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Histopathological features of low-dose organophosphate exposure

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Abstract

Organophosphate (OP) use remains largely available worldwide despite more strict regulatory measures, in agriculture, parks or households, leading to a daily low-dose exposure. The systemic dysfunction appears partly due to acetylcholinesterase inhibition, exhibiting a primary toxic effect on the endocrine system but also on the liver and kidneys, which are responsible for products metabolization and elimination. Prolonged OP exposure can be responsible for histopathological (HP) changes that can either evolve or worsen pre-existing conditions. We conducted an experimental study including six male Wistar rats divided into two groups (four rats in the study group and two in the control group). The subjects in the first group were administered 100 mg/kg Chlorpyrifos half median lethal dose (LD_{50}) at baseline and at 48 hours, under general anesthesia. Organ harvesting was achieved after one week. HP modifications were discovered in all kidney samples, with dystrophic changes and vacuolization of mesangial cells, dilation of renal tubules and epithelial atrophy. Congestion of vascular structures also occurred. The liver samples showed severe alteration in both vessels and hepatocytes. Adrenal gland impairment was confirmed through an increase in vacuole number in all areas, while a decrease in colloid content was noted in the thyroid gland simultaneously with a modified foamy aspect. This study is the first to certify the extent of organ injury induced by OP exposure, describing both glomerular and tubular involvement in the kidneys, liver necrosis and endocrine disturbances.

Keywords: organophosphate exposure, liver injury, glomeruli atrophy, endocrine glands histopathology.

Introduction

Organophosphate (OP) toxicity remains a prevailing topic despite all the measures dedicated to decrease the worldwide use of these compounds [1]. Although epidemiological data suggests a higher incidence of severe OP poisoning in least developed countries, extensive use of these insecticides in agriculture and public parks, led to daily exposure to low doses even in the industrialized countries [2, 3].

OP exposure induces a systemic dysfunction through acetylcholinesterase irreversible inhibition [1], which determine a persistent depolarization through acetylcholine accumulation at the end-plate level [3]. The cyclic adenosine monophosphate (cAMP) synthesis is also altered, leading to modified cellular signaling processes. OP-induced oxidative stress is associated with lipids and proteins oxidation, reactive oxygen species production and deoxyribonucleic acid (DNA) alteration, causing various tissue damage [4]. Primary toxic effects are exerted on the nervous system, but due to the high liposolubility, it is assumed that all organs can suffer toxic injuries [5]. OP cytotoxicity is responsible for the appearance of an increased number of necrotic cells in the blood sampled from poisoned patients [6].

Considering the OP toxicokinetic properties, the most affected organs are the liver and the kidneys. The liver is the main organ involved in metabolizing all xenobiotic substances [7]. Various histopathological (HP) modifications result from induced-accelerated glycogenolysis, proteolysis and lipolysis processes, with secondary reduction in liver size [8]. After passing the liver, OP metabolic

products are eliminated mainly through the kidneys on a rate dependent on accumulation in the fat tissue, relying on their liposolubility [9]. The exact mechanism of OP-related renal dysfunction is not fully understood, exacerbated oxidative stress being the most incriminated process [10]. Current reported HP changes are glomerular sclerosis, tubular degeneration, and inflammatory infiltration [4].

Endocrine impairment has recently become of interest to the scientific community [11, 12]. Glucocorticoid and catecholamine hypersecretion were proved to be caused through an exaggerated cholinergic stimulation and a response to stress [13]. Existing literature data show a high sensitivity of the thyroid function to OP compounds [14]. Histological changes (such as focal necrosis) were detected in the thyroid follicles [14, 15].

Aim

Starting from current researches, we performed a study that aimed to simulate daily repeated low-dose exposure to OPs, to discover and to describe the specific HP lesions of the main organs involved in metabolizing and eliminating these compounds, as well as the modifications of the main endocrine glands.

Materials and Methods

We performed an experimental study that included six male Wistar rats. During the study, they have had adequate food intake and *ad libitum* water.

The six rats were divided into two groups: group 1, the study group, included four rats, and group 2 was the control group. The subjects in the first group were administered 100 mg/kg Chlorpyrifos through oral gavage (Reldan™ 22 EC, Dow Agrosciences). The dose is equivalent to half of median lethal dose (LD_{50}). Subjects in the control group were administered saline through oral gavage. In both groups, the gavage was performed under general anesthesia, according to the *Institutional Animal Care and Use Committee* (IACUC) recommendations, with 1% Isoflurane and Ketamine 40 mg/kg–Xylazine 5 mg/kg.

The protocol was repeated at 48 hours and seven days later, the subjects were euthanized with Isoflurane overdose to perform organ harvesting (liver, kidney, thyroid gland, adrenal glands, and pancreas) for HP examination.

The euthanasia technique follows the *IACUC* regulatory guidelines on this topic [16].

Organ sampling was carried out in an adequate period to avoid prolonged ischemia (less than 15 minutes).

After collection, the samples were fixed in 20% neutral buffered formalin and transported to the Laboratory of Histopathology, Victor Babeş National Institute for Research and Development in Pathology and Biomedical Sciences, Bucharest, Romania. The tissues were embedded in paraffin and these samples were cut and stained with Hematoxylin–Eosin (HE). Moreover, thyroid and adrenal gland samples were immediately prepared after harvesting in order to be evaluated under electron microscopy. These samples were finely cut and were quickly fixed with glutaraldehyde.

Tissue samples were examined using a Leica DM750 microscope by independent observers, highly experienced in the optical microscopy field. Ultrastructural examination was performed using Philips EM 3101 transmission electron microscope.

The study protocol was approved by the Local Ethics Committee of the Emergency Clinical Hospital of Bucharest, respecting the ethical regulations regarding experimental research on laboratory animals.

Results

All subjects from the study group survived after Chlorpyrifos administration, although two presented mild cholinergic symptoms (sialorrhea and diarrhea).

The most frequent HP lesions quantified in the liver samples were sinusoidal dilation (Figure 1, A and B), often accompanied by centrilobular vein dilatation (Figure 2, A and B). Inflammation of the hepatic tissue was also present and mononuclear cell infiltrates were highlighted (Figure 3, A and B). Furthermore, Kupffer cells hyperplasia appeared to be a reiterated HP feature (Figure 4, A and B). Considering the low-dose regimen of Chlorpyrifos, it is surprising that confluent necrotic areas were frequently identified (Figure 5, A and B). Cytoplasmic vacuolization of the hepatocytes was also noticed (Figure 6, A and B).

During microscopic examination, HP changes were discovered in all the kidney samples from the exposed rats. The most interesting findings were dystrophic changes, vacuolization of mesangial cells (Figure 7) and glomeruli atrophy. The most frequent renal tubules histological modifications were dilatation and epithelial atrophy (Figure 8, A and B). Dystrophic epithelial areas were usually associated with the presence of pyknosis and karyolysis. Areas with pronounced tubular dilatation, acquiring the aspect of 'thyroidization' (thyroid-like appearance), were found in the exposed kidneys (Figure 8C). Multiple tubular areas with turbid dystrophy (cloudy swelling) were observed during microscopic examination (Figure 8D). Vascular structures from the analyzed samples were mostly congested (Figure 8, A and B). Like the hepatic tissue, the kidney was observed to be infiltrated with mononuclear cells (Figure 9).

