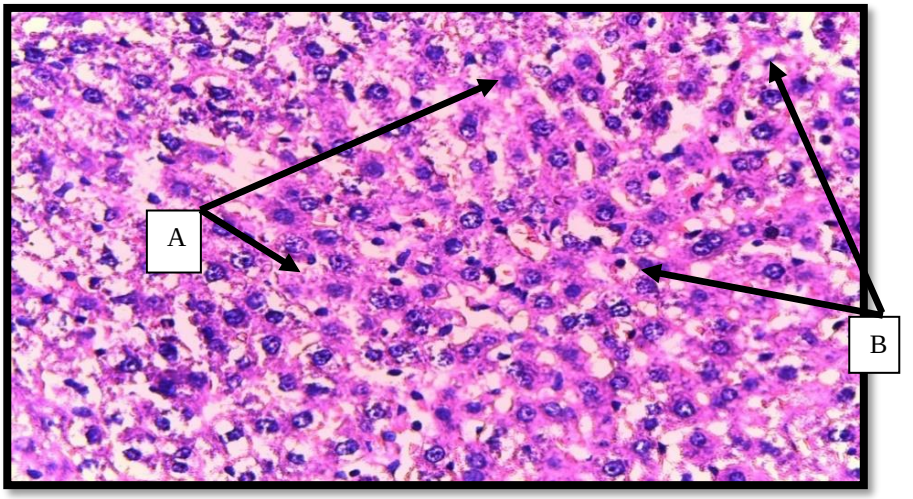

Figure 5. A section of the liver tissue of rats treated with a concentration of 30 mg/kg shows A nucleolysis of hepatocytes B cells (400X) (H&E).

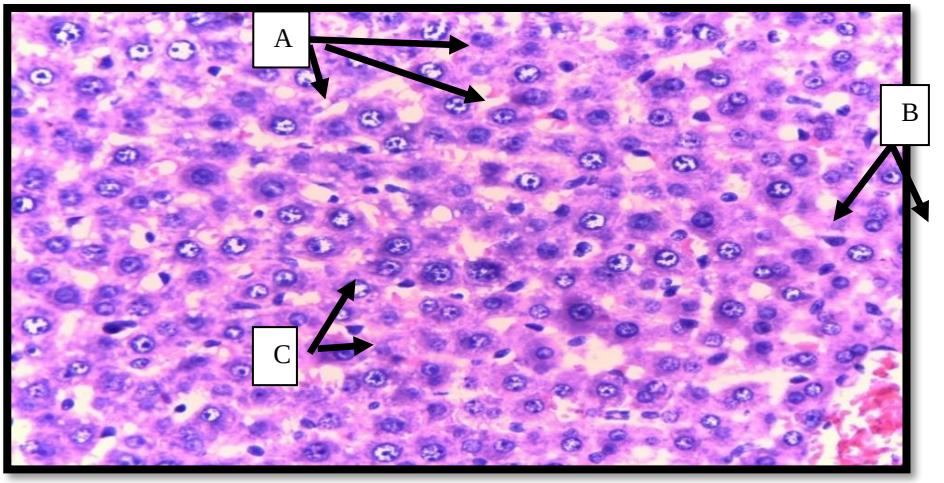

Figure 6. A section of the liver tissue of rats treated with a concentration of 40 mg/kg shows A hepatocyte nucleolysis B nuclei doubling C sporulation of hepatocytes (400X) (H&E)

