EGYPTIAN PHARMACEUTICAL JOURNAL EPJ

Original Article 147

Efficiency of $NiFe_2O_4$ nanoparticles in treatment hospital effluent toxicity on liver, kidney, and testis of Wistar rats: CYP450 and FABP1 mechanisms

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Received: 19 May 2025
Revised: 31 May 2025
Accepted: 11 June 2025
Published: 19 October 2025

Egyptian Pharmaceutical Journal 2025, 24: 147-159

Background
Hospital effluents (HEs) include a diverse range of pathogens. Water pollution is a major environmental challenge worldwide, emphasizing the importance of creative, dependable, and sustainable solutions.
Objective
The effects of nanotechnology on human health as it relates to HEs treatment were the primary focus of the current work. This study investigates the impact of recently emerging nanotechnology solutions for treating HEs toxicity on tissue damage.
Material and methods
Male experimental rats in 3 groups, 8 rats per each, were designed: Group 1 received tap water as a control, group 2 received HEs, and group 3 received $NiFe_2O_4$ nanoparticles treated hospital effluents (N-HEs) for 4 weeks, each group received an intragastric injection of each water type (2.5 ml/100 g body weight/six hours). At the end of the experiment, blood and tissues samples were collected. Biochemical and histopathological assessments were measured.
Results and conclusion
The exposure to HEs treated with $NiFe_2O_4$ nanoparticles may reduce the dangers of HEs's tissue-damaging effects. This amelioration was shown by enhancing hematological parameters like erythrocyte, white blood cells, platelets count, hemoglobin content and the blood indices (MCV, mean corpuscular volume and MCH, mean corpuscular hemoglobin). Whereas an elevation of the level of cytochrome P450(CYP450) and total antioxidant activity (TAA), while decreasing contents of lipid peroxidation and fatty acid binding protein-1 (FABP1) in liver, kidney and testis tissues were noticed. Also, the augmentation of the histopathology of the selected tissues was recorded. In summary, the $NiFe_2O_4$ nanoparticles are appropriate to remove 95% pharmaceutical residues from HEs entirely with low-cost-effectiveness, which in turn reflected on the reduction of toxicity in the liver, kidney, and testis of the investigated rats.

Keywords: Hospital effluents, nanotechnology, FABP1, CYP450, toxicity, and total antioxidant activity

Egypt Pharmaceut J 24: 147-159

1687-4315

Introduction

Chemically active substances utilized for prevention, diagnosis, and treatment are referred to as pharmaceuticals. They are crucial to the general wellbeing of the populace. The increased use of these medications worldwide because of the increasing frequency of viral illnesses releases pharmaceutically energetic chemicals continually into the aquatic ecosystem. Hospital effluents (HEs) are among the most significant causes of this kind of contamination [1]. Prior research [1] has demonstrated that HEs contains an infinite variety of hazardous materials, such as pharmaceutical composites, byproducts of research and laboratory work, radioactive indicators, different vehicles mediators, sterilants, heavy metals, and disinfectants, in addition to a high concentration of pathogens like viruses, bacteria, and parasites that are resistant to drugs. But in many nations, these wastewaters are often regarded as household wastewaters, and as such, they are gathered alongside municipal waste waters and released into the receiving environment without any prior treatment[2]. Therefore, it is common to find pharmaceutical substances in the environment (e.g., drinking water, fish, mussels, and vegetables). These substances and/or their interface

products may have a long-term hazardous effect on aquatic creatures due to their pseudo-determined nature and depending on their physio-chemical characteristics. Consequently, these contaminants pass through the food chain and enter the human body [3].

By developing innovative nanomaterials intended for the treatment of wastewater, surface water, and groundwater polluted by hazardous metals ions, microbes, organic and inorganic solutes, we can enhance access to cleanse drinking water. Nano-adsorbents, nano zero-valent iron, nano biocides, nanofiltration, and magnetic nanoparticles are some of the products and techniques developed because of nanotechnology breakthroughs that are used in wastewater treatment [4]. According to earlier research, metal oxide nanoparticles are a promising new option for improving the aquatic ecosystem's quality and raising humankind's standard of life [1].

A crucial route in cellular metabolism is the redox reaction. Peroxidative stress, which results in a significant build-up of active oxygen free radicals and causes a variety of damage to the organism. It is one of the harmful processes of hazardous chemicals on aquatic species and has expanded to human [5]. Hematological parameters, such as white blood cells (WBCs), red blood corpuscles (RBCs), and platelet (PLT) count as well as hemoglobin (HB) concentration are critical markers of physiological and pathologic alterations in the body. They shed important light on how hazardous chemicals affect leukopoiesis, erythropoiesis, and thrombopoiesis. When these characteristics change, it can reveal information on the systemic impacts of harmful chemicals, such as toxicity on different organs[6]. On the other hand, superfamily ofCYP450 comprised of heme enzymes, catalyzes the oxidative conversion of substances, performing a vital role in metabolism of xenobiotic and alteration in animals [7]. Zhao et al. [8] found that 50% of the total clearance of popular therapeutic medicines and almost 80% of oxidative metabolism in humans are associated with one or more CYP450 enzymes. Furthermore, various clinical disorders have been linked to certain forms of FABPs, which have been suggested as indicators of tissue damage [9].

The intent of the current study is to investigate the effects of treated nanocomposite ($NiFe_2O_4$) HEs on the toxicity caused by HEs influents in blood hematology of Wistar rats. In addition to analyzing the oxidation of lipids, TAA, and other specific biomarkers (e.g., CYP450 and FABP1) inthe liver, kidney, and testistissues with monitoring thehistopathology in these tissues.

Materials and methods

Experimental animals

Wistar male rats weighing up 120 ± 10 g were selected formanimal housing of the National Research Center. The rats in the experiment were kept in ordinary polypropylene boxes, each holding eight rats. The rats were housed in a twelve-hour dark-light rotation with a measured room temperature of 21±2°C and a moisture content of 55%. Seven days prior to the research, they started becoming used to the lab setting. The rats were fed on commercial neutrality pallets and given unrestricted access to food. Following the sci1332410001 permission code, the Ethical Agreement Commission at Ain Shams University made sure that every animal research followed their standard protocols for the handling and care of experimental animals.

Hospital effluents sample collection

Sewage pipelines at Ain Shams Specialty Hospital in northern Egypt were used to collect HEs before it was disposed of. It was collected in February and March 2023. By the time the hospital was running at full capability, the samples were taken. Furthermore, water usage in all these devices and analyzers was estimated using the test results, as well as the water consumption resulting from the main hospital procedures such as analysis, sterilization, and laboratory hemodialysis. All the facility's wastewater was collected and disposed of at two sizable disposal sites on the hospital grounds. Following that, the effluent was mixed and kept at 4^oC in a sterile 2-liter glass container for the duration of the study. Analgesics such as diclofenac and antibiotics (sulfamethoxazole, ofloxacin, oxytetracycline, and amoxicillin) and their quantities in HEs are screened in our earlier study by Mostafa et al.[1] using the HPLC/UV system.

Synthesis of nickel iron oxide ($NiFe_2O_4$) nanocomposite

Aqueous nickel and ferric chloride solutions were mixed at a 1 to 2 molar ratio and stirred for thirty minutes. NaOH 2.0 M was used to gradually raise the pH to 11. After sixty minutes of constant mixing and heating (100 °C), a brownish precipitate was formed. The mixture was allowed to stay all night to ensure constancy. The pH of the filtrate became 7 by repeatedly washing the precipitate with cleansed water. The residual substance was collected and dried for six hours at 110°C. Later, being crumpled into adequate gunpowder, the substance was heated at 600°C for five hours after being stored in a desiccant [10].