Regarding the endocrine system, adrenal and thyroid alteration were observed. Despite careful pancreas harvesting, no sample was adequate for examination. The HP feature identified in the adrenal gland samples was cytoplasmic vacuolization of the glandular cells in the *zona fasciculata* (Figure 10, A and B). These changes have also been confirmed by electron microscopy, identifying a marked vacuolization of the chromaffin cells after the intoxication (Figure 11, A and B). An interesting observation was represented by the inhomogeneous aspect of lipid droplets inside the chromaffin cells (Figure 12). Another particular aspect was that all nuclei were identified with chromatin margination (Figure 13).

After thyroid gland examination, decreased colloid in the thyroid follicles (Figure 14A) and changes in the colloid aspect, like a foamy transformation (Figure 14B), were described. Ultrastructural examination revealed decreased colloid in the thyroid follicles, as well as a significant alteration of the follicular cells' microvilli (Figure 15). Moreover, an intense damage of the mitochondria was confirmed in the follicular cells (Figure 16).

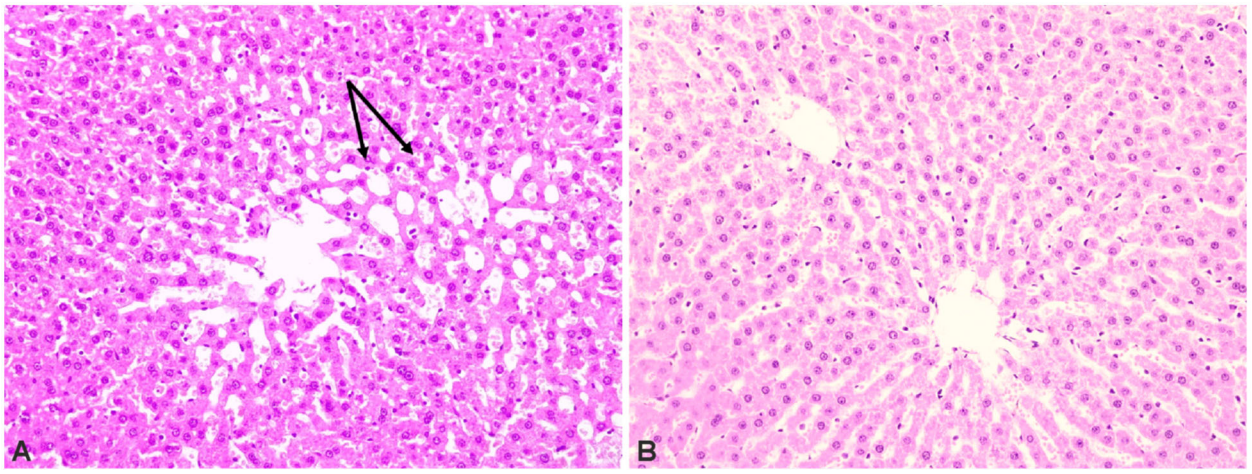

Figure 1 – *(A and B) Liver after OP exposure with sinusoidal dilatation. HE staining: (A) ×200; (B) ×100. OP: Organophosphate; HE: Hematoxylin–Eosin.*

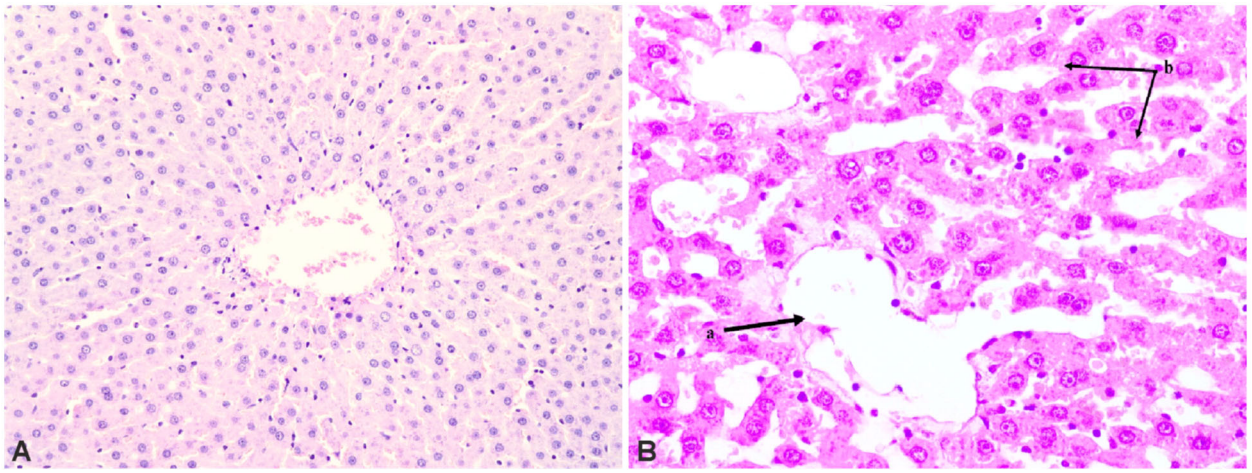

Figure 2 – *(A) Liver after OP exposure with centrilobular vein dilatation; (B) Liver after OP exposure with centrilobular vein dilatation (a) and sinusoidal dilatation (b). HE staining: (A) ×100; (B) ×400.*

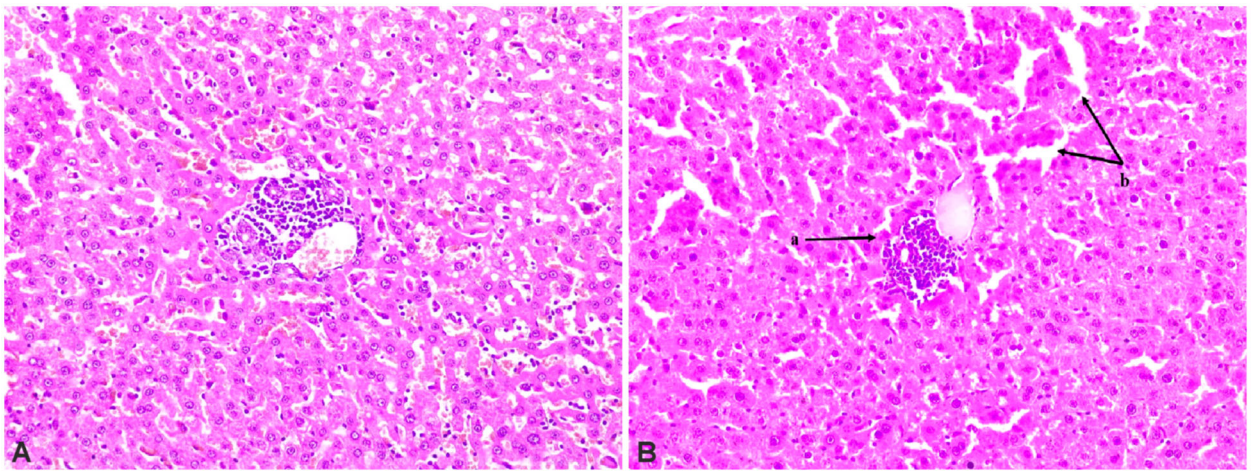

Figure 3 – *(A) Liver after OP exposure with mononuclear cells infiltrate in portal space; (B) Liver after OP exposure with mononuclear cells infiltrate (a) and sinusoidal dilatation (b). HE staining, ×200.*