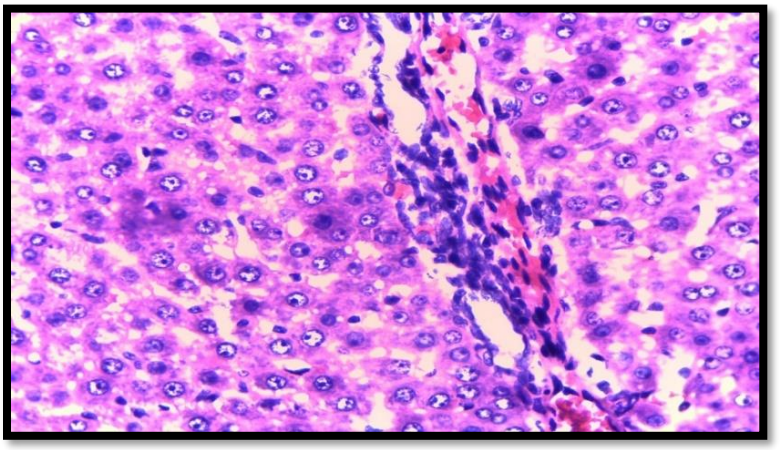

Figure 7. A section of the liver tissue of rats treated with a concentration of 40 mg/kg shows A hepatocyte doubling B infiltration of inflammatory cells C swelling of hepatocytes (400X) (H&E

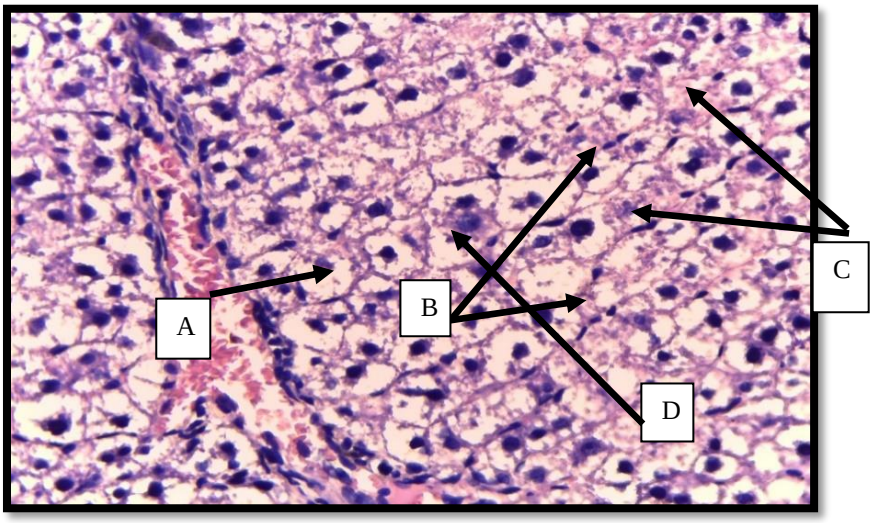

Figure 8. section of the liver tissue of rats treated with a concentration of 50 mg/kg shows A hemorrhage B hepatocyte exudation C thickening of hepatocyte nuclei D hepatocyte hemolysis (400X) (H&E)

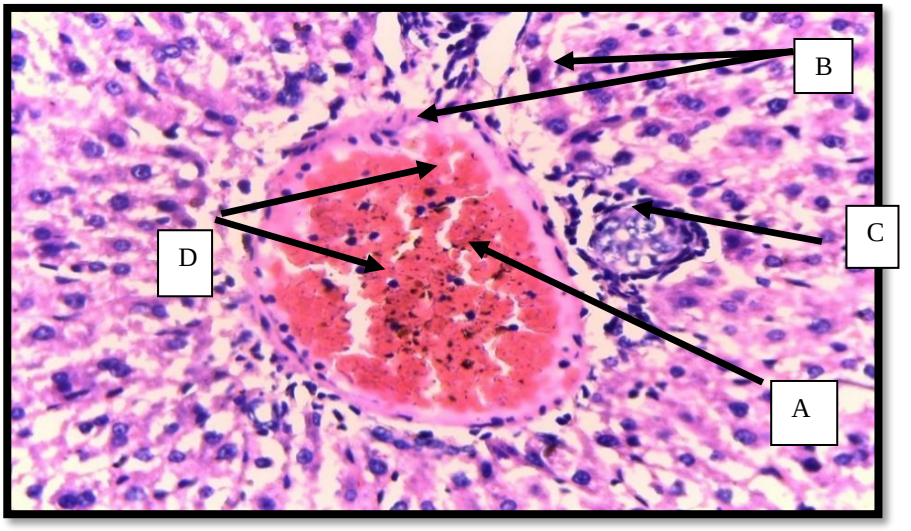

Figure 9. is a section of the liver tissue of rats treated with a concentration of 50 mg/kg A shows severe central venous congestion B Expansion of hepatic sinusoids C Infiltration of inflammatory cells D Hepatocyte necrosis (400X) (H&E)

