In vivo toxicities of the hospital effluent in Mahdia Tunisia
Sabrine Afsa, Ons Fekih Sallem, Nouha Ben Abdeljelil, Anouar Feriani, Mohamed Fadhel Najjar and Hedi Ben Mansour

ABSTRACT
Hospital effluent (HE) is one of the most important sources of pharmaceuticals released into the environment. This kind of pollution is a recognized problem for both human health and aquatic life. Consequently, in the present study, we assessed the effects of untreated hospital effluent on mice via biochemical and histopathological determinations. Female mice were given free access to water bottles containing untreated HE at different dilutions for 21 days. Then clinical biochemistry and histopathology evaluation were conducted. Serum biochemistry analysis showed the presence of significant increase in cholesterol, triglycerides, glycaemia and total bilirubin. However, phosphatase alkaline and urea activities have been significantly decreased compared to the control group. No significant variation was observed for the rest of the studied parameters (high-density lipoproteins; low-density lipoproteins and uric acid). Additionally, multiple alterations, including cellular necrosis, leucocyte infiltration and congestion, were observed in different tissues of mice exposed to the tested HE.

Key words | biochemical perturbation, histopathology, hospital effluent, mice toxicity, pharmaceuticals

HIGHLIGHTS
- Hospital effluent (HE) exposure did not influence the total body weight of mice.
- Sub-chronic exposure to the HE disturbed biochemical parameters in mice.
- Histological abnormalities were observed in mice liver, kidney and heart sections.
- Effluent toxicology.
- Water contamination.

INTRODUCTION
Pharmaceuticals are a group of chemically active compounds used for diagnosis, treatment and prevention. They play an important role in the population's well-being. Pharmaceutically active compounds (PhACs) are continuously released into the aquatic environment (Seifrtová et al. 2009) due to the increase of these drugs' consumption in human populations especially in industrialized countries (Mendoza et al. 2015). One of the most important sources of this type of pollution is hospital effluents (HEs), which are generated in high quantities (between 200 and 1,200 L bed⁻¹ day⁻¹) (Oliveira et al. 2017). In the last decades, many research studies have been focusing on the characterization of HEs (Verlicchi et al. 2022; Santos et al. 2013; Mendoza et al. 2015; Oliveira et al. 2015), which are of particular concern due to their specific chemical, physical and biological properties. Previous studies have shown the
presence of an unlimited number of hazardous substances in hospital wastewaters, including pharmaceutical compounds, products of laboratories and research activities, contrast media agents, radioactive markers, heavy metals, disinfectants and sterilant, as well as the high presence of pathogens such as multi-drug resistant bacteria, viruses and parasites (Kümmerer 2001; Carraro et al. 2016; El-Ogri et al. 2016; Oliveira et al. 2017). Nevertheless, in most countries, these effluents are usually considered as domestic wastewaters and are, therefore, collected with municipal effluents without any pre-treatment and discharged into the received environment after conventional treatment in wastewater treatment plants (WWTPs) (Carraro et al. 2016). However, previous studies showed a low effectiveness of WWTPs, which are not designed to remove complex chemicals, namely pharmaceuticals (Verlicchi et al. 2012b; Lindholm-Lehto et al. 2015). As a result, pharmaceutical compounds are detected ubiquitously in the environment (e.g., mussels, fish, vegetables and drinking water). Due to their pseudo-persistent character and depending on their physio-chemical properties, these compounds and/or their interaction products may pose a long-term toxic effect on the aquatic organisms (Santos et al. 2010) and consequently, the human body is the final target of these pollutants via the food chain. In Tunisia, there are no quality references or HE management methods imposed by the competent authorities, and thus, HEs are directly released into the sewerage system, and thereafter discharged into the waterbody after conventional treatment in WWTP. Recently, our research team has observed that wastewater coming from the university hospital of Mahdia (centre of Tunisia) contains high levels of pharmaceuticals ranging from a few nanograms to hundreds of micrograms as well as heavy metals (Nasri et al. 2017; Afsa et al. 2019). These compounds were also detected in the seawater, even after treatment in a municipal WWTP, which may affect negatively the aquatic environment and human health (Afsa et al. 2019).