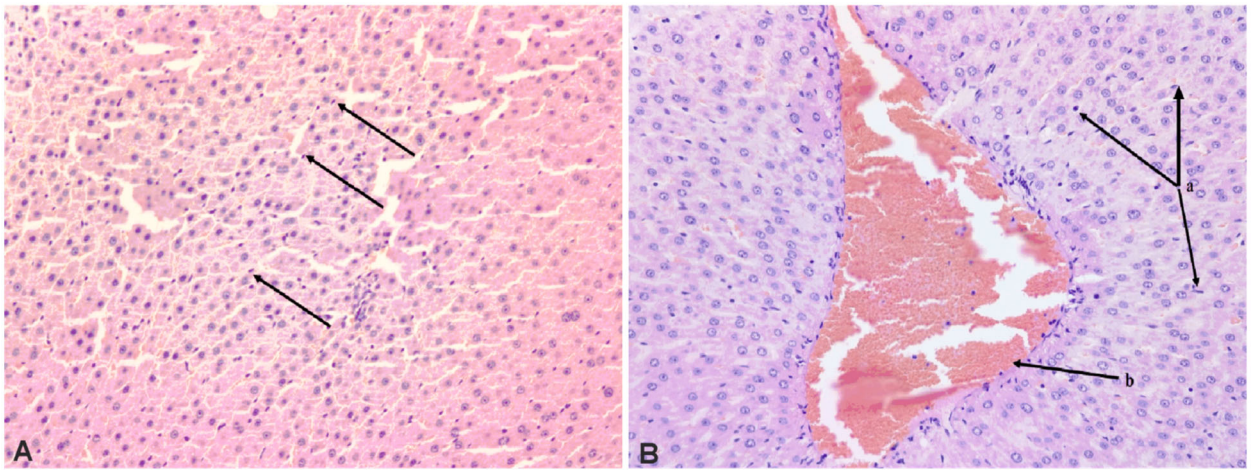

Figure 4 – ***(A) Liver after OP exposure with Kupffer cells hyperplasia; (B) Liver after OP exposure with Kupffer cells hyperplasia (a) and centrilobular vein dilatation (b). HE staining, ×100.***

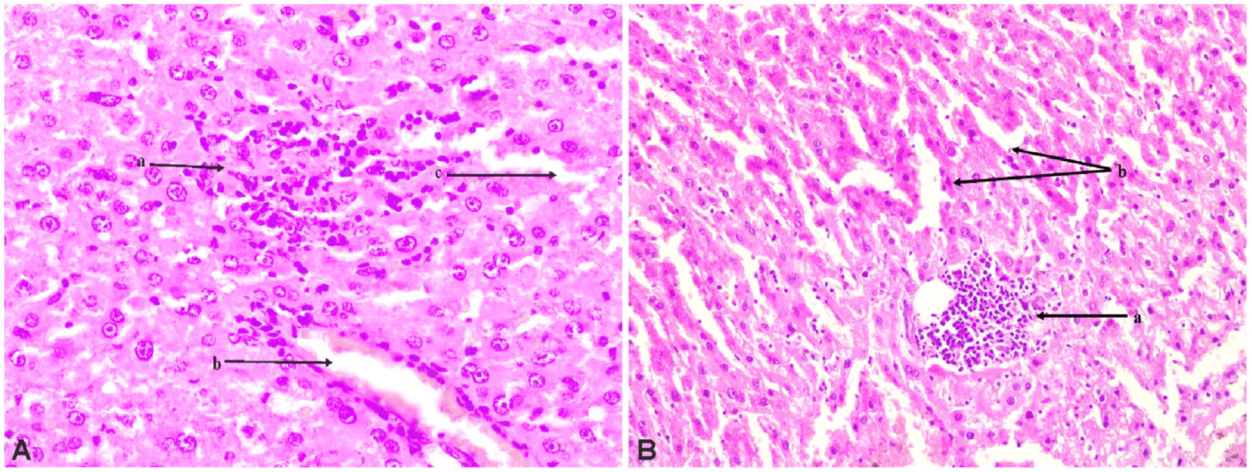

Figure 5 – ***(A) Liver after OP exposure with intralobular confluent necrosis (a), vascular congestion (b) and sinusoidal dilatation (c); (B) Liver after OP exposure with intralobular confluent necrosis (a) and sinusoidal dilatation (b). HE staining: (A) ×400; (B) ×200.***

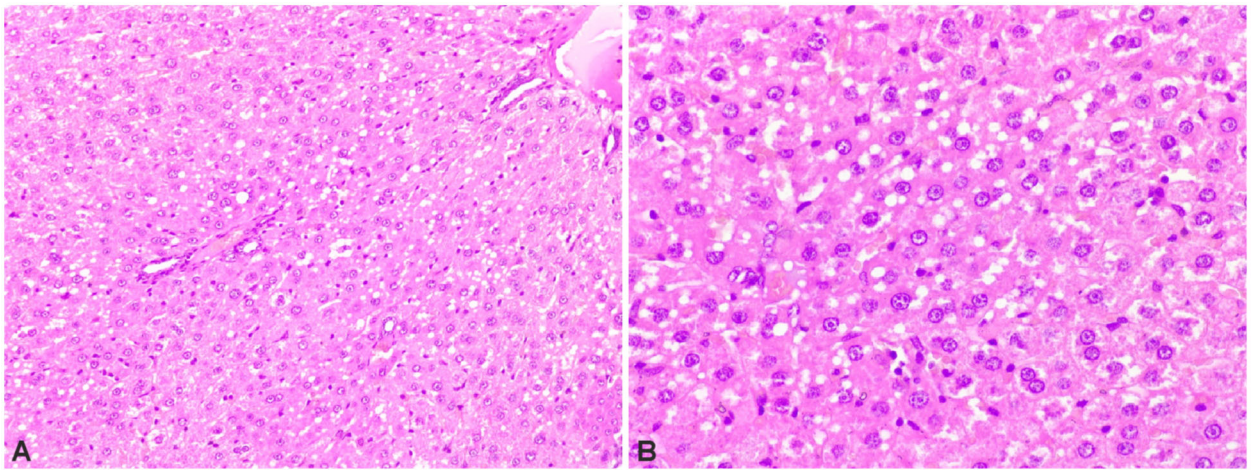

Figure 6 – ***(A and B) Liver after OP exposure with cytoplasmic vacuolization. HE staining: (A) ×100; (B) ×400.***

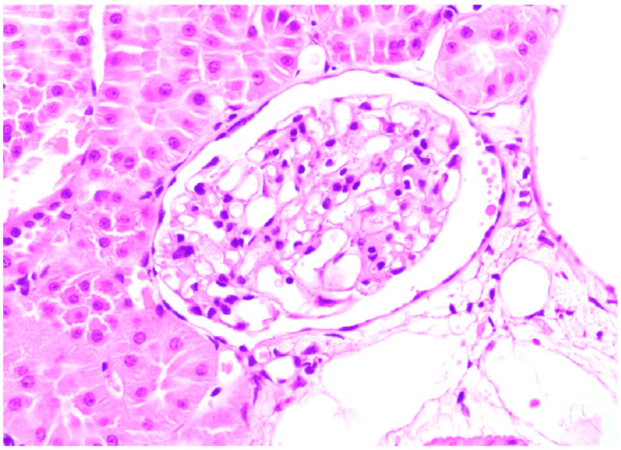

Figure 7 – ***Kidney after OP exposure with dystrophic changes on the glomeruli and mesangial cells vacuolization. HE staining, ×400.***

