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RESEARCH ARTICLE

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Investigating the protective effects of *Elaeagnus angustifolia* fruit extract on hematological parameters and damage of different tissues of male mice exposed to graphene oxide nanoparticles

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Abstract

The aim of this study is to investigate the protective effects of *Elaeagnus angustifolia* fruit extract (EAFE) on hematology, biochemical parameters, liver enzymes and damage of different tissues of male mice exposed to graphene oxide nanoparticles (GO NPs). In this research, 48 mice were divided into six groups. Hematology parameters including red blood cells (RBC), white blood cells (WBC), hematocrit (Ht), and hemoglobin (Hb), biochemical parameters such as cholesterol, triglyceride, glucose, protein, albumin, creatinine and bilirubin, liver enzymes including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) and histological damage of liver, kidney, lung and testis of mice were investigated. The results show that GO NPs in both concentrations (30 and 60 mg/kg/bw) decrease the number of RBC compared to the control group ($p < 0.05$). EAFE has no effect on RBC count while EAFE in combination with both concentrations of 30 and 60 mg/kg/bw GO NPs significantly ($p < 0.05$) improves the RBC count of mice. Also, the results show that EAFE improves the number of WBC, Ht, Hb, and biochemical parameters of mice exposed to different concentrations of GO NPs. A series of morphological changes were observed in the RBC of rats exposed to 60 mg/kg/bw GO NPs, including Dacrocyte, Poikilocyte and Schistocyte, which EAFE can improve the morphological changes caused in RBC. A series of histological damages such as degeneration and dilatation in the central vein in liver tissue, thickening and inflammatory cell infiltration in the renal capsule, peribronchiolar inflammatory cell infiltration and congestion in peribronchiolar blood vessels in lung tissue and decrease in the height of the germinal epithelium and a decrease in the population of spermatozoid cells in spermatogenic cells

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were observed in the testis of mice exposed to 60 mg/kg/bw GO NPs. The results show that EAFE can improve the damage caused by GO NPs in the liver, kidney and lung tissues, while it has no effect on the damage in the mice testis tissue. Therefore, GO NPs cause damage in the blood and different tissues of mice, and the use of EAFE can be effective in improving these damages.

KEYWORDS
biochemical parameters, dacrocyte, degeneration, liver, medicinal plants

1 | INTRODUCTION

Nanotechnology is one of the sciences that has received wide attention since the beginning of this century and has made it possible to produce different nanomaterials with different characteristics, and their demand is expanding in various sectors of industry, medicine and agriculture.[1] Nanomaterials are divided into several categories based on their shape, dimensions or chemical composition and are considered a serious threat to the environment due to their toxicity and persistence.[2] Recently, graphene nanoparticles (G NPs) have been used for many applications in medicine, such as drug release, biosensors, imaging and antimicrobial compounds, DNA sequencing, cancer treatment, and biomarkers due to their special properties such as high cross-sectional area, electrical conductivity, adaptability, and low production cost.[3,4] Although the insolubility of graphene in water limits its application, but GO NPs have exceptional properties that are of great interest in medicine. In the pharmaceutical industry, GO NPs are used as a biological material. The physicochemical properties of GO NPs have made it a very useful material for the delivery of anticancer drugs to biological cells, cell imaging, bioassays and gene transformation. In addition, scientists have shown that graphene and its derivatives are useful for extracting and identifying food toxins and organic pollutants and have antibacterial properties that have improved food packaging applications.[5] Therefore, excessive use of GO NPs in different parts leads to their wide distribution in different ecosystems and different effects on living organisms.[6] Various studies have proven that GO NPs show different behaviors when faced with environmental pollutants and cause damage to different tissues of living organisms. Patlolla et al. (2017) stated that oral administration of graphene oxide (GO) causes liver damage and oxidative stress in male rats.[7] Amrollahi-Sharifabadi et al. (2018) stated that GO NPs cause necrosis and degeneration in mice brain tissue.[8] Xiong et al. (2020) stated that G NPs had the ability to enter zebrafish cells and caused fish liver dysfunction.[9] Abd-Alsahib and Faris stated in (2022) that GO NPs cause histopathological damage in liver tissue such as cell rupture, thickening of nuclei in some cells, bleeding, dissolution and necrosis of cells in laboratory mice.[10] Therefore, investigating the toxicity effects of GO NPs and finding a substance to reduce these toxicity effects on living organisms is very important.

Today, the use of herbal medicines is increasing with the aim of obtaining low-risk medicines with minimal side effects, and this is due to the fact that many herbal medicines have been proven to be low-risk in scientific societies. *Elaeagnaceae* is a plant in the form of a tree or shrub, which is often thorny and has three genera and more than 20 species, and the *elaeagnaceae* tree (*Elaeagnus angustifolia*) is included in this family. *Elaeagnaceae* fruit contains potassium, magnesium, sodium, iron, calcium, zinc and copper. The medicinal properties of *elaeagnaceae* include strengthening the body's immune system, treating the digestive system, strengthening the heart and nervous system, improving the function of blood cells, strengthening cartilage and bones, treating wounds, and relieving stress. In addition to the mentioned properties, *elaeagnaceae* is also a natural diuretic, which helps to cleanse and detoxify the liver.[11,12] Any herbal product before it is used as a medicinal form it should also be examined in terms of toxicological studies. Since it seems necessary to use herbal treatment in order to reduce the effects of poisoning caused by various nanoparticles, and there are few studies on the protective effects of the EAFE in different tissues of living organisms. Therefore, the purpose of this research is to investigate the restorative effects of EAFE in reducing the damages caused by GO NPs in the liver, kidney, lung and testis tissues of mice and also its effect on the hematological, biochemical parameters and liver enzyme of mice.

2 | MATERIALS AND METHODS

2.1 | Synthesis of GO NPs

A modified Hammers method has been used to make GO NPs.[13] In this method, 80 mL of sulfuric acid was added

to 2 gr of sodium nitrate and heated at 70°C. Then 2 gr of graphite powder was added to the solution and the mixing continued for 15 minutes. The solution was placed in an ice bath at 5°C. Then, 12 gr of potassium permanganate was slowly added to the solution during 1.5 hours and the mixing process continued for half an hour. At this stage, the color of the solution changes from black to green. The solution was heated at 35°C for 5 hours to obtain a brown paste then, to dilute the dough, 100 mL of distilled water is added to the container and 300 mL of distilled water is poured into a larger container and the contents of the first container are slowly transferred to the larger container and stirred for half an hour. At this stage, the temperature of the solution should not be more than 40°C. After diluting the solution with distilled water, 20 mL of hydrogen peroxide is slowly added to the container and stirred for half an hour. At this stage, the color of the solution changes to light yellow for a short time, then 10% hydrochloric acid was used to increase the pH. The solution was centrifuged at 14,000 rpm for 15 minutes. Then, the settled materials are separated and placed in an oven at a temperature of 70°C for 2 days to dry. The formed powder is GO NPs.

2.2 | Preparation of EAFE

To prepare EAFE, the required *elaeagnaceae* fruit was first bought from stores in Birjand city (South Khorasan, Iran) and poured into a mortar and ground slowly to separate the fruit from the core. Then 100 gr of ground powder was boiled with 400 mL of distilled water and poured into an Erlenmeyer until it was completely paste-like. Due to the presence of carbohydrates in the *elaeagnaceae* fruit and water absorption by it, sufficient amount of boiling water was added to it in several stages to cover its surface with water and for 72 hours, it remained inside the rotary at a temperature of 55°C until it was completely mixed and the extract was taken out. Then the aqueous extract in Erlen was filtered by filter paper (Whatman grade 40, Nikoshimi Co, Iran) and in order to concentrate, the resulting solution was placed at a temperature of 55°C. Finally, the amount of dry matter of the concentrated extract was measured.

2.3 | Design of experiments

In this research, 48 male mice with an average weight of 33–35 gr were obtained from the animal breeding and maintenance center of Birjand University of Medical Sciences (South Khorasan, Iran). Mice were kept with a standard diet (standard pellet food and free access to water and food in room conditions, 12 hours of light and 12 hours of darkness, temperature of 20–25°C). After 10 days of adaptation to the laboratory environment, the mice were randomly grouped into 6 groups of 8 as follows.[14]

Group control: Recipient of regular food and water.
Group B: Recipient 30 mg/kg/bw of GO NPs.
Group C: Recipient 60 mg/kg/bw of GO NPs.
Group D: Recipient 40 mg/kg/bw of EAFE.
Group E: Recipient 30 mg/kg/bw GO NPs + 40 mg/kg/bw EAFE.
Group F: Recipient 60 mg/kg/bw of GO NPs + 40 mg/kg/bw of EAFE.

All groups received GO NPs and EAFE by gavage for 21 days and at a certain time (every day at 8 am). According to past studies, concentrations less than 30 mg kg^{-1} do not have harmful effects on hematological and histological parameters of mice, but concentrations higher than 50 mg kg^{-1} can cause disturbances in hematological and histological parameters of mice. It can be said that the induction of oxidative stress increases with increasing the dose of graphene oxide nanoparticles.[10–15,16]

2.4 | Blood sampling and collection of serum and plasma from mice

After the end of the experimental period (21 days), blood sampling was done from the heart tissue of 3 mice in each group that were anesthetized by ketamine-xylazine as an intraperitoneal injection. To collect serum, the blood sample was kept in Eppendorf vials without anticoagulant for 10 minutes at 4°C. After the blood clotted, the samples were centrifuged at 3000 rpm for 10 minutes, then the serum was collected. They were frozen at −20°C for biochemical analysis and liver enzymes. Also, to collect plasma, blood samples were poured into heparin vials and after 24 hours they were subjected to hematology analysis.

2.5 | Hematology studies

After 21 days, RBCs were counted manually after diluting with isotonic sodium chloride solution (0.85 g of sodium chloride and 100 mL of distilled water) by Neuobuer slide using (Equation 1).

Thus, in order to count RBCs, first the vial containing the sample was completely shaken to make the blood completely uniform. Then, by using a melange pipette for counting RBCs and a diluting solution, a dilution of 1:200 was obtained. RBCs were counted on a Neuobuer slide (in 5 out of 25 houses) under a light microscope.[17]

In order to count WBCs, a 1:20 dilution was prepared using a white melange pipette and Marcano's dilution

solution (2 mL of CH_3COOH and 1 mL of 1% dolegensin violet solution, and 100 mL of distilled H_2O). Then, the number of WBCs was calculated on the Neuobuer slide (in 4 cells for WBCs) under the light microscope using (Equation 2).

$$\begin{aligned}&\text{Number RBCs in per mm}^3\text{of blood}\\&= \text{Total number of RBCs counted in}\\&\quad 5 \text{ small squares of Neuobuer slide}\\&\quad \times 200 \times 10 \times 5\end{aligned} \tag{1}$$

where number 10 is a correction factor to account for the distance between slide and slider and number 200 is a dilution factor.

The number of WBCs on the Neuobuer slide (in 4 cells for WBCs) was then calculated under a microscope using (Equation 2).

$$\text{Number of WBCs per mm}^3\text{of blood} = \frac{w}{4} \times 20 \times 10 \tag{2}$$

where W is the number of WBCs in each Neuobuer slide house, number 10 is a correction factor of the distance between slide and slider, and number 20 is a dilution factor.

To calculate the amount of Ht, 2/3 of the capillary tube was filled with blood sample and its lid was closed with hematocrit-paste (Pars Payvand Co.) and centrifuged at 3000 rpm for 15 minutes and using (Equation 3). the amount of Ht was calculated as a percentage.[18]

$$\text{Ht\%} = \frac{100 \times \textit{Erythrocyte height}}{\textit{Erythrocyte height and plasma}} \tag{3}$$

The amount of Hb was calculated in mg/dL according to (Equation 4).

$$\text{Hb (mg/dL)} = \frac{Ht}{3} + 1 \tag{4}$$

Globular indices such as Mean corpuscular volume erythrocyte (MCV), mean corpuscular hemoglobin erythrocyte (MCH) and mean corpuscular hemoglobin concentration erythrocyte (MCHC) were calculated using (Equations 5, 6, and 7).

$$\text{MCV} = \frac{10 \times Ht}{\text{RBC}} \ (fl) \tag{5}$$

$$\text{MCH} = \frac{10 \times (Hb)}{R} \ (pg) \tag{6}$$

$$\text{MCHC} \times \frac{10 \times (Hb)}{Ht} \times 100 \tag{7}$$

2.6 | Investigating changes in the morphology of erythrocytes

In order to check the changes in the morphology of the erythrocytes of the tested mice, first blood smears were prepared from them on a slide and dried at ambient temperature, then the blood smears were fixed using methanol and stained with giemsa 10%. Finally, the morphology of erythrocytes was observed under the light microscope.[19]

2.7 | Calculation of biochemical parameters and liver enzymes

Measurement of biochemical parameters (Triglyceride, glucose, cholesterol, albumin, protein, creatinine and bilirubin) and liver enzymes ALP, AST, ALT, and LDH were performed using a biochemical autoanalyzer device (Hitachi 917, Hitachi Company, Japan). Serum preparation was described earlier. Commercial kits (Pars Azmoun company, registration code 34998, Iran) were used to measure biochemical and enzymatic parameters.[20–21]

2.8 | Histopathological examination

After the end of the experimental period (21 days), the abdomens of animals anesthetized by ketamine-xylazine were opened, and liver, kidney, lung and testis tissues were removed and fixed in 10% formalin for 24 hours. Then, 50, 70, 80, 90, and 96 percent alcohols were used to dehydrate the samples. The samples were placed in each of the mentioned alcohols for 1 hour.