Characterization of nanocomposite

By way of reported in earlier work [1], EDX (energy dispersive X-ray) examination was done to validate $NiFe_2O_4$ chemically (Fig. 1). Additionally, Bruker AXS-D8, Germany, and Cu Kα radiation (λ = 1.5406 Å) were used for XRD (diffraction of X-ray) measurements around the 2θ span (10 to 80 ^{o} C). The infrared spectra were assessed using FTIR, a Fourier-transform infrared spectrometer, Pye-Unicam Sp-3-300, which was equipped with the KBr and 8101 PC method. The morphological descriptions of $NiFe_2O_4$ were examined using an electronic transmission and scanning microscope (TEM, SEM) as shown in Fig. 2. Utilizing a JEOLJEM-1400EX TEM (Osaka, Japan), pictures were taken. For TEM investigations, the granules were dissolved in ethanol to distribute them throughout the samples. After that, the sample was cautiously placed on a copper net made of lacey carbon, which served as a support for TEM investigation. The grid was exposed to lower pressure after two hours of room temperature drying. The TEM's acceleration voltage was 20 kilovolts.

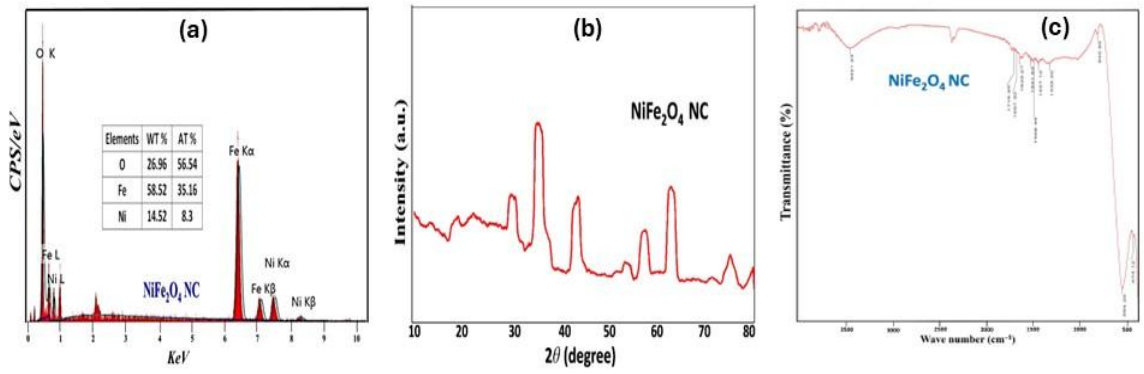

Fig. 1. (a) Energy dispersive X-ray (EDX) spectra, (b)X-ray diffraction (XRD) forms, and (c) Fourier-transform infrared spectrometer (FTIR) spectra of $NiFe_2O_4$ nanocomposites (available with authorization from Mostafa et al.[1].

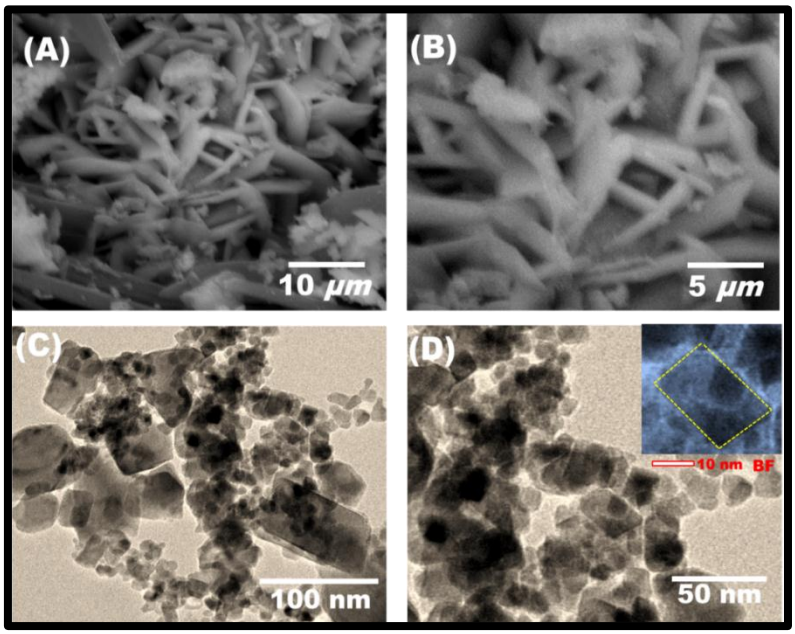

Fig. 2. (A, B) Scanning electronic microscope (SEM) and (C, D) Transmission electronic microscope (TEM) pictures of $NiFe_2O_4$ nanocomposite at various magnifications.

Hospital effluents treatment by nanocomposite

A magnetic stirrer was then used to agitate the mixture for one hour at room temperature after mixing 1.0 liter of HEs and 0.1 g of nanocomposite ($NiFe_2O_4$) . Once filtered, the HEs treatment sample was stored at –20°C for the duration of the experimental inquiry [1].

Experimental design

According to Claassen [11] 9 - 12 ml/100 g of body weight of rats is the daily water consumption. Following one week of acclimatization, each group received an intragastric injection of each water kind (2.5 ml/100 g b. wt./six hours) for 4 weeks [12,13]. In this experiment, 24 rats were separated into 3 groups: control group received standard tap water, the 2nd group received hospital effluent (HEs), and the 3rd group received HEs treated with $NiFe_2O_4$ nanoparticles (N-HEs).

Blood and tissues sampling

The test rats were sacrificed by fast decapitationand blood was collected into tubes having heparin for hematological investigation. The samples of the liver, kidney, and testis were then taken from the animal bodies, cleaned in ice-cold saline, and dried. Each of them was homogenized separately in 0.05 M phosphate buffer, pH 7. The homogenates were centrifuged at 5°C and5000 ×g for 5 minutes, and they were then kept for biochemical analyses. For additional histological analysis, the liver, kidney, and testis of the rats have been fixed in formalin.

Hematological analysis

The automatic hematology analyzer Swelab Alfa Standard (Boule Medical A.B., Sweden) was used to determine the clinical hematological parameters; RBCs count, HB concentration, WBCs count, PLT count, MCV, and MCH.

Colorimetric assay

Malondialdehyde (MDA) was utilized as a marker of the lipid peroxidation index, it was measured at 532 nm using the thiobarbituric acid reactive chemicals assay, as described by Draper and Hadley [14]. The determination of TAA was measured using Koracevic et al. [15] technique at a wavelength of 532 nm.

Enzyme Linked Immunosorbent Assay (ELISA)

Techniques for identifying rat total CYP450kit (catalog number: MBS730881) and rat FABP1 kit (catalog number:LS-F19487) are acquired from My BioSource Co, Southern California, San Diego, USA and LS-Bio Co, New York, USA, respectively. Within 450 nm of the optical density, the ELISA plate reader (USA, Florida-based Technologies Notice delivers Stat Fax 2200) assessed the absorbance color.

Histological investigation

The process to get histology samples is outlined in detail by Bancroft and Stevens [16]. The leftover tissue was cut into thick (3–4 mm) sections and then placed back into 10% neutral buffered formalin. After that, it was dried using different ratios of ethyl alcohol, cleaned in xylene, and lastly placed in the paraffin wax. The blocks of paraffin wax were censored into 4-6 µm substantial slices using a microtome to evaluate the structure of all the tissues. The slices were then stained with hematoxylin and eosin. Leica Microsystems in Switzerland is the source of the Leica microscope (CH9435 Hee56rbrugg, Leica, Switzerland).

