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Ameliorative effect of vitamin D on CPF toxicity by evaluation of Wistar rat liver enzymes and kidney biomarkers

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ABSTRACT

Chlorpyrifos (CPF) is a hepatotoxic agent that adversely affects multiple organ systems. This study investigated how vitamin D mitigates liver and kidney damage caused by CPF exposure. Fifteen Wistar rats were divided into three groups: CPF (3 mg kg^{-1} BW), Control (PBS), and vitamin D (1 µg g^{-1} BW). Serum total antioxidant capacity (TAC) and total oxidative stress (TOS) were measured, along with enzyme levels, to assess liver and kidney function. CPF treatment resulted in elevated biomarkers indicating organ damage, while vitamin D treatment decreased these biomarkers and enzyme levels. The protective effects of vitamin D may stem from its antioxidant and anti-inflammatory properties. Notably, blood urea nitrogen (BUN), serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), and alkaline phosphatase (ALP) levels were significantly lower in the vitamin D group than in the CPF one. TAC levels in the treated group were lower than in control, indicating increased oxidative stress, while TOS levels rose significantly, suggesting oxidative damage. These results highlight CPF role in renal and hepatic injuries and emphasize the importance of enzyme analysis in assessing hepatotoxicity potentially influenced by vitamin D. The research concludes that vitamin D supplementation significantly mitigates liver and kidney damage caused by CPF exposure. This is evidenced by lower levels of liver enzymes and kidney biomarkers in the vitamin D group compared to the CPF one, indicating a protective role against organ damage.

Keywords: Antioxidant, Chlorpyrifos, Liver, Kidney, Vitamin D.
Article type: Research Article.

INTRODUCTION

Pesticides are essential components of agricultural practices and public health initiatives, as they serve to manage agricultural pests, minimize food wastage, and regulate disease-carrying organisms that threaten both human and animal well-being. The continuous rise in the global population has escalated the need for enhanced food

production, resulting in an increased reliance on pesticide use (Blain 2011). Organophosphate pesticides (OPs), which are synthesized from phosphorus-containing compounds such as phosphoric and phosphorothioic acids, rank among the most widely employed pesticides. Annually, approximately 2 million tons pesticides are applied worldwide, with forecasts suggesting an increase to 3.5 million tons by 2020, of which nearly 40% are OPs. Although OPs have been utilized in pest management for over five decades, their usage has surged following the prohibition of organochlorine pesticides. This increase can be attributed to the relatively brief environmental persistence of OPs and their comparatively lower health hazards when juxtaposed with organochlorine alternatives. Nonetheless, inadequate practices concerning the use, storage, transportation, application, and disposal of pesticide residues pose risks to non-target organisms. OPs can infiltrate living organisms through three primary routes of exposure: ingestion, inhalation, and skin contact (Mansukhani *et al*. 2024). Chlorpyrifos, scientifically referred to as O, O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate, manifests as white or colorless crystalline solids. This compound is employed for the management of various pests, such as termites, mosquitoes, and nematodes. Since its approval for use in 1965, it has been permitted in both agricultural and non-agricultural environments. Human exposure to chlorpyrifos may arise through ingestion, inhalation, or dermal contact with mucous membranes (Nandi *et al*. 2022). As a result, chlorpyrifos is frequently found in environmental samples, which raises considerable public health issues. In Iran, the use of chlorpyrifos (CPF) in agricultural activities is widespread; however, the presence of its residues on crops presents substantial health hazards to humans, as demonstrated by a multitude of scholarly research. CPF has been detected in diverse environmental settings, including surface waters, seawater, and rainfall (Kermani *et al*. 2021). Current therapeutic approaches exhibit limited success in halting or preventing the symptomatic advancement of degenerative diseases, primarily concentrating on symptom alleviation rather than tackling the underlying pathologies. This scenario highlights the urgent necessity for the development of alternative pharmacological agents aimed at preventing and safeguarding against these age-related disorders (Lips 2006). Vitamin D is classified as a lipid-soluble compound, existing primarily in two notable forms: D_2 and D_3. This vitamin plays a crucial role in promoting health by safeguarding the cardiovascular and skeletal systems, modulating cell growth and differentiation, and bolstering immune function. A deficiency in vitamin D has been linked to a range of health issues, including various forms of cancer, diabetes, and diseases affecting the pulmonary, cardiovascular, and musculoskeletal systems (Bargagli *et al*. 2021; Adamantidi *et al*. 2024). Recent studies underscore the promising role of vitamin D and its analogs in alleviating inflammatory mechanisms that are pivotal in the development of numerous chronic conditions, such as glomerulosclerosis and other renal disorders. Specifically, vitamin D analogs, including calcitriol, have been shown to inhibit cell proliferation, reduce glomerular expansion, and diminish both glomerulosclerosis and albuminuria. Understanding the anti-inflammatory benefits of vitamin D could open new therapeutic avenues for managing glomerulosclerosis and promoting better outcomes in kidney and liver disease due to toxin effects (Mizobuchi *et al.* 2007; Adamantidi *et al*. 2024). The application of pesticides has markedly enhanced product quality. Nonetheless, the potential impacts on various essential organs remain largely uncharted, prompting researchers to explore and assess these adverse effects. Consequently, this study aimed to examine the possible effects and underlying mechanisms of chlorpyrifos (CPF) exposure on the liver and kidney. Researchers are investigating innovative therapeutic approaches, particularly in developed countries, to improve the quality of life for patients suffering from lung diseases attributed to the inhalation or ingestion of toxins. Consequently, the current study sought to evaluate the therapeutic impact of vitamin D on organ alterations induced by CPF poisoning in rats, to identify potential adjunctive treatments following such poisoning events.

