Alqalam Journal of Medical and Applied Sciences. 2026;9(9):2709-2713
https://doi.org/10.54361/ajmas.269911

Original article

Ameliorative Effect of Vitamin E Against Chlorpyrifos-Induced Nephrotoxicity in Adult Male Rabbits: A Histological Study

Fatma Emrage[1]*, Hanan EL Daffri[1], Najat Mohammed[2], Fyrouz Khalid[3]

[1]Department of Histology, Faculty of Medicine, University of Omar Al-Mukhtar, Al-Bayda, Libya
[2]Department of General Nursing, Faculty of Nursing, University of Omar Al-Mukhtar, Al-Bayda, Libya
[3]Department of Chemistry, Faculty of Science, University of Omar Al-Mukhtar, Al-Bayda, Libya
Corresponding Email. sohilaissa@gmail.com

Abstract
Chlorpyrifos (CPF) is a widely used organophosphate pesticide whose long-term exposure has been linked to renal oxidative stress and structural kidney damage. This study aimed to evaluate the chronic histopathological effects of CPF on the renal cortex and medulla of adult male rabbits and to assess the ameliorative antioxidant efficacy of Vitamin E against this nephrotoxicity. Twenty adult male New Zealand rabbits (mean body weight 2.76 ± 0.19 kg) were randomly allocated into three unequal groups: a Control group (n = 6) receiving distilled water; a CPF group (n = 7) receiving chlorpyrifos orally at 33.3 mg/kg/day; and a CPF + Vitamin E group (n = 7) receiving chlorpyrifos (33.3 mg/kg/day) together with Vitamin E (100 mg/kg/day). Treatments were administered once daily by gastric tube for 12 consecutive weeks. Kidneys were then excised, fixed in 10% neutral buffered formalin, processed routinely, sectioned at 5 µm, stained with Hematoxylin and Eosin (H&E), and examined by light microscopy. Control kidneys showed normal glomerular and tubular architecture. The CPF group exhibited marked tubular epithelial degeneration, intraluminal cast formation, and interstitial inflammatory cell infiltration across both the cortex and medulla. Co-administration of Vitamin E markedly attenuated these changes, with visibly reduced inflammatory infiltration, milder tubular degeneration, and fewer intraluminal casts compared with the CPF-only group. Chronic CPF exposure induces significant histopathological nephrotoxicity in rabbits, and co-administration of Vitamin E substantially mitigates this renal damage, supporting its potential use as a protective antioxidant against organophosphate-induced kidney injury.
Keywords: Chlorpyrifos, Vitamin E, Renal Tissue, Histopathological Damage.

Introduction

Chlorpyrifos (CPF) is a broad-spectrum organophosphate (OP) pesticide extensively applied globally to control pests in agricultural crops, public health sectors, and domestic environments [1]. Like other OPs, the classic mechanism of action of CPF involves the inhibition of acetylcholinesterase (AChE), leading to the accumulation of acetylcholine at synaptic junctions and subsequent cholinergic hyperstimulation [3, 5]. Beyond its neurotoxic properties, recent investigations have revealed that CPF exposure targets non-target metabolic and excretory organs, particularly the liver and kidneys [1, 2, 9]. The kidney is highly susceptible to xenobiotic-induced toxicity due to its high blood flow and physiological role in filtering, concentrating, and excreting toxic metabolites [11, 12]. The operational toxicity of CPF in renal tissues is predominantly mediated via the excessive generation of reactive oxygen species (ROS) [2, 4]. This severe oxidative stress promotes lipid peroxidation of cellular membranes, alters cellular biochemical and enzymatic profiles, damages DNA, and initiates apoptotic or necrotic pathways, particularly within the renal tubules [5, 8, 9]. Consequently, this structural disintegration leads to advanced histopathological changes, such as the degeneration of the tubular epithelium, the formation of obstructive intraluminal casts, and the recruitment of localized inflammatory cells [2, 5, 11, 12].

Antioxidants serve as a vital defense mechanism against pesticide-induced oxidative injury by modulating cellular signaling and transcription pathways [4, 6]. Vitamin E (α-tocopherol) is a lipid-soluble antioxidant located in cell membranes, where it scavenges free radicals and prevents lipid peroxidation [7, 13, 14]. It acts efficiently to scavenge free radicals, terminate lipid peroxidation chain reactions, and sustain membrane integrity under chemical stress [4, 7, 8]. By stabilizing cellular membranes and maintaining enzymatic defense systems, Vitamin E has the potential to minimize epithelial cell desquamation and downregulate the subsequent inflammatory response within the renal parenchyma [2, 8].