Statistical analysis

The statistical platform for societal science research, Windows form 23.1, from SPSS, Chicago, IL, was utilized for this work. Results are shown as mean 8 rats ± standard errors. Following the achievement of a significant F test and the realization of comparisons at $P< 0.05$, the least significant differences (LSD) between the groups were evaluated.

Results

Hematological results

Table 1, the rats treated with HEs and N-HEs showed a significant ($P<0.05$) decrease in RBCs count, HB content, MCV and MCH, in comparison with the control group. Moreover, the HEs-treated group showed a significant decrease in PLT and WBCs count when compared with the group of controls. But the treatment with N-HEs demonstrated a significant rise in the all values of hematological parameters in relation to the HEs-treated group and normalized the PLT and WBCs count.

Biochemicalresults

In the present study, the administration of HEs for 4 weeks induced a significant decrease and increase intotal antioxidant activity and MDA concentration at $P<0.05$, respectively of the liver, kidney, and testis tissues in comparison to the control group. Also, the treatment of HEs with nanocomposite (N-HEs) produced a significantdecline in TAA of liver, kidney, and testis, as well as a significant rise in kidney MDA content in comparison to the control group. While as compared to HEs group,N-HEs treated rats appeared to have a significant reduction in the MDA concentrations with a significant improvement in TAA in all investigated tissues in comparison to the HEs-treated rats as shown in Table 2.

Table 1. The influence of Hospital effluents (HEs) and Nanocomposite treated- Hospital effluents (N-HEs) on hematological parameters.

Parameters	Control	HEs	N-HEs
RBCs (10^6 cells/ml)	8.69 ±0.17	6.29*±0.22	7.69*♦±0.22
HB (g/dl)	16.20±0.23	13.16*±0.11	14.43*♦±0.33
MCV (fl)	63.26±0.94	51.30*±0.69	56.66*♦±1.83
MCH (pg)	19.61±0.15	17.21*±0.21	18.10*♦±0.22
PLT (10^3/ml)	600.33±13.42	506*±10.28	588.50♦±6.37
WBC (10^3cells/ml)	12.10±0.40	9.53*±0.73	11.60♦±0.14

The results are for 8 rats' means ± SE. At *P*-value <0.05, * refers to a significant difference from the group of control and ♦ means a significant difference from the group of HEs. (RBCs: Red blood corpuscles, HB: Hemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, PLT: Platelet, WBCs: White blood cells)

Table 2. The influence of Hospital effluents (HEs) and Nanocomposite treated-Hospital effluents (N-HEs) on liver, kidney, and testis Malondialdehyde (MDA, μmol/g) and Total Antioxidant Activity (TAA, μmol/g).

Tissue	Parameters	Control	HEs	N-HEs
Liver	**MDA**	38.98±1.34	52.02*±1.78	40.44♦±1.56
	TAA	23.09±0.92	5.50*±0.61	15.18*♦±1.36
Kidney	**MDA**	25.68±0.61	70.55*±2.68	36.92*♦±1.32
	TAA	16.03±0.57	4.43*±0.35	13.45*♦±0.66
Testis	**MDA**	16.01±0.31	24.77*±1.19	18.50♦±1.972
	TAA	14.97±0.70	6.90*±0.75	12.53*♦±0.81

The results are for 8 rats' means ± SE. At *P*-value <0.05, * refers to a significant difference from the group of control and ♦ means a significant difference from the group of HEs.

Inthe current study the administration of HEsand N-HEs induced asignificant decrease in liver, kidney, and testis total CYP450 concentrations and an increase in FABP1 concentrations in comparison to thecontrol group. While administration of N-HEs produced a significant increase in CYP450 concentrations but revealed a significant (P<0.05) reduction in the FABP1 concentrations incomparison to the HEs-treated rats (Figs 3,4).

Histological results

In the present study, the hepatic tissue from a control rat, presenting a normal liver section, shows hepatic cells radiating around the central vein, which is lined with endothelial cells and blood sinusoids. . Some hepatocytes appeared bi-nucleated (Fig. 5A). The liver sections of HEs group delineated destruction of the normal pattern of the hepatic strands, vacuolar hepatic degeneration and some hepatocytes nuclei appeared pyknotic, dilated hepatic sinusoids stuffed with inflammatory cells. Besides the appearance of the central vein and hepatic tissue stuffed with erythrocytes along with the activation of Kupffer cells (Fig.5B, C, D). Hepatic tissue from N-HEs group showed restoration of the arrangement of hepatic cords, hepatocytes appeared near normal shape, the central vein appeared clear, although hepatic tissue was still stuffed with some erythrocytes, and dilated hepatic sinusoids (Fig.5E). Moreover, in kidney cortex sections revealed the preserved kidney structure and normal tubular epithelial cells morphology standard histological construction of standard renal parenchyma, round glomeruli, distal convoluted tubules with extensive lumen, and proximal convoluted tubules with thin lumen wrinkled through cuboidal epithelial cells (Fig. 6A). The Renal cortex section from HEs group showed atrophied glomerular cluster with expansion of Bowman's capsule, renal tissue stuffed with erythrocytes, degenerative epithelial lining of renal tubules and convoluted tubules lumen filled with cell debris (Fig.6B). While renal tissue from N-HEs group maintained a normal morphology with slight segmentation compared with the HEsgroup, someproximal convolute tubules appeared with cell debris in their lumen (Fig.6C).

Additionally, the current study showed normal testicular architecture with distinct series of spermatogenic cells and sperms was evident in the testicular tissue from the control group (Fig. 7A). Testicular tissue from HEs group showed deformed seminiferous tubules (arrows), loss and degeneration of all types of the spermatogenic cells (*) and sperms (arrows) within the seminiferous tubules, blood stagnation (orange arrow) in the interstitial spaces between seminiferous tubules (Fig.7B); while restoration of the spermatogenic cells, which are found in an arranged series and the presence of the sperms, nevertheless blood stagnation still appeared in the interstitial spaces in testis sections from N-HEs group (Fig.7C).

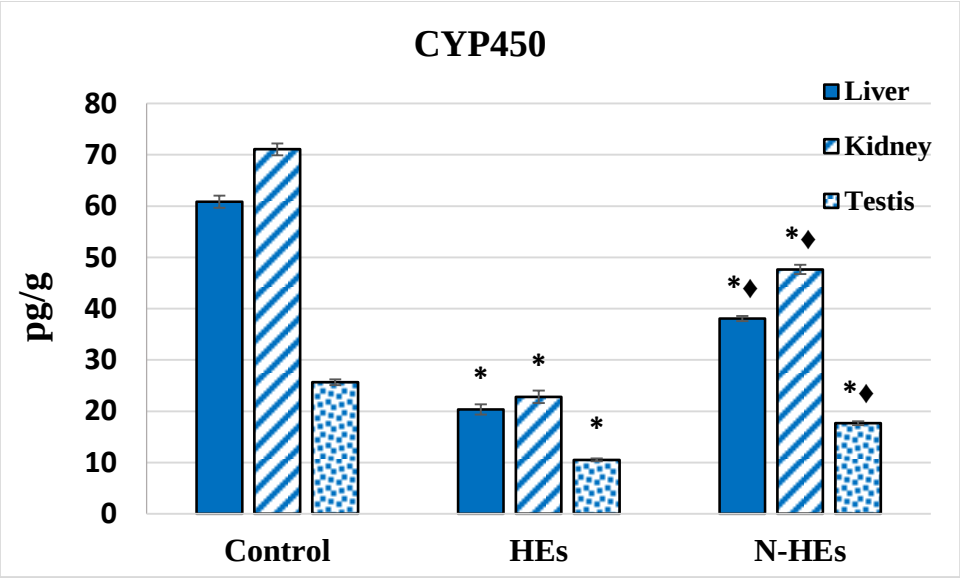

Fig. 3. The influence of Hospital effluents (HEs) and Nanocomposite treated- Hospital effluents (N-HEs) on liver, kidney, and testis total cytochrome P450 concentrations (pg/g).The results are for 8 rats' means ± SE. At P-value <0.05, * refers to a significant difference from the group of control and ♦ means a significant difference from the group of HEs.