In the next step, the samples were placed in Xylene solution for 20 minutes to make them clear. After 9 hours of storage in liquid paraffin (in an oven with a temperature of 60°C), the samples were molded and 5 micrometer sections were prepared from them using a microtome. In order to deparaffinize the samples, Xylene solution was used. Then, 96%, 80%, and 50% alcohols were used for 10 minutes for the hydration of the samples, and they were placed in Xylene for 20 minutes to make them clear. Hematoxylin-eosin dye was used to stain the samples and acid-alcohol solution was used to remove the excess color of the samples. Finally, the samples were pasted on a glass slide and kept in an oven at 37°C to dry and were studied and photographed with a light microscope (Nikon, eclipse-E200).[1]

2.9 | Statistical analysis

Kolmogorov-Smirnov test was used to check the normality of the data. Due to the non-normality of the data,

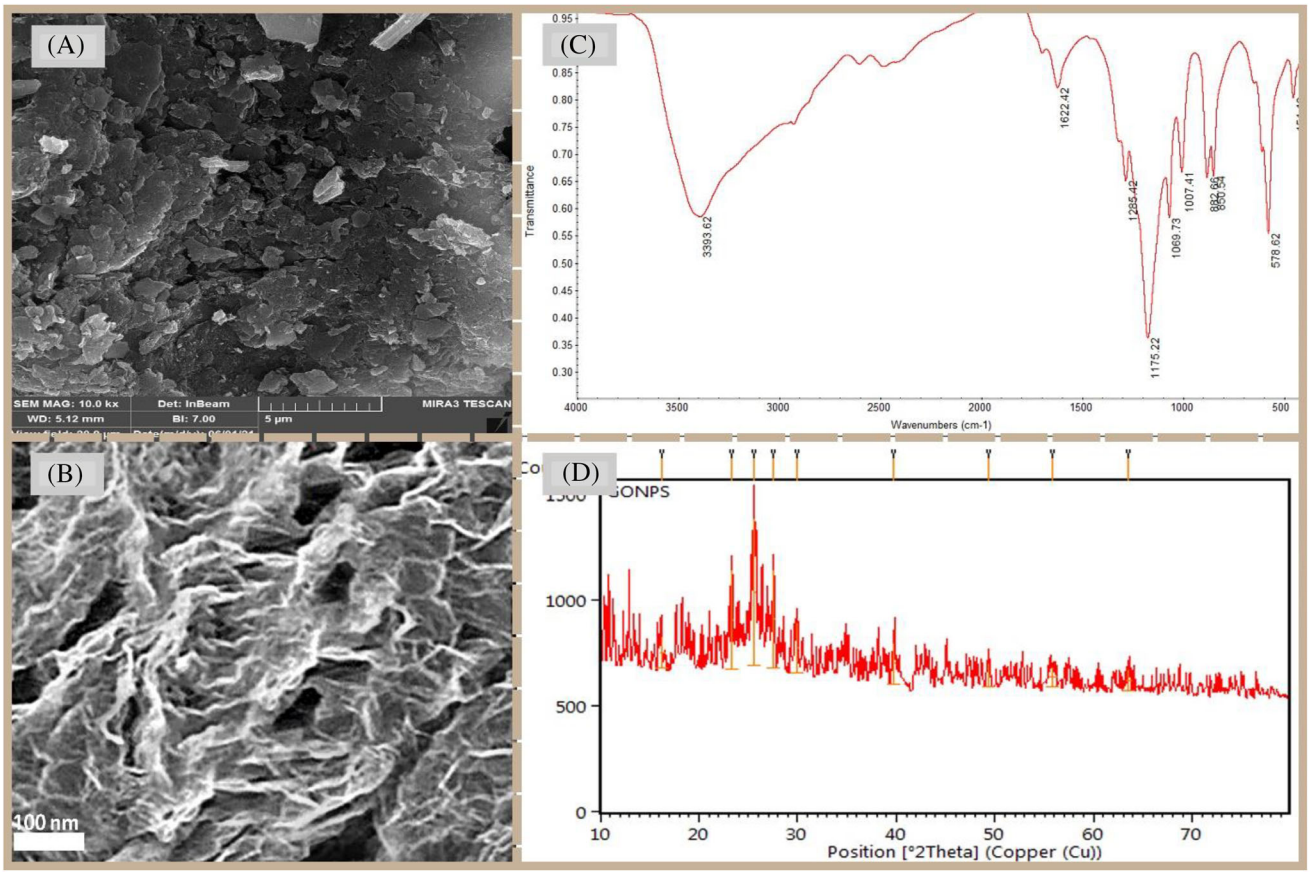

FIGURE 1 Characteristics of GO NPs SEM image (A), TEM image (B), FTIR pattern (C), and XRD pattern (D).

the non-parametric Kruskal–Wallis test was used to determine the significant difference between the treated groups at the probability level of $p < 0.05$. All data obtained are presented as mean ± standard deviation (SD). Statistical analysis was performed using SPSS version 20 software.

3 | RESULTS

3.1 | Properties of synthesized GO NPs

The properties of GO NPs evaluated by scanning electron microscopy (SEM), transmission electron microscopy (TEM), fourier transform infrared spectroscopy (FTIR), and X-Ray diffraction analysis (XRD) are shown in (Figure 1). Based on the SEM image, GO NPs have a layered structure, which is extremely thin graphene layers. Sometimes the graphene layers are folded, and the edges of the individual sheets including the twisted and wrinkled regions, can be recognized in (Figure 1A,B). Based on the FTIR spectrum, it is possible to determine the amount of presence and type of functional groups on GO. Therefore, FTIR analysis was used to prove the separation of functional groups from GO. According to (Figure 1C), it can be seen that the intensity and width of the broad peak in the range of 3000 to 3600 cm^{-1} is related to OH groups. Also, the peak area of 1069.73 cm^{-1} is related to (C–O) groups, including epoxide and hydroxyl groups of the oxide structure, and the peaks of 1622.42 cm^{-1} and 1723.31 cm^{-1} are related to (C–C) and (C = O) groups which is part of the main and hexagonal structure of graphene. Therefore, in general, it confirms the structure of GO. The results obtained from the FTIR spectrum are consistent with previous studies.[22–24] Figure 1D shows XRD patterns of GO NPs. The carbon (001) peak features for GO sheets appeared at 10°, which corresponds to definite d-spacing of 0.7 nm reported by Marcano et al.[25] After reduction, (001) eventually disappear and the new peak (002) was observed at $2\Theta = 25.3^{\circ}$ corresponding to the of inter layer distance about 0.5 nm which is consistent with the results of other studies.[26] and showedgood arrangement of interlayer distance of grapheme. The size of GO NPs was calculated based on dynamic light scattering (DLS) analysis. DLS analysis shows that the zeta potential of the sheets was around −20 +3 mV and the size of the nanoparticles varied between 25.3 and 143 nm, which is consistent with the results of other studies.[24]

TABLE 1 Chemical composition of EAFE.

Compositions of EAFE	Percent
Total sugar	57 ± 7.3
Pectic polysaccharide	3.24 ± 0.2
Flavonoids and polycarboxylic acids	1.15 ± 0.17
Tannin	4.81 ± 0.03
Protein	5.49
Fat	0.6
Fiber	3.82

3.2 | Chemical composition of EAFE

The chemical composition of the EAFE is presented in Table 1. The amount of total sugar, pectic polysaccharide, flavonoids and polycarboxylic acids, tannin, protein, fat and fiber of the EAFE used in the research were 57 ± 7.3, 3.24 ± 0.2, 1.15 ± 0.17, 4.81 ± 0.03, 5.49, 0.6, and 3.82%, respectively.

3.3 | Changes in hematology parameters

The changes in the hematology parameters of the different studied groups are shown in the (Figure 2). Based on the obtained results, the number of RBCs (millions/mm^3) of groups B (9.61 ± 1.05) and C (8.40 ± 0.58) significantly ($p < 0.05$) is lower than the control group (11.9 ± 0.95). The maximum decrease in the number of RBCs is related to group C.

The number of RBCs in group D (10.98 ± 1.53) had no significant difference with the control group. In groups E (10.24 ± 1.22) and F (9.72 ± 1.06) there was a significant increase ($p < 0.05$) in the number of RBCs compared to groups B (9.61 ± 1.05) and C (8.40 ± 0.58), respectively.

The results show that GO NPs in both concentrations (30 and 60 mg/kg/bw) decrease the number of RBCs in mice. The EAFE alone has no effect on the number of RBCs in mice While in the EAFE in combination with both concentrations of 30 and 60 mg/kg/bw of GO NPs significantly ($p < 0.05$) improves the number of RBCs in mice.

The number of WBCs (thousands/mm^3) of group B (7.56 ± 0.99) is significantly ($p < 0.05$) lower than the control group (8.88 ± 1.33). While the number of WBCs in group C (9.81 ± 1.07) is significantly ($p < 0.05$) more than the control group. The WBCs of group D (8.62 ± 1.46) had no significant difference with the control group. The number of WBCs in group E (7.94 ± 1.19) increased compared to group B (7.56 ± 0.99), but this increase was not significant, while there was a significant decrease in the number of WBCs in group F (8.83 ± 1.23) compared to group C

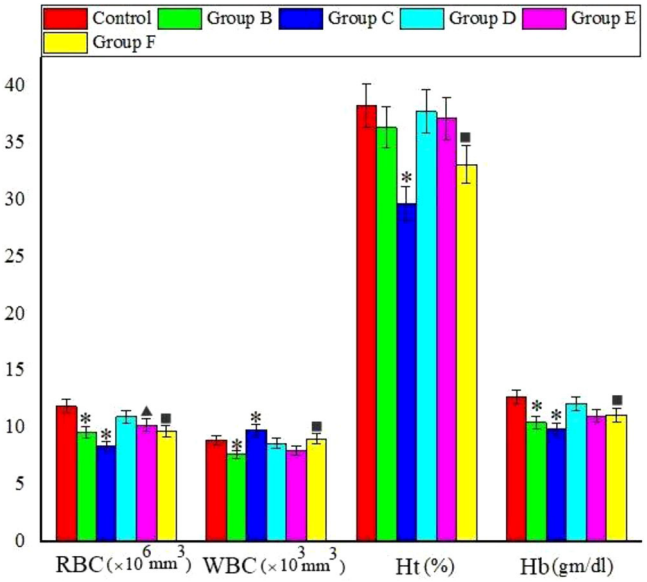

FIGURE 2 Changes in the number of RBCs, WBCs, hematocrit and hemoglobin in the blood of mice exposed to GO NPs and EAFE alone and in combination with each other after 21 days. Control group: normal food and water, group B: 30 mg/kg/bw GNO NPS, group C: 60 mg/kg/bw GNO NPS, group D: 40 mg/kg/bw EAFE, group E: 30 mg/kg/bw GNO NPS + 40 mg/kg/bw EAFE, group F: 60 mg/kg/bw GNO NPS + 40 mg/kg/bw EAFE. *Significant difference ($p < 0.05$) with the control group. ▲There is a significant difference ($p < 0.05$) with group B. ▬There is a significant difference ($p < 0.05$) with group C.

(9.81 ± 1.07). The results show that the EAFE improves the number of WBCs in mice exposed to GO NPs.

The Ht (%) of group B (36.4 ± 2.54) had no significant difference with the control group (38.3 ± 4.59) while the Ht of group C (29.7 ± 3.56) is significantly ($p < 0.05$) lower than the control group. The Ht of group D (37.8 ± 4.15) was not significantly different from the control group. The Ht of group E (37.2 ± 1.86) increased compared to group B (36.4 ± 2.54), but there was no significant difference with each other, while the Ht of group F (33.1 ± 2.64) was It increased significantly ($p < 0.05$) compared to group C (29.7 ± 3.56). The results show that the EAFE improves the Ht of mice exposed to GO NPs.