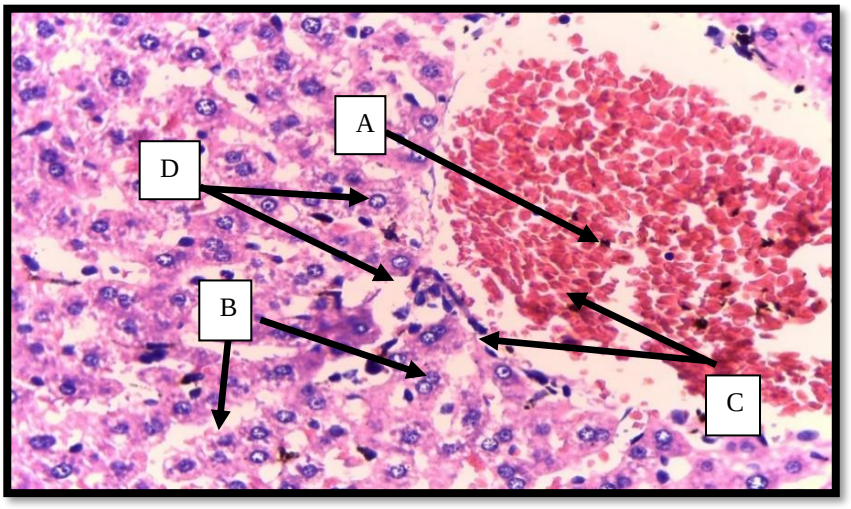

Figure 10. a section of the liver tissue of rats treated with a concentration of 60 mg / kg A shows central venous congestion B Expansion of hepatic sinusoids C Swelling of hepatocytes D Kupffer cells (400X) (H&E)

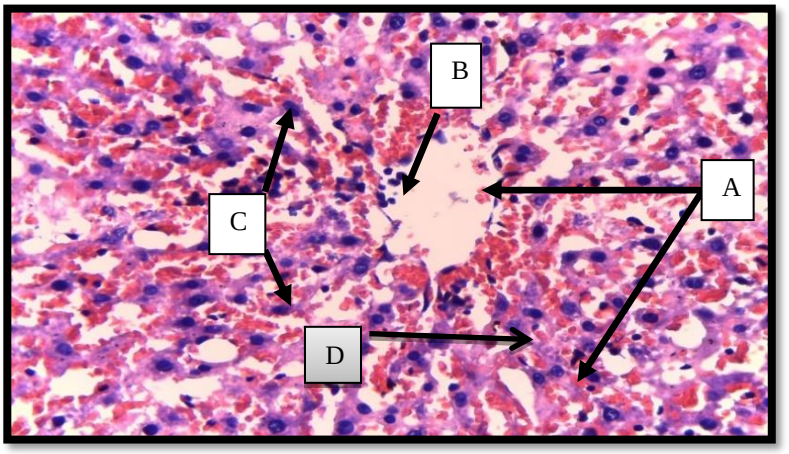

Figure 11. A section of the liver tissue of rats treated with a concentration of 60 mg/kg shows A hemorrhagic bleed B hepatocyte C thickening of the nuclei of hepatocytes D expansion of hepatic sinusoids (400X) (H&E)