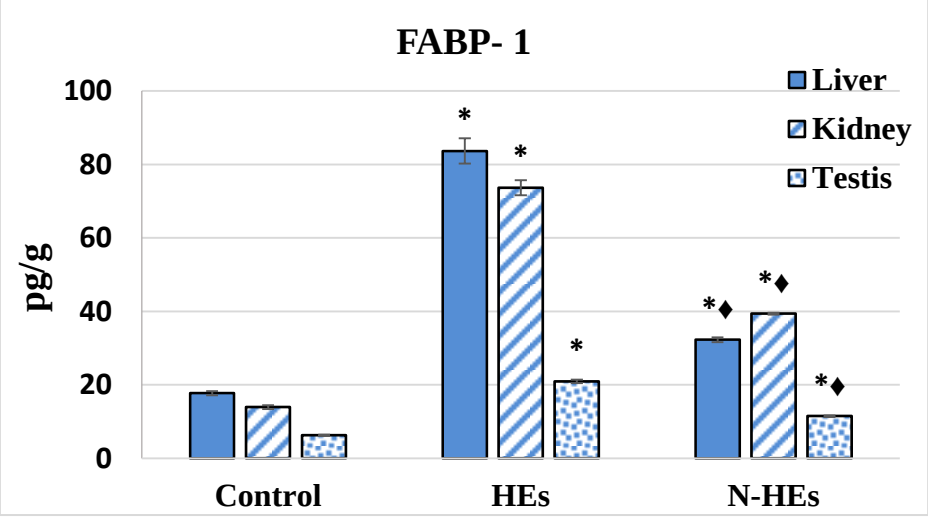

Fig. 4. The effect of Hospital effluents (HEs) and Nanocomposite treated-Hospital effluents (N-HEs) on liver, kidney, and testis fatty acid binding protein 1 concentrations (FABP1, pg/g).The results are for 8 rats' means ± SE. At P-value <0.05, * refers to a significant difference from the group of control and ♦ means a significant difference from the group of HEs.

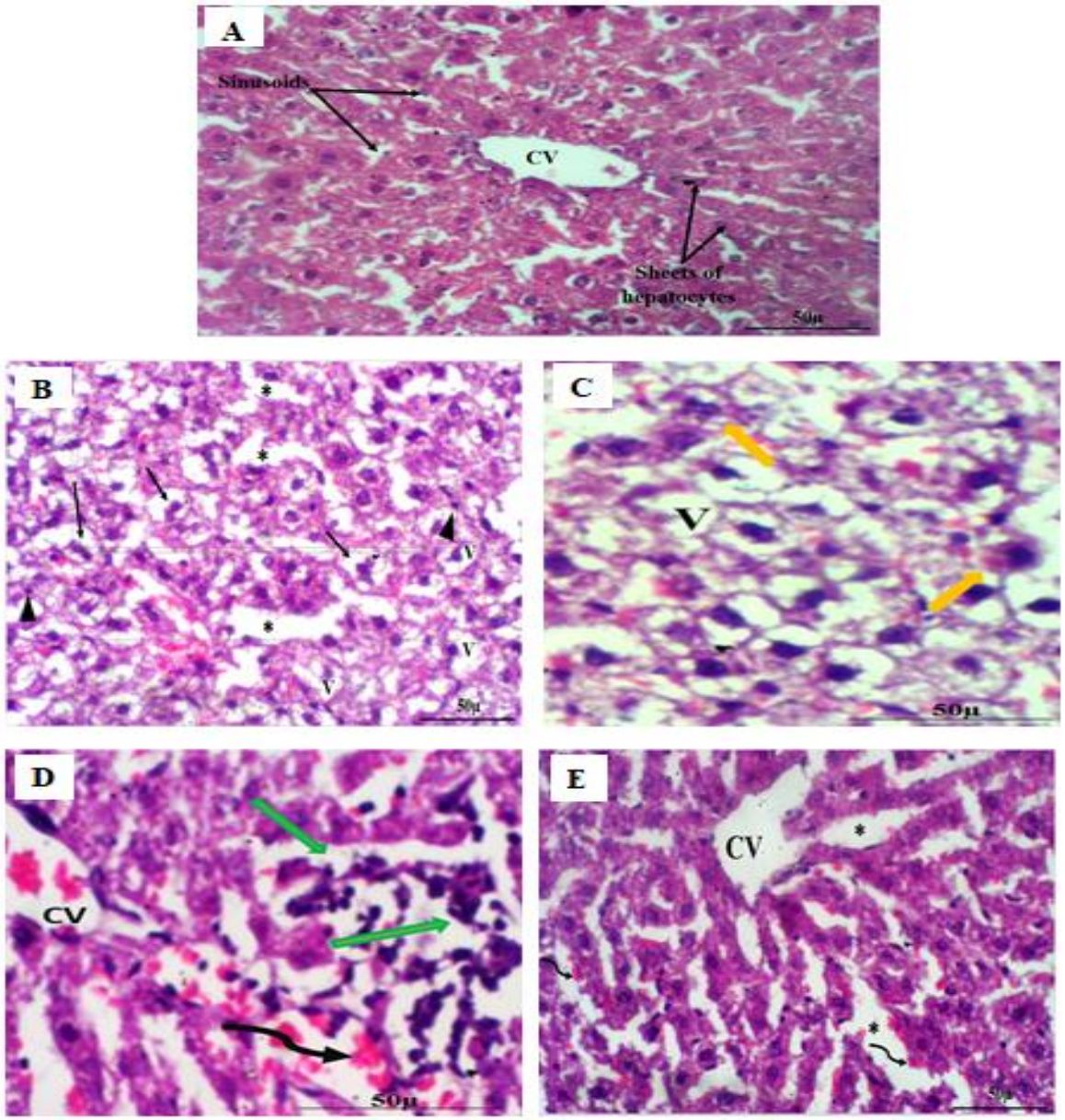

Fig. 5. (A) Photomicrograph of liver from control rat presenting normal liver section, showing hepatic cells radiating around the central vein (CV) that is lined with endothelial cells and blood sinusoids are also present (H-E, X 400). (B, C, D) Photomicrographs of liver section of HEs rat showing destruction of the normal pattern of the hepatic strands, vacuolar hepatic degeneration (V) and some hepatocytes nuclei appeared pyknotic (head arrows), dilated hepatic sinusoids (*) stuffed with inflammatory cells (green arrow). Central vein and hepatic tissue stuffed with erythrocytes (curved arrows),along with the activation of Kupffer cells (yellow arrows). (H-E, X 400). (E) Photomicrograph of liver section from N-HEs rat showing restoration of the arrangement of hepatic cords, hepatocytes appeared near to normal shape, central vein (CV) appeared clear, although hepatic tissue still stuffed with some erythrocytes (curved arrow) and dilated hepatic sinusoids (*). (H-E, X 400).

