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Environnement et Santé

Chronic toxicity of six pesticides mixture traces on biochemical, hematological, and histological parameters of liver and kidney in female Wistar rats

Toxicitéchronique d'un mélange de six pesticides sur les paramètres biochimiques, hématologiques, et histologiques du foie et reins de rat Wistar femelle.

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Abstract ***Introduction.*** The modern world uses several toxic chemicals, among them, pesticides which present a potential risk to humans when they are exposed to many pesticides at the same time through diet. ***Objective***. The present study aimed to evaluate the toxic effect of six pesticides mixture on liver hematological, biochemical and histopathological parameters, in rats. ***Material and methods***. Female Wistar rats, aged two months and weighed 150±5 g, were divided into three groups of ten rats. Pesticides mixture (Chloroperiphos-methyl, Deltamethrin, Methidathion, Cypermethrin, Acetamiprid, and Abamectin), at Acceptable Daily Intake (ADI and ADI×2), was administered orally for 7 months. ***Results***. Pesticides mixture at ADI exposed rats exhibited severe damage in liver and kidney structure and function. At dose of ADI×2, more severe effects, with notable changes and damage, were observed in liver and kidney histology by several forms of inflammation, denaturation, and necrosis of cells and tissues. This showed direct impact on functioning and metabolism of these organs by significant changes in biochemical and haematological parameters (total cholesterol, total proteins, aspartate-aminotransferase, alanine-aminotransferase, alkaline phosphatase, gamma-glutamiltransferase, total bilirubin, potassium, iron, urea, creatinine, uric acid, hemoglobin and hematocrit). ***Conclusion***. The six pesticides mixture is toxic at ADI×2, and even at ADI that represents security dose of one pesticide. However, in a mixture, this dose causes deleterious effects on liver and kidney structure and function in rats.

Key words: *Pesticides, Toxicity, Biochemistry, Histopathology, Liver, Kidney, Rat*

Résumé ***Introduction*****.** Le monde moderne utilise plusieurs produits chimiques toxiques, parmi lesquels les pesticides qui présentent un risque potentiel pour l'homme lorsqu'il est exposé à de nombreux pesticides en même temps par l'alimentation. ***Objectif***. La présente étude visait à évaluer l'effet toxique d'un mélange de six pesticides sur les para-mètres hématologiques, biochimiques et histopathologiques du foie et des reins, chez le rat. ***Matériel et méthodes***. Des rats Wistar femelles, âgés de 2 mois et pesant 150±5 g, ont été divisés en trois groupe de dix rats chacun. Le mélange de pesticides (chloro-périphos-méthyl, deltaméthrine, méthidathion, cyperméthrine, acétamipride et abamectine) à une Dose Journalière Admissible (DJA et DJA×2) a été administré par voie orale pendant 7 mois aux rats. ***Résultats***. Le mélange de pesticides chez les rats traités à la DJA a présenté de graves altérations de la structure et la fonction du foie et des reins. A DJA×2, des effets plus graves, avec des changements notables et des dommages ont été observés dans l'histologie du foie et des reins, par plusieurs formes d'inflammation, dénaturation et nécrose des cellules et des tissus. Ceci a montré un impact direct sur le fonctionnement et le métabolisme de ces organes par des modifications significatives de paramètres biochimiques et hématologiques (cholestérol total, protéines totales, aspartate-aminotransferase, alanine-aminotransferase, phosphatase alkaline, Gamma-glutamiltransferase, bilirubine totale, potassium, fer, urée, créatinine, acide urique, hémoglobine et hématocrite). ***Conclusion***. Le mélange des six pesticides est toxique à la DJA×2 et même à la DJA qui représente la dose de sécurité d'un pesticide. Cependant, dans un mélange, cette dose provoque des effets délétères sur la structure et la fonc-tion du foie et des reins chez les rats Wistar.

Mots clés : *Pesticides, Toxicité, Biochimie, Histopathologie, Foie, Rein, Rat*

Introduction

Today, the demand for food will only increase with an increasing world population. Around 1000 pesticides are accessible in different arrangements in present day agriculture and sanitary fields. These chemicals are widely used all over the world fat has its impor-tance, and these benefits to humanity in increasing the production and quality of food products of plant origin [1-3]. Pesticides are synthetic compounds bio-cides likewise equipped for slaughtering all types of life, deliberately added to environment for the purpo-se of killing or injuring pests (insects, rodents, weeds, and other unwanted organisms) and the era-dication and control of diseases that result in increa-sed food production. Major classes of pesticides are insecticides, herbicides, fungicides, and rodenticides. While many pesticides are toxic only to target species, many are not highly selective [3-5]. Acute and chronic serious problems on the ecological system and human health are observed because of the massive and uncontrolled use of pesticides [3, 6].

The greater part of the human population is exposed to pesticides. Pesticides are evaluated to be answe-rable for about 4% of the considerable number of passings from all incidental poisonings, essentially in the developing world. As for long-term and chronic effects [7, 8]. Human exposure is fairly normal with significant levels happening in word related settings (creation and showering exercises in farming), low levels in family units, and as residues in food. Human exposure may bring about intense and postponed wellbeing impacts. Intense pesticide harming repre-sents critical dismalness and mortality around the world [6]. A major pathway for exposure to many pesticides at the same time is through diet [9].