While our companion paper characterized renal susceptibility to CPF using standard histological processing techniques [1, 10], the present study expands the evaluation to the renal system using the same experimental rabbit model [1, 9]. This study aims to determine the chronic histopathological impacts of CPF on both the cortical and medullary regions of rabbit kidney tissues, and to assess the therapeutic efficacy of Vitamin E in preventing or ameliorating this specific nephrotoxicity.

Methods

Chemical Reagents

The pesticide utilized in this investigation was a commercial-grade formulation of chlorpyrifos (CPF, 50 % EC), manufactured by the Pesticides & Chemicals Company (China) [1]. Dietary antioxidant supplementation was achieved using Vitamin E tablets (100 mg), sourced directly from Sigma Aldrich (India) [1].

Received: *01-07-2026 - **Accepted:** 29-08-2026 - **Published:** 05-09-2026*

https://doi.org/10.54361/ajmas.269911

Animal Welfare and Experimental Protocols

A total of twenty adult male New Zealander rabbits, maintaining a mean body weight of (2.76 ± 0.19 Kg), were selected for the current study [1]. Prior to the initiation of the experiment, all animals were housed under standardized environmental and laboratory conditions, with free, unrestricted access to standard pellet food and water (ad libitum) [1]. To evaluate the nephrotoxic impacts and potential recovery, the rabbits were randomly allocated into three experimental groups of unequal size (n = 6, 7, and 7, respectively) as follows [1]:

- **Control Cohort (n = 6):** Animals within this group were given daily oral doses of distilled water using a gastric tube [1].
- **CPF-Exposed Cohort (n = 7):** Rabbits in this category received a daily oral regimen of chlorpyrifos at a concentration of 33.3 mg/kg body weight, a sub-lethal dose consistent with the sub-chronic exposure model used in our companion hepatotoxicity study [1, 9].
- **Ameliorated Cohort (CPF + Vitamin E) (n = 7):** Animals in this combined group were concurrently administered identical daily doses of CPF (33.3 mg/kg) alongside Vitamin E (100 mg/kg), a supplementation dose within the protective range reported in comparable organophosphate-nephrotoxicity models [1, 2, 19].

The designated treatments for all experimental groups were administered once every morning for a continuous duration of 12 weeks [1].

Histological and Tissue Processing:

Upon the completion of the 12-week experimental timeline, all rabbits were euthanized under anesthesia [1]. The kidneys were meticulously dissected, removed, and immediately immersed in a solution of 10% neutral buffered formalin for a duration ranging between 24 and 48 hours to ensure optimal tissue fixation [1]. Following the fixation window, the specimens underwent a standardized sequence of histological processing [1,10]:

- **Washing and Dehydration:** Tissues were washed to remove excess fixative and subsequently dehydrated via a graduated series of increasing ethanol concentrations [1, 10].
- **Clearing and Embedding:** The samples were cleared using xylene and securely embedded within blocks of paraffin wax [1], [10].
- **Sectioning and Staining:** Microtome sectioning was performed to obtain serial tissue slices at a thickness of 5 µm [1, 10].

Finally, these cross-sections were mounted and stained utilizing the classic Hematoxylin and Eosin (H&E) staining technique [1, 10]. Structural and histopathological changes across the renal cortex and medulla were examined, evaluated, and photographed under a standard light microscope [1].

Results

Light microscopic inspection of the renal sections revealed distinct histopathological profiles among the experimental groups:

Control Group

Renal sections from the control group displayed a well-preserved, normal renal parenchyma architecture. Intact renal corpuscles were clearly observed, comprising a normal glomerulus (G) encapsulated within a distinct Bowman's capsule (B) and surrounded by an orderly arrangement of proximal and distal renal tubules (RT). Blood vessels (BC) within the interstitium appeared structurally normal without signs of congestion or cellular leakage.