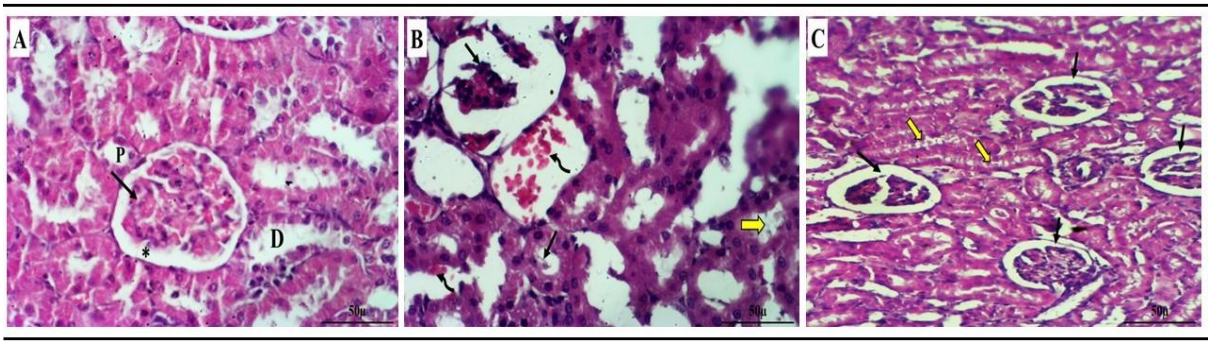

Fig. 6. (A) Renal cortex Photomicrograph of control rat, show the standard histological construction of standard renal parenchyma, round glomeruli, distal convoluted tubules (D) with widespread lumen and proximal convoluted tubules (P) with thin lumen wrinkled by cuboidal epithelial cells. (H-E, X 400).(B) Renal cortex Photomicrograph of HEs rat showing atrophied glomerular tuft (black arrow) with distension of Bowman's capsule (*), renal tissue stuffed with erythrocytes (curved arrow), degenerative epithelial lining of renal tubules (arrow) and convoluted tubules lumen filled by cell debris (yellow arrow). (C) Photomicrograph of the renal cortex, from N-HEs rat presentation seeming standard renal parenchyma, but still some glomeruli occurred with insignificant segmentation (arrows). The proximal convoluted tubules showed with cell debris (yellow arrows) in their lumen. (H-E, X400).

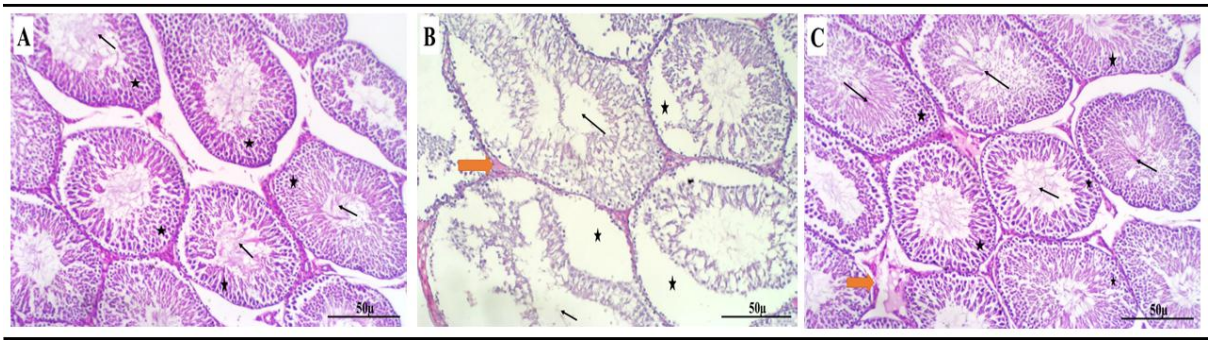

Fig. 7. Photomicrographs from rat testis section (A) The group of control demonstrating ordinary testicular architecture with different series of spermatogenic cells (*) and sperms (arrows). (B) from HEs group showing deformed seminiferous tubules (arrows), loss and degeneration of all types of he spermatogenic cells (*) and sperms (arrows) within the seminiferous tubules, blood stagnation (orange arrow) in the interstitial spaces between seminiferous tubules. (C) from rat treated with N-HEs showing restoration of the spermatogenic cells (*), which are found in an arranged series and the presence of the sperms (arrows), nevertheless blood stagnation (red arrow) still appeared in the interstitial spaces (H-E, X 400).

Discussion

The HEs are among the most significant causes of ecosystem contamination. Mostafa et al. [1] demonstrated that HEs contains an infinite variety of hazardous materials, such as pharmaceutical composites and byproducts of research and laboratory works. Among these pharmaceuticals are analgesics like diclofenac and antibiotics (sulfamethoxazole (SMZ), amoxicillin (AMX), fluoroquinolones antibiotics (ofloxacin) and oxytetracycline). The seantibiotics, SMZ, AMX, and oxytetracycline are three common antibiotics used in medication, which have limited biodegradability and considerable environmental toxicity and affect human health [17-19].

In the present study, the alternations in the hematological parameters (HB concentration, WBCs, RBCs, and PLT count) shed important light on how hazardous chemicals affect. Where pharmaceuticals such as diclofenac may result in bone marrow inhibition and thymocyte developmental abnormalities by reducing the generation of leukocytes [20]. Mitrašinović-Brulić et al. [21] explained that the nonsteroidal anti-inflammatory drug-induced oxidation of hemoglobin into methemoglobin and sulfhemoglobin, as well as the decreased erythrocyte life span, can also affect the reduction of erythrocyte count and hemoglobin concentration. Also, the fluoroquinolones antibiotic (ciprofloxacin) demonstrated that the decrease in HB content, PLT, WBCs, and RBCs count might be

caused by bone marrow activity being suppressed, which could lead to anemia such as microcytic anemia and thrombocytopenia [22].

Cellular and organ dysfunction is a result of free radicals, oxidant species, and toxic and harmful consequences. Excess of these species may cause damage to proteins, lipids, and DNA systems. Though functional responses contain defense and signaling mechanisms,which use little to modest amounts of reactive nitrogen species (RNA) or reactive oxygen species (ROS). The common method that contaminants of the environment affect damage is oxidative stress [23].

The analysis alterations in the group that received HEs compared to the group of control, such as the increase of lipid peroxidation with a decrease in total antioxidant activity, was confirmed with Sharif et al. [24] who found an elevation of oxidative stress markers in liver and kidney of rats exposed to pharmaceutical HEs. Ramírez-Moreno et al. [25] discovered that the oxidative environment within cells causes a reduced response in antioxidant activity during extended exposure durations to HEs. Hepato-renal and testicular toxicity are among the many adverse consequences linked to the administration of analgesics like diclofenac [26-28]. In rats with testicular dysfunction, diclofenac increased lipid peroxidation and decreased plasma total antioxidant capacity. Diclofenac biotransformation causes an excess of reactive oxidants, which are associated to DNA fragmentation, caspase activation, and cytochrome c release [28]. In addition, Hou et al. [29] indicated that the antibiotics caused testis toxicity by several mechanisms, for instance; inhibition of testis enzyme activity, disturbance in the balance of redox, and disturbance in the hypothalamic-pituitary–gonadal axis normal function. On the other hand, the pharmaceuticals in HEs like diclofenac-induced hepatotoxicity accompanied by the invasion of inflammation in the liver, lead to depleteperipheral leucocytes [20].

Moreover, the present work recorded a significant decrease in liver, kidney, and testis total CYP450 concentrations in HEs treated rats when compared with the control one. The primary route of drug metabolism is oxidation, and about 80% of drug redox is catalyzed by CYP450 enzymes [30]. Many thousands of molecules, including medications, carcinogens, and endogenous chemicals, are directly metabolized by CYP450, and conjugated enzymes degrade xenobiotics to aid in their removal from the body's tissues [7].

Because CYP enzymes are varied in both structure and function, they can catalyze the metabolic oxidization of pharmaceuticals by a variety of methods, such as different proton-coupled electron transfer mechanisms and singular electron transmission [31]. According to Mostafa et al. [1], HEs are overflowing with antibiotics, analgesics, and other medications that may consume CYP450 and reduce their concentration. Where enzymes inhibition especially CYP450 enzymes were involved in drugs metabolism and transport [32]. Also, CYP relative's members were significantly reduced in antibiotics-treated rats. Furthermore, antibiotics treatments caused a significant decrease in enzyme activities and the protein expressions of CYP2E1 and CYP3A1 in rat liver [33].