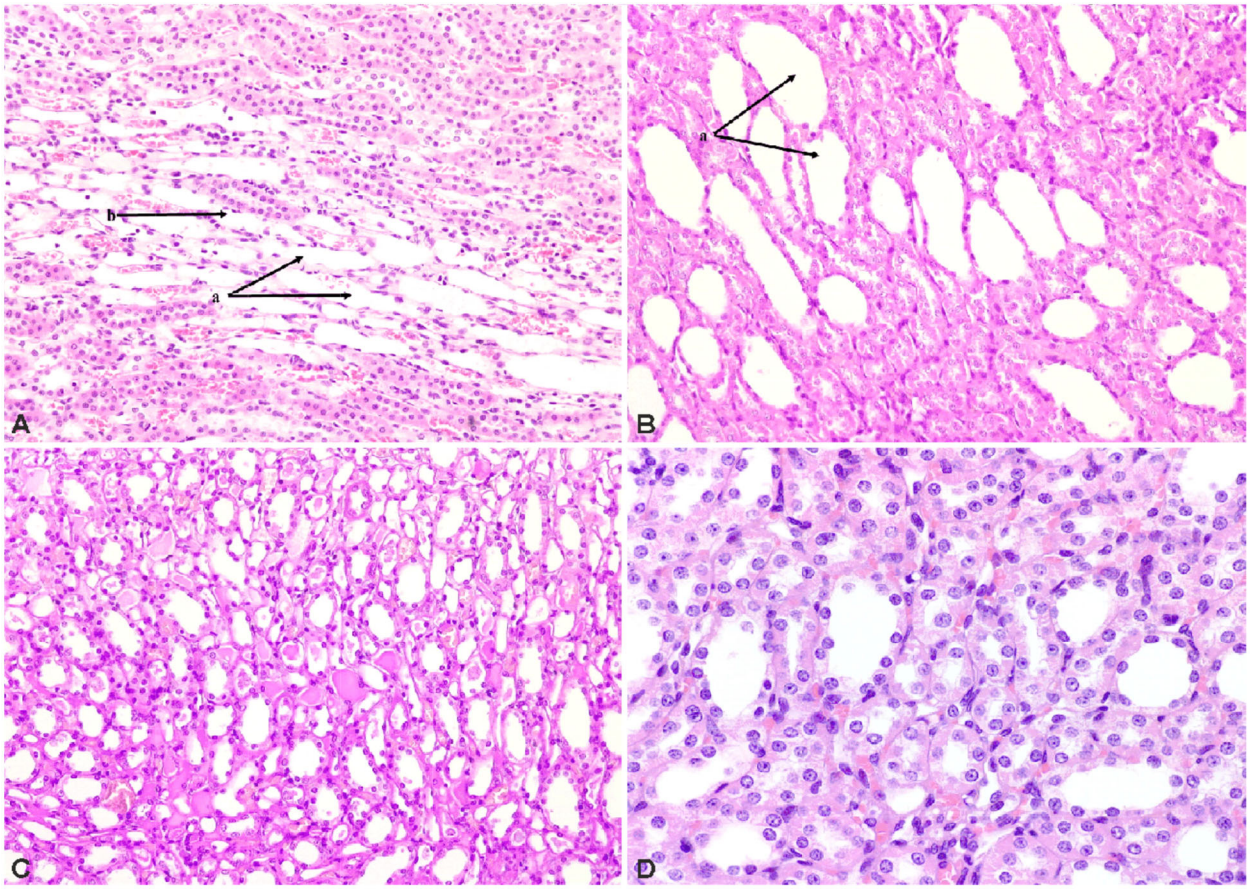

Figure 8 – ***(A and B) Kidney after OP exposure with dilatation of the tubuli (a) and epithelial atrophy (b); (C) Kidney after OP exposure with dilatation of the tubuli, with the aspect of 'thyroidization'; (D) Kidney after OP exposure with dilatation of the tubuli with turbid dystrophy (cloudy swelling). HE staining, ×400.***

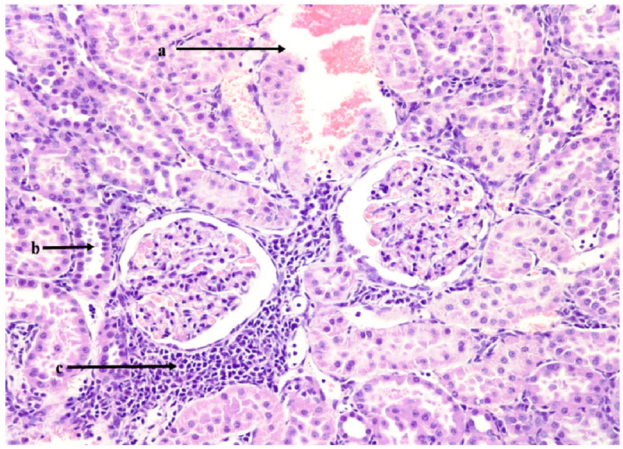

Figure 9 – ***Kidney after OP exposure with vascular congestion (a), dystrophic aspect of the tubular epithelial cells (b) and mononuclear cell infiltration (c). HE staining, ×100.***

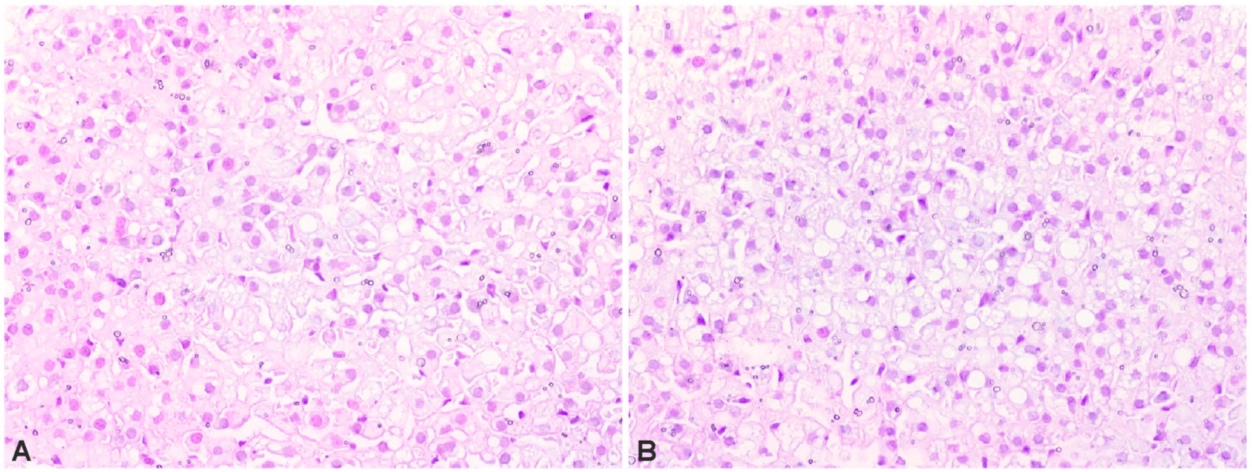

Figure 10 – *(A and B) Adrenal glands after OP exposure with cytoplasmic vacuolization. HE staining, ×400.*