The Hb (mg/dL) of groups B (10.46 ± 0.74) and C (9.9 ± 1.08) was significantly ($p < 0.05$) lower than the control group (12.7 ± 1.14). The highest amount of Hb decrease was observed in group C. The amount of Hb in group D (12.1 ± 0.96) had no significant difference with the control group. The amount of Hb in group E (11.02 ± 0.99) increased compared to group B (10.46 ± 0.74), but there was no significant difference with each other while the amount of Hb of group F (11.1 ± 0.77) has increased significantly compared to group C (9.9 ± 1.08). The results show that

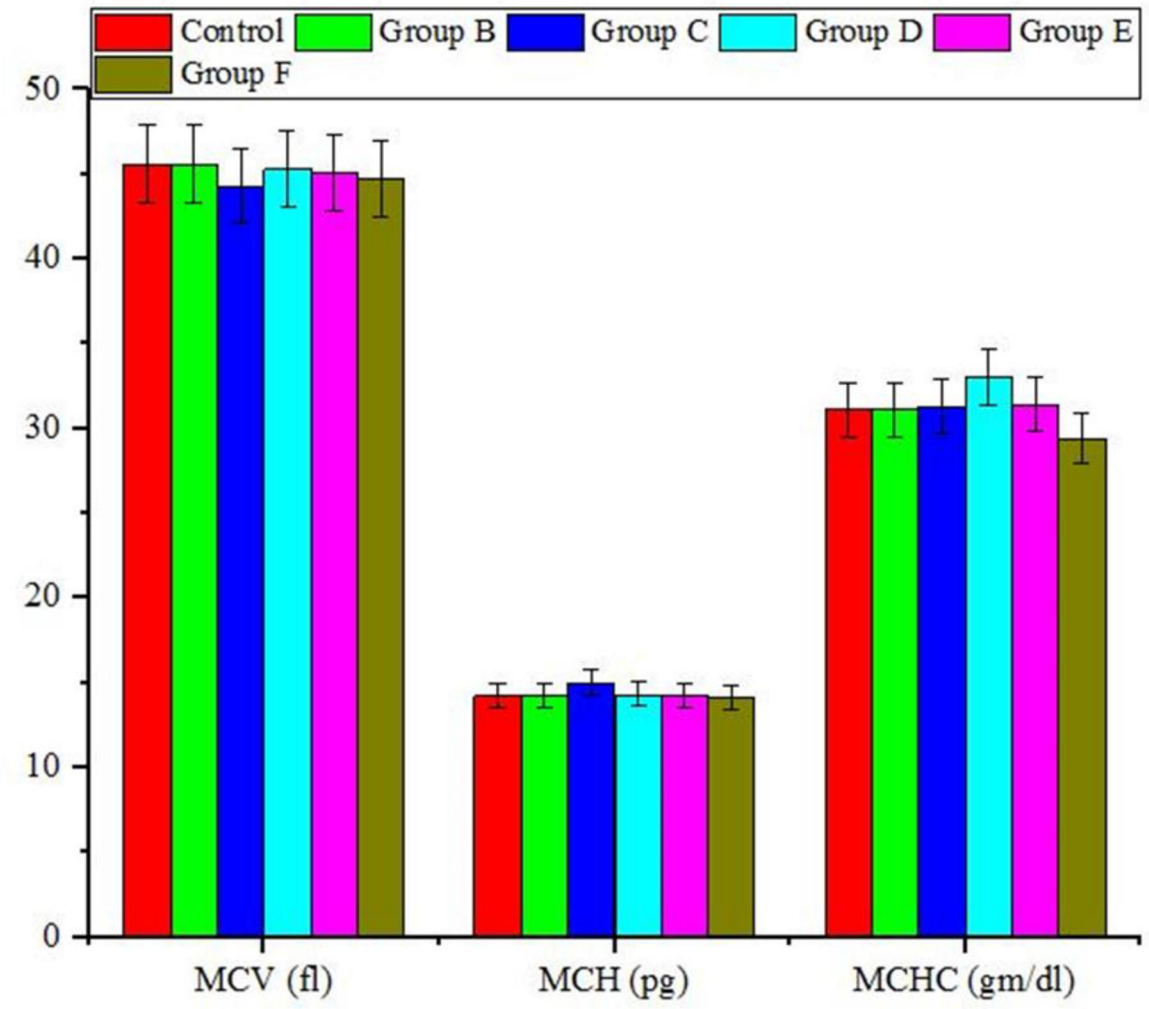

FIGURE 3 The changes in MCV, MCH, and MCHC blood indices of mice exposed to GO NPs and EAFE alone and in combination with each other after 21 days. (Control group: normal food and water, group B: 30 mg/kg/bw GNO NPS, group C: 60 mg/kg/bw GNO NPs, group D: 40 mg/kg/bw EAFE, group E: 30 mg/bw kg/bw GNO NPS + 40 mg/kg/bw EAFE, group F: 60 mg/kg/bw GNO NPs + 40 mg/kg/bw EAFE.

the EAFE improves the amount of Hb in mice exposed to GO NPs.

3.4 | Changes in MCV, MCH, and MCHC blood indices

The changes of MCV, MCH, and MCHC blood indices in different studied groups are presented in (Figure 3). Based on the results obtained in all groups, no significant difference ($p < 0.05$) was found in the amount of MCV, MCH and MCHC blood indices compared to the control group.

3.5 | Erythrocyte changes

A series of morphological changes in erythrocytes of the studied groups is shown in (Figure 4). Morphological changes observed in erythrocytes include dacrocyte, ovalocyte, keratocyte, stomatocyte, elliptocyte, poikilocyte, schistocyte, and echinocyte. The intensity of damage to erythrocytes of group C is much higher than other groups while the intensity of damage to erythrocytes of group B is much lower than group C. The effect of EAFE on the erythrocytes of mice (group D) is very low. Based on (Figure 4) shows the results that the EAFE can reduce the intensity of morphological damage to erythrocytes group C and D.

3.6 | Changes in biochemical parameters

3.6.1 | Cholesterol, triglycerides, and glucose

Changes in the concentration of various biochemical parameters in the blood serum of mice in different studied groups are presented in Table 2. Based on the obtained

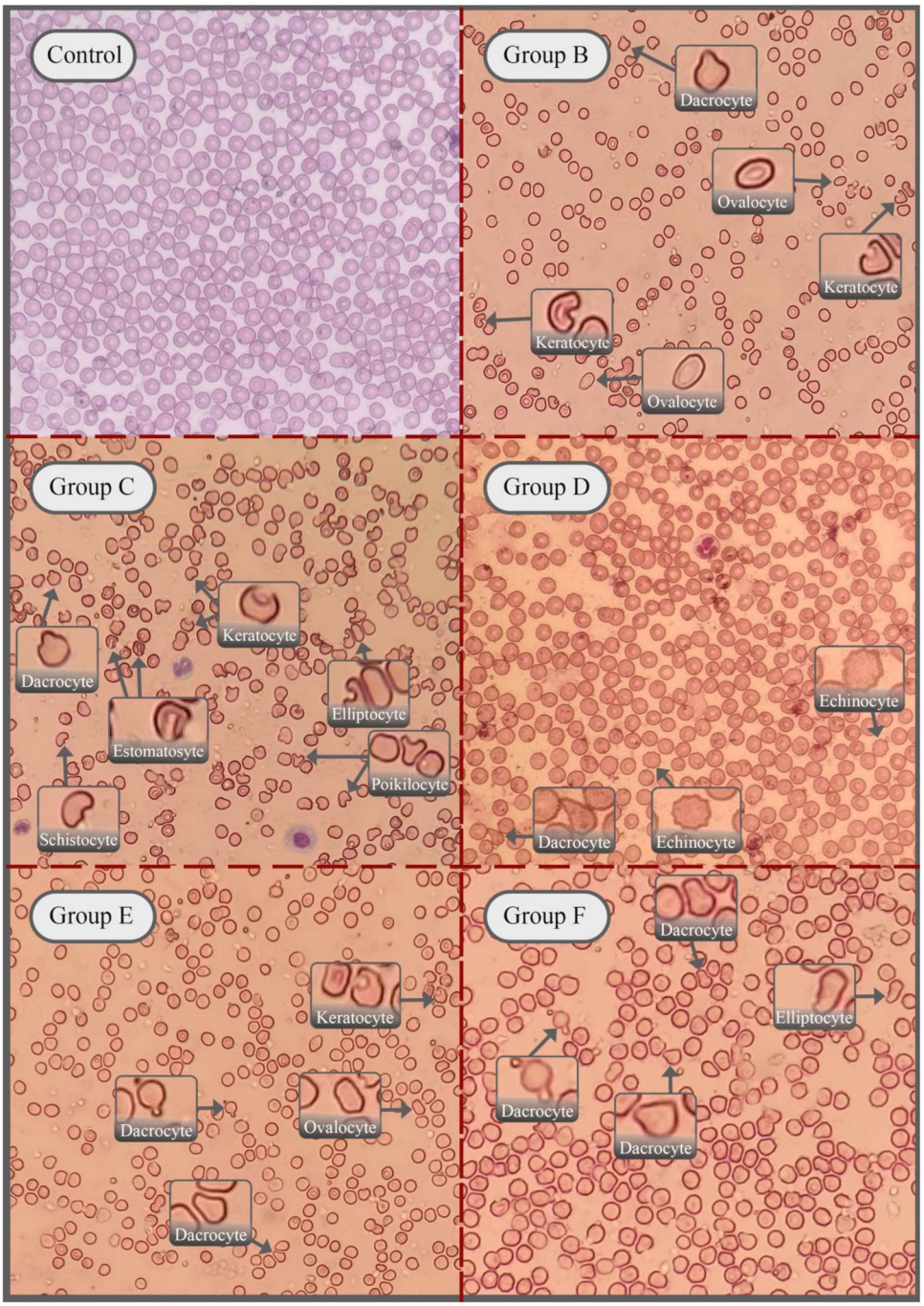

FIGURE 4 The changes in the morphology of erythrocytes of mice exposed to GO NPs and EAFE alone and in combination with each other after 21 days. (Control group: normal food and water, group B: 30 mg/kg/bw GNO NPS, group C: 60 mg/kg/bw GNO NPS, group D: 40 mg/kg/bw EAFE, group E: 30 mg/bw kg/bw GNO NPS + 40 mg/kg/bw EAFE, group F: 60 mg/kg/bw GNO NPS + 40 mg/kg/bw EAFE.

results, the serum cholesterol level (mg/dL) of the mice in the group B increased compared to the control group, but it was not significant ($p < 0.05$), while the serum cholesterol level of the group C is significantly ($p < 0.05$) higher than the control group. The cholesterol level of group D has no significant difference with the control group. The cholesterol level of group E was reduced compared to group B but, it was not significant, while the cholesterol level of group F was significantly ($p < 0.05$) compared to group C has decreased. The results show that the EAFE improves the cholesterol level of mice exposed to a concentration of 60 mg/kg/bw of GO NPs.

The results show that the serum triglyceride level (mg/dL) of mice in the group B was reduced compared to the control group, but it was not significant ($p < 0.05$), while the serum triglyceride level Group C is significantly

TABLE 2 Changes in the biochemical parameters of the blood of mice exposed to GO NPs and EAFE alone and in combination with each other after 21 days. (Control group: Normal food and water, group B: 30 mg/kg/bw GNO NPS, group C: 60 mg/kg/bw GNO NPS, group D: 40 mg/kg/bw EAFE, group E: 30 mg/bw kg/bw GNO NPS + 40 mg/kg/bw EAFE, group F: 60 mg/kg/bw GNO NPS + 40 mg/kg/bw EAFE.

Parameters	Days	Control	Control	Group B	Group C	Group D	Group E	Group F
Cholesterol (mg/dL)	21	157 ± 10.3	157 ± 10.3	141 ± 11.2	110 ± 8.7*	152 ± 13.2	148 ± 11.1	137 ± 12.3 ▬
Triglyceride (mg/dL)	21	185 ± 12.9	185 ± 12.9	174 ± 10.2	106 ± 12.7*	180 ± 24.2	179 ± 11.2▲	153 ± 18.6 ▬
Glucose (mg/dL)	21	95 ± 5.7	95 ± 5.7	143 ± 17.2*	234 ± 28.0*	129 ± 14.9	118 ± 8. 2	152 ± 12.1 ▬
Protein (mg/dL)	21	4.4 ± 0.2	4.4 ± 0.2	3.9 ± 0.3	3.1 ± 0.2*	4.2 ± 0.2	4.1 ± 0.3	4.0 ± 0.4 ▬
Albumin (mg/dL)	21	3.1 ± 0.2	3.1 ± 0.2	2.8 ± 0.2	2.2 ± 0.3*	2.9 ± 0.3	2.8 ± 0.2	2.8 ± 0.2 ▬
Creatinine (mg/dL)	21	0.9 ± 0.0	0.9 ± 0.0	0.8 ± 0.1	0.5 ± 0.1*	0.9 ± 0.2	0.8 ± 0.1	0.7 ± 0.2 ▬
Bilirubin (mg/dL)	21	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.8 ± 0.0*	0.3 ± 1.7	0.3 ± 0.0	0.5 ± 0.1 ▬

▲There is a significant difference ($p < 0.05$) with group B.
▬There is a significant difference ($p < 0.05$) with group C.
*Significant difference ($p < 0.05$) with the control group.

lower than the control group. The triglyceride level of group D has no significant difference with the control group. The triglyceride level of group E increased compared to group B but, it was not significant, while the triglyceride level of group F significantly ($p < 0.05$) compared to Group C has increased. The results show that the EAFE improves the triglyceride level of mice exposed to a concentration of 60 mg/kg/bw of GO NPs.

Based on the obtained results, the serum glucose level (mg/dL) of group B and C increased significantly ($p < 0.05$) compared to the control group while the serum glucose level of group D had no significant difference with the control group. The glucose level of group E (118 ± 8.2) increased significantly ($p < 0.05$) compared to group B (143 ± 17.2). The glucose level of group E decreased significantly ($p < 0.05$) compared to group B. But it was not significant, while the glucose level of group F decreased significantly ($p < 0.05$) compared to group C. The highest increase in glucose level was observed in group C. The results show that the EAFE improves the glucose level of mice exposed to a concentration of 60 mg/kg/bw of GO NPs.

3.6.2 | Protein and albumin

The serum protein level (mg/dL) of group B decreased compared to the control group, but it was not significant ($p < 0.05$), while the serum protein level of group C was significantly ($p < 0.05$) lower than the control group. The protein level of group D has no significant difference with the control group. The protein level of group E increased compared to group B, but it was not significant, while the protein level of group F increased significantly ($p < 0.05$) compared to group C. The highest decrease in protein level was observed in group C. The results show that the EAFE improves the protein level of mice exposed to a concentration of 60 mg/kg/bw of GO NPs.

The serum albumin level (mg/dL) of group B decreased compared to the control group, but it was not significant ($p < 0.05$), while the serum albumin level of group C was significantly ($p < 0.05$) lower than the control group. The albumin level of group D (2.9 ± 0.3) has no significant difference with the control group. The albumin level of group E had no change in group B, while the albumin level of group F increased significantly ($p < 0.05$) compared to group C. The highest decrease in albumin level was observed in group C. The results show that the EAFE improves the albumin level of mice exposed to a concentration of 60 mg/kg/bw of GO NPs.