Pesticides residues determination in food is beco-ming an especially imperative and challenging issue. Generally, it is very complex because it necessitates the search for several pesticides trace of numerous chemical class differences [10]. Approaches to testing the safety of pesticides are summarized as the basis of setting Acceptable Daily Intake (ADI), and Maxi-mum Residue Limit (MRL). There is an increasing awareness that the global reliance on pesticides should be reduced due to their cost, and environ-mental and health effects [11].

Interactions among pesticides and different xenobio-tics happen, as often as possible through the reactive intermediates formation. In liver, interactions of pesticides with other xenobiotics and harmful meta-bolites cause inhibition or induction of the respon-sible enzymes of xenobiotics biotransformation in the liver [12]. Residential pesticide exposure was asso-

ciated with cancer risk for both adults and children. This effect is related to the way of exposure and metabolism of pesticides, as well as their mechanism of action on the organism. [13,14].

Several studies have tested toxicological interactions in pesticides mixtures, at molecular level, and their impact on human health. A number of cocktail effects examples, such as the toxicity potentiation of some pesticides by others, e.g. malathion by isomalathion, pyrethroids by anticholinesterases insecticides, organophosphorus by organochlorine insecticides, were found [15,16].

In practice, several types of pesticides were consumed at the same time with doses that can even shift the ADI in the same meal and the same day, presenting a danger to the consumer. The present study aimed to evaluate the chronic toxic effect of six pesticides mixture, administered orally for 7 months, and the possible synergy between them, even at very low doses which present the security dose of one pesticide at ADI and ADI×2, on biochemical, hematological, and histopathological parameters of liver and kidney tissues in female Wistar albino rats.

Material and methods

Animals and diets

Healthy females albino Wistar rats, two months old with an average body weight of 150±5 g were obtained from Pasteur Institute, Algiers, Algeria. Animals were maintained under standard laboratory conditions of temperature (25±2°C), relative humidity (50±15%), 12h light/dark cycle, and received standard diet and water *ad libitum*. This study has been carried out in accordance with the Directive 2010/63/EU for animal experiments ethics, and approved by the scientific committee of Blida 1 University (Algeria).

Rats received throughout experimentation period a mixture of corn, bran, middlings, soya, and multivitamin complex. Rats had free access to food and each rat consumed diet amount according to these needs (total nitrogenous matter: 158.10 g/kg, Fat: 54.40 g/kg, Calcium: 12.80 g/kg Phosphorus: 3.80 g/kg).

Pesticides choice and doses

After the investigation approach about the most used pesticides in agriculture, the six commercial pesticides used in this study were purchased from Phytosanitary Chemical (Tipaza, Algeria). Pesticides tested were Chloroperiphos-methyl (insecticide, Reldan 40 EC, 400g/L; Dow Agro Science, king Lin Great Britain), Deltamethrin (insecticide, decis 25 EC, 25g/L; Bayer Crop Science, Valence, Spain), Methidathion (insecticide, Limacide 40, 400g/L; George-Daras S.A, Marseille, France), Cypermethrin (insecticide, Cyatrin 10 EC, 100 g/L; Golden Field, Amane, Jordan), Acetamiprid (insecticide, PICADOR 20%, 200g/L; Vitagro, China) and Abamectin (insecticide, acaricide and nematicide, VAPCOMIC EC, 18g/L; VAPCO, Amane, Jordan).

The six pesticides were used as mixture suspended in water, administered orally *ad libitum* at low doses, ADI and ADI×2, respecting animal body weight and daily water quantity consumed by rats. Pesticides doses were given to rats daily for 7 months. According to the Codex Alimentarius, the doses of pesticides were : Chloroperiphos-methyl ADI : 0.01 mg/kg, ADI×2 : 0.02mg/kg ; Deltamethrin : ADI : 0.01 mg/kg, ADI×2 : 0.02mg/kg ; Methidathion : ADI : 0.001mg/kg, ADI×2 : 0.002 mg/kg ; Cypermethrin : ADI : 0.05mg/kg, ADI×2 : 0.1mg/kg ; Acetamiprid : ADI : 0.07mg/kg, ADI×2 : 0.14mg/kg ; Abamectin : ADI : 0.0025mg/kg, ADI×2 : 0.005mg/kg [17].

Experimental design

Rats were divided into three groups, namely control, pesticide at ADI, and pesticide at ADI×2. Each group consisted of six animals. Control rats were given only water, group 2 rats were given daily oral doses of pesticide mixture (ADI), and group 3 rats were given daily oral doses of pesticides mixtures ADI×2 *ad libitum* for 7 months, a sufficient time for the appearance of the chronic toxic effects of these low doses of pesticides. The safe doses of individual pesticides can be toxic in the mixture.

At the end of the experiment, and after overnight fast, all the animals were sacrificed by decapitation under light ether anesthesia. Blood samples were collected by arterial decapitation in specific tubes with anticoagulant substance, ETDA-2K was used for the hematological analysis, and lithium heparin was used for the biochemical analysis. Serum was collected using a centrifugal separator at 4000 rpm for 10 min. The liver was carefully dissected out, weighed, and fixed in 10% buffered formalin to study the histology.