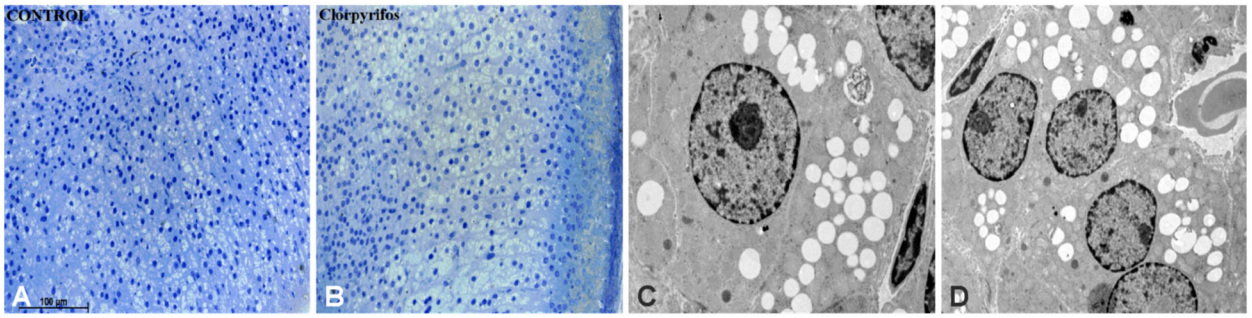

Figure 11 – *(A–D) Marked vacuolization of the chromaffin cells after the intoxication. Toluidine Blue staining: (A and B) Scale bar 100 µm. Transmission electron microscopy (TEM): (C and D) ×4500.*

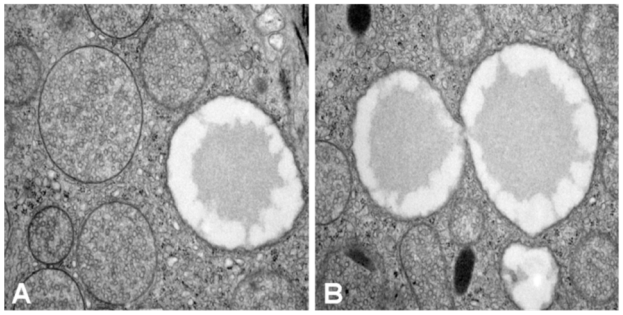

Figure 12 – *(A and B) Inhomogeneous aspect of lipid droplets inside the chromaffin cells. TEM, ×6000.*

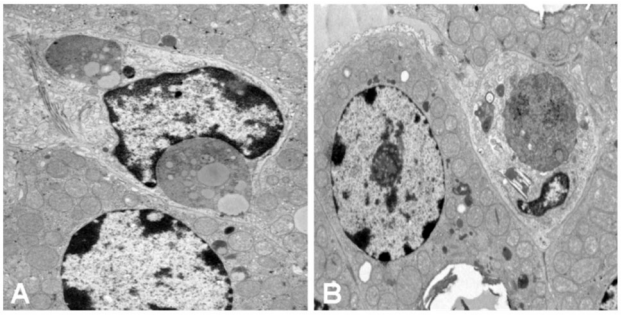

Figure 13 – *(A and B) Chromatin margination of the nuclei. TEM, ×4500.*

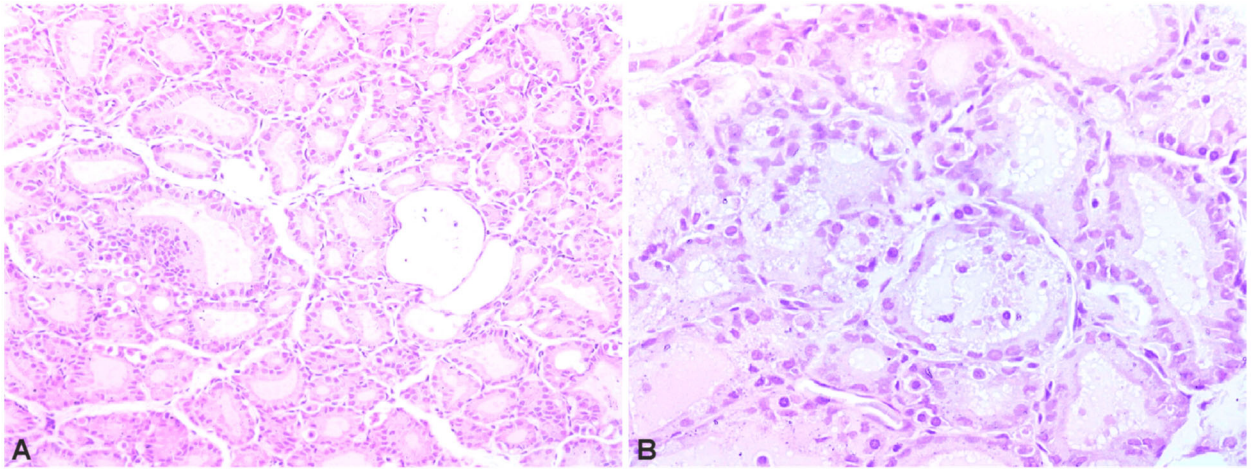

Figure 14 – *(A) Thyroid gland after OP exposure with reduced colloid; (B) Thyroid gland after OP exposure with foamy transformation of the colloid. HE staining, ×400.*

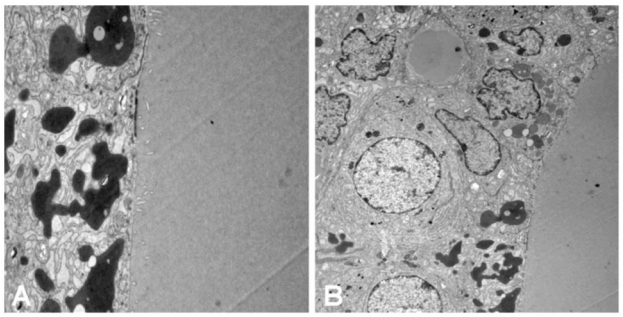

Figure 15 – *(A and B) Significant alteration of the follicular cells' microvilli. TEM, ×4500.*

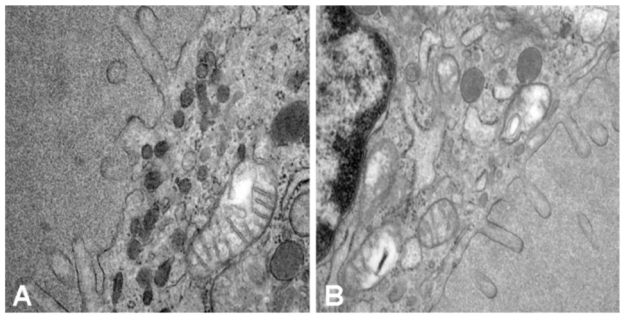

Figure 16 – *(A and B) Intense damage of the mitochondria. TEM, ×4500.*

Discussions

The toxicity of OP compounds has raised global concern knowing the harming effects on health, in both acute and long-term exposure [17]. Present concerns are justified, considering the raising exposure that has become almost continuous because of external and inside use of these substances [18]. Our experimental research had as primary objective the evaluation of tissue changes caused by OP low-dose exposure in the main organs involved in metabolization and elimination, as well as in the adrenal and thyroid glands. The mechanism of OP toxicity has proven to be extremely complex, through inhibition of acetylcholinesterase and other types of enzymes or direct toxic effects [19]. The latter are due to the initial compound direct toxicity, but it can also act through its metabolites [7].

The liver is the main site of activation and detoxification from OPs, involving various enzymatic complexes, like P450 cytochrome, paraoxonase-1 and B-esterases [20]. Thiophosphate-like OP compounds become active in the liver through oxidative desulfurization to oxon compounds and the resulted substances are metabolized through hydrolyzation of the esteric bond, resulting fewer toxic compounds [9, 21]. Regarding HP changes, experimental studies have shown that various types of OPs lead to accelerated apoptosis of the hepatocytes together with structural and vascular damage [22].