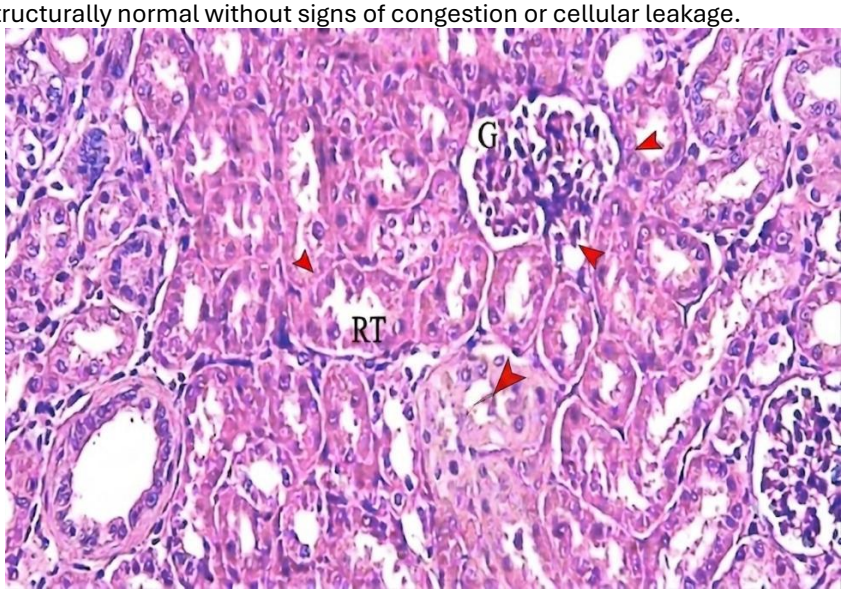

Figure 1. Light micrograph of a histological section in the kidney tissue of a control rabbit (H&E stain, x400), showing normal renal parenchyma with an intact glomerulus (G), proximal tubules, distal tubules (RT), and a normal interlobular artery

Received: *01-07-2026 -* ***Accepted:*** *29-08-2026 -* ***Published:*** *05-09-2026*

https://doi.org/10.54361/ajmas.269911

CPF-Treated Group

Chronic administration of chlorpyrifos induced severe, multifocal histopathological lesions spanning across both the renal cortex and medulla. The most prominent alterations highlighted in the tissue sections included:

- Degeneration of Tubular Epithelium (Black arrows): Widespread vacuolization, swelling, and degenerative changes in the lining epithelial cells of the renal tubules in both the cortical and medullary regions.
- Intraluminal Casts (Green arrows): Distinct accumulation of proteinaceous or cellular casts filling the lumina of several damaged renal tubules, indicating compromised tubular filtration and secretory function.
- Inflammatory Cell Infiltration (Red arrows): Dense, focal aggregates of mononuclear inflammatory cells infiltrating the interstitial spaces between the degenerated tubules, confirming a notable localized chronic inflammatory response.