In this study, HEs exposurecaused a significant rise in liver, kidney, and testis FABP1 concentrations when compared with the control one.FABP1 is essential in the absorption of fatty acids and intracellular transportation, as well as controlling lipid metabolism and pathways of cell signaling**[**34]. FABP1 is a significant intrinsic cytoprotectant. FABP1 is commonly located in the cytoplasm of hepatic cells and a variety of tissues [35], e.g., tubular cells of the kidney[36]. Several disorders have been associated with FABP1 deficiency or dysfunction. FABP1 also affects the proliferation of cells through the regeneration of the liver, therefore may be used as ananalytical factor in hepatic surgeries [37].FABP1 binds to besides regulates the activity of several substances, including heme, fatty acids, and supplementary metalloporphyrins. FABP1 involved availability and protection from oxidative stress, FABP1 has a critical role throughout intracellular infections via decreasing inflammation and the negative consequences of hunger [37]. Moreover, Yabut et al. [38] found that FABP1's ability to bind various drug ligands and modify drug metabolism through direct protein-protein interactions with CYPs may make it a determinant of drug metabolism in the liver.

Furthermore, in cases of acute transplanted rejection, hepatocyte damage is linked to FABP1. It has been demonstrated that lowering FABP1 levels in mice's livers can effectively prevent nonalcoholic fatty liver disease [39]. Moreover, Panduru et al. [40] discovered that FABP1 concentrations rose in individuals with normal albumin levels compared to the control participants and increased with the development of diabetic nephropathy. On the other hand, the present study agreed with Imai et al. [41] who found that the increase in FABPs is associated with hemoglobin reduction and anemia. Whereas anemia causes a reduction in blood capacity on carrying oxygen, thenleads to hypoxia in tissues such as the kidneys, and liver. Hypoxia increases gene expression of FABPs, particularly Liver-type FABP.

The damage of tissue in the present investigation was evaluated histologically, via alterations recorded in liver, kidney, and testis's histoarchitecture. These results were confirmed with Sasi et al. [42] who found that the ciprofloxacin antibiotic recorded liver histopathological transformations in rats including diminished hepatocytes numbers, pyknotic hepatocyte nuclei and dilated sinusoids. Also, Hameed et al. [43] proved that antibiotics cause glomerular atrophy and reduce epithelial cells of convoluted tubule in the kidney. On the other hand, the decrease in RBCs count and HB concentration (anemia) in our results may be one of the reasons that caused damage occurred in liver, kidney, and testis in the present study. A Previous study proved that any injury to the glomerular or tubular tissue of the kidneys can prevent the synthesis of erythropoietin and result in hypoxia and renal tissue infarction. Generally, the feedback regulation of hemoglobin contents and RBCs count controls erythropoietin serum levels; if this feedback mechanism seems out of balance, this will result in injury to the kidneys [44]. Also, there are several hematological abnormalities associated with hepatocyte injury [45]. For example, hematolysis in blood vessels due to RBCs reduction, will cause liver cell degradation[43]. In line with Awad et al. [46], cells may suffer impairment from oxidative stress if there is an inequity between the production of ROS and antioxidant protection mechanisms. ROS damage DNA and causes lipid peroxidation when they target molecules of cell and the plasmic membrane, which causes cell disruption and tissue damage.

Necrosis is the least desirable kind of cell death since it breaks up the cell and spills its contents into the surrounding tissue, exposing neighboring cells to proteases as well as the proinflammatory mediators and initiating a cycle of positive feedback that results in tissue damage from inflammation. Antibiotics have been linked to DNA damage, cell apoptosis, seminiferous tubule atrophy, and necrosis of germ cells [29]. Moreover, Yeniocak et al. [47] provedthat the increasing FABP levels in testiscorrelated with testicular necrosis.

Merely 50–70% of the recently identified contaminants in the HEs may be eradicated by traditional treatment methods. Nonetheless, many countries continue to lack suitable HEs treatment techniques [48]. Nanoparticles treatment to HEs adjusted the hematological parameters, and TAA and CYP450 concentrations while decreasing lipid peroxidation and FABP1 concentrations. Also, the histopathology of the liver, kidney and testis improved, it may have resulted from the HEs losing a large amount of chemical and medicinal ingredients. Based on the $NiFe_2O_4$ nanocomposite, XRD, FTIR, TEM and SEM descriptionoutcomes in this study, Mostafa et al. [1] reported that the $NiFe_2O_4$ nanoparticles have anidentical, cube structure alongside spreading collections with typical dimension (13.8 - 16.7) nm and fine size (2 -6 nm) in TEM. According to previous research, $NiFe_2O_4$ has a particular surface with a cluster of tiny features that include protrusions and insertions that improve the adsorption areas [49,50]. As a result, in less than 40 minutes equilibrium, the antibiotics such as ofloxacin, SMZ, AMX, and oxytetracycline derivatives were successfully removed with an effectiveness of around 88% [1].

The $NiFe_2O_4$/activated carbon magnet complex material removed 95% of pharmaceuticals effluents in sixty minutes [1]. Moreover, Nawaz et al. [51] and Makofane et al. [52] demonstrated that it successfully transformed SMZ into numerous complexes, which may create mineral acids besidesbasic ions. $NiFe_2O_4$ may be used as a photographic reagent to remove contaminants while also withdrawing behind high thermodynamic energy. This can accelerate the redox reactions that split medications like oxytetracycline besides ofloxacin [53,54].

Additionally, magnetized $NiFe_2O_4$ nanoparticles exhibited great extremeability for the adsorption of penicillin G, and amoxicillin derivatives [55]. Employing nanotechnology filtration membranes is from the furthermost effective techniques in removing ionized heavy metals from sewer water[56]. Researchers [1, 57] proved that the Langmuir isotherm construction and the pseudo-additional order dynamical model through the ability of adsorption layer were agood fit for the process of adsorption. Also, $NiFe_2O_4$ nanoparticles have the possibility to substitute expensive adsorbents in removing the metal divalent ions from polluted water.

Conclusion

The treatment of Hospital effluents **(**HEs) is an important target to eliminate dangerous contaminants and pharmaceuticals from water before it is discharged into the ecosystem. This article recorded the significance of HEs administration on the induction of hematological impairments with redox toxicity and histopathological alternation in liver, kidney, and testis in male rats. While administration of $NiFe_2O_4$ nanoparticles treated HEs reversed hematological parameters change and showed reduction of toxicity and improved the histological architectureof liver, kidney, and testis tissues of the investigated rats. Hence,the present study explores a better understanding of the vital function of $NiFe_2O_4$ nanoparticle treatment in health protection. While this study provides valuable insights into the

toxicological influences of HEs and its treatment with $NiFe_2O_4$ nanoparticle on experimental animals, some limitations must be taken into consideration in future studies; like the evaluation of different times or concentrations of HEs. Also, immunotoxicity, genotoxicity studies would provide a wide toxicological picture.

Conflicts of interest

The authors declare no conflicts of interest.

Funding

This study was supported by funding from the Faculty of Science, Ain Shams University among a project titled "Assessment of hospital wastewater disposal hazards with a special scope on designing biological model and nanocomposite for treatment."

Authors' contributions

HAS performed the experiment and the analysis of parameters and wrote the draft. ABM designed and synthesized the nanocomposite in this study and reviewed the manuscript. MMZ performed the experiment and reviewed the manuscript. AAEI reviewed the manuscript. EHAA suggested the idea, performed data analysis, and reviewed the manuscript and all the authors approved the final article.