In the present study, alterations in both hepatocytes and vessels were seen after a week from intoxication. The dilation of the centrilobular and sinusoidal veins were identified in all study liver specimens. The sinusoidal damage usually occurs in conditions of an increased portal pressure being linked to severe hepatic diseases, like cirrhosis. However, in our study, the sinusoidal dilatation is most probably due to a hepato-portal sclerosis that generates an intra-hepatic shunt. These pathological liver modifications are often described as the result of toxic effects of various exogenous substances [23].

Another vascular damage observed in the poisoned rat group was the dilation of the portal vein that probably occurred in the same context as the sinusoidal vein dilation. A hypertrophy of the Kupffer cells was detected indicating an intensive phagocytic activity secondary to Chlorpyrifos exposure. These cells are the first barrier in the liver's process of detoxification, being frequently injured after exposure to exogenous toxic substances, like endotoxins or Acetaminophen, hence being the main source for local inflammatory mediators [24]. Their hypertrophy also contributes to the impairment of blood flow through the sinusoidals.

Additionally, we noticed an increased number of cytoplasmic vacuoles in the hepatocytes. The occurrence of this vacuolization might be explained either by an alteration in lipid metabolism in the liver due to OP exposure or by a mechanism of collecting and isolating toxic products. The presence of an abundant inflammatory infiltrate and areas of necrosis indicate the proinflammatory status consecutive to OP exposure [25]. These changes are similar with those suggested by Ezzi *et al.* that evaluated the toxic effects of Chlorpyrifos, which pointed out the correlation between the inflammatory process in the liver and the one in the bone marrow [8]. Cakici & Akat also confirmed similar HP changes in the liver after acute poisoning with diazinon [10].

Among all renal injuries caused by the exacerbated oxidative stress, the OP exposure may decrease the level of some amino acids, like methionine or arginine, precipitating renal dysfunction and finally leading to renal impairment [7]. Other data suggest that the inhibition of butyryl-cholinesterase in the renal distal convoluted tubules may be responsible [26]. Nevertheless, studies on human embryonic kidney cells revealed that OPs might produce DNA breaks and non-inflammatory apoptosis proportional with the exposure time and dosage. Once the apoptosis process has reached a certain level, a secondary inflammatory response appears to aggravate kidneys lesions [27]. Current reported HP changes are glomerular sclerosis, tubular degeneration, and inflammatory infiltration [4].

In our study, renal HP alterations were localized in the glomeruli and tubules. In the glomeruli, the most frequent changes were atrophic, as well as vacuolization of the mesangial cells, while in the tubules epithelial atrophy and dilation prevailed. It has already been shown that during mechanical or chemical aggression, cilia from the epithelial cells play a primary role [28]. As a result, epithelial cell atrophy induced by OP exposure may lead to further severe renal dysfunction. Current literature data show that the presence of tubular atrophy significantly correlates with serum creatinine levels and urine concentration capacity. Furthermore, the degree of tubular atrophy reflects the decrease of glomerular filtration rate in patients with diabetic nephropathy. Therefore, the extensive tubular atrophy indicates a possible association between renal function and the exposure to OPs. The mechanisms of this tubular impairment documented in the present research is probably related with the local inflammation caused by the OP compounds and the oxidative stress [29]. The role of the inflammatory process in the renal tissue changes after Chlorpyrifos intoxication is confirmed by the identi-

fication of the abundant inflammatory infiltrate and the congestive aspect. The increased number of macrophages at the levels of the tubules may play a role in promoting dilatation [30].

Like in our study, Cakici & Akat identified similar glomerular changes but he did not describe any tubular alterations, except a dilation of the Bowman capsule [10]. El-Shenawy *et al*. also noted the occurrence of glomerular atrophy and inflammatory infiltrate, with minimal tubules modification, after Diazinon exposure [31, 32]. Sarhan & Al-Sahhaf, in their study on rabbits intoxicated with Diazinon, highlighted the presence of both extensive glomerular atrophy and diffuse tubular alteration [33], while Badraoui *et al.* reported a decreased volume of glomeruli with increased number of sclerosed glomeruli after organochlorine exposure [34]. Although, 'thyroidization' aspect caused by hyaline cast formation of Tamm–Horsfall glycoprotein was a common finding in pyelonephritis and obstructive nephropathy [35], this is the first study to report such alteration after OPs exposure.

After OPs exposure, studies have shown injury of the adrenal gland parenchyma, both in the cortical and medullar areas, showing that adrenal glands are highly vulnerable at OP exposure, with extensive cytoplasmic lesions and hypertrophy of the fascicular zone as opposed to the reticular area. Migration of medullar cells in the cortical area and a widespread cellular atrophy was also observed in laboratory studied animals [15, 36]. We observed a diffuse vacuolization of the cortical area in the adrenal glands. Although lipid vacuoles are naturally present in the cortical zone, the intoxicated subjects presented an increased level. The raise in vacuole number, especially in the fascicular zone, is frequent after exposure to toxic substances. Moreover, an increase in vacuole density can occur because of extensive corticotrophin activity [37]. Similar to our findings, Jeong *et al.* noted an extensive vacuolization in all three areas of the adrenals in Chlorpyrifos-treated rats [15]. In contrast, De Angelis *et al.* reported the absence of histological alterations of the adrenal glands' tissues after Chlorpyrifos administration in adult animals and newborns from poisoned mothers [38].

In acute intoxications a decrease in triiodothyronine (T3) and thyroxine (T4) levels were confirmed, and in low-dose exposures appeared an alteration of the hypothalamic–pituitary–thyroid axis, even in the absence of a significant variation of the butyrylcholinesterase level [38, 39]. In thyroid samples, after optic microscope study, we discovered that the colloid appeared to be the most affected. A global decrease in colloid content was seen in the follicles and a change in its consistence, getting a foamy aspect. The reduction in follicles colloid quantity is usually associated with aging and does not always reflect the functional changes in the gland [2]. The foamy aspect of the colloid was also confirmed by De Angelis *et al.* in small-aged rats borne from mothers exposed to Chlorpyrifos [38]. Furthermore, many follicles presented many desquamated cells in the colloid, reflecting an enhancement of the apoptotic process in the thyroid after intoxication. This exfoliation process of the follicular epithelium was reported in the research of Pandey & Mohanty who evaluated toxic effects on the thyroid after exposure to a combination of pesticides [31]. Jeong *et al.* also identified that exposure to Chlorpyrifos-methyl can be responsible for a decrease in the colloid level and the exfoliation of the colloid follicular tissue [15].

The OP intoxication is characterized by secondary metabolic disturbances, like hyperglycemia and an increased insulin resistance [8], indirectly stimulating the pancreas to secrete more insulin [40]. The excessive cholinergic stimulation also favors insulin and glucagon release [41]. Although we were unable to study the pancreas, there are morphological analysis which reveal a hypertrophy of the Langerhans islets and a change in microscopic aspect of the α and β cells, in cases with OPs exposure [16].

Study limitations

One potential limitation of the present research study is the lack of serum parameters that could have contributed to the assessment of functional organ involvement in OP intoxication.