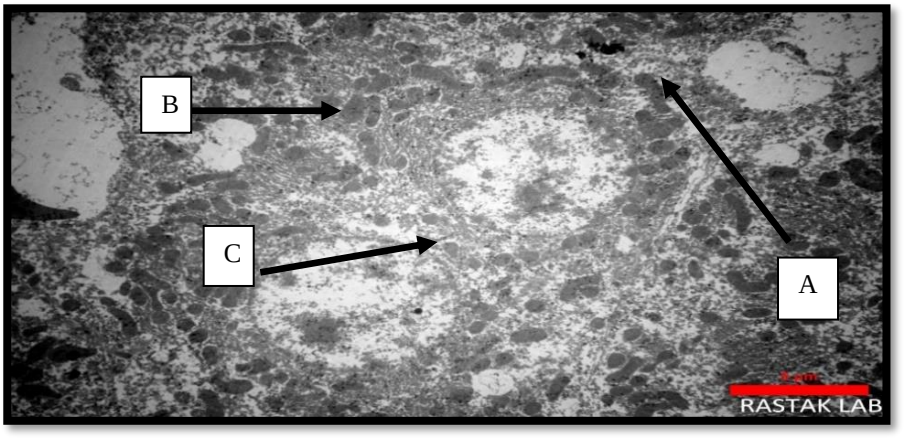

Figure 12. cross-section of liver tissue of control group rats showing A hepatocyte nuclei, B plasma network, C, mitochondrial transmission, transmission electron microscop

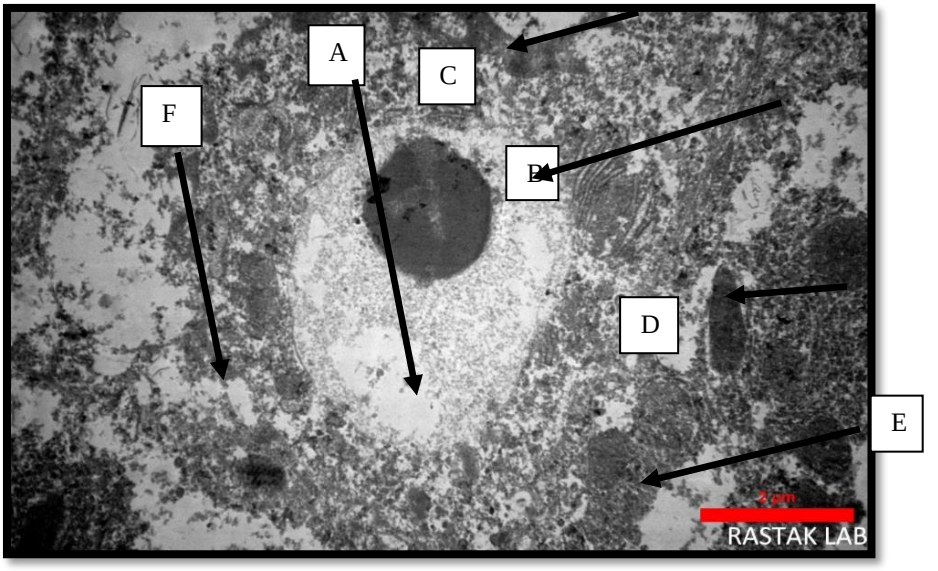

Figure 13. A cross section of the liver tissue of rats treated with a concentration of 20 mg/kg shows a vacuolar nucleus B nucleus C Golgi apparatus D plasma network E Mitochondria F vacuolated cytoplasm, transmission electron microscope

