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Histopathological changes in the kidney tissues of white Swiss mice (*Mus musculus* L.) exposed to alpha-cypermethrin

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ABSTRACT

Introduction: Alpha-cypermethrin is a pyrethroid insecticide. Chronic exposure to alpha-cypermethrin is mainly due to the usage in household pest control or through drinking water and contaminated fruits and vegetables. The kidneys are considered as an essential organ in human and animal bodies. One of the causes of nephrotoxicity is exposure to environmental pollutants.

Objectives: The present study was designed to investigate the deleterious effects of alpha-cypermethrin when administered in two doses in the kidney tissues of mice.

Patients and Methods: The kidneys of treated and control mice were removed and fixed in 10% neutral buffered formalin. Subsequently, the specimens were passed by a series of concentrations of ethyl alcohol and embedded in paraffin and finally stained with hematoxylin and eosin.

Results: Treated mice groups are being investigated with several histopathological changes in their kidney tissues even at low-concentration of dose. These changes varied in males and female laboratory mice. These effects included bleeding, congestion, edema, aggregation of fat cells in various sizes and inflammation and death renal cells. Additionally, vacuolization of cytoplasm, necrosis, loss of glomeruli, shrinkage of tubular cell nuclei, necrosis of some tubular cells, enlargement or death of these cells, congestion and dilation Bowman's space, hemorrhage and hyperplasia of cells were also existed.

Conclusion: This research confirmed that alpha-cypermethrin causes severe deterioration in kidney tissues of Swiss albino mice.

Original Article

Implication for health policy/practice/research/medical education:
The experimental study on the deleterious effects of alpha-cypermethrin on the kidney tissues of mice showed bleeding, congestion, edema, aggregation of fat cells in various sizes and inflammation and death renal cells. Additionally, we detected the vacuolization of cytoplasm, necrosis, loss of glomeruli, shrinkage of tubular cell nuclei, necrosis of some tubular cells, enlargement or death of these cells, congestion and dilation Bowman's space and hemorrhage and also hyperplasia of cells. Our study showed that α-CYP pesticide is an environmental hazard and the extent of its repercussions on human health should be investigated more. We found that, the mice treated with this pesticide showed several pathological changes in kidney tissues even at low- doses. These changes varied in both genders.

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Introduction

Pyrethroids are organic compounds used as household and commercial insecticides. Pyrethroids class II is distinguished from pyrethroids I by the presence of the cyano group, which is limited to the alpha position. Additionally, this α-cyano group increases the toxicity of pyrethroids II compared with pyrethroids I, and the most common compounds of pyrethroids II are cypermethrin (CYP) and alpha-cypermethrin (α-CYP) (1). The main mechanism of action of pyrethroids is the disruption of the peripheral nervous system, where exposure to second type (α-cyano) produces neurotoxicity in insects and mammals. After the effect of α-CYP on sodium channels in the nervous membrane, a long-term transient increase in membrane permeability to sodium accrue, leading to a change in nerve function, resulting in convulsions or paralysis (2).

Alpha-cypermethrin is widely used in crops as it is very effective in exterminating sucking insects and rodents, It has also a application in the field of veterinary treatments

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and public health. However, it is considered a hazardous water pollutant and toxic to all mammals, and it is one of the synonyms for CYP (3). Alpha-cypermethrin consists of two active and toxicologically effective cis-isomers among the eight isomers of cis and trans in CYP (4). Studies have shown a qualitative similarity in the metabolism and toxicity of α-CYP and CYP, with α-CYP making up the bulk of CYP up to about 20%-40% (5). Both cis and trans-isomers of CYP, including α-CYP, are metabolized by ester bond cleavage and a hydroxyl ether bridge. Extensive studies in humans, mice, and rats show that CYP is rapidly absorbed and metabolized in the body and then distributed to various tissues and organs.

Similarly, this mechanism applies to α-CYP; since the metabolic pathway and rate are similar in both compounds. Additionally, α-CYP is more active than CYP. Therefore, the assessment of CYP toxicity is taken from the toxicological effects of α-CYP. The major metabolites of α-CYP are phenoxybenzoic acid (PBA) and cyclopropane carboxylic acid (CPA), often PBA in conjugation form. These conjugates differ in animals but have the same metabolic pathway in humans and rats (6). Again, PBA is metabolized to hydroxyl derivatives OH-PBA and conjugated as sulfate or glucuronate, while CPA is derived from the metabolism of the parent compounds excreted mainly in the form of glucuronate, since the major metabolites of α-CYP identified are 4-OH-PBA(7).