Ethical considerations

The approval of the animal protocol was obtained from the Ethical Agreement Commission at Ain Shams University (approval number sci1332410001).

References

1. Mostafa AB, Ali EHA, Azzam AM, Abdel-Messih MF, Fathy M. A.,Mostafa SS, Khalil MT, El-Naggar MM. Hospital Wastewater Screening and Treatment Using Two Novel Magnetic Iron Nanocomposites. EJABF 2024; 28 (2): 273-298.
2. Gupta S, Gupta SK. Development and evaluation of an innovative Enhanced River Pollution Index model for holistic monitoring and management of river water quality. Environ Sci PollutRes 2021; 2: 27033–27046.
3. Khan MT, Shah IA,Ihsanullah I,Naushad M,Ali S,Shah SAHA.Mohammad A. Hospital wastewater as a source of environmental contamination: An overview of management practices environmentalrisks and treatment processes. J Water Process Eng 2021; 41: 101990.
4. Kumar NS. Smart and innovative nanotechnology applications for water purification. Hybrid Adv 2023; 3: 100044.
5. Matić MM, Milošević MD, Paunović MG, Ognjanović BI, Štajn AŠ, Saičić ZS. Paracetamol-induced changes of haemato-biochemical and oxidative stress parameters in rat blood: protective role of vitamin C and beta glucan. Kragujev J Sci 2016;38:135–146.
6. Ekeke PO, Iorhemba AP, Achukee CK, Valentine IM, Ayozie-Samuel VC, Nwokoro EC, Sani AM. Effects of Paracetamol Toxicity on Hematological Parameters of Wistar Rats. Int J Res Pub Rev 2025; 6 (2):1980-1986.
7. Munro AW, McLean KJ, Grant JL, Makris TM. Structure and function of the cytochrome P450 peroxygenase enzymes. BiochemSoc Trans 2018; 46(1): 183-196.
8. Zhao M, Ma J, Li M, Zhang Y, Jiang B, Zhao X, Huai C, Shen L, Zhang N, He L, Qin SY. Cytochrome P450 Enzymes and Drug Metabolism in Humans. Int J Mol Sci2021; 22(23):12808.
9. Fuseya T,Furuhashi M,Yuda S,Muranaka A,Kawamukai M,Mita T,Ishimura S,Watanabe Y,Hoshina K,TaTanaka M, Ohno K, AkasakaH,Ohnishi H, Yoshida H,SaitohS, ShimamotoK, MiuraT. Elevation of circulating fatty acid-binding protein 4 is independently associated with left ventricular diastolic dysfunction in a general population. Cardiovasc Diabetol. 2014; 13:126.
10. Fathy MA,Kamel AH, Hassan SS. Novel magnetic nickel ferrite nanoparticles modified with poly (aniline-co-o-toluidine) for the removal of hazardous 24-dichlorophenol pollutant from aqueous solutions. RSC advances 2022; 12(12):7433-7445.
11. Claassen V. Food and water intake. In Claassen V (ed) Neglected Factors in Pharmacology and Neuroscience Research Biopharmaceutics, Animal characteristics Maintenance, Testing conditions. Elsevier 1994; 12, 267-287.
12. Brahmi B, Nasri E, Moula A, Feriani A, Tlili N, Raldua D, Lopez-Maldonado, EA,Borgi MA. Assessment of Liver Toxicity in Wistar Rats after Chronic Exposure to Phosphate-Processing Wastewaters from Gafsa-Metlaoui Laundry in Tunisia. Water 2024; 16: 214.
13. Sabry HA, Ali EHA, Osman AA, Zahra MM. Nanotechnology strategy for inhibition of PARP1 and IL-17A-associated with neurotoxicity in rats exposed to hospital wastewater. Naunyn Schmiedebergs Arch Pharmacol 2025; 398(4):4149-4164.
14. Draper HH, HadleyM. A review of recent studies on the metabolism of exogenous and endogenous malondialdehyde. Xenobiotica 1990; 9: 901-907.
15. Koracevic D,Koracevic G,Djordjevic V,Andrejevic S,Cosic V. Method for the measurement of antioxidant activity in human fluids. J Clin Pathol 2001; 54: 356-361.
16. Bancroft JD, Stevens A. Theory and Practice of Histological Techniques. Churchill Livingstone, London. Edition:2016; 7th. P, 120:131.
17. NgigiAN,Magu MM, MuendoBM. Occurrence of antibiotics residues in hospital wastewater, wastewater treatment plant, and in surface water in Nairobi County, Kenya. Environ Monit Assess 2019; 192(1):18.
18. Aleksić S,ŽgajnarGotvajnA,PremzlK, Kolar M, Turk SŠ. Ozonation of Amoxicillin and Ciprofloxacin in Model Hospital Wastewater to Increase Bio treatability. Antibiotics (Basel)2022; 10(11):1407.
19. McCarthy B, Apori SO, Giltrap M, Bhat A, Curtin J,Tian F. Hospital Effluents and Wastewater Treatment Plants: A Source of Oxytetracycline and Antimicrobial-Resistant Bacteria in Seafood. *Sustainability* 2021; *13:* 13967.
20. GomaaS. Adverse effects induced by diclofenac, ibuprofen, and paracetamol toxicity on immunological and biochemical parameters in Swiss albino mice. *JoBAZ* 2018; 79(5).
21. Mitrašinović-Brulić M, Kulovac A, Suljević D. Chronic effects of tramadol hydrochloride/paracetamol oral administration on haematological parameters and glucose level in Wistar rats. JRes Pharm 2020; 24(3): 310-317.
22. Edem EO, Akpan HD, Etim OE, Akpan EJ. The Effect of Ciprofloxacin on Hematological and Biochemical Parameters in Albino Rats. Nigeri JPharmaceut Appl Sci Res 2020; *5*(1): 61–64.