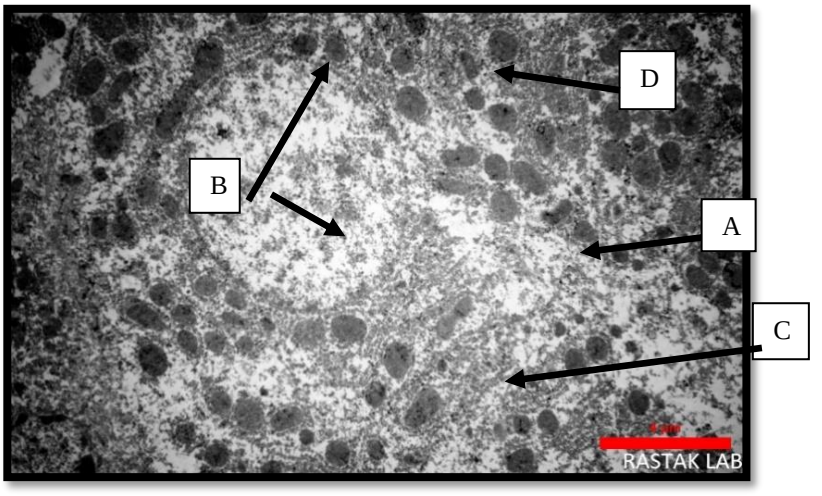

Figure 14. cross-section of liver tissue of rats treated with a concentration of 30 mg/kg showing cell organelles A, lysosomes, B, mitochondria, C, rough plasma, D, Golgi apparatus, transmission electron microscope

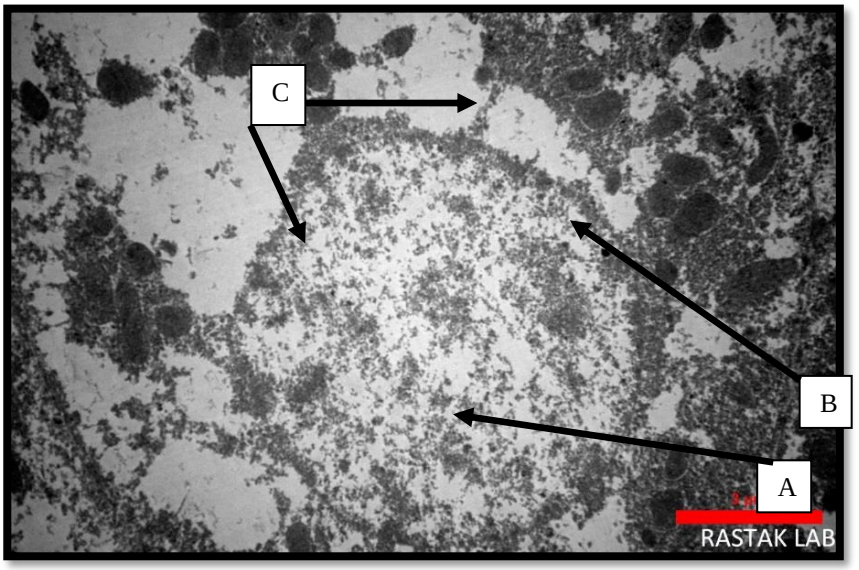

Figure 15. a cross section of liver tissue of rats treated with a concentration of 40 mg/kg A shows the nucleolar nucleus B Nuclear envelope C rupture and cytoplasm decomposition, transmission electron microscope

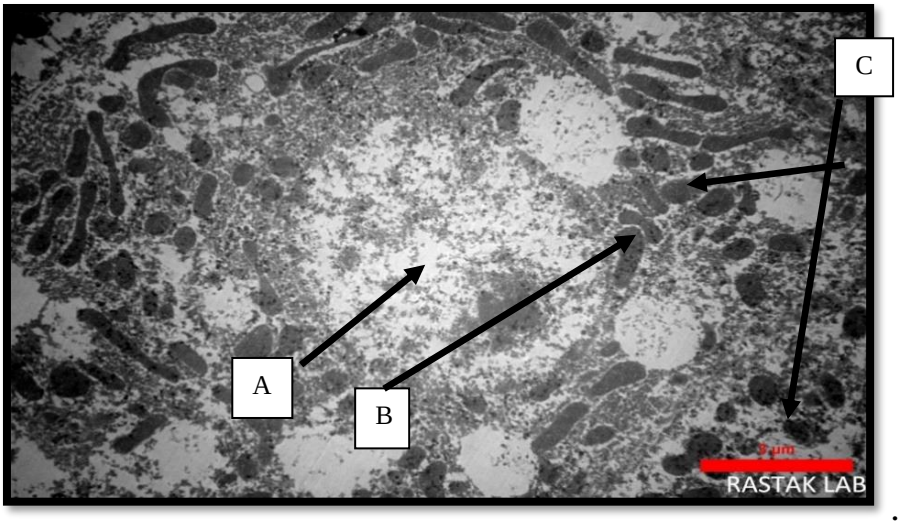

Figure 16. A cross section of the liver tissue of rats treated with a concentration of 50 mg/kg shows A nucleus B nucleolysis C rupture of the cytoplasm, transmission electron microscope