3.6.3 | Creatinine and bilirubin

The serum creatinine level (mg/dL) of group B had no change compared to the control group, while the serum creatinine level of group C was significantly ($p < 0.05$) higher than the control group. The creatinine level of group D has no changes with the control group. The creatinine level of group E had no change compared to group B, while the creatinine level of group F decreased significantly ($p < 0.05$) compared to group C. The highest increase in creatinine level was observed in group C. The results show that the EAFE improves the creatinine level of mice exposed to a concentration of 60 mg/kg/bw of GO NPs.

The level of bilirubin (mg/dL) of group B had no change compared to the control group, while the level of serum bilirubin of group C was significantly ($p < 0.05$) lower than the control group. The bilirubin level of group D has no changes with the control group. The bilirubin level of

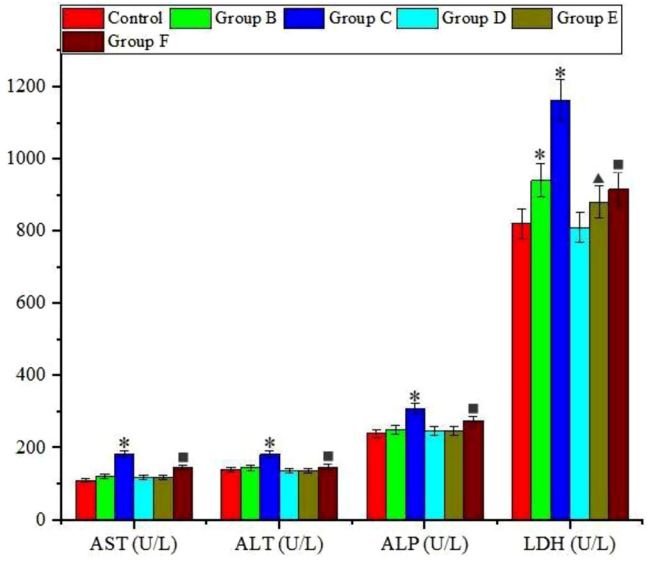

FIGURE 5 Change in the amount of liver enzymes of mice exposed to GO NPs and EAFE alone and in combination with each other after 21 days. (Control group: normal food and water, group B: 30 mg/kg/bw GNO NPs, group C: 60 mg/kg/bw GNO NPs, group D: 40 mg/kg/bw EAFE, group E: 30 mg/bw kg/bw GNO NPs + 40 mg/kg/bw EAFE, group F: 60 mg/kg/bw GNO NPs + 40 mg/kg/bw EAFE. AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase. *Significant difference ($p < 0.05$) with the control group. ▲There is a significant difference ($p < 0.05$) with group B. ▬There is a significant difference ($p < 0.05$) with group C.

groups E and F did not have any changes compared to groups B and C, respectively. Based on the results obtained, the EAFE has no effect on improving serum bilirubin level of mice exposed to GO NPs.

3.7 | Changes in liver enzymes

The effect of GO NPs and EAFE alone and in combination with each other on the activity of liver enzymes (AST, ALT, ALP and LDH) is shown in (Figure 5). According to the obtained results, the level of AST in group B (121 ± 9.68) has increased compared to the control group (110 ± 7.70), but it is not significant ($p < 0.05$), while the level of AST enzyme in Group C (183 ± 16.47) increased significantly ($p < 0.05$) compared to the control group. The amount of AST enzyme increased in group D (118 ± 7.08) compared to the control group, but there was no significant difference. The amount of AST enzyme in group E (119 ± 14.28) has decreased compared to group B (121 ± 9.68), but there is no significant difference, while the amount of AST enzyme in group F (146 ± 14.28) compared to group C (183 ± 16.47) has decreased significantly ($p < 0.05$). The results show that the EAFE improves the amount of AST enzyme in rats exposed to 60 mg/kg/bw of GO NPs.

The amount of ALT enzyme in group B (145 ± 11.60) has increased compared to the control group (140 ± 18.20), but no significant difference ($p < 0.05$) was observed, while the amount of ALT enzyme in group C (182 ± 25.48) has increased significantly ($p < 0.05$) compared to the control group. The amount of ALT enzyme in group D (136 ± 5.44) has decreased compared to the control group, but there is no significant difference. The amount of ALT enzyme in group E (137 ± 9.59) has decreased compared to group B (145 ± 11.60), but no significant difference was observed, while the level of ALT enzyme in group F (147 ± 13.23) compared to group C (182 ± 25.48) has decreased significantly ($p < 0.05$). The results show that the EAFE improves the amount of ALT enzyme in rats exposed to 60 mg/kg/bw of GO NPs.

The amount of ALP enzyme in group B (251 ± 30.12) has increased compared to the control group (240 ± 19.20), but no significant difference ($p < 0.05$) was observed, while the amount ALP enzyme in group C (308 ± 46.92) increased significantly ($p < 0.05$) compared to the control group. The amount of ALP enzyme increased in group D (246 ± 27.06) compared to the control group, but there was no significant difference. The amount of ALP enzyme in group E (248 ± 29.76) has decreased compared to group B (251 ± 3012), but no significant difference was observed, while the amount of ALP enzyme in group F (275 ± 35.75) compared to group C (308 ± 46.92) has decreased significantly ($p < 0.05$). The results show that the EAFE improves the amount of ALP enzyme in rats exposed to 60 mg/kg/bw of GO NPs.

The amount of LDH enzyme in groups B (941 ± 112.92) and C (1162 ± 92.96) increased significantly ($p < 0.05$) compared to the control group (821 ± 41.05) while the amount of LDH enzyme in group D (811 ± 56.77) has decreased compared to the control group, but there is no significant difference. The amount of LDH enzyme in groups E (881 ± 96.91) and F (917 ± 128.38) decreased significantly ($p < 0.05$) compared to groups B and C, respectively. The results show that the EAFE improves the amount of LDH enzyme in mice exposed to 30 and 60 mg/kg/bw of GO NPs.

3.8 | Changes in weight and behavior

All the studied groups lost weight at the end of the experiment, but this weight loss in group C has a significant difference at the 0.05 level with the control group (Table 3). In groups B and C, a decrease in motor activity and food consumption was observed in such a way that the decrease in activity and food consumption increases with the passage of time (Table 4).

TABLE 3 Weight changes of mice in different studied groups during 21 days of receiving GO NPs and EAFE alone and in combination with each other. (Control group: Normal food and water, group B: 30 mg/kg/bw GNO NPs, group C: 60 mg/kg/bw GNO NPs, group D: 40 mg/kg/bw EAFE, group E: 30 mg/kg/bw GNO NPs + 40 mg/kg/bw EAFE, group F: 60 mg/kg/bw GNO NPs + 40 mg/kg/bw EAFE (*significant difference ($p < 0.05$) with the control group).

	7 Days	14 Days	21 Days
Control	36 ± 2/45	35 ± 2/80	35 ± 2/10
Group B	40 ± 4/40	39 ± 3/51	37 ± 1/85
Group C	40 ± 4/80	37 ± 4/81	31 ± 1/86*
Group D	34 ± 4/08	34 ± 1/70	32 ± 2/56
Group E	37 ± 3/33	36 ± 2/78	34 ± 3/74
Group F	37 ± 5/12	36 ± 4/68	36 ± 6/12

3.9 | Histopathological examination

3.9.1 | Liver histological damage

Some histological damages in the liver tissue of mice after 21 days of exposure to GO NPs and EAFE alone and in combination with each other are shown in (Figure 6). These damages include dilatation in the central vien, dilatation in the portal vien, degeneration in hepatocytes, increase of inflammatory cells around the bile ducts in the portal area and inflammation of liver cells due to the penetration of GO NPs. The control group shows the normal tissue structure of the central vein. The intensity of the histological damage of GO NPs in group B is low and it causes dilatation of the central vein in the liver tissue. The intensity of the histological damage to GO NPs is severe in group C. GO NPs in group C caused dilatation in the portal vein, degeneration in the hepatocytes, increase of inflammatory cells around the bile ducts in the portal area and inflammation of liver cells due to the penetration of GO NPS. The EAFE in group D has no histological damage in the liver tissue. In group E, the EAFE had no effect on improving the severity of the damage caused by GO NPs in the liver tissue. In group F, the EAFE had a great effect in reducing the damage caused by the presence of GO NPs in the liver tissue. As the results show, the concentration of 30 mg/kg/bw of GO NPs does not cause much damage to the mice liver tissue, while the concentration of 60 mg/kg/bw GO NPs causes a lot of damage to the mice liver tissue. On the other hand, the EAFE alone does not cause damage in the liver tissue of mice and has the ability to greatly reduce the severity of damage caused by the concentration of 60 mg/kg/bw of GO NPs.

TABLE 4 Changes in food consumption and motional activity of mice in different studied groups during 21 days of receiving GO NPs and EAFE alone and in combination with each other. (Control group: Normal food and water, group B: 30 mg/kg/bw GNO NPS, group C: 60 mg/kg/bw GNO NPS, group D: 40 mg/kg/bw EAFE, group E: 30 mg/bw kg/bw GNO NPS + 40 mg/kg/bw EAFE, group F: 60 mg/kg/bw GNO NPS + 40 mg/kg/bw EAFE.

	Decreased motor activity	Reduce food consumption
Control	–	–
Group B	++	++
Group C	+++	+++
Group D	–	–
Group E	–	–
Group F	+	+

3.9.2 | Kidney histological damage

The histological damages caused by GO NPs and EAFE alone and in combination with each other after 21 days in kidney tissue are shown in (Figure 7). These damages include thickening and inflammatory cell infiltration in the renal capsule, perivascular inflammatory cell infiltration, degeneration in the lining epithelium of tubules and focal inflammatory cell infiltration in between the tubules in the perivascular area. The control group shows the normal structure of kidney tissue. The histological damage observed in group B is the thickening and inflammatory cell infiltration in the renal capsule which, is of low intensity. The intensity of the histological damage of GO NPs is severe in group C. GO NPs in group C cause thickening and inflammatory cell infiltration in the renal capsule, perivascular inflammatory cell infiltration, degeneration in the lining epithelium of tubules and focal inflammatory cell infiltration in between the tubules and in the perivascular area. In group D, the EAFE causes damage to perivascular inflammatory cell infiltration in the kidney tissue, which is of low intensity. The EAFE in group E had no effect on improving the severity of damage caused by GO NPs in the kidney tissue while the EAFE is very effective in reducing the damage caused by GO NPs in the kidney tissue (group F). As the results show, the EAFE alone does not cause much damage in the kidney tissue and has the ability to greatly reduce the severity of damage caused by the concentration of 60 mg/kg/bw of GO NPs.

3.9.3 | Lung histological damage

The effects of GO NPs and EAFE alone and in combination with each other after 21 days in lung tissue are shown in (Figure 8). A series of histological damages such as emphysema in air alveoli, peribronchiolar

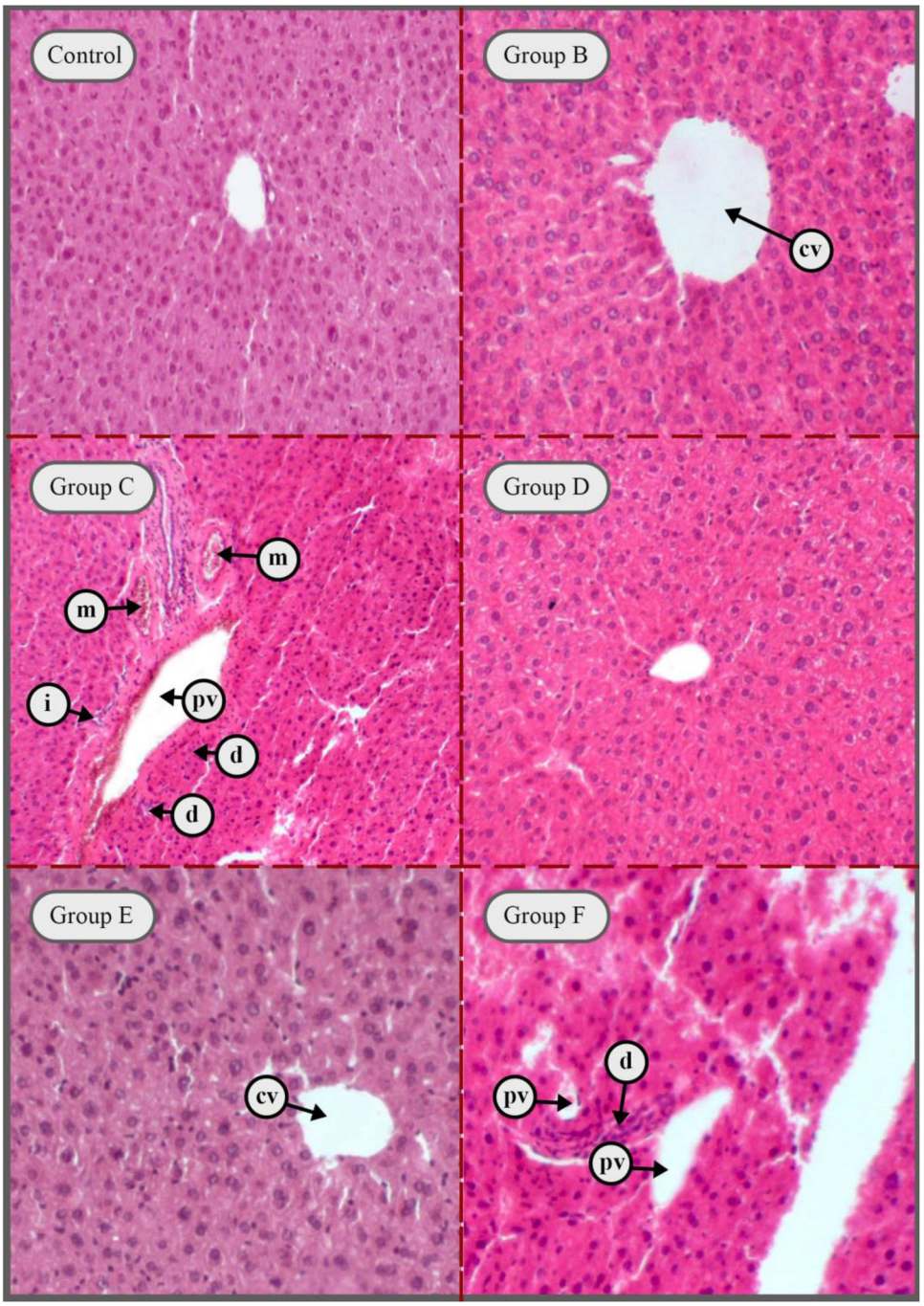

FIGURE 6 Histological damage of mice liver tissue exposed to GO NPs and EAFE alone and in combination with each other after 21 days. (Control group: normal food and water, group B: 30 mg/kg/bw GNO NPs, group C: 60 mg/kg/bw GNO NPs, group D: 40 mg/kg/bw EAFE, group E: 30 mg/bw kg/bw GNO NPs + 40 mg/kg/bw EAFE, group F: 60 mg/kg/bw GNO NPs + 40 mg/kg/bw EAFE). CV, dilatation in the central vein; pv, dilatation in the portal vein; d, degeneration in hepatocytes; m, increase in inflammatory cells around the bile ducts in the portal area and i: inflammation of liver cells due to penetration of GO NPs. H&E, 40×.