Several researchers have examined the harmful effects of insecticides on several types of experimental animals (8). A study was found that exposure of laboratory rats to oral doses of α-CYP leads to the induction of neurotoxicity resulting from histopathological effects in the sciatic and tibial nerves, with an increase in the activity of beta-galactosidase enzyme and occurrence of axonal degeneration as well as abnormal neuromuscular functions(6). Alpha-cypermethrin cause cardiotoxicity in male Wister rats because it damages muscle fibre tissue in the heart (9).

The kidneys are an essential organ in the human and animal body because they perform several main functions, including detoxification, elimination of toxic metabolites, regulation of extracellular body fluids and homeostasis. Renal system consist of numerous nephrons, which are the main functional unit in them(10). Studies have shown that the damage to kidney tissues of laboratory mice after exposure to insecticides may differ according to the dose exposure and the period of administration as well as the gender and age of the animal, however in general, contact with these compounds causes severe damage to the kidney tissues(11).

Objectives

The need for this study is due to the high use of pyrethroids in general and α-CYP in particular in agricultural fields in Iraq, where the current study was designed to investigate the pathological effects caused by the pesticide α-CYP when administered at two doses in the kidney tissues of Swiss albino mice. In this investigation we aimed to study the pathological changes to assess which part of the nephrons will be involved more.

Materials and Methods

The experimental design

Swiss laboratory mice (*Mus musculus* L.) were used in this study (n = 18) for both males and females and they were 10-12 weeks old and weighed 22-25 g. They were divided into a control group and two treatment groups (per group = 6). Our group supervised living conditions in the animal house, college of health and medical techniques ,in the southern technical university in Basra (Iraq). The temperature ranged between 20-25°C and a 12/12 hour light cycle was light and dark as provided with water and food *ad libitum.* The α-CYP $C_{22}H_{19}Cl_2NO_3$ was administered to the mice by intraperitoneally injection (i.p). Both treatment groups for each sex were administered with the pesticide solution at a rate of 4.75 mg/kg and 2.5 mg/kg at 2-3 times a week for nearly five weeks. The control groups were administered with 2.5 ml distilled water.

Histological preparation

The treated and control mice were anesthetized for both sexes, and kidneys were removed for all replicates to investigate the histopathological changes, fixed in 10% of neutral buffered formalin. Subsequently, the kidneys were washed, passed with a series of concentrations of ethyl alcohol, then embedded in paraffin and stained with hematoxylin and eosin (H&E).

Results

The histological examination of the kidney (glomerulus) of the control mice showed that its structure is intact. The glomerulus surrounded with Bowman's capsule and consists of a network of blood vessels intertwined with modified epithelial cells. The inside of the capsule is lined with capillaries that form the glomerulus, while the outer layer forms the outer surface of the capsule, and the space of Bowman's capsule separates the two layers. However, the kidney includes tubules lined by simple epithelial tissue that varies depending on the tubule type (Figure 1A).

Effects appeared in pathological changes that included all the renal glomeruli. Effects in the low-dose female group are the shrinkage of some tubular cells nuclei and necrosis of others with vacuolization in cytoplasm of cells in the glomerular shape (Figure 1B), the emergence of clumping of mesenchymal cells in various regions with increased

tubular cells enlargement(Figure 1C). The effects varied in the form of bleeding at one time, congestion and edema at other times (Figure 1D) and inflammation and death of renal cells (Figure 1E) and aggregation of fat cells of different sizes (Figure 1F).

In low-dose males, the effects varied in the form of loss of the glomerulus (Figure 2A, 2B and 2E), congestion and dilation of Bowman's space (Figure 2A and 2E), glomerular necrosis, or loss of glomerulus (Figure 2B and 2D), hemorrhage (Figure 2C), congestion (Figure 2D and 2F), renal cell death (Figure 2E) and cells hyperplasia (Figure 2F).

In females and males at high doses, the effects in females were represented by swelling of tubular cells, loss of the glomerulus, death of tubular cells again, and loss of the renal glomeruli (Figure 3A and 3B), while in males, they were represented by hemorrhage and death of the renal epithelium, loss of the glomerulus and congestion of the tubules (Figure 3C and 3D).