Over recent decades, different biological systems have been developed to deeply understand the single and mixture effects of various contaminants (e.g., pharmaceuticals) in wildlife as well as human health. Recently applied to wastewaters, these systems include small-scale whole organism-based bioassays, mainly aquatic organisms (e.g., fish and bivalve embryos, crustacean, algae and bacteria) as well as cell-based bioassays (Isidori et al. 2016; Neale et al. 2017; Oliveira et al. 2017; Altenburger et al. 2018; Escher et al. 2018). Rodent species such as rats and mice are one of the most important indicators to understand the adverse effects of wastewaters in mammals. Nevertheless, rats and mice have received less attention compared to aquatic organisms.

Toxicity data related to the adverse effects of hospital wastewaters on rodents are relatively scarce, and most of the research studies have focused on the impact of municipal and industrial wastewaters (Zhang et al. 2013; Adeoye et al. 2015; Zouiten et al. 2016). In Tunisia, only few studies have given interest to the impact of wastewaters in rodents (Zouiten et al. 2016; Tahrani et al. 2017). So far, only one study reported the adverse effects of HE in the livers of rats (Beltifa et al. 2019). Hence, the present study aimed to improve the knowledge about the ‘cocktail effect’ of HE in mice. Female mice were exposed to different dilutions (10, 25 and 50%) of untreated HE for 21 days. Thereafter, histopathological and biochemical analyses were conducted.

MATERIALS AND METHODS

Effluent collection and physico-chemical analysis

Hospital untreated wastewater samples were collected from the Tahar Sfar university hospital located in Mahdia city into a sterile glass bottle and stored at 4 °C during the study period. The studied characteristics of effluent were as follows: pH, BOD (biochemical oxygen demand), COD (chemical oxygen demand), TOC (total organic carbon), TSSs (total suspended solids) NO₃ (nitrate) and AOX (absorbable organically bound halogens).

Animals and experimental design

Twenty-four female mice (5-week old/18 ± 1.7 g) were acclimatized for 2 weeks in cages in the animal room, at the Higher Institute of Applied Sciences and Technology, University of Monastir. They were maintained in laboratory conditions of 12 h dark and light cycle and the temperature of 25 ± 3 °C. Dilutions of 10, 25 and 50% (v/v, effluent/tap water) were selected (Adeoye et al. 2015). Healthy mice
were randomly divided into four groups \((n = 6)\), three HE-treated groups and a control group. During the exposure period (21 days), mice of each group were given free access to clean drinking water bottles and food ad libitum, individually. For each mouse, the bottle was filled, weekly, with 28 mL of tap water (control group) or HE dilutions (treated groups). The volume of water was chosen referring to the average daily consumption of water per mice (4 mL) \((\text{Williams} 1959)\). Mice were given free access to water bottles because gavage could induce stress responses and adverse effects in these animals, which could confound the results \((\text{dos Santos Moysés et al.} 2014)\). Prior to dissection, treated and untreated mice were fasted overnight. Mice were sacrificed under light ether anaesthesia, and blood samples were drawn from the heart ventricle of anaesthetized animals immediately (by sterilized disposable syringe) for biochemical assays. All of the protocols were carried out in accordance with French standard ethical guidelines for laboratory animals (agreement 75-178, 5_16_2000).

**Histopathology and clinical biochemistry analyses**

To investigate the histopathological changes in the tissues of HE-treated mice, slices of heart, livers and kidneys were fixed in 10% neutral buffered formalin. In brief, the tissues were dehydrated in the ascending order of ethyl alcohol-water concentrations, cleared in xylene and embedded in paraffin. Sections of 3-μm thickness were obtained using a rotary microtome. Prepared slides were stained with haematoxylin and eosin (H&E) and observed at 400× magnification under a light microscope by a trained pathologist.