inflammatory cell infiltration and dilatation and congestion of the blood vessels were observed in the lung tissue. In the control group, the normal structure of air alveoli (a) and bronchioles (b) is shown. In group B, peribronchiolar inflammatory cell infiltration has been shown, which is low in intensity. The intensity of the histological damage of GO NPs is severe in group C. The GO NPs in group C caused peribronchiolar inflammatory cell infiltration and dilatation and congestion of the blood vessels. The aqueous extract of elderberry fruit causes emphysema in the air alveoli with low intensity (group D). As the results show, the EAFE in groups E and F improves the injuries.

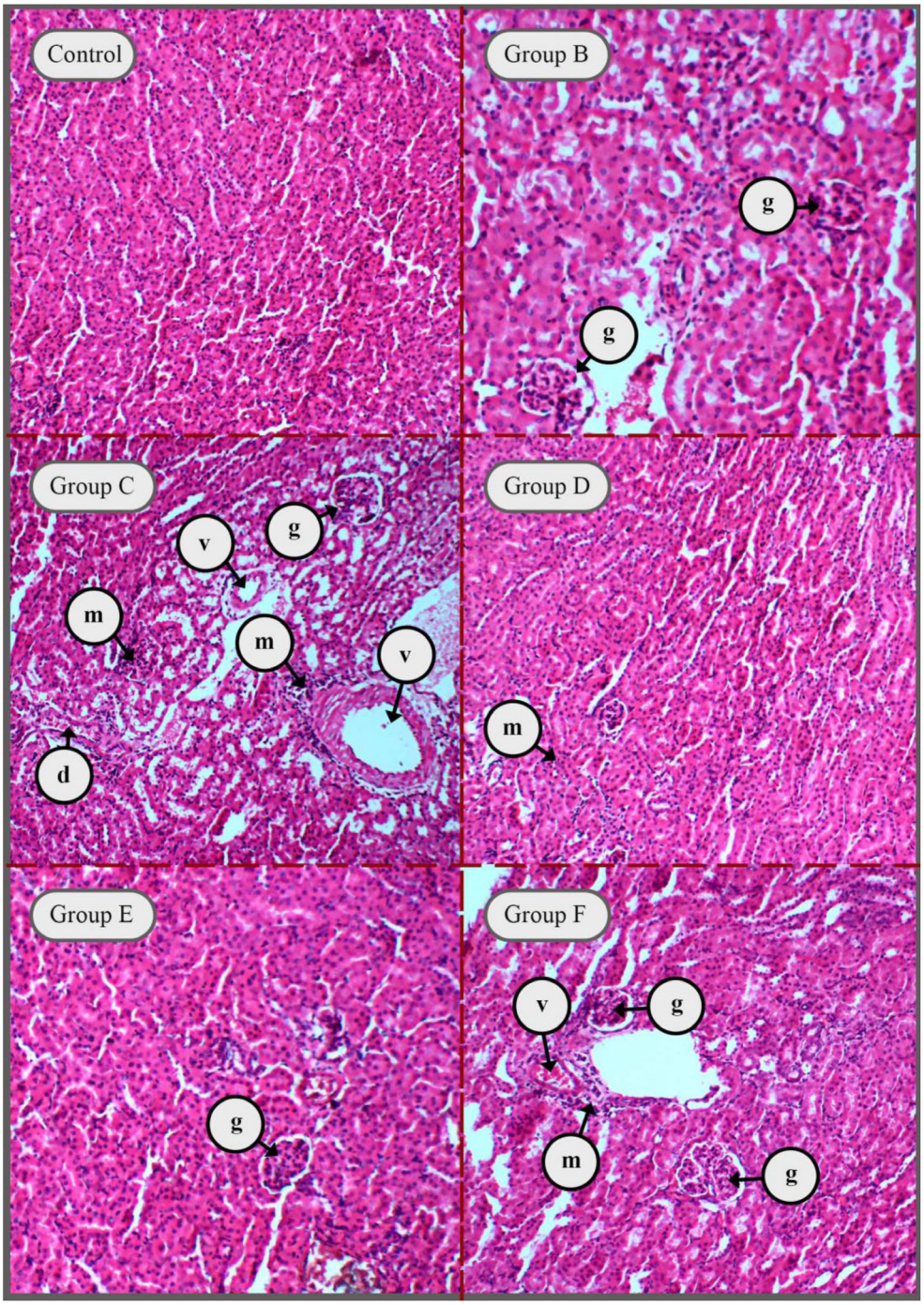

FIGURE 7 Histological damage of mice kidney tissue exposed to GO NPs and EAFE alone and in combination with each other after 21 days. (Control group: normal food and water, group B: 30 mg/kg/bw GNO NPs, group C: 60 mg/kg/bw GNO NPs, group D: 40 mg/kg/bw EAFE, group E: 30 mg/bw kg/bw GNO NPs + 40 mg/kg/bw EAFE, group F: 60 mg/kg/bw GNO NPs + 40 mg/kg/bw EAFE). g, thickening and inflammatory cell infiltration in the renal capsule; m, perivascular inflammatory cell infiltration; d, degeneration in the lining epithelium of tubules; v, focal inflammatory cell infiltration in between the tubules in the perivascular area. H&E, 40×.

3.9.4 | Testis histological damage

A series of histological damages caused by GO NPs and EAFE alone and in combination with each other after 21 days in the testis is shown in (Figure 9). These damages include decrease in the height of the germinal epithelium and decrease in the population of spermatozoid cells in spermatogenic cells, increased shrinkage of tubules, deformation in sperm tubes, mild edema in the intertubular space moderate number of sperm in the tubular lumen,

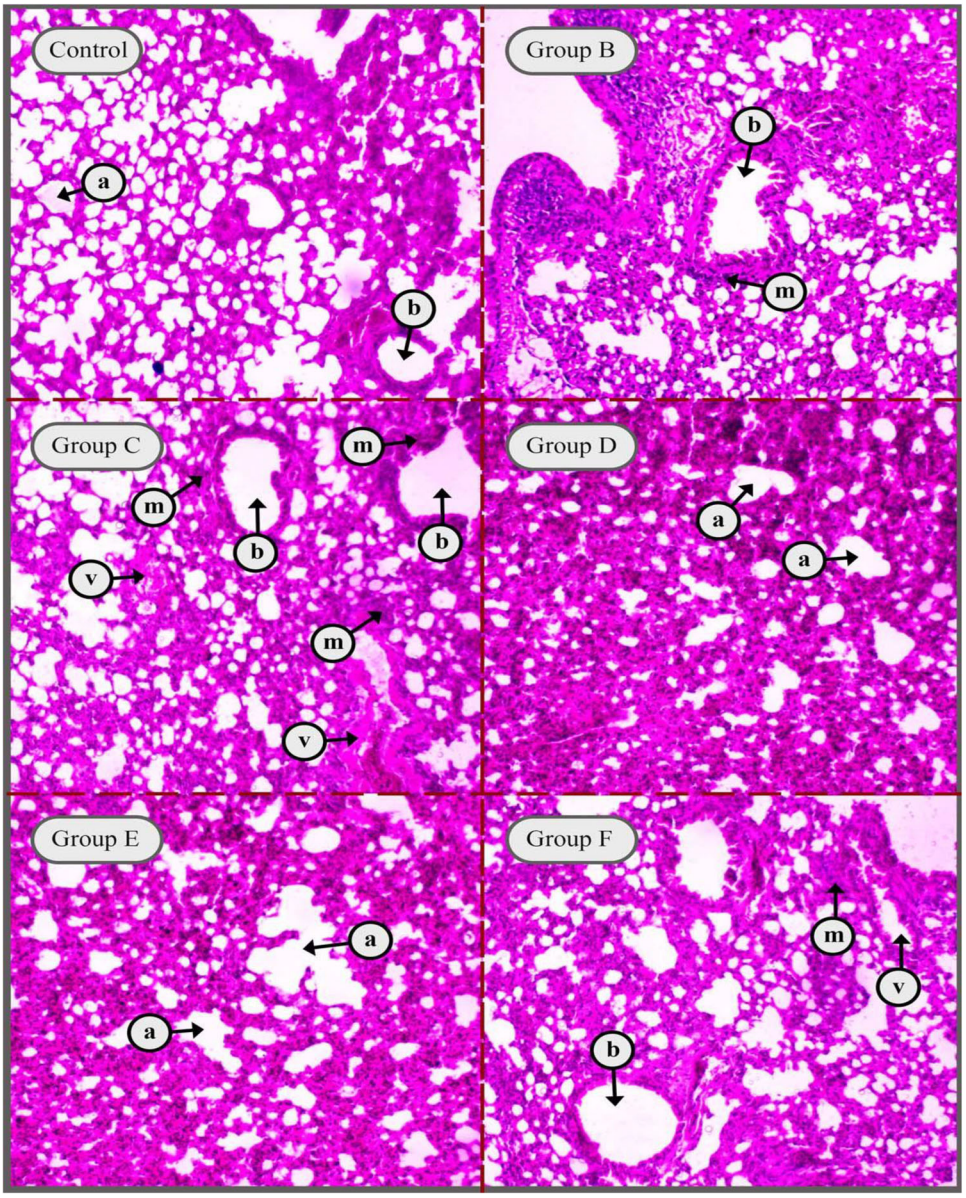

FIGURE 8 Histological damage of mice lung tissue exposed to GO NPs and EAFE alone and in combination with each other after 21 days. (Control group: normal food and water, group B: 30 mg/kg/bw GNO NPs, group C: 60 mg/kg/bw GNO NPs, group D: 40 mg/kg/bw EAFE, group E: 30 mg/bw kg/bw GNO NPs + 40 mg/kg/bw EAFE, group F: 60 mg/kg/bw GNO NPs + 40 mg/kg/bw EAFE). Control group: normal structure of air alveoli (a) and bronchioles (b). e, emphysema in air alveoli; m, peribronchiolar inflammatory cell infiltration; v, congestion of the blood vessels. H&E, 40×.

increasing the extent of interstitial tissue and vacuolation and increase in the number of Leydig cells. The control group shows the normal structure of testis tissue. The histological damage observed in group B is an increasing the extent of interstitial tissue and vacuolation and increased shrinkage of tubules with a low intensity. The intensity of the histological damage of GO NPs is severe in group C. GO NPs in group C cause damage to decrease in the height of the germinal epithelium and a decrease in the population of spermatozoid cells in spermatogenic cells, increased shrinkage of tubules, deformation in sperm tubes, mild edema in the intertubular space moderate number of sperm in the tubular lumen and increase in the number of Leydig cells. The EAFE causes damage to increasing the extent of interstitial tissue and vacuolation with low intensity. The EAFE in groups E and F had no effect on the healing of the damage caused by GO NPs in the testis tissue. As the results show, the concentration of 60 mg/kg/bw of GO NPs causes a lot of damage in the testis and the EAFE has no effect on the healing of the effects caused by GO NPs in the testis tissue.