Body weight and relative organ weight of rats

Body weight (BW) of each animal was measured once a week or once two weeks throughout the experimental period. Liver was weighed, and relative organ weight of each animal was calculated by the equation of Romero-Sarmiento *et al*., 2012.

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat (g)}} \times 100$$

Hematological analysis

Blood samples were analyzed for white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), platelet count (PLT), using a full-automatic blood cell counter model PCE-210N (Tipaza, Algeria).

Biochemical analysis

Blood serum was collected and biochemical indicators of liver function were analyzed: blood glucose (Glu), total cholesterol (TC), triglycerids (TG), total proteins (TP), albumin (ALB), aspartate aminotransferase (ASAT), alanine Aminotransferase (ALAT), alkaline phosphatase (ALP), Gamma-glutamil-transferase (GGT), total bilirubin (TB), potassium (K), iron (I). Biochemical indicators of kidney function: urea (U), creatinine (Cre), uric acid (UA) were determined by colorimetric methods (spectrophotometer model BIOLIS 24i) (kits DiaSys Germany laboratory).

Histological analysis

Liver and kidney from control and experimental rats were removed, and preserved in formalin 10% until processed for histology, then washed under running water to remove formalin pigments and ascending grades of alcohol which can be dehydrated. After, paraffin blocks were made by impregnation with paraffin wax. They were processed and sections were cut with 5µm in thickness using Spencer Lens, rotatory microtome (LEICA RM 2125 RT) and then, stained with hematoxylin and eosin stain. They were examined *via* routine light microscopy [18].

Statistical analysis

Statistical analysis was made using BioStat version 09.Ink. Data were calculated by using One-way ANOVA and expressed as mean ± standard deviation (SD) followed by Dunnet's t-test to determine the difference between experimental groups and control. Values were considered significant at $p<0.05$.

Results

The present study showed that daily oral administration of pesticide mixtures at ADI and ADI×2 for 7 months causes deleterious effects on biochemical and histological parameters compared to control group. Data from various groups of rats are presented in **Tables 1-3** with mean ± SD.

Body weight

Mean changes in rats BW are shown in **Fig. 1**.

No significant change in BW gain of rats was observed at both doses of pesticides mixtures at ADI and ADI×2.

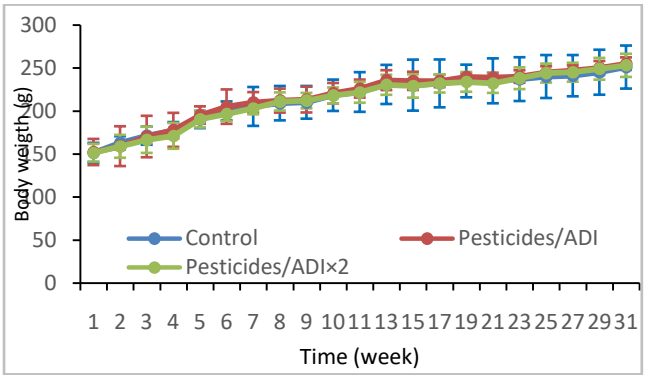

Fig. 1. Body weight of control and exposed rats to pesticides mixture

Values are presented as mean±SD of 10 rats per group. Control group fed standard diet. Two groups of rats exposed to pesticides mixture at doses ADI or ADIx2 for 7 months.

Relative organ weight

Liver and kidney relative organ weights of females rats are shown in **Table 1**. Liver relative weight increased slightly by 71.5% at ADI×2 pesticide dose, while kidney relative weight was comparable in control and exposed rats with no significant change.

Table 1. Relative organ weight of female rats orally administered pesticides mixture for 7 months

	Liver relative weight	Kidney relative weight
Control	3.413 ± 0.571	0.647 ± 0.071
Pesticides/ADI	2.930 ± 0.500	0.570 ± 0.019
Pesticides/ADI×2	2.095 ± 0.332*	0.562 ± 0.015

*Values are presented as mean±SD of 10 rats per group. Control group fed standard diet. Two groups of rats exposed to pesticides mixture at doses ADI or ADIx2 for 7 months. Values differed significantly compared to control : * p<0.05.*

Hematomogical analysis

The effects of different exposures on haematological parameters are shown in **Table 2**. A significant decrease was found in hematocrit (32.80%) ($p<0.01$), and haemoglobin (11.32g/dL) ($p<0.05$) in exposed rats at ADI×2 compared to control group. However, no significant change was shown in other haematological parameters.

Biochemical analysis

Plasma biochemical parameters of females rats which had received pesticides mixture are shown in **Table 3**. Exposure to pesticides at ADI and ADI×2 doses induced significant changes in liver activity levels biomarkers with a very significant increase in serum level of ALAT, ASAT, PAL, GGT, and total bilirubin. A decrease

in total cholesterol, and total proteins values was noted with ADI×2. There was a significant decrease in urea, uric acid and potassium in kindey, whereas, a significant increase was noted in creatinine level. No significant change in blood glucose, triglycerids, and albumin, was observed in exposed rats compared to control group.