23. El-Demerdash FM, Tousson EM, Kurzepa J, Habib SL. Xenobiotics, Oxidative Stress, and Antioxidants. Oxid Med Cell Longev 2018; 2018: 9758951.
24. Sharif A, Ashraf M, Javeed A, Anjun AA, Akhtar MF, Akhrar B, Saleem A, Oxidative stress responses in Wistar rats on subacute exposure to pharmaceutical wastewater. Environ Sci Pollut Res 2016; 23: 24158–24165
25. Ramírez-Moreno FJ, Gómez-Oliván LM, Islas-Flores H, García-Medina S, Aguirre-Garrido JF, Hernández-SotoLM. A comprehensive approach to how hospital effluents lead to oxidative stress and shift the gene expression in target organs of *Danio rerio*. Science of The Total Environment 2023;887: 164057.
26. Nouri A, Heidarian E. Ameliorative effects of N-acetyl cysteine on diclofenac-induced renal injury in male rats based on serum biochemical parameters, oxidative biomarkers, and histopathological study. J Food Biochem 2019;43(8): e12950.
27. El-Hadary AE, Ramadan MF. Anti-oxidant traits and protective impact of Moringa oleifera leaf extract against diclofenac sodium-induced liver toxicity in rats. J Food Biochem 2019;;43(2): e12704.
28. Adeyemi WJ, Omoniyi JA, Olayiwola A, Ibrahim M, Ogunyemi O, Olayaki LA. Elevated reproductive toxicity effect of diclofenac after withdrawal: Investigation of the therapeutic role of melatonin. Toxicol Rep 2019; 6:571–577.
29. Hou L, Fu Y, Zhao C, Fan L, Hu H, Yin S. The research progress on the impact of antibiotics on the male reproductive system. Environ Int 2024; 187: 108670,
30. NikzadN, Rafiee M. Electrochemical study of drug metabolism. Curr Opin Electrochem2024; 44:101446.
31. Bussy U, Boujtita M. Advances in the electrochemical simulation of oxidation reactions mediated by cytochrome p450. Chem Res Toxicol2014; 27(10):1652-68.
32. Guengerich FP. Inhibition of Cytochrome P450 Enzymes by Drugs&mdash, Molecular Basis and Practical Applications. Biomolec & Therapeut2022; 30:1-18.
33. Yang H, Zhang Y, Zhou R, Wu T, Zhu P, Liu Y, Zhou J, Xiong Y, Zhou H, Zhang W, Shu Y, Li X, Li Q. Antibiotics-Induced Depletion of Rat Microbiota Induces Changes in the Expression of Host Drug-Processing Genes and Pharmacokinetic Behaviors of CYPs Probe Drugs. Drug Meta Disposition 2023; 51 (4): 509-520
34. Yabut KCB, Martynova A, Nath, A, Zercher BP, Bush MF,Isoherranen N. Drugs Form Ternary Complexes with Human Liver Fatty Acid Binding Protein 1 (FABP1) and FABP1 Binding Alters Drug Metabolism. Mol Pharmacol 2024; 105(6):395-410.
35. Mukai T, Egawa M, Takeuchi T, Yamashita H,Kusudo T. Silencing of FABP1 ameliorates hepatic steatosis, inflammation, and oxidative stress in mice with nonalcoholic fatty liver disease. FEBS Open Bio 2017; 7(7):1009-1016.
36. Uhlén M, Fagerberg L, Hallström BM, Lindskog C,Oksvold P,Mardinoglu A, Sivertsson Å, Kampf C, Sjöstedt E, Asplund A. Proteomics. Tissue-based map of the human proteome. Science 2015; 347: 1260419.
37. Wang G, Bonkovsky HL, de Lemos A, Burczynski FJ. Recent insights into the biological functions of liver fatty acid binding protein 1. J Lipid Res 2015; 56(12):2238-47.
38. Yabut KB, Zercher B, Bush MF, Isoherranen N. Characterization of Drug Binding to Liver Fatty Acid Binding Protein (FABP1) and Its Effect on Diclofenac Metabolism by CYP2C9. The Journal of Pharmacology and Experimental Therapeutics ASPET Annual Meeting Abstract - Drug Metabolism and Disposition 2023; 385 (Supplement3):589
39. Lu YC, Chang CC, Wang CP, Hung WC, Tsai IT, Tang WH, Wu CC, Wei CT, Chung FM, Lee YJ, Hsu CC. Circulating fatty acid-binding protein 1 (FABP1) and nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus. Int J Med Sci2020; 17(2):182-190.
40. Panduru NM, Forsblom C, Saraheimo M, Thorn L,Bierhaus A, Humpert PM, Groop PH, Urinary Liver-type fatty acid-binding protein and progression of diabetic nephropathy in type 1 diabetes. Diabetes Care 2013; 36:2077–2083.
41. Imai N, Yasuda T, Kamijo-Ikemori A, Shibagaki Y, Kimura K. Distinct roles of urinary liver-type fatty acid-binding protein in non-diabetic patients with anemia. PLoS One 2015; 10(5): e0126990.
42. Sasi S, Alghoul N, Toumi S, Awayn N, Saadawi W, Ragil Prastiya R. Ciprofloxacin Affect Sperm Quality and Induced Testicular and Hepatic Injury in Male Mice. Alq J Med App Sci 2024;7(3):477-483.
43. Hameed BK, Hameed MA, Hussein HM, Wadee SA. Histological Study of Antibiotic Effect on Liver, Kidney, and Breast Muscle in Poultry. Egyptian Journal of Veterinary Sciences (EJVS)2025; 56(1):1, 39-46.
44. Xing X, Zhang W, Sun H. Role of Erythropoietin in Renal Anemia Therapy.Trop J Pharm Res, May 2016; 15(5): 1083.
45. Gkamprela E, Deutsch M, Pectasides D. Iron deficiency anemia in chronic liver disease: etiopathogenesis, diagnosis and treatment. Ann Gastroenterol. 2017;30(4):405-413.
46. Awad A, Ahmed ZSO, AbuBakr HO, Elbargeesy GAEH, Moussa MHG, Oxidative stress, apoptosis and histopathological alterations in brain stem and diencephalon induced by subacute exposure to fipronil in albino rats. Environ Sci Pollut Res 2022; 29: 936–948.
47. Yeniocak S, Sarac F, Erbn A,Yazicioglu M,OlgacV, Koldas M, Uzun O, Akkoc I. The diagnostic role of testicular fatty acid-binding protein in testicular ischemia: A randomized controlled experimental study. Arch Esp Urol 2021; 74(3):328-334.
48. ParidaVK, Sikarwar D, Gupta AK. An assessment of hospital wastewater and biomedical waste generation, existing legislations, risk assessment, treatment processes, and scenario during COVID-19. J Environ Manage 2022; 308: 114609.
49. Shao L, Ren Z, Zhang G, Chen L. Facile synthesis, characterization of a MnFe 2O 4/activated carbon magnetic composite and its effectiveness in tetracycline removal. Mater Chem Phys 2012;135: 16e24.
50. Alamier WM, Hasan N, Nawaz MS, Ismail KS,Shkir M, Malik MA,Oteef MD. Biosynthesis of $NiFe_2O_4$ nanoparticles using Murayyakoenigii for photocatalytic dye degradation and antibacterial application. J Materials Res and Technol 2023; 22: 1331-1348.
51. Nawaz M, Shahzad A, Tahir K, Kim J,Moztahida M, JangJ, Alam MB, Lee SH, Jung HY, Lee DS. Photo-Fenton reaction for the degradation of sulfamethoxazole using a multi-walled carbon nanotube-NiFe2O4 composite. Chem Eng J 2020; 382: 123053.
52. Makofane A, Maake PJ, Mathipa MM, Matinise N, Cummings FR, Motaung DE, Hintsho-Mbita NC. Green synthesis of $NiFe_2O_4$ nanoparticles for the degradation of Methylene Blue, sulfisoxazole and bacterial strains. Inorg Chem Commun 2022; 139:109348.
53. ZhaoG, Ding J, Zhou F, Chen X, Wei L, Gao Q, Wang K, Zhao Q. Construction of a visible-light-driven magnetic dual Z-scheme BiVO4/g-C3N4/$NiFe_2O_4$ photocatalyst for effective removal of ofloxacin: mechanisms and degradation pathway. Chem Eng J 2021; 405: 126704.
54. Song J, Zhang J, Zada A, Ma Y, Qi K. CoFe2O4/NiFe2O4 S-scheme composite for photocatalytic decomposition of antibiotic contaminants. Ceram Int 2023; 49(8): 12327-12333.

55. Derakhshani E, Naghizadeh A, Mortazavi-Derazkola S. Phyto-assisted synthesis of magnetic NiFe2O4 nanocomposite using the Pulicariagnaphalodes methanolic extract for the efficient removal of an antibiotic from the aqueous solution: a study of equilibrium, kinetics, isotherms, and thermodynamics. AQUA—Water Infrastructure Ecos and Soci 2023; 72(11): 2035-2051.
56. Covaliu-Mierlă CI, Păunescu O, Iovu H. Recent Advances in Membranes Used for Nanofiltration to Remove Heavy Metals from Wastewater: A Rev*Memb* 2023; 13(7): 643.
57. Ahmed HA, Soliman MSS, Othman S. Synthesis and Characterization of Magnetic Nickel Ferrite-Modified Montmorillonite Nanocomposite for Cu (II) and Zn (II) Ions Removal from Wastewater. *E J Chem* 2021,64(10): 5627-5645.