For biochemical analysis, one millilitre of venous blood of each mouse was collected in plastic tubes. Blood sera were obtained by cooling centrifuge 3,000 rpm for 15 min at \(+4^\circ\text{C}\) and stored at \(-80^\circ\text{C}\) prior to biochemical analysis. All measurements, including CHOL (cholesterol), HDLc (high-density lipoproteins), LDLc (low-density lipoproteins), TG (triglycerides), urea, uric acid, ALP (alkaline phosphatase), TB (total bilirubin) and GLY (glucose), were performed using automated analyser ‘cobas 6000 roche diagnostics R c 501’ at the Department of Biochemistry in Fattouma Bourguiba Hospital of Monastir. Statistical analyses were conducted using GraphPad Prism 5.0 computer programmes. Data were analysed using one-way ANOVA followed by the Dunnett multiple post hoc test. \(P\)-values less than 0.05 were considered statistically significant.

**RESULTS**

**Physico-chemical parameters of untreated HE**

Physico-chemical parameters of HE are presented in Table 1. All the measurements were below the national allowable limits (NT 106.0021989).

**Effect of untreated HE on body and organ relative weight**

The total body weight has not significantly increased in the treated groups during the exposure period compared to the control group (Table 2). Organ relative weights have also been evaluated, and the obtained results have shown a significant decrease in the relative weight of heart in the group treated with HE diluted to 50% (Table 3). A mortality percentage of 17% was observed in treated groups with dilutions of 25 and 50%. There was no mortality found in...
the group treated with the dilution of 10% and the control group.

Effect of untreated HE on biochemical parameters

Compared to the control group, the exposure of mice to HE at different dilutions has induced a significant variation in some serum biochemical parameters, mainly in the 50% treated group. The obtained results have shown an increase in CHOL, TG, GLY and TB, whereas the values of URA and ALP have decreased (Table 4).

Histopathological assessment

Sections from the negative control showed normal tissues. Histological changes were observed in the different treated groups. Cell necrosis, hepatic sinusoidal dilatation, leucocyte infiltration and congestion of the central vein were observed in liver sections (Figure 1). Additionally, hepatic steatosis was detected in the liver tissue of 50% treated group. Kidney sections have shown congestion, cell necrosis, leucocyte infiltration, degenerated epithelia of renal tubules, hypertrophied glomerulus and tubular dilatation (Figure 2). Concerning heart sections, enlargement and disruption of connective tissue have been observed as well as leucocyte infiltration and necrosis (Figure 3). Additionally, in the present study, we have detected cancerous cells in skin tissue of only one mouse exposed to HE diluted to 50% (Figure 4).

**DISCUSSION**

In this study, physico-chemical parameters were within the permissible limits of the Tunisian authorities (NT 106.0021989). Nevertheless, based on our previous results, HEs represent a major source of the release of pharmaceuticals and other contaminants into the environment (Nasri

| Parameters/Groups | NC group | 10% group | 25% group | 50% group |
|-------------------|----------|-----------|-----------|-----------|
| CHOL (mg/dL)      | 1.17 (±0.074) | 1.13 (±0.201) | 1.13 (±0.274) | 1.74* (±0.637) |
| TG (mg/dL)        | 0.53 (±0.057) | 0.65 (±0.123) | 0.48 (±0.058) | 0.67* (±0.083) |
| HDLc (mg/dL)      | 0.80 (±0.107) | 0.79 (±0.152) | 0.76 (±0.303) | 0.69 (±0.134) |
| LDLc (mg/dL)      | 0.17 (±0.093) | 0.07 (±0.058) | 0.15 (±0.088) | 0.20 (±0.139) |
| GLY (mg/dL)       | 3.15 (±0.251) | 4.583 (±0.911) | 4.6 (±0.509) | 6.38* (±2.787) |
| URA (mg/dL)       | 9.32 (±2.432) | 6.72 (±1.28) | 5.96* (±1.326) | 9.86 (±2.311) |
| UAC (mg/dL)       | 133.7 (±33.18) | 119.8 (±31.42) | 138 (±31.22) | 118.4 (±19.11) |
| TB (mg/dL)        | 1.37 (±0.306) | 2.73* (±0.898) | 1.64 (±0.329) | 2.42* (±0.823) |
| ALP (IU/L)        | 115.5 (±19.11) | 70.83* (±10.07) | 81* (±22.58) | 67.4* (±13.28) |