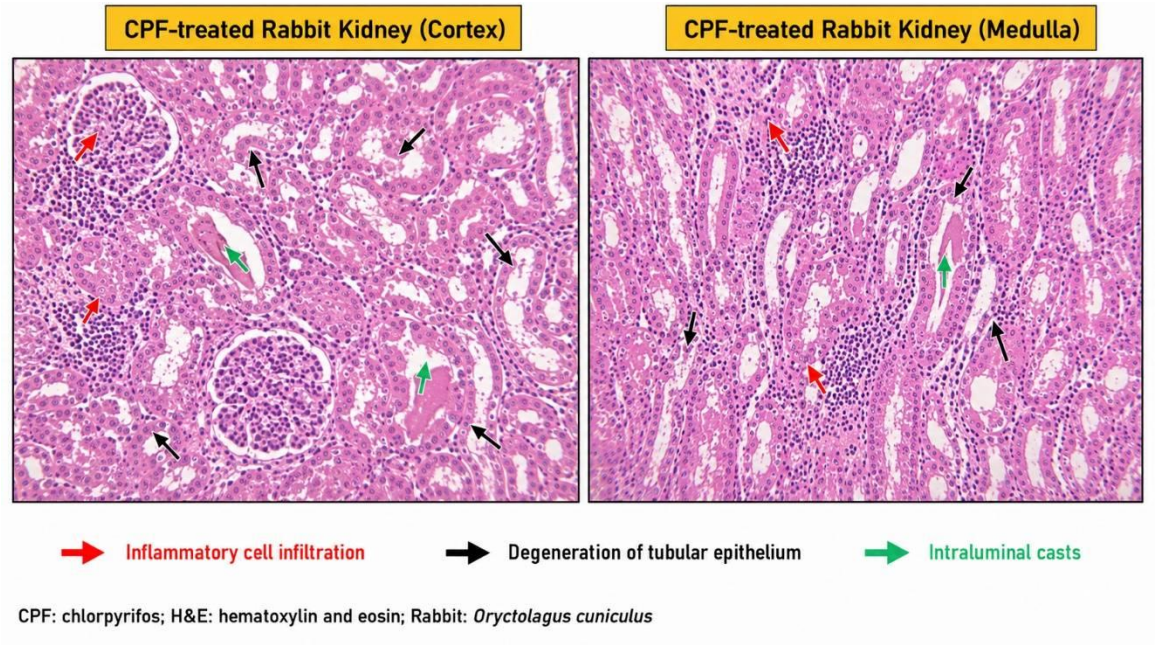

Figure 2. Light micrographs of histological sections from CPF-treated rabbit kidney (H&E stain) showing severe histopathological alterations in both the Cortex and Medulla. Alterations include dense interstitial inflammatory cell infiltration (red arrows), advanced degeneration of tubular epithelium (black arrows), and the formation of obstructive intraluminal casts (green arrows) within the tubular lumina

CPF + Vitamin E Group

Renal sections from rabbits co-treated with Vitamin E and CPF displayed an improved tissue architecture across both the renal cortex and medulla compared to the CPF-alone group. Microscopic examination revealed distinct signs of amelioration, which included:

- **Reduced Inflammatory Cell Infiltration (Arrows 1):** A marked reduction in the density and extent of leucocytic aggregates within the interstitial spaces, presenting only mild inflammatory cell infiltration.
- **Mild Degeneration of Tubular Epithelium (Arrows 2):** Noticeable improvement in the lining of the renal tubules, where the degenerative alterations of the tubular epithelium were visibly less severe and predominantly mild.
- **Fewer Intraluminal Casts (Arrows 3):** A significant decrease in the presence of proteinaceous or cellular casts filling the tubular lumina, indicating a recovery in tubular clearance and integrity.

Overall, these findings present a better preservation of the renal architecture, validating the potent antioxidant and protective capabilities of Vitamin E against CPF-induced nephrotoxicity.

Received*: 01-07-2026 -* ***Accepted****: 29-08-2026 -* ***Published****: 05-09-2026*

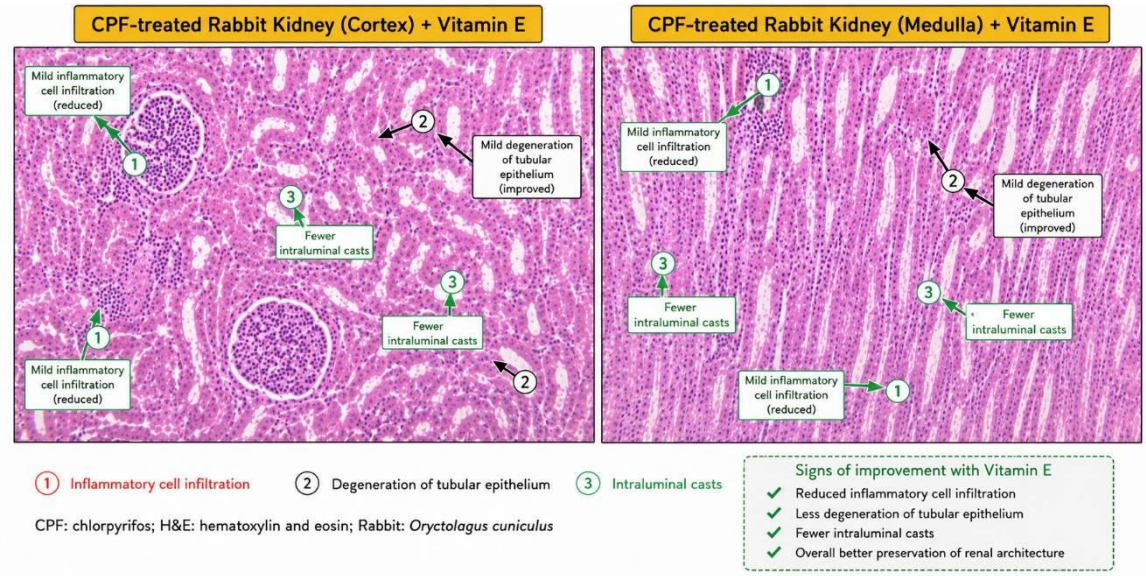

Figure 3. Light micrographs of histological sections from rabbit kidney co-treated with CPF and Vitamin E (H&E stain), showing remarkable improvement in tissue architecture across both the Cortex and Medulla. The protective signs include a significant reduction in interstitial inflammatory cell infiltration (1), milder and improved degeneration of tubular epithelium (2), and a distinct decrease in the formation of intraluminal casts (3).