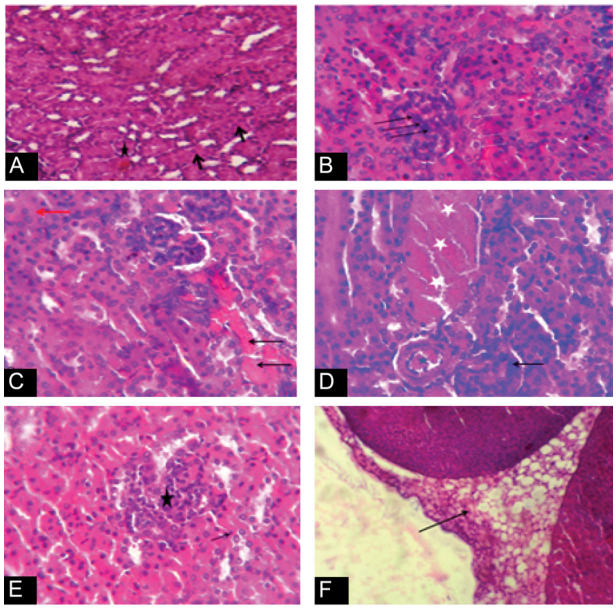

Figure 1. (A) Histological section of the kidney of control group represents the cells hyperplasia and congestion of the renal glomerulus (arrows), H & E stain, magnification power 400 ×. **(B)** Histological section of the kidney of the low-dose group of female laboratory mice demonstrating nuclei contraction with necrosis of tubular cells (black arrows) with vacuolization in cytoplasm of cells in the glomerular shape (white arrows) H & E stain,magnification power 400×. **(C)** Histological section of the kidney of the low-dose group of female laboratory mice showing the aggregation of the mesenchymal cells (white arrow), endothelial cells increase (red arrow) and tubular cells hypertrophy (black arrows)(H & E stain), magnification power 400×. **(D)** Histological section of the kidney of the low-dose group of females laboratory mice showing calcium deposition in part of the glomerulus (black arrow), hemorrhage (white stars), hyperemia (black star) and edema (white arrow), (H & E stain), magnification power 400×. **(E)** Histological section of the kidney of the low dose group of females laboratory mice demonstrates inflammation and death of tubular cells, H&E stain, magnification power 400×. **(F)** Histological section of the kidney of the low-dose group of females laboratory mice demonstrates the fat cell pool, (H & E stain), magnification power 400×.

Discussion

Nephrotoxicity is the imbalance and rapid deterioration in renal function and often occurs due to its exposure to environmental pollutants and therapeutic drugs (12). This defect includes direct injury to kidney tissues and cells,