CHOL, cholesterol; TG, triglycerides; HDLc, high-density lipoproteins; LDLc, low-density lipoproteins; GLY, glucose; URA, urea; UAC, uric acid; TB, total bilirubin; ALP, alkaline phosphatase. Asterisks (*) represent statistically significant differences (p < 0.05) compared to the negative control group.
Figure 1 | Sections of the liver from mice exposed to untreated HE at different dilutions (H&E, ×400). NC: liver section from mouse in the negative control group showing apparently normal tissue. G10% (a) and (b): liver sections of mice exposed to 10% diluted HE. G25% (a) and (b): liver sections of mice exposed to 25% diluted HE. G50% (a) and (b): liver sections of mice exposed to 50% diluted HE. CV, central vein; SD, hepatic sinusoidal dilatation; CN, cell necrosis; LI, leucocyte infiltration; C, congestion of the central vein; HS, hepatic steatosis.
Figure 2 | Sections of the kidney from mice exposed to untreated HE at different dilutions (H&E, ×400). NC: kidney from mouse in the negative control group showing apparently normal tissue. G10% (a) and (b): kidney sections of mice exposed to 10% diluted HE. G25% (a) and (b): kidney sections of mice exposed to 25% diluted HE. G50% (a) and (b): kidney sections of mice exposed to 50% diluted HE. GL, glomerulus; BS, Bowman’s space; RT, renal tubules; C, congestion; GC, glomerular congestion; LI, leucocyte infiltration; HG, hypertrophied glomerulus; CN, cell necrosis; ED, degenerated epithelia of renal tubules; TD, tubular dilatation.
Figure 3 | Sections of the heart from mice exposed to untreated HE at different dilutions (H&E, ×400). NC: normal heart muscle cells from mouse in the negative control group showing apparently normal tissue. G10% (a) and (b): heart sections of mice exposed to 10% diluted HE. G25% (a) and (b): heart sections of mice exposed to 25% diluted HE. G50% (a) and (b): heart sections of mice exposed to 50% diluted HE. CT, connective tissue; ESCT, enlarged and separated connective tissue; LI, leucocyte infiltration; N, necrosis; C, congestion; DCT, disruptive and destroyed connective tissue.
et al. 2017; Afsa et al. 2019), which may represent a threat to environmental and human health. Therefore, this research work aimed to assess the toxic effects of the untreated HE on mice. Our results did not show a significant increase in body weight during the treatment period, which is in agreement with previous studies that have shown that wastewaters (e.g., municipal wastewaters and tannery effluents) did not induce body weight variation (Zhang et al. 2013; Amin et al. 2016).

The here obtained results showed a disturbance of several biochemical parameters compared to the control group, which is probably due to the impairment of both kidney and liver function (Zhang et al. 2013). The elevation of TB as well as the decrease of ALP can be attributed to the impairment of hepatic function and biliary excretion (Amin et al. 2016). One of the adaptive mechanisms to exogenous invasion is the regulation of different liver gene expressions such as hepatic transporter genes involved in the regulation of bile acid efflux and bilirubin (Yang et al. 2017; Bai et al. 2018). Therefore, the increase of bilirubin in our study probably reflects a change in the liver gene profile as an adaptive response to this invasion in order to minimize the toxic effects. Additionally, it is also possible to attribute this disorder to the alteration of gene expression as a result of DNA damage, given that most of the pollutants can react with DNA, which eventually results in various genetic mutations (Gao et al. 2015). Research studies focusing on molecular analysis have reported transcriptomic alterations in liver genes of mice/rats treated with wastewaters. These alterations include lipid, energy, nucleotide, amino acid metabolism as well as different signalling pathways and cell biological processes (Zhang et al. 2010; Zhang et al. 2013; Yin et al. 2015), which probably explain the observed biochemical phenotype. However, this possible explanation should be supported by complementary molecular analysis.