Discussion

The kidney is one of the primary targets for organophosphate pesticide toxicity due to its role in the metabolism and excretion of xenobiotics. Chronic administration of chlorpyrifos (CPF) in this study induced extensive histopathological damage spanning both the renal cortex and medulla.

The observed degeneration of the tubular epithelium (Black arrows) represents a direct cytotoxic consequence of CPF exposure. This finding is consistent with previous studies showing that organophosphates induce a marked elevation in reactive oxygen species (ROS), leading to oxidative stress and subsequent lipid peroxidation of cell membranes. This cellular stress disrupts membrane permeability and ion regulation, causing cell swelling, vacuolization, and eventual desquamation of the tubular epithelial lining. These histopathological patterns closely mirror those reported by Tripathi and Srivastav [16] and Oncu et al. [17], who documented comparable tubular epithelial degeneration and structural renal damage following chronic exposure to chlorpyrifos and its analogues.

Furthermore, the presence of obstructive intraluminal casts (Green arrows) within the tubular lumina indicates a significant impairment in renal filtration and clearance mechanisms. These proteinaceous and cellular casts are formed from the accumulation of desquamated epithelial cells and plasma proteins leaking through damaged filtration barriers, leading to tubular blockage and functional nephron compromise. Comparable histomorphometric alterations, including tubular casts and impaired renal architecture, were reported by Sakinah et al. [18] following oral chlorpyrifos exposure in Wistar rats, further corroborating the filtration-compromising effect of CPF on the nephron.

The severe inflammatory cell infiltration (Red arrows) observed in the interstitial spaces of both cortical and medullary regions further confirms the chronic toxic insult of CPF. This extensive mononuclear cell infiltration is a vital physiological response to tissue injury, where cellular necrosis and cellular leakage provoke a localized inflammatory cascade. These pathological manifestations align with the work of Tanvir et al. [11] and Heikal et al. [12], who reported significant tubular degeneration and structural alterations in the renal architecture of animals subjected to organophosphate poisoning.

Conversely, oral supplementation of Vitamin E alongside CPF demonstrated an outstanding protective and ameliorative role, yielding an improved tissue architecture in both the cortex and medulla. Vitamin E (α -tocopherol) is a premier lipid-soluble antioxidant that acts as a membrane stabilizer and a potent scavenger of free radicals. By terminating the chain propagation of lipid peroxidation within cell membranes, Vitamin E prevents intracellular biochemical injury, stabilizes the tubular epithelium, and subsequently reduces epithelial cell desquamation and cast formation. Similar membrane-protective effects of Vitamin E have been documented by Ben Amara et al. [19], who demonstrated that Vitamin E supplementation restored membrane-bound ATPase activity and attenuated oxidative renal injury in rats exposed to the organophosphate dimethoate.

Consequently, the degeneration of tubular epithelium was visibly milder (Arrows 2), and fewer intraluminal casts (Arrows 3) were formed. Furthermore, by mitigating oxidative damage and suppressing cellular necrosis, Vitamin E effectively down-regulated the localized inflammatory response, resulting in a marked reduction in interstitial inflammatory cell infiltration (Arrows 1). These findings are in strong agreement with Ma et al. [2], who demonstrated that Vitamin E efficiently protects renal tissues from oxidative injury and structural destruction induced by chlorpyrifos, and with Oncu et al. [17], who similarly reported that antioxidant co-administration substantially reduced tubular degeneration and inflammatory infiltration in chlorpyrifos-ethyl-exposed rat kidneys.

Received*: 01-07-2026 -* ***Accepted****: 29-08-2026 -* ***Published****: 05-09-2026*

Conclusion

In conclusion, chronic exposure to the organophosphate chlorpyrifos (CPF) exerts severe, multi-focal nephrotoxicity in adult male rabbits, characterized by advanced tubular epithelial degeneration, obstructive intraluminal cast formation, and significant interstitial inflammation within both the renal cortex and medulla. However, dietary supplementation with Vitamin E effectively mitigates these pathological alterations, reduces cellular damage, and preserves the overall structural integrity of the renal parenchyma. These results emphasize the potential of utilizing Vitamin E as a powerful antioxidant therapy to counteract pesticide-induced organ damage.