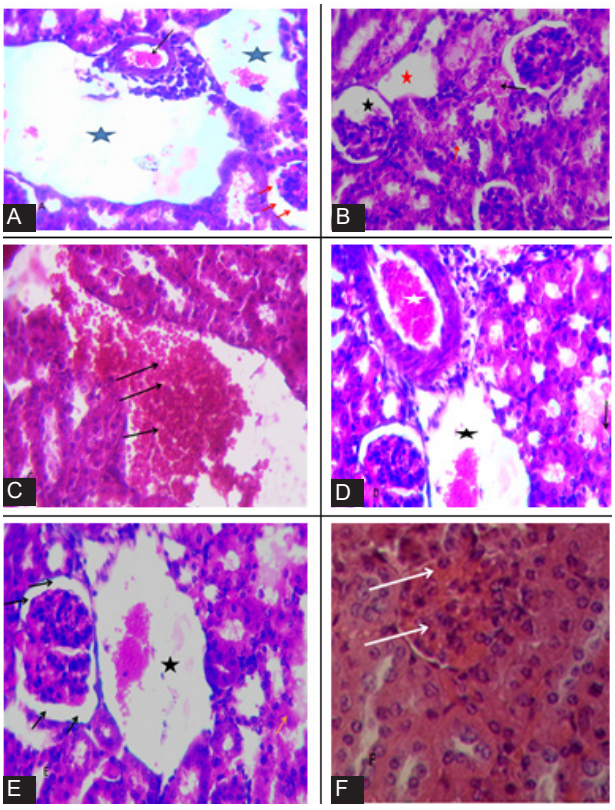

Figure 2. (A) Histological section of kidney of the low-dose group of males laboratory mice illustrates the loss of glomerulus (stars), congestion (black arrow), Bowman space expansion (red arrows), H &E stain, magnification power 400×. **(B)** Histological section of kidney of the low-dose group of males laboratory mice illustrates glomerular necrosis (black star), loss of glomerulus (red star), hemorrhage (black arrow), with vacuolization in the cytoplasm of cells (red arrow), (H & E stain), magnification power 400×. **(C)** Histological section of kidney of the low-dose group of males laboratory mice demonstrates an acute haemorrhage (arrows), H &E stain, magnification power 400×. **(D)** Histological section of kidney of the low-dose group of males laboratory mice show congestion (white star) and loss of glomerulus (black star) H&E stain, magnification power 400×. **(E)** Histological section of kidney of the low-dose group of males laboratory mice demonstrates Bowman space expansion (black arrows), loss of glomerulus (black star) and cells death (orange arrow), (H & E stain), magnification power 400×. **(F)** Histological section of kidney of the low-dose group of males laboratory mice demonstrates cells hyperplasia and glomerular congestion (arrows), H&E stain, magnification power 400 ×.

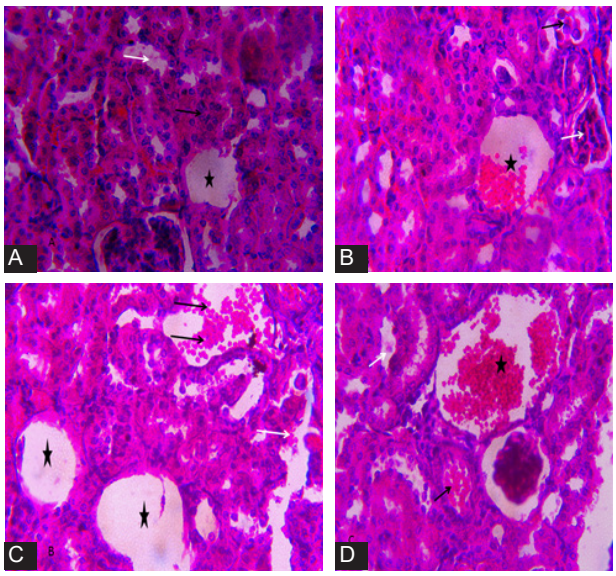

Figure 3. (A) Histological section of kidney of a high dose group for females laboratory mice show swelling of tubular cells (black arrow), loss of glomerulus cells (star) and death of tubular cells (white arrow), H&E stain, magnification power 400×. **(B)** Histological section of kidney of a high dose group for females laboratory mice illustrates the loss of glomerulus (black star) and death of tubular cells (white arrow), (H & E stain), magnification power 400×. **(C)** Histological section of kidney of a high dose group for males laboratory mice demonstrates loss of glomerulus (black stars) hemorrhage (black arrows), and death tubular epithelial cells (white arrow), (H & E stain), magnification power 400×. **(D)** Histological section of kidney of a high dose group for males laboratory mice demonstrates loss of glomerulus (black star) and congestion (black arrow), and death of tubular cells (white arrows), (H & E stain), magnification power 400×.

pathological changes and changes in blood circulation, in addition to obstruction in the renal excretion (13). The kidneys are more susceptible to damage from exposure to toxic chemicals because their metabolites are excreted through them, including exposure to lethal or sub-lethal doses of CYP that may cause various histological changes (14). A previous study on six human volunteers who received α-CYP in oral administration, showed that the rate of metabolism and excretion is same for both α-CYP and CYP. Their study showed α-CYP is excreted in the urine up to 43% in the form of free or conjugated carboxylic acid within the first 24 hours (15). Furthermore, the kidneys of experimental animals are among the most important target organs attacked by pesticides (16). . The results of the current study showed several clear histopathological changes in the kidney of males and females experimental animals treated with two doses of the pesticide α-CYP. These changes are represented by the occurrence of infections, including bleeding, congestion and edema in different areas of the kidney. These results are consistent with a study of Kemabonta and Akinhanmi on toxic effects of α-CYP vapour on the kidney of mice, as pesticide causes interstitial nephritis and chronic inflammation (17). Correspondingly, histopathological changes were observed in various organs of rats, including kidneys, following percutaneous mode of administration was performed with α-CYP at two doses. The changes observed in the kidney tissues were single-cell parenchymal degeneration in the proximal renal tubules, dilation of the Golgi apparatus and endoplasmic reticulum of epithelial cells in these tubules, and the absence of cell membrane and thickening of the basement membrane (18). Moreover, the largest quantities of α-CYP residues are located in the kidneys and other organs due to CYP's lipophilic nature, they may be more hazardous than the parent substance, as the elimination of cis-isomers from these tissues occurs three or four times slower than trans-isomers. The α-CYP residues are stored in muscle tissue, kidneys, liver and fat for up to 6 months (19). A study conducted on rats poisoned with medium and high doses of CYP 50 mg/kg and 150 mg/kg, respectively, showed significant damage in the kidney tissues, including multiple bleeding in both cortical and medullary tubules as well as interstitium tissue (20). It is believed that any process interfering with the structure of glomeruli and renal tubules results from a severe nephrotoxic effect. Toxic substances, including the pesticide α-CYP, also produce oxidative stress, which is caused by free radicals (21), which may cause disturbances in the chain of vital reactions in the cell and its systems, such as antioxidant scavenging system. All this may explain the damages in the kidney tubules and glomeruli from swelling, hypertrophy, degeneration, necrosis and others that were observed in the present study.