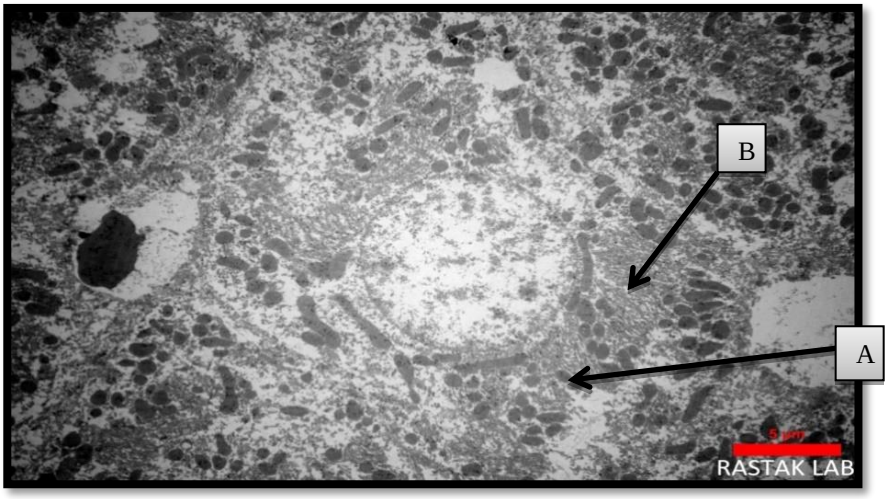

Figure 17. Section of liver tissue of rats treated with a concentration of 60 mg/kg A shows the nucleolar nucleus B nuclear envelope, transmission electron microscope

Discussion

Effect of graphene oxide on phenotypic and behavioral trait

The results of the current study showed a set of phenotypic and behavioral changes in animals treated with graphene oxide, represented by an increase in aggression among animals, especially in the first days of treatment with graphene oxide, 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 stool changed color as it became dark black compared to the control group animals, and this may be caused by trauma or the increased load from dosing with nanographene oxide. In the treated animals, it led to a change in walking behavior, lethargy, and an increase in aggression, and these changes were associated with dose levels.

What supports this result is what was shown (Zheng et al., 2016), where he showed that nanoparticles with diameters of 10-200 nm can penetrate the blood-brain barrier BBB and can enter into cells and thus affect the central nervous system, as well as these results agree with what was mentioned (Zhang et al., 2016) showed that nanomaterials such as gold nanoparticles can pass through the blood-brain barrier. These results do not agree with what was mentioned (Li et al., 2016), where he showed that the functional and motor behaviors of animals treated with graphene oxide did not change, as he showed that graphene oxide cannot cross the blood-brain barrier, as he confirmed that graphene oxide is not toxic to the central nervous system and therefore It does not affect the animals treated by it.

Effect of graphene oxide on body weight

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. This result is consistent with what was mentioned (Kim et al., 2016), which showed a significant decrease in body weight in the animals of the group treated with this substance compared to the control group. This result is also consistent with what was mentioned (Liu et al., 2015), where it was shown that graphene oxide causes toxicity by destroying the lipid bilayers and penetrating the lipid membranes through the edges and corners of the nanosheets, and this result is also consistent with what was mentioned (Tabish et al., 2018). Where 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. Also, this result is consistent with what was mentioned (Radhi and Al-Bairuty, 2019), where they showed that feeding rats with zinc oxide nanoparticles causes a decrease in body weight. In the function of the digestive system and consequently weight loss, and this result agrees with what was mentioned (Ko et al., 2o15), where it was shown that oral administration of zinc nanoparticles to rats led to a loss of appetite for food and consequently a decrease in the animals' weights. This result is also consistent with what was mentioned (Zhang et al., 2010), where he showed that taking gold nanoparticles orally causes a decrease in body weight.