Histological analysis

Fig. 2-7 illustrate the histopathological changes in liver and kidney of experimental rats, respectively. Histological results revealed that liver of control rats showed normal histological architecture with normal hepatocytes around the central vein and sinusoids (**Fig. 2A,3A**). Histopathological alterations were observed in rats exposed to pesticides mixture compared to controls. Microscopic observations of liver parenchyma in rats exposed to ADI dose are shown in **Fig. 2-3:B,C**. These figures showed an appearance of leukocytic infiltrates throughout the liver tissue, sinusoidal dilations, congested blood sinusoids, and necro -tic hepatocytes. For rats exposed to ADI×2 pesticides, **Fig. 2:D,E,F** and **Fig. 3:B,C** showed severe alterations of pesticides on hepatic histology, manifested by the high number of hepatocytes that had strong pink colors with eosinophilic cytoplasm throughout hepatic parenchyma. These were hepatocytes in necrosis course, and several zones of hepatocytes totally necrotic with denaturation of cellular organelles. Even, the plasma membrane, with the presence of multiple sites of leukocytic infiltrates, sinusoidal dilations and blood congestion with hemorrhagic capillaries were observed. In kidney, the histological results revealed in the control rats a normal kidney tissue with normal glomerulus, renal corpuscle, proximal tubules, loops of Henle and distal tubules (**Fig. 4: A,B**). In **Fig. 4:C**, and **Fig. 5:A,B**, microscopic observation of renal parenchyma in rats exposed to pesticides mixture ADI, showed the presence of multiple zones of leukotytic infiltrates in renal interstitium around glomerulus, denaturing and even denaturing proximal and distal tubes with total denaturation of cytoplasm and cellular organelles, and only nuclei and carcases of tubes remained, blood congestion and hemorrhagic glomerulus, swelling of glomerulus due to hyperplasia of mesangial cells of glomerulus. Thus for rats exposed to ADI×2 (**Fig. 4:D,E,F**, and **Fig. 5:C,D**), the six pesticides mixture caused several important changes on renal histology: blood congestion, hemorrhagic blood vessels with hemosiderin deposition in proximal tubes, presence of several areas of leukocytic infiltrates in renal interstitium, atrophy, dilatation of proximal renal tubes with flattened epithelium and denatured cells with a total denaturation of cytoplasm and cellular organelles, and damaged. distal renal tubes with denatured cells, hemorrhagic glomerulus, presence of denatured glomerulus with swelling of glomerulus due to hyperplasia of mesangial cells.

Discussion

The present study demonstrated the toxic effect of six pesticides mixture on liver and kidney function and histology in an experimental rat model. Modern agriculture uses a high number of pesticides for one agricultural product to obtain the highest possible quantitative and qualitative productivity. The ingestion of a diversity of fruits and vegetables everyday exposes the consumer to pesticides and to the danger of consuming several types of pesticides at the same time, even at low doses or in trace quantities, and can cause severe long-term damage to human health [19,20]. Liver and kidney represent a major target for pesticides, and because of their important roles in the body, their damage causes negative reparations on the proper functionary of the human body [21]. Our results showed that the 7 months of rats oral exposure to pesticides mixtures at ADI and ADI×2

Table 2. Effect of pesticide mixture (ADI and ADI×2) on hematological parameters

Parameters	Control	Pesticides/ADI	Pesticides/ADI×2
Red blood cells (10^6/µL)	7.34 ± 0.29	7.29 ± 0.25	7.17 ± 0.15
Hemoglobin (g/dL)	14.40 ± 0.27	14.32 ± 0.14	11.32 ± 0.31*
Hematocrit (%)	37.20 ± 1.76	37.15 ± 1.20	32.80 ± 1.17**
White blood cells (10^3/µL)	6.60 ± 0.82	6.70 ± 0.91	6.70 ± 1.28
Platelets count (10^3/µL)	1221.50 ± 82.85	1224.50 ± 51.98	1258.50 ± 56.57

*Values are presented as mean±SD of 10 rats per group. Control group fed standard diet. Two groups of rats exposed to pesticides mixture at doses ADI or ADIx2 for 7 months. Values differed significantly compared to control : * p<0.05.** p<0.01.*

Table 3. Effect of pesticides mixture (ADI and ADI×2) on biochemical parameters

Parameters	Control	Pesticides/ADI	Pesticides/ADI×2
Glucose (g/L)	1.67 ± 0.10	1.59 ± 0.16	1.74 ± 0.12
Total Cholesterol (g/L)	1,00 ± 0.10	0.90 ± 0.14	0.82 ± 0.09*
Triglycerids (g/L)	1.09 ± 0.09	1.05 ± 0.04	1.10 ± 0.10
ASAT (µl/L)	129 ± 2.06	155,00 ± 10,00***	194,00 ± 15.01***
ALAT (µl/L)	84.25 ± 5.98	96.25 ± 9.34***	102.75 ± 5.11***
PAL (µl/L)	64,00 ± 2.50	93,00 ± 3.70***	112 ± 11.33***
GGT (µl/L)	6.25 ± 0.68	11.33 ± 1.86***	11.50 ± 1.41**
Total Bilirubin (mg/L)	4.40 ± 0.48	5.50 ± 0.50*	7.25 ± 0.43***
Total protein (g/L)	89.20 ± 4.70	86,00 ± 0.01*	81.75 ± 5.21**
Albumin (g/L)	36.80 ± 1.32	37,00 ± 2.74	36,40 ± 1.92
Iron (µg/dL)	265.20 ± 12.10	332.75 ± 37.61***	331.8 ± 38.57***
Potassium (mmol/L)	4.06 ± 0.51	3.73 ± 0.38*	3.78 ± 0.59*
Urea (g/L)	0.51 ± 0.05	0.45 ± 0.06*	0.41 ± 0.02**
Creatinine (g/L)	7.60 ± 0.48	9.50 ± 0.63**	9.60 ± 0.48**
Uricacid (mg/L)	15.60 ± 1.31	9,00 ± 1.05***	10.20 ± 1.32***