Swelling and hyperplasia of epithelial cells of the renal tubules may cause a narrowing of lumen of these tubules, and this swelling occurs as a result of inhibiting the processes of glycolysis and oxidative phosphorylation, leading to a decrease in ATP molecules and ultimately decreasing in O_2 level (a decrease in aerobic respiration). In order to maintain ATP levels, the cells must accustom to the glycolysis process, which leads to the accumulation of lactic acid and thus low pH (acidic environment inside the cells). As a result, an imbalance occurs in the Na^+/K^+ ATPase pump, which causes the flow of H_2O and Na^+ into cells and exit of K^+ from them, causing swelling of mitochondria; the endoplasmic reticulum and the cells appear swollen. Where it was found that CYP pesticide causes swelling of mitochondria, expansion of the rough endoplasmic reticulum, which causes the separation of ribosomes and a decrease in the number of smooth endoplasmic reticulum (22). Finally, the energy-dependent protein synthesis process will change and therefore the use of lipids in the formation of lipoproteins will decrease or may stop and as a result, lipids accumulate in the form

of fat droplets within the cytoplasm of cells (23). In the present study, necrosis led to the death some tubular and glomerular cells in some kidney tissue sections, causing a glomerular shrinkage, the damage is progressing to a complete loss of glomeruli in other sections, and these results are consistent with several studies (24). The expansion of Bowman's space is due to the shrinkage of necrotic glomeruli, in contrast, narrowing of Bowman's space was observed in glomeruli in which there was congestion of blood and hyperplasia of endothelial cells and mesangium cells; all these changes in Bowman's space cause a decrease in the efficiency of the kidneys in the glomerular filtration process (25).

Conclusion

Our study showed that α-CYP pesticide is an environmental hazard and the extent of it's repercussions on human health should be investigated more. We found that, the mice treated with this pesticide showed several pathological changes in kidney tissues even at low- doses. These changes varied in both genders. Among these changes are shrinkage of tubular cell nuclei, necrosis of them, vacuolization of cytoplasm, necrosis or loss of glomeruli, enlargement and death tubular cells, congestion and dilation of Bowman's space, hemorrhage and cells hyperplasia. We also found that, the toxicity of compounds to humans and animals is estimated by studying the histopathological changes that may occur in the tissues of organs, and these changes may lead to changes in the biochemical parameters of these organs and vice versa. Therefore, the current study suggests conducting a study to verify the effects of α-CYP on biochemical criteria of kidneys.

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Authors' contribution

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Supervision: Maysoon Hussein Zain, Saphia Ali Aitte.
Validation: Maysoon Hussein Zain, Saphia Ali Aitte.
Visualization: Maysoon Hussein Zain, Saphia Ali Aitte.
Writing–original draft: Maysoon Hussein Zain, Saphia Ali Aitte.
Writing–review & editing: Maysoon Hussein Zain, Saphia Ali Aitte.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

The research and protocol of this study adhered to the guidelines for animal studies and received approval from the Ethics Committee of the Health and Medical Techniques College at Southern Technical University in Iraq. We followed the animal experiment guidelines established by the United States National Institutes of Health (NIH, 1978).

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