The increase of glycaemia observed in our study could be related to the disruption of the glucose uptake at the cellular level, and it could also suggest a high-energy demand of organism metabolic pathways to cope with stressors. The elevated level of cortisol is one of the physiologically conserved responses to stress in vertebrates, which maintains an increased plasma glucose level to fuel the necessary energy and re-establish body homoeostasis. Previously, it has been reported that municipal wastewater perturbs cortisol response in fish (rainbow trout) (Ings et al. 2012a, 2012b). Other authors have linked the disruption of these parameters by the impairment of the expression of liver metabolism key genes in exposed mice, which probably induce the alteration of glucose and lipid homoeostasis (Yin et al. 2015; Buron et al. 2017). Our results are in accordance with previous studies focusing on the impact of wastewaters on mammals, where multiple alterations in mice and rats after exposure were reported. The disruption of haematological and biochemical parameters as well as the induction of histopathological lesions are the most reported adverse effects (Zhang et al. 2010, 2013; Adeoye et al. 2015). The impairment of biochemical function may be related to cellular and tissue alterations. Thus, the histopathological study was conducted to better understand the effects of untreated HE in several organs of exposed mice. As can be seen in Figures 1–3, several abnormalities have been detected in livers, kidneys and hearts of HE-treated mice compared to the control group. The liver is the central organ for detoxification processes in vertebrates. It contains several metabolizing enzymes that play an important role in the biotransformation process of xenobiotics. This process leads to the production of fewer or more toxic metabolites (Sharif et al. 2016). Thus, the observed damage in both liver and kidney may be attributed to the occurrence of oxidative stress in mice tissue, which is probably generated by the contaminants present in the tested effluent and their toxic metabolites (e.g., pharmaceuticals and their transformation products, metals, unknown mixture and other environmental contaminants). On the one hand, several metals, including mercury, lead, cadmium and arsenic, have been reported to enhance the production of reactive

![Figure 4](http://iwaponline.com/jwh/article-pdf/19/3/499/902735/jwh0190499.pdf)

Figure 4 | Section from a mouse exposed to untreated HE diluted to 50% showing the presence of tumour necrosis without viable tumour cells (arrow) and with many polynuclear neutrophils often altered (asterisk). (a) H&E, ×100, (b) H&E, ×400.
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dant species (ROS) (e.g., superoxide radicals, hydroxyl and hydrogen peroxide) (Ercal et al. 2001). Plumb, lead and arsenic have been associated with oxidative stress and cell damage by altering the enzymatic activities of the anti-
oxidant system. As an example, a decrease of both superoxide dismutase (SOD) and catalase (CAT) activities has been observed when exposing rats to lead (Lakshmi et al. 2013) and arsenic (Santra et al. 1999; Xu et al. 2015). These inhibitory effects of metals were associated with oxi-
dative injuries in the liver and kidneys of exposed animals. On the other hand, pharmaceutical compounds may largely participate in the observed damage since HEs were reported to contain high concentrations of drugs (Verlicchi et al. 2012a; Santos et al. 2013; Oliveira et al. 2015). Due to their fre-
quent detection in the aquatic environment, the adverse effects of pharmaceuticals have been largely evaluated on aquatic organisms. Previous studies have evidenced the occurrence of oxidative stress in a multiplicity of aquatic species (e.g., bivalves, fish and crustacean) after being exposed to several compounds, mainly NSAI (non-steroidal anti-inflammatories) analgesic drugs and antibiotics, at environmental doses (Islas-Flores et al. 2013; Daniel et al. 2019; Freitas et al. 2019, 2020; Dionísio et al. 2020; Nunes et al. 2020).