Effect of graphene oxide on the liver

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. Which causes an increase in their concentration in the body, and these results are consistent with what he mentioned (Patlolla et al., 2011), where he showed that the liver is 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 lead to liver poisoning. These results are also consistent with what was mentioned (Oberdorster et al., 2005), where he explained that liver cells are disintegrated by a variety of harmful factors and products, and the accumulation of graphene in the liver causes changes in liver functions and thus leads to degeneration, necrosis and inflammation in the liver tissue .

Also, this result is consistent with what was mentioned (Patlolla et al., 2017), where it was shown that graphene oxide significantly increased the activity of ALP

enzyme in the serum of treated rats compared to the control group, which indicates the occurrence of injuries in the liver tissues, as explained (Murakami et al. 2004) ALP enzyme is used as an indicator of cholestatic liver disease, as it is produced in the liver by the cells lining the small bile ducts (ducts) in the liver, and an increase in it indicates liver damage or a defect in the filtering of the liver . 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 (Nel et al. al., 2006), where he showed that indirect contact between nanomaterials and cells causes toxic reactions that lead to the release of reactive oxygen species and oxidative stress that is followed by the release of cytokines and inflammation, which is primarily a response to reactive oxygen species.

These results are also consistent with what was mentioned by (Ramaiah, 2007), where he showed that severe organ damage leads to an increase in the release of ALT and AST, which is an indication of liver toxicity as it affects the toxicity of hepatocytes, and (Ozer et al., 2008; Huldani et al.,2022) showed that the increase in enzyme activity ALT reflects hepatocyte injury. These results are also consistent with what was mentioned (Tabish et al., 2018; Mohammed and Qasim , 2021), where he showed that graphene nanoparticles GNPs cause significant damage to the liver, which is observed by increasing the levels of ALT and ALP in the serum, as graphene nanoparticles reduce the activity of antioxidant enzymes and contribute in oxidative stress and hepatocyte damage. These results agree with what was mentioned (Nirmal et al., 2020; Hafsan et al.,2022), where it was shown 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, while 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 (Chatterjee et al., 2014), where he showed that the main mechanisms of graphene oxide toxicity are oxidative stress, physical destruction, inflammatory response, programmed cell death, necrosis, and autophagy.

Discussing the results of the meta-study

The results of the over-the-top study by transmission electron microscopy (TEM) revealed changes at the cellular level in hepatocytes represented by rupture of the nucleus and cytoplasm, nucleolysis and disappearance, in addition to the lysis of the cytoplasm. The reason for this may be attributed to the ability of graphene oxide sheets to penetrate the cell membrane as a result of their nanoscale, which causes damage or damage to cell components by several mechanisms, including direct interaction with large biomolecules in the cell, or through oxidative stress that causes cell damage and this was confirmed by many Some of the studies showed that graphene oxide easily moves across the cell membrane due to its nanoscale size and shape, which leads to the destruction of large biomolecules

such as lipids, proteins and nucleic acid molecules (Lin et al., 2015). Graphene and reduced graphene oxide cause DNA damage through the generation of reactive oxygen species, as it was shown that there is a relationship between the oxygen content and the genotoxicity of these materials (Ou et al., 2021; Bokov et al.,2022). Some researchers have shown that the toxicity of carbon nanomaterials to the cell is usually induced by the oxidative stress that arises either through the generation of reactive oxygen species (ROS), where it has been observed that the production of reactive oxygen species is directly related to the concentration of graphene oxide and the exposure time, or it may produce oxidative stress in a non-dependent manner. ROS where nanomaterials damage or oxidize certain components in the cell and this leads to oxidative stress (Liu et al., 2011; Zadeh et al.,2022), and another study reported that the accumulation of excessive reactive oxygen species leads to lipid oxidation, lysosome membrane damage and DNA damage (Zhao et al., 2021; Ansari et al.,2022).

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