*Values are presented as mean±SD of 10 rats per group. Control group fed standard diet. Two groups of rats exposed to pesticides mixture at doses ADI or ADIx2 for 7 months. Values differed significantly compared to control : * p<0.05,** p<0.01, *** p< 0.001. ASAT: Aspartate aminotransferase; ALAT: Alanine Aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma-glutamiltransferase.*

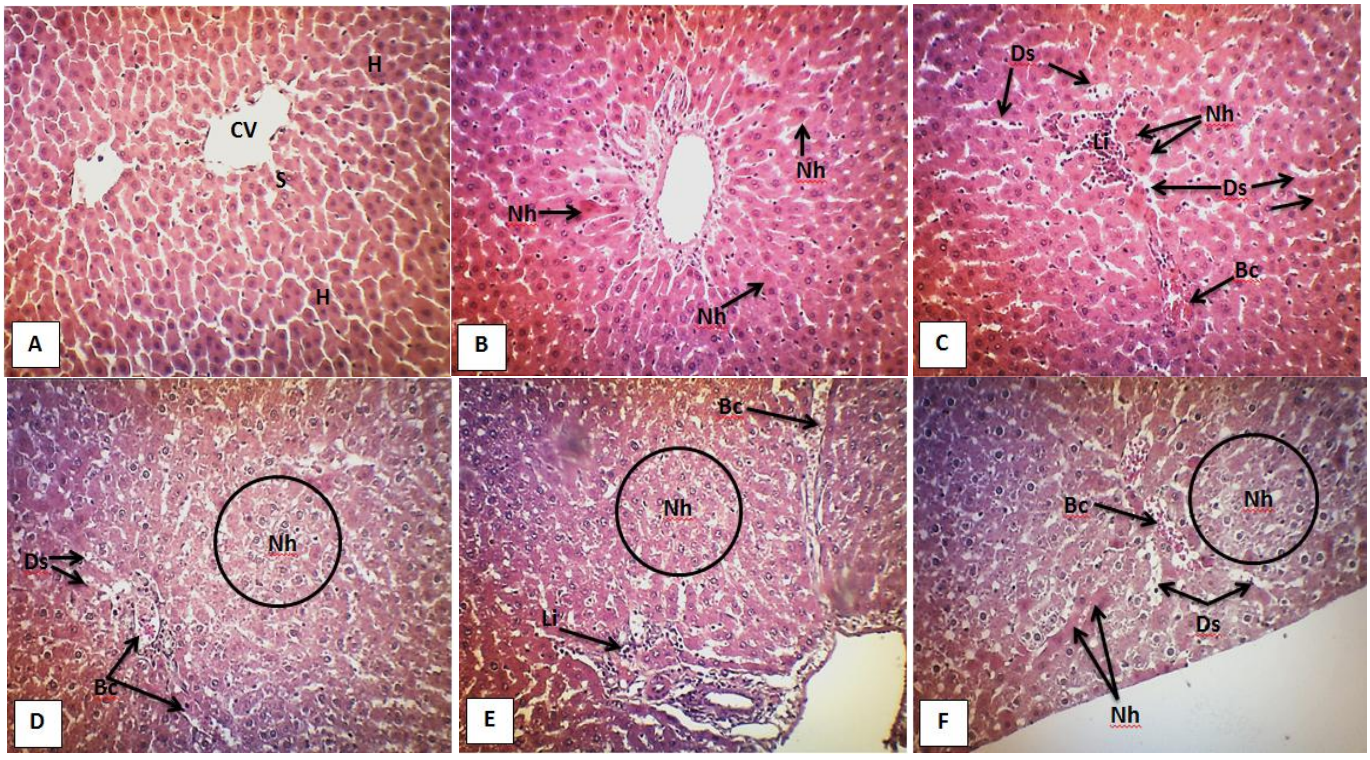

Fig. 2. Histological sections of the liver of exposed and control rats

A: Normal histological structure of control liver. B, C: Section of liver exposed to pesticides mixture ADI. D,E,F: Section of liver exposed to pesticides mixture ADI×2. Magnification: × 400. Stain: Hematoxylin and Eosin. H: Normal hepatocyte. Nh: Necrotic hepatocyte. S: Sinusoids. Ds: Dilated sinusoids. CV: Central vein. Bc: Blood congestion. Li: Leukocytic infiltration.