Zivna et al. (2015) have reported that salicylic acid (NSAI), at environmental doses, induced a reduction in GPx (glutathione peroxidase), GR (glutathione reductase) and CAT activities in early life stages of fish Cyprinus carpio. The inhibition of these enzymes was associated with a lipid peroxidation (increase in TBARS values), which may reflect a cellular damage. The inhibitory trend of salicylic acid may be linked to its metabolite gentisic acid (Szwaigier 2013). Furthermore, salicylates have also induced hepatotoxic effects in mammals (rats liver). The authors related the toxic effects of salicylic acid to its oxidative metabolism mediated by cytochrome P450s pathway, which resulted in oxidative damage (lipid peroxidation) (Doi et al. 2002; Doi & Horie 2010). In addition, the analgesic paracetamol has also been reported to induce oxidative damage as well as histopatho-
logical abnormalities in liver cells of the fish Oreochromis mossambicus (hepatocellular vacuolization and congestion). The hepatotoxic effects of paracetamol may be related to its highly toxic P450 metabolite N-acytely-p-benzoquinone imine (NAPQI), which may lead to hepatic necrosis at toxic doses (Kavitha et al. 2011). Histochemical analysis has shown that the β-blocker propranolol, at environmental con-
centrations (0.1 μg L⁻¹), induced a depletion of hepatic glycogen reserves in exposed fish (Phalloceros harpagos), which may indicate an increased demand for glucose in order to enhance the metabolic capacities of animals to cope with stressors (Matus et al. 2018). Based on the foregoing, exposure to untreated HE may lead to the overproduction of ROS by different substances and their oxidant metabolites, which probably overwhelm the enzymatic antioxidant defence mechanisms of the cell such as CAT, SOD and GST (glutathione-S-transferase) activities (Ercal et al. 2001; Adeoye et al. 2015). Consequently, these undetoxi-
fied oxidant substances could react with cell membrane and induce the loss of membrane integrity and fluidity thereby probably damaging proteins, lipids and DNA structure (Ercal et al. 2001), as well as inducing the activation of pro-
inflammatory cytokines and consequently triggering cell death (Elbanna et al. 2017; Beltifa et al. 2019). Recently, Sharif et al. (2016) have reported a decrease of T-SOD and CAT in plasma, kidney, and liver of male Wistar rats exposed to pure and 10% diluted pharmaceutical wastewater for 60 days. In the same study, different histopathological lesions have been recorded in kidney (e.g., cellular necrosis and swel-
ling; peritubular congestion) and liver (e.g., cellular degeneration and swelling; coagulative necrosis). These reported abnormalities corroborate our study and again jus-
tify the toxic potential of environmental pharmaceuticals. The here obtained histological results are in agreement with previous studies focusing on the effects of wastewaters on rodents (Zhang et al. 2013; Adeoye et al. 2015; Sharif et al. 2016). In the present work, histopathological data support partially the biochemical results (discussed above). The presence of lipid droplets in liver tissue corroborates the increased levels of biochemical parameters, namely CHOL and TG. Similarly, the nephrotoxic lesions observed in the kidneys may induce a depression of the glomerular filtration, therefore a loss of renal function, which may lead to the dis-
ruption of biochemical parameters, namely urea levels (Adeoye et al. 2015). Thus, the mixture of contaminants pre-
sent in untreated effluent may interfere with the antioxidant system and metabolic pathways in exposed animals (such as lipid and glucose metabolism), which may explain the toxicity of HE. Given that liver and kidney are the most sensitive
indicators of chemical toxicity and well correlate with the histological studies, most of the research studies have focused on those organs, although, in this study, we have also evaluated the impact of the HE on the heart tissue of mice. Under stress conditions, the heart increases blood flow as an adaptive response to enhance the body’s capacities to get rid of invasion (Ings et al. 2022a). Thus, the heart plays an important role in protecting the body; however, its function could also be affected and unbalanced by different environmental toxicants. Therefore, this organ could be a useful indicator, giving an insight into the state of toxicity. Interestingly, the obtained results showed leucocyte infiltration, necrosis, congestion, disruption and destruction of connective tissue. These results suggest that HE could influence heart tissue and its function. It is well known that increased levels of cholesterol could be considered as a risk factor of cardiovascular diseases (Zhang et al. 2019), and thus, the observed damage in heart tissue probably is linked to the disruption of lipid metabolism. Additionally, histological abnormalities, such as cellular infiltration and necrosis, were observed in heart tissues of fish (e.g., Catfish) treated with effluents. In rodents, exposure to pharmaceutical effluent has induced cellular breakdown and infiltration of inflammatory cells in endocardium (Sharif et al. 2016). The bioaccumulation of toxicants in heart tissues as well as high detoxification capacities required by fish organs might impede heart function and cause cell degeneration, thus necrosis of heart cells (Olaniyi et al. 2017), which probably explains the results obtained in our study.