Conflict of interest. Nil

References

[1] El Daffri H, Mohammed NMH, Amraje F, Khaled F, Ali AM. Histological changes induced by organophosphate chlorpyrifos in liver tissue of adult male rabbits. Sci J Univ Benghazi. 2022;35(2):207-11.
[2] Ma P, Wu Y, Zeng Q, Gan Y, Chen J, Ye X, et al. Oxidative damage induced by chlorpyrifos in the hepatic and renal tissue of Kunming mice and the antioxidant role of vitamin E. Food Chem Toxicol. 2013;58:177-83.
[3] Bartles E, Nachmansohn D. Organophosphate inhibitors of acetylcholine-receptor and -esterase tested on the electroplax. Arch Biochem Biophys. 1969;133(1):1-10.
[4] Gultekin F, Delibas N, Yasar S, Kilinc I. In vivo changes in antioxidant systems and protective role of melatonin and a combination of vitamin C and vitamin E on oxidative damage in erythrocytes induced by chlorpyrifos-ethyl in rats. Arch Toxicol. 2001;75(2):88-96.
[5] Chargui I, Grissa I, Bensassi F, Hrira MY, Haouem S, Haouas Z, et al. Oxidative stress, biochemical and histopathological alterations in the liver and kidney of female rats exposed to low doses of deltamethrin (DM): a molecular assessment. Biomed Environ Sci. 2012;25(6):672-83.
[6] Owuor ED, Kong ANT. Antioxidants and oxidants regulated signal transduction pathways. Biochem Pharmacol. 2002;64(5-6):765-70.
[7] Burton GW, Ingold KU. Vitamin E as an in vitro and in vivo antioxidant. Ann N Y Acad Sci. 1989;570:7-22.
[8] Amin KA, Hashem KS. Deltamethrin-induced oxidative stress and biochemical changes in tissues and blood of catfish (Clarias gariepinus): antioxidant defense and role of alpha-tocopherol. BMC Vet Res. 2012;8(1):1-8.
[9] Yassin MM, Adas TO, Yasin MM. Serum glucose, bilirubin, liver enzymes, renal parameters, protein profile and some electrolytes in adult male domestic rabbits intoxicated with chlorpyrifos. J Toxicol Risk Assess. 2021;7(1):037.
[10] Bancroft JD, Gamble M. Theory and practice of histological techniques. 6th ed. Philadelphia: Churchill Livingstone Elsevier; 2008. p. 126-7.
[11] Tanvir E, Afroz R, Chowdhury MA, Gan SH, Karim N, et al. A model of chlorpyrifos distribution and its biochemical effects on the liver and kidneys of rats. Hum Exp Toxicol. 2016;35(9):991-1004.
[12] Heikal T, Mossa A, Marei G, Rasoul MA. Cyromazine and chlorpyrifos induced renal toxicity in rats: the ameliorating effects of green tea extract. J Environ Anal Toxicol. 2012;2(5).
[13] Machlin LJ. Vitamin E: a comprehensive treatise. New York: Marcel Dekker; 1991.
[14] Kamal-Eldin A, Appelqvist L. The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids. 1996;31(7):671-701.
[15] Al-Omar MS, Naz M, Mohammed S, Mansha M, Ansari M, Rehman N, et al. Pyrethroid-induced organ toxicity and anti-oxidant-supplemented amelioration of toxicity and organ damage: the protective roles of ascorbic acid and α-tocopherol. Int J Environ Res Public Health. 2020;17:1-27.
[16] Tripathi S, Srivastav AK. Nephrotoxicity induced by long-term oral administration of different doses of chlorpyrifos. Toxicol Ind Health. 2010;26(7):439-47.
[17] Oncu M, Gultekin F, Karaoz E, Altuntas I, Delibas N. Nephrotoxicity in rats induced by chlorpyrifos-ethyl and ameliorating effects of antioxidants. Hum Exp Toxicol. 2002;21(4):223-30.
[18] Sakinah EN, Wisudanti DD, Abrori C, Supangat S, Ramadhani LR, Putri IS, et al. The effect of chlorpyrifos oral exposure on the histomorphometric and kidney function in Wistar rat. Indian J Pharmacol. 2024;56(3):186-90.
[19] Ben Amara I, Karray A, Hakim A, Ben Ali Y, Troudi A, Soudani N, et al. Dimethoate induces kidney dysfunction, disrupts membrane-bound ATPases and confers cytotoxicity through DNA damage: protective effects of vitamin E and selenium. Biol Trace Elem Res. 2013;156(1-3):230-42.

Received*: 01-07-2026 -* ***Accepted****: 29-08-2026 -* ***Published****: 05-09-2026*