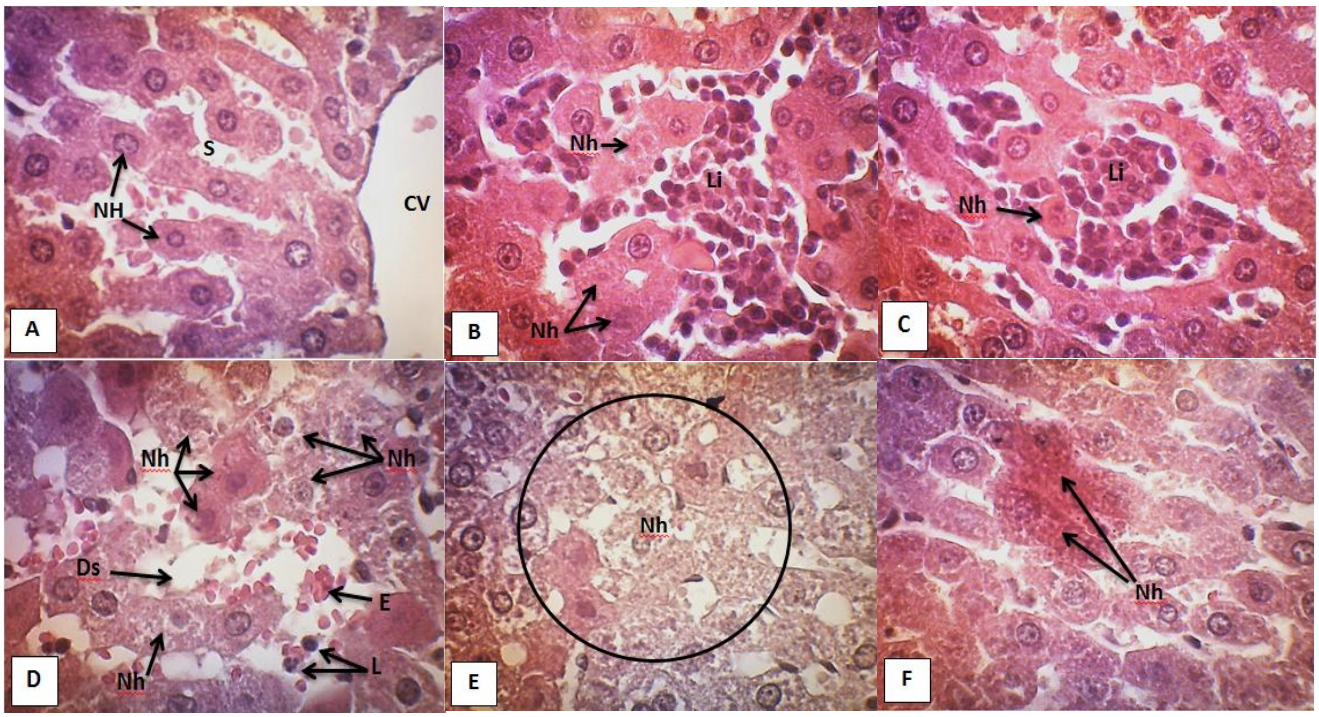

Fig. 3. Liver histological sections of control and exposed rats

A: Normal histological structure of control liver. B, C: Section of liver exposed to pesticides mixture DAI. D,E,F: Section of liver exposed to pesticides mixture ADI×2. Magnification: × 1000. Stain: Hematoxylin and Eosin. H: Normal hepatocyte. Nh: Necrotic cells. S: Sinusoids. Ds: Dilated sinusoids. CV: Central vein.Bc: Blood congestion. Li: leukocytic infiltration. L: Leukocytes. E: Erythrocytes.