In the present study, we have detected sporadic cancerous cells in skin tissue, which may translate to the high genotoxicity of untreated HE. It is not surprising to obtain these results, since the same tested effluent has been recently reported to be highly cytotoxic and genotoxic in exposed rat (liver) and human breast cancer cell line MDA-231 (Nasri et al. 2017; Beltifa et al. 2019). Additionally, it has been reported that wastewaters could alter various signalling pathways in mice, which play an important role in cell proliferation and differentiation (e.g., MAPK, Jak-STAT and Wnt signalling pathways). The aberrant regulation of these pathways could result in carcinogenic effects in various tissues including skin (Zhang et al. 2020, 2023). Furthermore, the occurrence of oxidative stress in exposed organisms may lead to DNA damage, which may promote cancer development (Sharif et al. 2016). Thus, the genotoxic potential of HE may be due to oxidant/genotoxic substances such as pharmaceuticals. As an example, Pomati et al. (2007) have reported transcriptional changes of several genes after exposing zebrafish liver (ZFL) cells to a mixture of 13 pharmaceuticals (atenolol, bezafibrate, carbamazepine, cyclophosphamide, ciprofloxacin, furosemide, hydrochlorothiazide, ibuprofen, lincomycin, ofloxacin, ranitidine, salbutamol and sulfamethoxazole) at environmentally relevant doses for 72 h. Namely, the expression of genes involved in DNA-repair mechanisms, cell cycle regulation, oestrogenic pathways and inflammation was altered in ZFL cells. These alterations, as reported by the same authors, could be associated with the insurgence of liver cancer in zebrafish. A similar response has been observed when exposing human embryonic cells HEK293 to the same drugs (Pomati et al. 2006, 2007). Similarly, a genotoxic potential has been reported for ciprofloxacin (antimicrobial drug) in daphnids, as justified by an increase of genetic damage index in a dose dependent manner. Quinolones such as ciprofloxacin inhibit cell replication by binding to DNA gyrase and to topoisomerase II in prokaryotic and eukaryotic cells, respectively (Nunes et al. 2018). Genotoxic effects were also observed in human lymphocytes and in rodent astrocytes exposed to ciprofloxacin (Gorla et al. 1999; Gürbay et al. 2006).

**CONCLUSION**

Based on our findings, the toxicity of HE cannot be ruled out even after a short (sub-chronic) exposure term. Consequently, more attention should be given to HE management by the concerned authorities. Given the high variability of HEs, future analyses should be undertaken to consolidate our results and to better understand the mechanism by which complex mixtures could negatively affect mammals (e.g., chemical analysis of HE, chronic exposure of mice, use of biomarkers of oxidative stress defence system and molecular biomarkers of DNA damage).

**ETHICAL APPROVAL**

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they will refrain from misrepresenting research results, which could damage the trust in the journal.

CONSENT TO PARTICIPATE

This work was carried out in collaboration with all authors. All authors read and approved the final manuscript.

CONSENT TO PUBLISH

The authors declare that there is no conflict of interest regarding the publication of this paper.

AUTHORS CONTRIBUTIONS

All authors contributed to this work: S.A. was involved in the writing of article, intoxication and histological analysis. O.F.S. was involved in the intoxication of mice by effluent. N.B.A. was involved in the reading of slides of histological sections. A.F. was involved in the reading of slides of histological sections. M.F.N. was involved in the serum biochemistry analysis. H.B.M. was involved in the writing of article and structuration of results.

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COMPETING INTERESTS

Not applicable.

DATA AVAILABILITY STATEMENT

All relevant data are available from an online repository or repositories (http://www.issatmh.rnu.tn/fra/pages/144/APAE: Recherche).

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