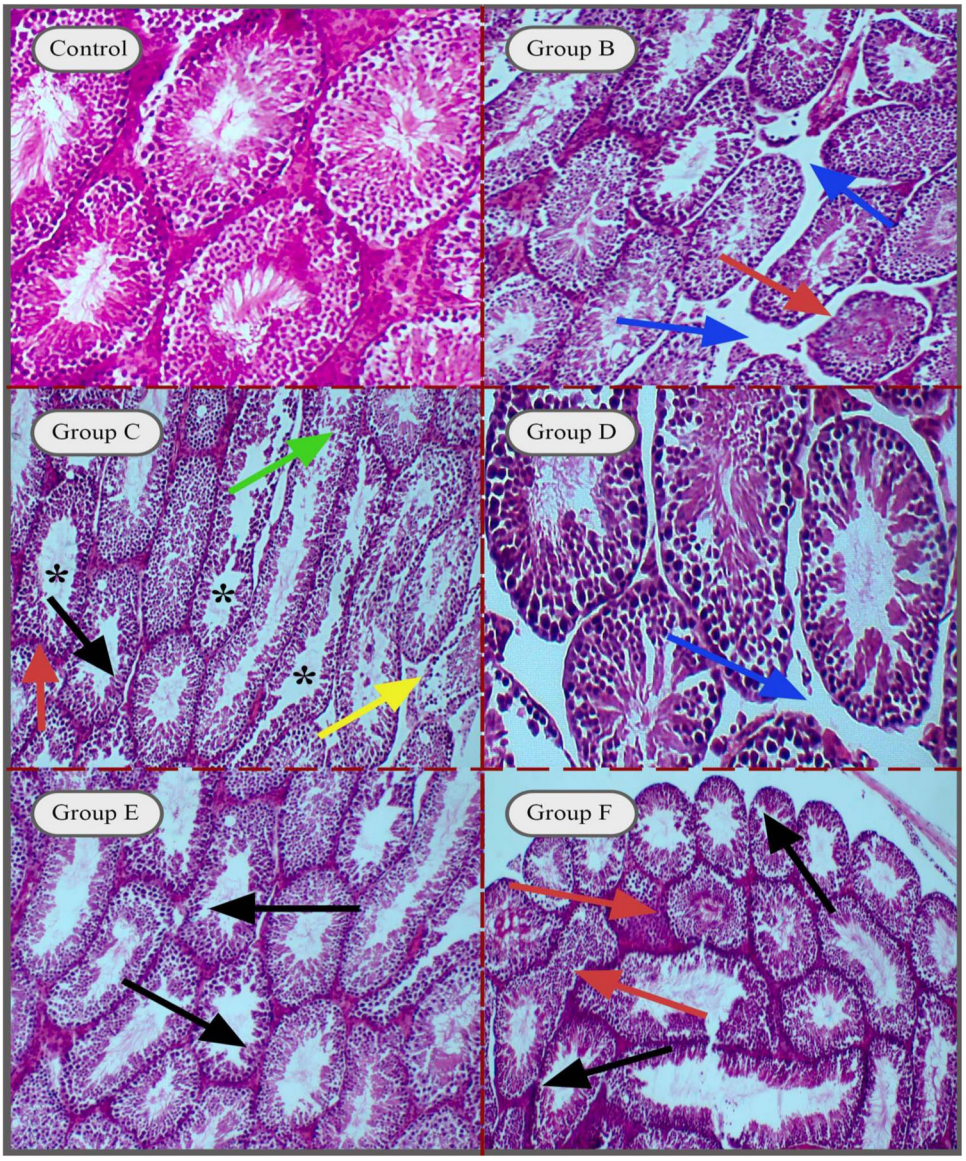

FIGURE 9 Histological damage of mice testis tissue exposed to GO NPs and EAFE alone and in combination with each other after 21 days. (Control group: normal food and water, group B: 30 mg/kg/bw GNO NPs, group C: 60 mg/kg/bw GNO NPs, group D: 40 mg/kg/bw EAFE, group E: 30 mg/bw kg/bw GNO NPs + 40 mg/kg/bw EAFE, group F: 60 mg/kg/bw GNO NPs + 40 mg/kg/bw EAFE). Black arrow: decrease in the height of the germinal epithelium and a decrease in the population of spermatozoid cells in spermatogenic cells, red arrow: increased shrinkage of tubules, *Deformation in sperm tubes, green arrow: mild edema in the intertubular space moderate number of sperm in the tubular lumen, blue arrow: increasing the extent of interstitial tissue and vacuolation and yellow arrow: increase in the number of Leydig cells. H&E, 40×.

4 | DISCUSSION

One of the most important problems in human life is environmental pollution. Today, due to the widespread use of nanoparticles and their dangers, the resulting pollution has been raised as a serious issue. In fact, the concern about the dangerousness of nanoparticles is due to their persistence in the environment and food chain.[27] In this research, after treating rats with GO NPs along with the EAFE, the protective ability of this natural compound against the damages caused by GO NPs on hematological, biochemical parameters, liver enzymes and histological damages was investigated.

4.1 | Changes in hematology parameters

Nanoparticles may cause toxicity to the organism by binding to specific receptors on the cell surface, cytoplasm or membrane of cell organelles, which lead to cellular

changes at the structural and functional levels and disrupt the normal functioning of the cell. Blood parameters can be considered as a pathophysiological index of the whole body, so it is a very useful tool in diagnosing the health of fish exposed to various toxins. Currently, hematological parameters are an important tool in toxicological and pharmacological research to monitor and evaluate the health status of organisms.[28,29] The results show that GO NPs in both concentrations (30 and 60 mg/kg/bw) reduce the number of RBCs, Ht and Hb in the blood of mice compared to the control group, also GO NPs in a concentration of 30 mg/kg/bw reduce WBCs and in The concentration of 60 mg/kg/bw causes a significant increase in the number of WBCs compared to the control group. The EAFE has no significant effect on the number of RBCs, WBCs, Ht, and Hb in the blood of mice while the EAFE in combination with both concentrations of 30 and 60 mg/kg/bw of GO NPs improves the number of RBCs, WBCs, Ht, and Hb in the blood of mice. Hematology studies show that GO NPs in both concentrations (30 and 60 mg/kg/bw) cause anemia in mice, since factors such as MCV, MCH and MCHC do not have any significant changes with the control group and the number RBCs, Ht, and Hb decreased. The anemia created is of normocytic type. Anemia may be caused by two causes: hemolysis and reduction of RBC production by marrow and bone. If the cause of anemia is hemolysis, the amount of MCV and bilirubin should increase, which the results obtained do not show this. Therefore, the anemia caused is probably caused by the disruption of RBC production in the bone marrow.[30] The EAFE in combination with both concentrations of 30 and 60 mg/kg/bw of GO NPs improves the number of RBCs, WBCs, Ht, and Hb in the blood of mice. The use of medicinal plants to strengthen immune parameters has been considered as a low-cost and effective alternative in the prevention and treatment of various diseases.[31] Plants have various chemical substances such as alkaloids, flavonoids, phenols, steroids and terpenoids that stimulate the body's immunity.[32,33] *Elaeagnaceae* extract is used in the treatment of many diseases such as anti-inflammatory, muscle relaxant and anti-ulcer agent in traditional medicine. In traditional Iranian medicine, elderberry fruit is used to relieve the pain of patients with rheumatoid arthritis.[34] It is also reported that the use of *elaeagnaceae* fruit is useful in the treatment of diarrhea.[35] Flavonoids and phenols play an important role in regulating blood pressure.[36] Therefore, the result of this study shows that the EAFE has been able to improve the number of RBCs exposed to GO NPs compared to the control group. The increase in the number of WBCs exposed to a concentration of 60 mg/kg/bw of GO NPs may have occurred due to the inflammation and dysfunction of the liver tissue, the results of this study show severe damage in the liver tissue exposed to a concentration of 60 mg/kg/bw of GO NPs (Figure 6). The significant decrease in blood Ht and Hb exposed to GO NPs may be due to the accumulation of these nanoparticles in the RBCs of fish, which caused a change in its structure and function and had destructive effects on them.[37] On the other hand, the results of this research showed that the EAFE improves the Ht and Hb of mice exposed to GO NPs. In similar studies, Samim and Vaseem in (2021) stated that nickel oxide nanoparticles (NiO NPs) significantly decrease the number of RBCs, WBCs, Hb and Ht in the blood of fish (*Heteropneustes fossilis*) compared to the control group and stated that the bioaccumulation of NiO NPs in the blood of fish has caused the reduction of RBCs.[38] Ali stated in (2019) that nickel and cobalt oxide nanoparticles alone cause a significant decrease in the number of RBCs and Hb in the blood of mice, while in combination with each other, there is no change in the amount of RBCs in mice.[39] Srivastav et al. (2016) stated that zinc oxide nanoparticles (ZnO NPs) decreased the number of RBCs, Ht, Hb, and blood platelets in westar mouse.[40] Grissa et al. (2015) stated that titanium oxide nanoparticles (TiO_2 NPs) significantly decrease the number of RBCs and Ht and increase the number of WBCs in westar mouse.[41] Mohammadi et al. (2020) used ginger extract (*Zingiber officinale*) in the diet of carp fish, and the results show that ginger extract improves the health status and hematological parameters of the fish.[42] Rashidian et al. (2022) investigated the effect of nutmeg extract (*Myristica fragrans*) on hematological parameters of carp fish. The results showed that nutmeg extract does not cause any significant changes in the hematological parameters of fish blood and stated that this extract can be used as an immune stimulant in carp fish.[43] Ameh and Alafi in (2018) investigated the effectiveness of *Moringa oleifera* leaf ethanol extract in the treatment of anemia caused by aluminum chloride ($AlCl_3$). The results showed that $AlCl_3$ causes a significant decrease in the number of RBCs, Hb and Ht in the blood of rats. However, *M. oleifera* leaf extract increased the number of hematological parameters in rats, so they stated that *M. oleifera* leaf extract has the potential to improve blood parameters damaged in anemia caused by AlCl3 in rats.[44] El-Naggar et al. (2021) investigated the restorative effects of *Phoenix dactylifera* seed extract on the antioxidant parameters of mice exposed to silver nanoparticles (Ag NPs). The results showed that *P. dactylifera* seed extract alone did not cause any changes in RBCs, Hb, and Ht. Ag NPs alone reduce the hematological parameters of mice, while the combination of Ag NPs and *P. dactylifera* seed extract improves the hematological parameters of mice. The results of this research are consistent with all the above studies.[45]

4.2 | Changes in MCV, MCH and MCHC blood indices

Changes in RBC, Hb, and Ht may have caused changes in calculated indices such as MCV, MCH, and MCHC because they are calculated using RBC, Hb, and Ht values. Based on the results obtained in all groups, no significant difference ($p < 0.05$) was found in the amount of MCV, MCH, and MCHC blood indices compared to the control group (Figure 3). In similar studies, Vali et al. (2020) did not report a significant difference in MCH and MCV values in common carp exposed to Ag NPs.[46] Samim and Vaseem reported in (2021) that the amount of MCV and MCH blood of fish (*H. fossilis*) exposed to different concentrations of NiO NPs (12, 36, and 48 mg L^{-1}) did not differ significantly compared to the control group.[38] Mustarichie et al. (2016) stated that the plant *Dendrophthoe pentandra L.* does not cause any significant difference in the amount of MCV, MCH and MCHC in the blood of mice.[47] Balta et al. (2019) investigated the protective effect of fruits (*Prunus spinosa*) against tartrazine toxicity in wistar rats. The results showed that tartrazine significantly decreased the amount of MCV, MCH, and MCHC in the blood of mice compared to the control group while the fruit of *Prunus spinosa* improves the amount of MCV, MCH, and MCHC in the blood.[48] Vidya et al. (2022) stated that cyclophosphamide significantly reduced the amount of MCV, MCH, and MCHC in the blood of mice, while *Cucumis melo* fruit extract improved the amount of MCV, MCH, and MCHC.[49] Ramadhan et al. (2021) stated that ZnO NPs cause a significant decrease in the amount of MCV, MCH, and MCHC in the blood of mice, while *M. oleifera* extract has the ability to improve the amount of MCV, MCH, and MCHC.[50]

4.3 | Erythrocyte changes

Today, morphological studies of erythrocytes are one of the most important indicators in toxicological research to investigate the possible effects of toxins on living organisms. Morphological changes observed in erythrocytes include dacrocyte, ovalocyte, keratocyte, stomatocyte, elliptocyte, poikilocyte, schistocyte and echinocyte. The severity of the damage to erythrocyte exposed to 60 mg/kg/bw is much higher than other groups. The results show that the EAFE can reduce the severity of morphological damage of erythrocytes exposed to GO NPs. Erythrocyte dacrocytosis occurs when Erythrocytes form teardrops. keratocyte occurs when the fibrin strands cut a small part of the erythrocyte. ovulocyte and elliptocyte make erythrocytes oval in shape. Stomatocytes are erythrocytes whose central pale area is in the form of a slit. Poikilocyte is a mature erythrocyte that has a clear area on one side of the cell and a dense area of hemoglobin on the other side of the cell. schistocyte are parts of erythrocytes that are the result of some hemolytic processes. These parts can vary in size, usually schistocyte are triangular in shape. Echinocytes are reversible and it is the result of the effect of the environment in which the cell is placed, and the red blood cells have central paleness.[51] In similar studies, Pleskova et al. (2018) stated that magnetite nanoparticles can enter the membrane of erythrocytes and cause structural damage such as poikilocytosis and anisocytosis in erythrocytes.[51] Soliman et al. (2021) stated that copper sulfate ($CuSO_4$) causes abnormalities such as sickle cells, schistocytes and elliptocytes in the erythrocyte structure of tilapia fish (*Oreochromis niloticus*).[52] Mekkawy et al. (2019) reported that Ag NPs caused abnormalities in the structure of erythrocytes of African catfish (*Clarias gariepinus*), such as swollen cells, sickle cells and vacuole cells.[53] Zulfahmi et al. (2021) reported that lead nitrate ($Pb(NO_3)_2$) causes swelling cell abnormality, hemolysis and cell vacuolation in erythrocytes of fish (*Chanos chanos*). All of these studies show that different nanoparticles have the ability to cause different damages in the structure of erythrocytes.[54] Tortora et al. (2019) stated that mercury causes structural damage in human erythrocytes while *Feijoa sellowiana* fruit extract protects erythrocytes against mercury toxicity.[55] Raman et al. (2016) stated that flavonoid extracted from mulberry fruit (*Morus alba L.*) reduces erythrocyte hemolysis.[56] Antonio et al. (2015) stated that hawthorn extract (*Crataegus Mexicana*) provides significant protection of human erythrocytes against oxidative damage for up to 28 days, which is the result of the presence of various phenolic compounds in hawthorn extract.[57]