Conclusions

The present study showed that repeated low-dose OP exposure might be responsible for important HP changes, long-term exposure leading to severe functional and structural damage. Considering the extensive use of OP compounds, early exposure of children to low doses of these substances may be the cause for chronic illnesses, such as cancer, diabetes, or other metabolic disturbances. The liver, as the main activation and metabolization site of the OP compounds, presented severe alteration in both vessels and hepatocytes. The modifications of the hepatic structure through repeated exposure to OPs probably will lead to a vicious circle in which toxic effects will raise progressively concomitant with hepatic dysfunction. Regarding the kidney, extensive changes that occurred after OP intoxications affected the entire renal parenchyma, with alterations of the glomerular, tubular, and interstitial structures. From our knowledge, this is the first study that certified this degree of renal lesions severity caused by OP exposure. Adrenal gland research showed an increase in vacuole number in all glandular areas correlated to data obtained in previous experiments, suggesting that OP exposure is associated with a hypothalamic–pituitary–adrenal hyperfunction. In the thyroid, the observed follicles alterations are similar with changes secondary to the inflammatory process associated to OP exposure. Further studies are required to validate the results of our research.

Conflict of interests

The authors declare that they have no conflict of interests.

References

[1] Slotkin TA, Brown KK, Seidler FJ. Developmental exposure of rats to chlorpyrifos elicits sex-selective hyperlipidemia and hyperinsulinemia in adulthood. Environ Health Perspect, 2005, 113(10):1291–1294. https://doi.org/10.1289/ehp.8133 PMID: 16203236 PMCID: PMC1281268

[2] Mew EJ, Padmanathan P, Konradsen F, Eddleston M, Chang SS, Phillips MR, Gunnell D. The global burden of fatal self-poisoning with pesticides 2006-15: systematic review. J Affect Disord, 2017, 219:93–104. https://doi.org/10.1016/j.jad.2017.05.002 PMID: 28535450

[3] Georgiadis N, Tsarouhas K, Tsitsimpikou C, Vardavas A, Rezaee R, Germanakis I, Tsatsakis A, Stagos D, Kouretas D.

Pesticides and cardiotoxicity. Where do we stand? Toxicol Appl Pharmacol, 2018, 353:1–14. https://doi.org/10.1016/j.taap.2018.06.004 PMID: 29885332

[4] Oncu M, Gultekin F, Karaöz E, Altuntas I, Delibas N. Nephrotoxicity in rats induced by chlorpyrifos-ethyl and ameliorating effects of antioxidants. Hum Exp Toxicol, 2002, 21(4):223–230. https://doi.org/10.1191/0960327102ht225oa PMID: 12099624

[5] Selmi S, Rtibi K, Grami D, Sebai H, Marzouki L. Malathion, an organophosphate insecticide, provokes metabolic, histopathologic and molecular disorders in liver and kidney in prepubertal male mice. Toxicol Rep, 2018, 5:189–195. https://doi.org/10.1016/j.toxrep.2017.12.021 PMID: 29854588 PMCID: PMC5977160

[6] Gundogan K, Donmez-Altuntas H, Hamurcu Z, Akbudak IH, Sungur M, Bitgen N, Baskol G, Bayram F. Evaluation of chromosomal DNA damage, cytotoxicity, cytostasis, oxidative DNA damage and their relationship with endocrine hormones in patients with acute organophosphate poisoning. Mutat Res Genet Toxicol Environ Mutagen, 2018, 825:1–7. https://doi.org/10.1016/j.mrgentox.2017.11.005 PMID: 29307370

[7] Deng Y, Zhang Y, Lu Y, Zhao Y, Ren H. Hepatotoxicity and nephrotoxicity induced by the chlorpyrifos and chlorpyrifos-methyl metabolite, 3,5,6-trichloro-2-pyridinol, in orally exposed mice. Sci Total Environ, 2016, 544:507–514. https://doi.org/10.1016/j.scitotenv.2015.11.162 PMID: 26674679

[8] Ezzi L, Belhadj Salah I, Haouas Z, Sakly A, Grissa I, Chakroun S, Kerkeni E, Hassine M, Mehdi M, Ben Cheikh H. Histopathological and genotoxic effects of chlorpyrifos in rats. Environ Sci Pollut Res Int, 2016, 23(5):4859–4867. https://doi.org/10.1007/s11356-015-5722-x PMID: 26545888

[9] Vale JA. Toxicokinetic and toxicodynamic aspects of organophosphorus (OP) insecticide poisoning. Toxicol Lett, 1998, 102–103:649–652. https://doi.org/10.1016/s0378-4274(98)00277-x PMID: 10022329

[10] Cakici O, Akat E. Effects of oral exposure to diazinon on mice liver and kidney tissues: biometric analyses of histopathologic changes. Anal Quant Cytopathol Histpathol, 2013, 35(1):7–16. PMID: 23469619

[11] Rezg R, Mornagui B, Benahmed M, Chouchane SG, Belhajhmida N, Abdeladhim M, Kamoun A, El-Fazaa S, Gharbi N. Malathion exposure modulates hypothalamic gene expression and induces dyslipidemia in Wistar rats. Food Chem Toxicol, 2010, 48(6):1473–1477. https://doi.org/10.1016/j.fct.2010.03.013 PMID: 20233601

[12] Wallig MA. Chapter 20 – Endocrine system. In: Wallig MA, Haschek WM, Rousseaux CG, Bolon B (eds). Fundamentals of toxicologic pathology. 3rd edition, Academic Press–Elsevier, London, 2018, 565–624. https://doi.org/10.1016/B978-0-12-809841-7.00020-4

[13] Clement JG. Hormonal consequences of organophosphate poisoning. Fundam Appl Toxicol, 1985, 5(6 Pt 2):*S*61–*S*77. https://doi.org/10.1016/0272-0590(85)90115-0 PMID: 3005101

[14] Slotkin TA, Cooper EM, Stapleton HM, Seidler FJ. Does thyroid disruption contribute to the developmental neurotoxicity of chlorpyrifos? Environ Toxicol Pharmacol, 2013, 36(2):284–287. https://doi.org/10.1016/j.etap.2013.04.003 PMID: 23686008 PMCID: PMC3745805

[15] Jeong SH, Kim BY, Kang HG, Ku HO, Cho JH. Effect of chlorpyrifos-methyl on steroid and thyroid hormones in rat F0- and F1-generations. Toxicology, 2006, 220(2–3):189–202. https://doi.org/10.1016/j.tox.2006.01.005 PMID: 16472551

[16] Institutional Animal Care and Use Committee (IACUC). Animal use forms. Washington State University IACUC, USA. https://iacuc.wsu.edu/forms/

[17] Garí M, González-Quinteiro Y, Bravo N, Grimalt JO. Analysis of metabolites of organophosphate and pyrethroid pesticides in human urine from urban and agricultural populations (Catalonia and Galicia). Sci Total Environ, 2018, 622–623:526–533. https://doi.org/10.1016/j.scitotenv.2017.11.355 PMID: 29220776

[18] Tripathi S, Suzuki N, Srivastav AK. Response of serum minerals (calcium, phosphate, and magnesium) and endocrine glands (calcitonin cells and parathyroid gland) of Wistar rat after chlorpyrifos administration. Microsc Res Tech, 2013, 76(7):673–678. https://doi.org/10.1002/jemt.22217 PMID: 23564468

[19] Foxenberg RJ, Ellison CA, Knaak JB, Ma C, Olson JR. Cytochrome P450-specific human PBPK/PD models for the organophosphorus pesticides: chlorpyrifos and parathion. Toxicology, 2011, 285(1–2):57–66. https://doi.org/10.1016/j.tox.2011.04.002 PMID: 21514354 PMCID: PMC7275873