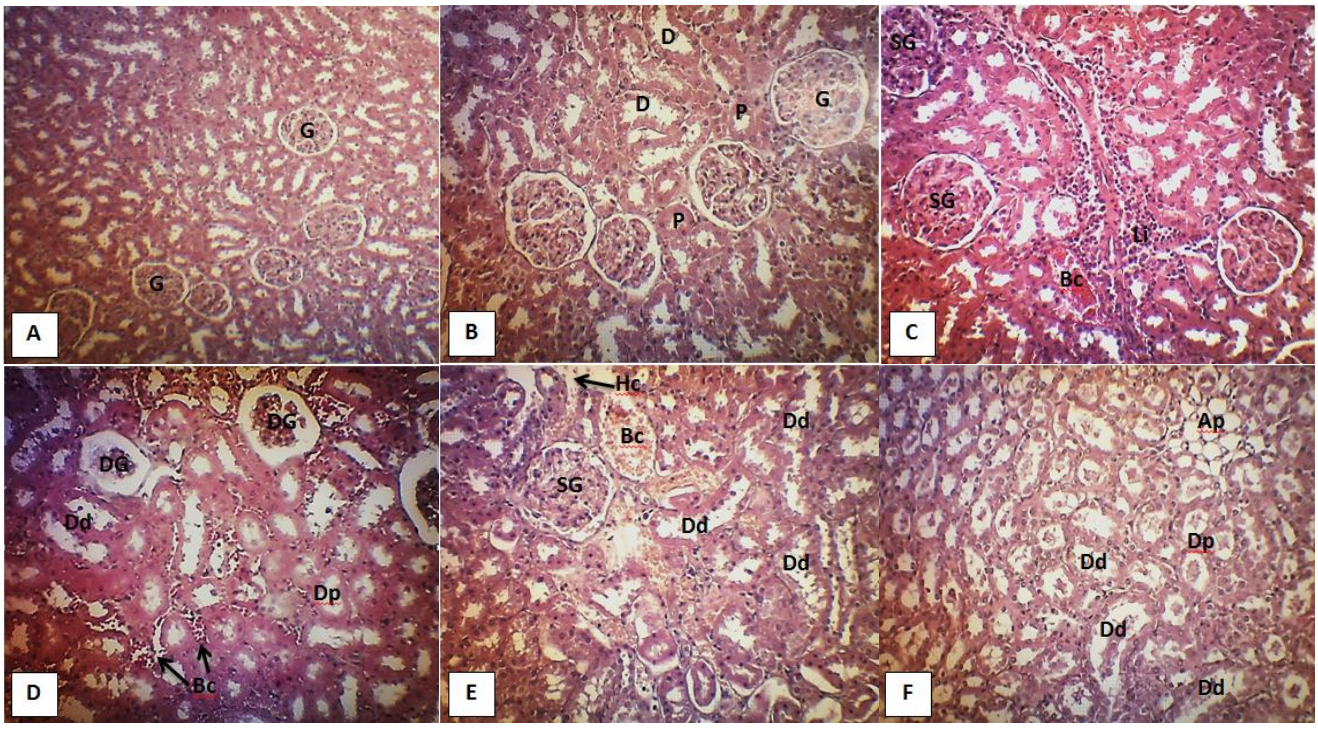

Fig. 4. Kidney histological sections of exposed and control rats

A, B: Normal histological structure of control kidney. C: Section of kidney exposed to pesticides mixture DAI. D,E,F: Section of kidney exposed to pesticide mixture ADI×2. Magnification: A :×100, and B, C, D, E, F : ×400. Stain: Hematoxylin and Eosin. G: Glomerulus. P: Proximal convoluted tubules. D: Distal convoluted tubules. DG: Denatured Glomerulus. SG: Swollen Glomerulus. HG: hemorrhagic glomerulus. Dp: Denatured proximal tubule. Dd: Denatured distal tubules. Ap: Atrophic proximal tubule. Bc: Blood congestion. Hc: Hemorrhagic capillary. Li: leukocytic infiltration.

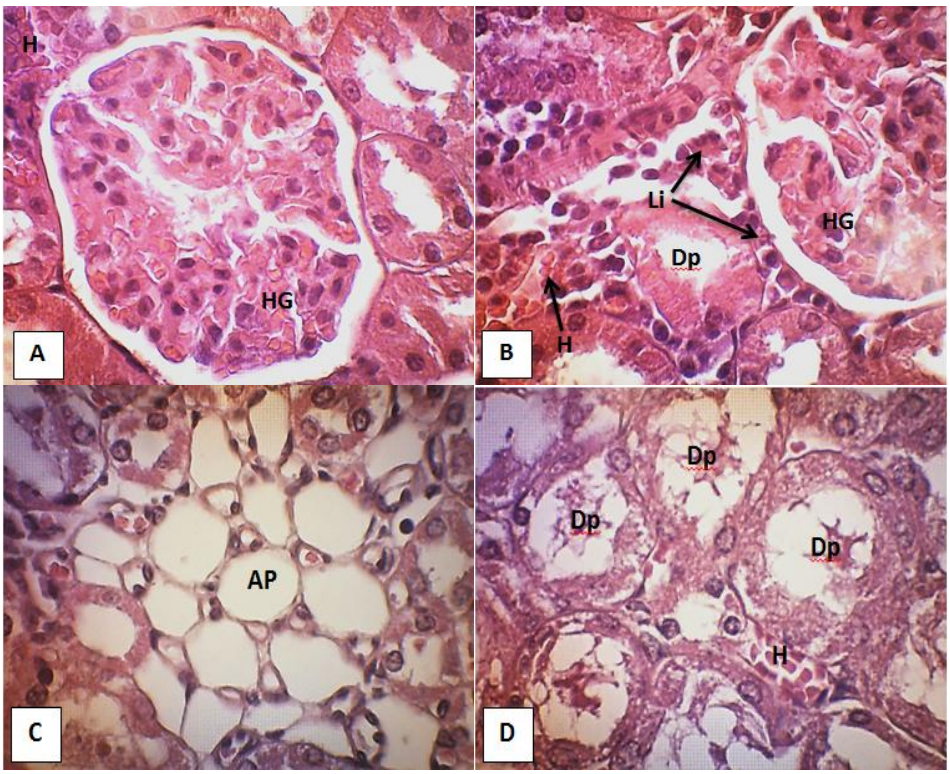

Fig. 5. Kidney histological sections of exposed and control rats

A, B: Section of kidney of rats exposed to pesticides mixture DAI. C, D: Section of kidney exposed to pesticides mixture ADI×2. Magnification: ×1000. Stain: Hematoxylin and Eosin. Dp: Denatured proximal tubule. Dd: Denatured distal tubules. Ap: Atrophic proximal tubule. Li: leukocytic infiltration. E: Erythrocytes.