4.4 | Changes in biochemical parameters

4.4.1 | Cholesterol, triglycerides, and glucose

In toxicology research, cholesterol is an important biomarker for determining stress conditions that are produced in response to various toxins. Based on the obtained results, the cholesterol level (mg/dL) of mice exposed to a concentration of 30 mg/kg/bw of GO NPs increased, but it was not significant ($p < 0.05$), while the cholesterol level of mice exposed to a concentration of 60 mg/kg/bw of GO NPs is significantly higher than the control group. Cholesterol level of mice exposed to EAFE has no significant difference with the control group. The results show that the EAFE improves the cholesterol level of mice exposed to concentrations of 30 and 60 mg/kg/bw of GO NPs. The increase in cholesterol levels after exposure to

60 mg/kg/bw of GO NPs may indicate liver dysfunction that causes the release of cholesterol into the blood.[38] Liver dysfunction in the present study has been observed in terms of changes in the activity of various enzymes such as AST, ALT and ALP, as well as the damage caused to the liver tissue (Table 4 and Figure 3). High levels of cholesterol have also been reported in previous articles in the serum of *H. fossilis*,[38] *Labeo rohita*,[58] and *Channa punctatus*,[59] which were exposed to NiO NPs, metals in wastewater, and chromium, respectively. Al-Attar and Alsalmi in (2019) stated that streptozotocin increases cholesterol levels in the blood of mice, while olive leaf extract (*Olea europaea*) has the ability to improve blood cholesterol levels.[60] Mohammadi et al. (2020) stated that the ethanolic extract of *P. dactylifera* has the ability to reduce blood cholesterol levels in mice.[61] Aldulaimi et al. (2021) stated that $CuSO_4$ causes a significant increase in the blood cholesterol level of mice compared to the control group, while the aqueous extract of *Utrica dioica* has the ability to reduce the blood cholesterol level.[62] Moradi et al. (2019) stated that the aqueous extract of *Allium saralicum* has the ability to improve blood cholesterol levels in mice.[63]

The results show that the triglyceride level (mg/dL) of mice exposed to 30 mg/kg/bw of GO NPs decreased compared to the control group, but it was not significant ($p < 0.05$), while the triglyceride level of mouse serum exposed to 60 mg/kg/bw of GO NPs is significantly lower than the control group. Triglyceride level of mice exposed to EAFE has no significant difference with the control group. The results show that the EAFE has the ability to improve blood triglyceride levels of mice exposed to concentrations of 30 and 60 mg/kg/bw of GO NPs. These changes may also occur due to liver damage and dysfunction.[38] Osman in (2019) stated that triglyceride levels decreased in African catfish (*C. gariepinus*) after exposure to copper nanoparticles (Cu NPs) compared to the control group and also reported liver damage.[64] Sulaiman et al. (2015) stated that Ag NPs reduce triglyceride levels in rats.[65] Abd El-Maksoud et al. (2019) stated that Ag NPs reduce the level of triglycerides in the blood of mice, while *Ginkgo biloba* plant extract improves blood triglyceride levels.[66] Moradi et al. (2019) stated that aqueous extract of *A. saralicum* has the ability to reduce blood triglyceride levels in mice.[63]

Glucose is one of the most sensitive parameters to evaluate the stress state of an organism. Its high concentration in the blood indicates that the organism is under stress and uses its energy reserves to control the stress level.[67] Based on the obtained results, the level of glucose (mg/dL) in the serum of mice exposed to concentrations of 30 and 60 mg/kg/bw of Go NPs increased significantly ($p < 0.05$) compared to the control group While the serum glucose level of mice exposed to EAFE had no significant difference with the control group. The results show that the EAFE has the ability to improve blood serum glucose levels in mice exposed to concentrations of 30 and 60 mg/kg/bw of Go NPs. The increase in blood glucose levels exposed to different concentrations of graphene oxide nanoparticles may be due to the breakdown of stored glycogen or the high rate of gluconeogenesis in order to meet the additional energy demand in stress conditions.[38] In previous studies, Vali et al. (2020) stated that Ag NPs cause a significant increase in the blood glucose level of common carp (*Cyprinus carpio*) compared to the control group.[46] Ibrahim stated in (2020) that Ag NPs increase the glucose level in the blood of fish (*O. niloticus*).[68] Mansour et al. (2022) stated that chlorpyrifos significantly increased blood glucose levels in African catfish (*C. gariepinus*), while plant extract (*Carica papaya*) had the ability to improve blood glucose levels.[69] Fagbohun et al. (2020) stated that fruit extract (*Kigelia Africana*) has the ability to improve blood glucose levels in mice.[70]

4.4.2 | Protein and albumin

The level of total protein and albumin are one of the most important indicators to check the health of living organisms. Proteins are very important components of all cells and tissues for the growth and development of the body. Albumin is a type of protein that plays a role in the transport of vitamins, hormones, drugs and toxic substances. These proteins are mainly produced and used in liver tissues.[38] Based on the obtained results, the level of protein and albumin (mg/dL) in the serum of rats exposed to 30 mg/kg/bw of GO NPs decreased compared to the control group, but it was not significant ($p < 0.05$), while the level of protein and The albumin of mice exposed to 60 mg/kg/bw of GO NPs is significantly ($p < 0.05$) lower than the control group. The protein and albumin levels of the serum exposed to the EAFE have no significant differences with the control group. The results show that the EAFE has the ability to improve the protein and albumin levels in the blood of mice exposed to different concentrations of GO NPs. The observed hypoalbuminemia is due to hypoproteinemia. The decrease in protein and albumin levels in groups exposed to GO NPs may be the result of gluconeogenesis, which is used to produce energy in the form of glucose.[71] Liver and kidney damage caused by different concentrations of GO NPs may decrease protein synthesis.[68] In similar studies, Alkaladi et al. (2015) reported a decrease in total protein and albumin levels in fish (*O. niloticus*) exposed to ZnO NPs.[71] Ali et al. (2021) stated that NiO NPs decrease the level of total protein and albumin in male rats (*Rattus norvegicus*) compared to the

control group.[72] Mansour et al. (2022) stated that chlorpyrifos causes oxidative stress in fish (*Mature Clarias*) and decreases the protein and albumin in the blood of the fish, while papaya extract has the ability to improve the protein and albumin levels in the blood.[69] Mohammadi et al. (2020) stated that the seed extract (*P. dactylifera*) improves blood protein and albumin levels.[61] Al-Obaidi et al. (2021) stated that lead nanoparticles (Pb NPs) decrease protein and albumin levels in the blood of mice, while *M. oliefera* seed extract improves blood protein and albumin levels.[73]

4.4.3 | Creatinine and bilirubin

The serum creatinine level (mg/dL) of mice exposed to 30 mg/kg/bw of GO NPs has no change compared to the control group, while the serum creatinine level of mice exposed to 60 mg/kg/bw of GO NPs significantly ($p < 0.05$) is more than the control group. The serum creatinine level of mice exposed to EAFE has no changes with the control group. The results show that the EAFE has the ability to improve the creatinine level of mice exposed to 60 mg/kg/bw of GO NPs. The results show that the serum bilirubin level (mg/dL) exposed to 30 mg/kg/bw of GO NPs does not have any changes compared to the control group, while the level of bilirubin in the serum exposed to 60 mg/kg/bw of GO NPs is significantly ($p < 0.05$) lower than the control group. The serum bilirubin level of mice exposed to EAFE has no changes with the control group. The results show that the EAFE has the ability to improve the blood bilirubin level of mice exposed to 60 mg/kg/bw of GO NPs. Serum creatinine and bilirubin levels are used as a rough indicator of glomerular filtration rate and renal dysfunction. Increased serum creatinine may be a sign of kidney dysfunction due to exposure to high concentrations of GO NPs.[38] Abdel-Latif et al. (2021) reported that silicon dioxide nanoparticles (SiO_2 NPs) increase creatinine levels in Nile tilapia fish (*O. niloticus*) compared to the control group.[74] Samim and Vaseem in 2021 reported an increase in creatinine levels in fish (*H. fossilis*) exposed to NiO NPs compared to the control group.[38] El-Naggar et al. (2021) stated that Ag NPs increase the level of creatinine in the blood of mice, while the seed extract (*P. dactylifera*) improves the level of blood creatinine.[45] Opris et al. (2017) stated that the extract of *Sambucus nigra* has the ability to improve creatinine levels in the blood of mice.[75] Jahanbakhsh et al. (2019) stated that the extract of *Capparis spinosa* has the ability to improve the blood bilirubin level of mice.[76] Azadbakht et al. (2021) stated that the hydroalcoholic extract of *Pistacia khinjuk* reduces the level of bilirubin in the blood of rats.[77]

4.5 | Changes in liver enzymes

In toxicological studies, liver enzymes such as AST, ALT, ALP, and LDH can be a suitable indicator against pollutants.[78] Pollutants such as GO NPs, due to their very small size, can enter the body of organisms and deposit in the liver. AST and ALT are mainly found in the liver and are intracellular enzymes involved in gluconeogenesis and amino acid metabolism. Due to the toxicity caused by GO NPs, there may be disturbances in the liver that cause the release of AST and ALT in the blood. ALP and LDH is another important enzyme that any change in the liver cell membrane can change its activity in the serum.[79] The results show that the amount of AST, ALT and ALP enzymes in the blood of mice exposed to 30 mg/kg/bw of GO NPs increased compared to the control group, but it is not significant ($p < 0.05$), while the amount of AST, ALT and ALP in mice exposed to 60 mg/kg/bw of GO NPs increased significantly ($p < 0.05$) compared to the control group. The amount of AST, ALT and ALP enzymes in mice exposed to EAFE increased compared to the control group, but there was no significant difference. The results show that the EAFE has the ability to improve the level of AST, ALT and ALP enzymes in the blood of mice. The amount of LDH enzyme in rats exposed to 30 and 60 mg/kg/bw of GO NPs increased significantly ($p < 0.05$) compared to the control group while, the amount of LDH enzyme in mice exposed to EAFE decreased compared to the control group, but there is no significant difference. The results show that the EAFE has the ability to improve the LDH level in the blood of mice exposed to different concentrations of GO NPs. In similar studies, Samim and Vaseem in (2021) reported that different concentrations of NiO NPs increase ALP levels in fish (*H. fossilis*) compared to the control group.[38] Pedarpour and Farrokhi in (2018) stated that the herbicide paraquat increases the level of AST and ALT in the blood of mice, while the hydroalcoholic extract of saffron (*Crocus sativus*) improves the level of AST and ALT in the blood of mice.[80] El-Naggar et al. (2021) stated that Ag NPs increase the level of AST, ALT and ALP enzymes in the blood of mice, while the seed extract (*P. dactylifera*) has the ability to improve the level of AST, ALT and ALP enzymes.[45] Opris et al. (2017) stated that *S. nigra* extract has the ability to improve the level of AST, ALT in the blood of mice.[75] Jahanbakhsh et al. (2019) stated that *C. spinosa* extract has no effect on AST and ALT enzymes in rat blood.[76] Hamed and Abdel-Tawwab in (2021) stated that Ag NPs increase the level of AST and ALT enzymes in the blood of Nile tilapia fish (*O. niloticus*), while pomegranate peel (*Punica granatum*) has the ability to improve liver enzyme levels.[81] All the above studies show that different nanoparticles have the ability to cause toxicity on liver

enzymes, while the extracts of different plants can improve the level of liver enzymes in different organisms.

4.6 | Histological damage

4.6.1 | Liver histological damage

Some of the histological damages in mice liver include dilatation in the portal vien, degeneration in hepatocytes, increase of inflammatory cells around the bile ducts in the portal area and inflammation of liver cells due to the penetration of GO NPs. The severity of histological damage in the concentration of 30 mg/kg/bw of GO NPs is low and includes the dilatation in the portal vien in the liver, while the severity of histological damage in the concentration of 60 mg/kg/bw of GO NPs is severe. The results show that the EAFE does not cause any histological damage in the liver, while it has the ability to greatly reduce the severity of histological damage in the liver of rats exposed to 60 mg/kg/bw of GO NPs. Studies show that various nanoparticles can cause severe damage to liver cells in the long term and are metabolized by liver enzymes and microsomes, and this in turn causes damage to hepatocytes, apoptosis and necrosis of liver cells. Contamination with graphene oxide nanoparticles causes degeneration and inflammation of liver cells (hepatic fibrosis).[82] Referring to the findings of a scientist named Del Monte and his colleagues in 2005, the researchers state that the reason for such conditions in liver cells is the disruption of membrane function and the entry of large amounts of water and sodium ions into the cell caused by nanoparticles. This cell swelling can be accompanied by the leakage of lysosomal hydrolyzing enzymes and lead to the destruction of the cytoplasm and the production of macromolecules.[83] As a proposed mechanism to justify these observations, the theory can be put forward that when nanoparticles enter the body, they accumulate in the liver, spleen, and testicles after entering, which leads to cellular absorption. The removal of nanoparticles by liver macrophages leads to the production of free radicals. It is possible that the free radicals produced by GO NPs cause peroxidation of the lipids of the liver membrane and as a result, decrease the fluidity of the membrane and destroy the cell structure and eventually destroy the liver cells. On the other hand, the restoration of liver cells and return to the normal state by means of EAFE can seek to maintain the integrity of the membranes of damaged cells due to the prevention of the leakage of intracellular enzymes.[84]