[20] Mutch E, Williams FM. Diazinon, chlorpyrifos and parathion are metabolised by multiple cytochromes P450 in human liver. Toxicology, 2006, 224(1–2):22–32. https://doi.org/10.1016/j.tox.2006.04.024 PMID: 16757081

[21] King AM, Aaron CK. Organophosphate and carbamate poisoning. Emerg Med Clin North Am, 2015, 33(1):133–151. https://doi.org/10.1016/j.emc.2014.09.010 PMID: 25455666

[22] Saraei F, Sadoughi M, Kaka G, Sadraie SH, Foaddodini M. Study of the effects of diazinon on fetal liver in Balb/c mice. Iran Red Crescent Med J, 2016, 18(4):*e*28076. https://doi.org/10.5812/ircmj.28076 PMID: 27330832 PMCID: PMC4913036

[23] Mathur P, Khanna AK, Cywinski JB, Maheshwari K, Naylor DF Jr, Papay FA. 2018 Year in review: machine learning in healthcare. Team BrainX, BrainX Community. https://www.brainxai.org/wp-content/uploads/2019/03/PDF-of-2018-Year-in-Review Machine-Learning-in-Healthcare..pdf

[24] Kolios G, Valatas V, Kouroumalis E. Role of Kupffer cells in the pathogenesis of liver disease. World J Gastroenterol, 2006, 12(46):7413–7420. https://doi.org/10.3748/wjg.v12.i46.7413 PMID: 17167827 PMCID: PMC4087584

[25] Durham SK, Brouwer A, Barelds RJ, Horan MA, Knook DL. Comparative endotoxin-induced hepatic injury in young and aged rats. J Pathol, 1990, 162(4):341–349. https://doi.org/10.1002/path.1711620412 PMID: 2290118

[26] Georgiadis G, Mavridis C, Belantis C, Zisis IE, Skamagkas I, Fragkiadoulaki I, Heretis I, Tzortzis V, Psathakis K, Tsatsakis A, Mamoulakis C. Nephrotoxicity issues of organophosphates. Toxicology, 2018, 406–407:129–136. https://doi.org/10.1016/j.tox.2018.07.019 PMID: 30063941

[27] Van Emon JM, Pan P, van Breukelen F. Effects of chlorpyrifos and trichloropyridinol on HEK 293 human embryonic kidney cells. Chemosphere, 2018, 191:537–547. https://doi.org/10.1016/j.chemosphere.2017.10.039 PMID: 29059561 PMCID: PMC7462251

[28] Kong MJ, Bak SH, Han KH, Kim JI, Park JW, Park KM. Fragmentation of kidney epithelial cell primary cilia occurs by cisplatin and these cilia fragments are excreted into the urine. Redox Biol, 2019, 20:38–45. https://doi.org/10.1016/j.redox.2018.09.017 PMID: 30292083 PMCID: PMC6172485

[29] Schelling JR. Tubular atrophy in the pathogenesis of chronic kidney disease progression. Pediatr Nephrol, 2016, 31(5):693–706. https://doi.org/10.1007/s00467-015-3169-4 PMID: 26208584 PMCID: PMC4726480

[30] Torres JA, Rezaei M, Broderick C, Lin L, Wang X, Hoppe B, Cowley BD Jr, Savica V, Torres VE, Khan S, Holmes RP, Mrug M, Weimbs T. Crystal deposition triggers tubule dilation that accelerates cystogenesis in polycystic kidney disease. J Clin Invest, 2019, 129(10):4506–4522. https://doi.org/10.1172/JCI128503 PMID: 31361604 PMCID: PMC6763267

[31] Pandey SP, Mohanty B. Disruption of the hypothalamic–pituitary–thyroid axis on co-exposures to dithiocarbamate and neonicotinoid pesticides: study in a wildlife bird, *Amandava amandava*. Neurotoxicology, 2017, 60:16–22. https://doi.org/10.1016/j.neuro.2017.02.010 PMID: 28237669

[32] El-Shenawy NS, Al-Eisa RA, El-Salmy F, Salah O. Prophylactic effect of vitamin E against hepatotoxicity, nephrotoxicity, haematological indices and histopathology induced by diazinon insecticide in mice. Curr Zool, 2009, 55(3):219–226. https://doi.org/10.1093/czoolo/55.3.219

[33] Sarhan OMM, Al-Sahhaf ZY. Histological and biochemical effects of diazinon on liver and kidney of rabbits. Life Sci J, 2011, 8(4):1183–1189. https://doi.org/10.7537/marslsj080411.145

[34] Badraoui R, Ben Nasr H, Louati R, Ellouze F, Rebai T. Nephrotoxic effect of tetradifon in rats: a biochemical and histomorphometric study. Exp Toxicol Pathol, 2012, 64(6):645–650. https://doi.org/10.1016/j.etp.2010.12.008 PMID: 21216578

[35] Ito S, Kobayashi A, Tsuchiya T, Moriyama Y, Kikuchi M, Deguchi T, Yamaguchi Y. Thyroidization in renal allografts. Clin Transplant, 2009, 23(Suppl 20):6–9. https://doi.org/10.1111/j.1399-0012.2009.01001.x PMID: 19594588

[36] Raees K, Ishfaq R, Ullah A, Tahir MZ, Abbas T, Tahir HM, Mukhtar MK, Arshad M, Khan SY, Ahmad KR. Histological and micrometric effects of diazinon exposure on adrenal

medulla and cortex in mice. J Appl Anim Res, 2012, 40(4): 267–722. https://doi.org/10.1080/09712119.2012.672309

[37] Hoernerhoff MJ, Hill GD, Gruebbel MM. Adrenal gland, cortex – vacuolization, cytoplasmic. In: ***. NTP nonneoplastic lesion atlas. National Toxicology Program (NTP), U.S. Department of Health and Human Services. https://ntp.niehs.nih.gov/nnl/endocrine/adrenal/cxvacuol/adrenal-gland-cortex-vacuolization-cytoplasmic-pdf_508.pdf

[38] De Angelis S, Tassinari R, Maranghi F, Eusepi A, Di Virgilio A, Chiarotti F, Ricceri L, Venerosi Pesciolini A, Gilardi E, Moracci G, Calamandrei G, Olivieri A, Mantovani A. Developmental exposure to chlorpyrifos induces alterations in thyroid and thyroid hormone levels without other toxicity signs in CD-1 mice. Toxicol Sci, 2009, 108(2):311–319. https://doi.org/10.1093/toxsci/kfp017 PMID: 19190125

[39] Ramirez-Vargas MA, Flores-Alfaro E, Uriostegui-Acosta M, Alvarez-Fitz P, Parra-Rojas I, Moreno-Godinez ME. Effects of exposure to malathion on blood glucose concentration: a meta-analysis. Environ Sci Pollut Res Int, 2018, 25(4):3233–3242. https://doi.org/10.1007/s11356-017-0890-5 PMID: 29235025

[40] Everett CJ, Matheson EM. Pesticide exposure and diabetes. In: Nriagu JO (ed). Encyclopedia of environmental health. 2nd edition, Elsevier, Burlington, 2019, 104–109. https://doi.org/10.1016/B978-0-12-409548-9.10647-5

[41] Pournourmohammadi S, Ostad SN, Azizi E, Ghahremani MH, Farzami B, Minaie B, Larijani B, Abdollahi M. Induction of insulin resistance by malathion: evidence for disrupted islets cells metabolism and mitochondrial dysfunction. Pestic Biochem Physiol, 2007, 88(3):346–352. https://doi.org/10.1016/j.pestbp.2007.02.001

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