(Chloroperiphos-methyl, Deltamethrin, Methidathion, Cypermethrin, Acetamiprid, and Abamectin) involved highly significant changes in biochemical, hematological, and histological parameters in liver and kidney. This toxic effect proved that the mixture of six pesticides was toxic to liver and kidney, even at low doses, and even at the acceptable daily intake (ADI) that is considered without any toxic effect only for one type of pesticide, but ADI×2 had a more severe toxic effect [11].

Data showed that pesticides interact in a variety of ways when they are combined, mainly in additive or synergistic or antagonistic effect, and that the effect of each mixture varies depending on the dose and the physiological target within cell or body, and the compound itself [22]. Firstly, about liver alteration, our results showed that pesticides mixture caused hepatotoxicity magnified by high number of necrotic hepatocytes and tissues, leukocytic infiltrates, sinusoidal dilations and blood congestion with hemorrhagic capillaries. These changes directly affected liver function, biochemical and hematological parameters of rat blood. Our results for ADI and ADI×2 confirmed histological findings, by a significant increase in liver enzymes level (ASAT, ALAT, PAL, GGT), total bilirubin and Iron, and a significant decrease in blood level of total proteins, total cholesterol, hemoglobin, and hematocrit. signs of liver damage, and injury with inflammation and liver insufficiency [23,24], the histological Increased ASAT and ALAT were linked to high oxidative stress effects of pesticides, leading to hepatocyte injury [25]. This serum increase in liver enzymes was primarily due to the loss of a large portion of these enzymes from the hepatocyte cytosol into the blood stream, as a result of pesticide attack on hepatocyte cell membranes, which is indicative of abnormal liver function [26]. The increase of PAL and GGT are related to the heaptotoxicity which can be explained by a lesion of the bile ducts and cholestasis [27]. The significant elevation of total bilirubin can be commented by hepatic insufficiency and dysfunction with damage to the bile ducts [28].

Liver is the main organ of protein metabolism, the significant decreases in total proteins level in exposed rats to the pesticides mixtures might be caused by liver damage, dysfunction, and disruption of protein metabolism [29]. The significant decreases in total cholesterol level at ADI×2 dose could be explained by the fact that pesticides mixture exhibited a toxic inter-action with lipids of Wistar rat through lipoperoxidation [30]. Its mechanism of action is based on the

induction of cytochrome P450 dependent mono-oxygenase system that is also responsible for the metabolism of fatty acids, vitamins, and steroids [31]. High level of iron and low level of hemoglobin and hematocrit at ADI×2 inexposed groups, observed in this study might be due to inhibitory impact of pesticides on numerous means of heme biosynthesis, and glycolysis in rat, and membrane permeability that was associated with cells damage [32-34].

Pesticides effects on cell activity could, in several ways, induce DNA damage, perturbation of mitochondrial activity, cell division and metabolism, oxydative stress, decreased capacity antioxidant defense, causes cell death by necrosis and apoptosis, inflammatory response modification activity of efflux ABC transporters or enzymes, the aterations of membrane protein cell [35-39].

Secondly, about kidney alteration, our results showed that pesticides mixture caused kidney toxicity magnified by high number of denaturing and even denaturing proximal and distal tubes, multiple zones of leukocytic infiltrates, blood congestion and hemorrhagic glomerulus, swelling of the glomerulus and denatured glomerulus. These observations are indicative of inflammation kidney insufficiency [40,41].

Kidney biochemical results confirmed histological findings. Indeed, exposureto pesticides mixture caused a significant increase in serum creatinine levels. These findings might indicate degenerative and necro-tic changes in kidney structure, decreased glomerular filtration, accompanied by renal dysfunction and insufficiency [42,43]. The low level in serum urea, uric acid, and potassium might be explained by increased nitrogen base degradation or by impaired renal function and hepatic insufficiency [44]. Decreased total proteins content in rat blood might indicate protein catabolism and renal impairment and dysfunction [45]. Our results are in accordance with several studies on a variety of pesticides mixtures [46-52].

Conclusion

Results of the present study demonstrate the chronic toxic effect of six pesticides mixture: Chloroperiphos-methyl, Deltamethrin, Methidathion, Cypermethrin, Acetamiprid, and Abamictin, administered orally for 7 months to females Wistar rats, at ADI and ADI×2, on biochemical, hematological and histological parameters of liver and kidney tissues. Pesticides mixture at ADI shows severe damage to structure and function of liver and kidney. At ADI×2 dose, more severe effects are noted and cause notable changes and damages in liver and kidney histology by several forms of inflammation, denaturation, and necrosis of cells and tissues, showing direct impacts on functioning and metabolism of the both organs by a significant change in biochemical and haematological para-meters. Hence, mixture of six pesticides types is toxic at ADI×2, even at ADI, which represents the security dose of one pesticide, but in a mixture, this dose causes harmful effects on structure and function of liver and kidney in Wistar rats.

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Conflict of interests

The authors declare that they have no conflict of interests.

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