According to the research of Shariatzadeh et al. (2019), plant extracts protect the liver against oxidative damage induced by nanoparticles, and this protective effect is related to the antioxidant properties of the extract.[85] Elderberry fruit has 80% polyphenol content compared to other fruits, which helps to improve health due to its high antioxidant and anti-inflammatory capacity. The results of previous studies show that the chemical compounds of elderberry polyphenol content strengthen the immune system. Polyphenol is a plant secondary metabolite, derived from the shikimate-derived phenylpropanoid and/or the polyketide pathway, having more than one phenolic ring, and devoid of any nitrogen-containing functionality. The medicinal properties of elderberries are reportedly attributable mostly to their high content of polyphenols, including anthocyanins and flavonols.[86] Abd-Alsahib and Faris in (2022) stated that GO NPs with a concentration of 60 mg/kg/bw caused damages such as dilation of liver sinuses, proliferation of the nucleus of liver cells, thickening of the nucleus in some liver cells, penetration Inflammatory cells, bleeding and dissolution of hepatocytes in mice liver tissue.[10] Rhazouani et al. (2023) stated that GO NPs cause inflammation in liver cells.[87] Sayadi et al. (2022) stated that G NPs cause the fusion of secondary lamellae, shortening of secondary lamellae and necrosis in the gill tissue and swelling of goblet cells and increasing the number of goblet cells in the intestinal tissue of black fish (*Capueta fusca*).[4] Zhang et al. (2012) stated that the physicochemical properties of graphene oxide (GO) such as shape and size cause different toxicity of GO on cells. Graphene oxide with a dose of less than 20 μg mL^{-1} has no toxicity for human fibroblast cells, while a dose of more than 50 μg mL^{-1} shows cytotoxicity, such as reducing cell adhesion, inducing cell apoptosis, and entering the lysosomes and the cell nucleus.[88] Abd El-Aziz et al. (2022) evaluated the protective effects of pomegranate peel extract on rat liver toxicity caused by iron oxide nanoparticles (Fe_2O_3 NPs). The results showed that Fe_2O_3 NPs cause histological damage such as changes in the morphology of liver cells and formation of inflammatory kupffer cells in the liver tissue of mice. On the other hand, they stated that Pomegranate peel extract alone does not have much effect on liver morphology and improves the histological damage caused by Fe_2O_3 NPs in the liver, which is due to the antioxidant and protective properties of pomegranate peel extract.[89] Mondal et al. (2021) stated that carbofuran causes changes in rat liver cells and fruit extract (*Citrus medica*) improves liver damage due to the presence of phenolic, flavonoid and antioxidant compounds in It is an extract.[90] El-Naggar et al. (2021) stated that Ag NPs cause inflammation in the liver tissue of mice, but the extract (*P. dactylifera*) improves the damage caused by Ag NPs in the liver, which is due to the presence of antioxidant compounds in the extract.[45]

4.6.2 | Kidney histological damage

A series of histological damages including include thickening and inflammatory cell infiltration in the renal capsule, perivascular inflammatory cell infiltration and degeneration in the lining epithelium of tubules were observed in the kidney tissue of the studied mice. The histological damage observed in the kidney tissue of mice exposed to 30 mg/kg/bw of GO NPs includes the thickening and inflammatory cell infiltration in the renal capsule, which is of low intensity. The severity of histological damage in the kidney tissue of mice exposed to 60 mg/kg/bw of GO NPs is severe and includes thickening and inflammatory cell infiltration in the renal capsule, perivascular inflammatory cell infiltration, degeneration in the lining epithelium of tubules and focal inflammatory cell infiltration in between the tubules in the perivascular area. The EAFE causes damage to perivascular inflammatory cell infiltration in the kidney tissue, which is of low intensity. As the results show, the concentration of 60 mg/kg/bw of GO NPs causes a lot of damage in the kidney tissue of mice (Figure 7) While, the EAFE alone does not cause much damage in the kidney tissue of mice and has the ability to reduce the severity of damage caused by the concentration of 60 mg/kg/bw of GO NPs in the kidney. Increasing the surface reaction of nanoparticles by creating a large surface per unit mass increases the absorption of these materials in biological membranes.[91] Since the kidney tissue is one of the important organs for the accumulation of pollutants such as nanoparticles, it is very important in terms of toxicological studies.[92] Lasagna-Reeves et al. (2010) showed that most of the nanoparticles are removed by the liver and spleen after entering the bloodstream, and kidney and heart tissues are at the next stages in terms of tissue accumulation of nanoparticles.[93] Different studies prove that different nanoparticles cause DNA damage, gene mutation and cell death by causing oxidative stress.[94,95] The toxicity of GO NPs has been fully confirmed in various cases, including oxidative stress and lipid peroxidation.[10–87] As a proposed mechanism to justify these observations, it can be suggested that the EAFE, due to its antioxidant properties, can neutralize free radicals caused by GO NPs and it has the ability to maintain oxidative homeostasis and combat oxidative stress in kidney, lung and testis tissues of mice.[96] Patlolla et al. (2016) stated that GO causes epithelial cell degeneration and necrosis in kidney tissue due to oxidative stress.[97] Chen et al. (2006) stated that CuO NPs in different doses cause structural and pathobiological changes on the kidney due to the increase of H^+ and HCO_3^-.[98] Meena and Paulraj (2012) stated that nanoparticles cause cell apoptosis in the kidney due to oxidative stress.[99] Sayadi and Kharkan in (2022) stated that various heavy metals cause the swelling and increase of goblet cells in the intestinal tissue of black fish (*C. fusca*). Recently, the role of different plants due to their antioxidant compounds on renal toxicity has been investigated by pharmacological research and it has been stated that plants can heal the damage caused by pollutants in the kidney tissue due to these compounds.[100] El-Masry et al. (2020) stated that hydroxyapatite nanoparticles cause apoptosis and decrease kidney function in mice and chicory extract can improve kidney damage.[101] Farhan stated in (2020) that cadmium causes damages such as renal cell inflammation, hyperplasia and necrosis in the kidneys of mice, while the alcoholic extract of *Tribulus terrestris* has the ability to improve the severity of these damages.[102] Hamed and Abdel-Tawwab in (2021) stated that pomegranate peel (*P. granatum*) improves kidney damage in Nile tilapia fish (*O. niloticus*) against Ag NPs.[81]

4.6.3 | Lung histological damage

A series of histological damages such as emphysema in air alveoli, peribronchiolar inflammatory cell infiltration and dilatation and congestion of the blood vessels were observed in rat lung tissue. The peribronchiolar inflammatory cell infiltration of low intensity was observed in the lungs of mice exposed to 30 mg/kg/bw of GO NPs. The severity of tissue damage in the lungs of mice exposed to 60 mg/kg/bw of GO NPs was severe and included peribronchiolar inflammatory cell infiltration and dilatation and congestion of the blood vessels. The EAFE causes emphysema in air alveoli with low intensity in the lung tissue. As the results show, the concentration of 60 mg/kg/bw of GO NPs causes a lot of damage in the liver tissue of mice, and the EAFE improves these damages. Inhalation is one of the simple ways of entering the body of pollutants, which is less controllable, while the skin and oral consumption are other ways of entering the body. Different nanoparticles have the ability to enter the lungs due to their small size.[103] The results show that the severity of the damage of GO NPs in the lung tissue increases with increasing dose. Fine particles have a larger active surface than larger particles and are more active in causing biological or toxicological responses. Therefore, due to their small size, graphene oxide nanoparticles have a great ability to pass into the alveoli and go to the deep parts of the respiratory system, which causes the absorption of these nanoparticles in the lungs.[104] In fact, the size and duration of nanoparticles in the alveolar space can cause emphysema of the cells. Emphysema includes bulky lungs with enlarged air sacs and destruction of the alveolar wall, which is caused by the imbalance of protease-antipotase

and oxidant and antioxidant, which is the result of increasing oxidative stress.[105] Free radicals including two types of reactive oxygen species (ROS) and reactive nitrogen species (RNS) cause oxidative stress and cause damage to the biomolecules of a living organism. Excessive production of free radicals can bind reactive oxygen species to lipids, proteins and DNA and ultimately increase oxidative stress. Like oxidative stress, nitrosative stress also causes relaxation of blood vessels and respiratory tracts, and its imbalance causes pathological damage in the lung tissue.[94] In similar studies, Bai et al. (2018) stated that Ag NPs cause damage such as parenchymal inflammation and fibrosis of lung cells in mice after 28 days, which is due to increased oxidative stress caused by Ag NPs.[106] Mo et al. (2019) stated that Ni NPs caused damage such as lung inflammation and fibrosis of mouse lung cells after 48 days due to penetration into the alveolar space.[107] Sun et al. (2012) stated that after 90 days, TiO_2 NPs cause inflammation and hemorrhage in the lungs of mice due to oxidative stress.[108] Karami et al. (2022) investigated the protective effect of *Absinthium artemisia* extract on lung damage caused by alumina nanoparticles in Wistar rats.[109] The results showed that alumina nanoparticles cause lung cell inflammation, while *Absinthium artemisia* extract improves the severity of injuries due to the plant's antioxidant properties. Sahyon et al. (2023) stated that avocado skin improves lung cancer in mice due to its antioxidant properties.[110] Zhang et al. (2022) stated that naringenin, which is a type of flavonoid in fruits, reduces inflammation and apoptosis in lung tissue caused by Ag NPs.[111] Chniguir et al. (2019) stated that (*Syzygium aromaticum*) extract ameliorates lipopolysaccharide-induced lung inflammation.[112] Bachoual et al. (2011) stated that *P. granatum* extract reduces lipopolysaccharide-induced lung inflammation in mice.[113]

4.6.4 | Testis histological damage

A series of testis damages caused by GO NPs and EAFE alone and in combination with each other were observed after 21 days (Figure 9). These damages include decrease in the height of the germinal epithelium and decrease in the population of spermatozoid cells in spermatogenic cells, increased shrinkage of tubules, deformation in sperm tubes, mild edema in the intertubular space moderate number of sperm in the tubular lumen, increasing the extent of interstitial tissue and vacuolation and increase in the number of Leydig cells. An increasing the extent of interstitial tissue and vacuolation and increased shrinkage of tubules with a low intensity were observed in the testis of mice exposed to 30 mg/kg/bw of GO NPs. The severity of damage to the testis of mice exposed to 60 mg/kg/bw of GO NPs is severe and includes decrease in the height of the germinal epithelium and decrease in the population of spermatozoid cells in spermatogenic cells, increased shrinkage of tubules, deformation in sperm tubes, mild edema in the intertubular space moderate number of sperm in the tubular lumen and increase in the number of Leydig cells. The EAFE increasing the extent of interstitial tissue and vacuolation with low intensity in mouse testis tissue. The results show that the EAFE does not have the ability to heal the damage caused by GO NPs in the testis tissue of mice. The toxicity effects of nanoparticles are accompanied by an increase in oxidative stress, which is caused by an imbalance between the production of free radicals and the amount of antioxidants.[114] Therefore, nanoparticles may cause various damages to reproductive organs such as ovaries and testicles. The testis is a tissue with high mitotic and meiotic division and despite having a testicular blood barrier, it is very quickly affected by toxic substances such as nanoparticles.[115] In this study, graphene oxide nanoparticles change the oxidative balance in the testis tissue, and with the increase in the concentration of nanoparticles, the severity of the damage caused in the testicle increases. In similar studies, Mozaffari et al. (2015) stated that ZnO NPs cause damage to mouse testis cells due to their small size.[116] Negahdary et al. (2015) stated that manganese oxide nanoparticles (Mn_2O_3 NPs) cause damage in testis tissue of mice.[117] Park et al. (2007) stated that Ag NPs cause DNA damage in Leydig cells, which results in cell death.[118] Krishnamoorthy et al. (2007) that the aqueous extract of the plant (*Terminalia chebula*) for 45 days causes morphological changes in the testis tissue of mice.[119] Alves et al. (2018) stated that *Luffa operculata* extract causes damage in testis tissue of mice.[120] Parhizkar et al. (2014) stated that *Phaleria macrocarpa* plant extract has no effect on the morphology of rat testis tissue.[121] Various studies prove that different nanoparticles can damage the tissues of the brain and heart of mice. For example, Wai et al. (2022) stated that SiO_2 nanoparticles can affect the brain tissue of mice through the olfactory and systemic pathways. They also stated that SiO_2 nanoparticles cause apoptosis and autophagy in mouse brain tissue.[122] Lozano et al. (2020) stated that the cumulative administration of nanoSiO_2 decreased the mechanical performance index of the rat heart with a half-maximal inhibitory concentration (IC50) of 93 μg mL^{-1}.[123] Namakin et al. (2023) stated that a diet containing elderberry can improve neurological disorders and inflammations in mice due to the presence of phenolic compounds.[86] Osman et al. (2023) stated that elderberry has the ability to reduce oxidative stress due to its high polyphenolic compounds and strengthens the body's immune system.[124]

5 | CONCLUSION

The present study shows the protective effect of EAFE on hematological, biochemical, liver enzymes and different tissues of mice against the damage caused by different concentrations of GO NPs. Oral consumption of EAFE can have beneficial effects in the prevention and regeneration of various tissues such as liver, kidney and lung exposed to GO NPs due to its antioxidant activity. In addition, it improves the hematological parameters of mice exposed to nanoparticles. This study was conducted on laboratory animals and its relevance to humans requires more studies that are clinical.

AUTHOR CONTRIBUTIONS

Mohammad hossein Sayadi: Conceptualization, methodology, software, supervision, and writing-review & editing. Nazanin Fahoul: Investigation, software, and methodology. Javad Kharkan: Data curation, conceptualization, methodology, software, supervision, and writing-review & editing. Masoume Khairieh: Methodology and investigation.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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