MATERIALS AND METHODS

Experimental animals study design

Fifteen female Wistar rats (6-8 weeks, 185 ± 15 g) were purchased. The animals were housed under specific conditions based on guidelines: Room temperature (22 ± 2 °C); Room light-dark cycles 12L:12D; Relative humidity 50-55%. Rats had access to adequate water in polycarbonate bottles and were fed with commercial rodent pellets. All mice were treated according to guidelines for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978) and kept under the ethical considerations of the Institutional Animal Care and Use Committee.

The selected rats were divided randomly into the following three groups (n = 5 / group):

Group 1: CPF (Model group) received CPF without treatment; (3 mg kg^{-1} for 4 weeks; Intraperitoneally; Gheibi *et al.* 2020, 2023).

Group 2: Control group in which healthy rats received PBS; (for 7 days; Gavage).
Group 3: CPF + Treatment (Treatment group) After induction the model with CPF (3 mg kg^{-1} for 4 weeks; Intraperitoneally), vitamin D prescribed (PO; Gavage; 1 µg g^{-1} BW; 7 days; Amanpour *et al*. 2024; Sharifnia *et al.* 2024).
The appropriate doses of CPF and vitamin D were obtained from previous studies (Barzi *et al*. 2020; Vahabi Barzi *et al*. 2022). At the end of the experimental period, rats were euthanized in a CO_2 +O_2 chamber. Blood samples were taken by cardiac puncture method, and the sera were separated by centrifugation at 4000 rpm, 10 min (Hettich-Germany), and refrigerated at -20 °C.

Measurement of oxidative stress biomarkers
Serum total oxidant status (TOS) concentration was determined by using an automated colorimetric method for measuring total oxidant status by Erel. Reagent 1 contained 150 µM xylenol orange, 140 mM sodium chloride, and 1.35 M glycerol in 25 mM sulfuric acid, pH = 1.75. Reagent 2 contained 5mM Fe^{3+}, and 5 mM 4-(4-amino-3-methoxyphenyl)-2- methoxyaniline in 25mM sulfuric acid. The absorbance was read at 650nm against blank after 3 min. To calculate TOS, a standard curve was constructed first, and then the regression equation y = mx ± b was used. The total antioxidant capacity (TAC) of serum samples was determined using a spectrophotometric method, developed by Erel. Reagent 1 contained 5 mM Fe^{3+}, and 10 mM 4-(4-amino-3-methoxyphenyl)-2-methoxy aniline in (75 mM, pH 1.8) Clark and Lubs solution. Reagent 2 contained 7.5 mM hydrogen peroxide in Clark and Lubs solution. The absorbance was read at 440 nm against blank after 3 min. To calculate TAC, a standard curve was constructed first, and then the regression equation y = mx ± b was used (Abod *et al.* 2021).

Evaluation of liver biomarkers
Following heart puncture to acquire blood samples, the concentrations of serum glutamic-oxaloacetic transaminase (SGOT) was measured spectroscopically using available kit (MyBioSource Co, Canada ; MBS703700), serum glutamic-pyruvic transaminase (SGPT) by kit obtained (MyBioSource Co, Canada; MBS2022063), alkaline phosphatase (ALP) by (MyBioSource Co, Canada; MBS264223), and BUN by (MyBioSource Co, Canada; MBS2611085; Amanpour *et al*. 2024).

Evaluation of kidney biomarkers
Creatinine levels in serum and urine were assessed using commercially available reagents. The estimation followed the Jaffe method, employing a pre-prepared solution consisting of equal volumes of sodium hydroxide and picric acid. For each serum sample and standard, 50 µL was combined with 1000 µL of the Jaffe working solution and incubated at 25 °C for 30 seconds. Urine creatinine was similarly evaluated, utilizing diluted urine mixed with distilled water at a 1:50 ratio. Quality control samples were implemented to ensure the reliability of the results. The absorbance of the reaction mixture was determined using an auto biochemistry analyzer. For the analysis of blood urea nitrogen (BUN) commercial reagents were utilized. The working reagent was prepared by combining five parts of reagent (R_1) with one part of coenzyme (R_2) and mixing thoroughly. A volume of 10 µL from each serum sample was introduced into the prepared working reagent (1000 µL), and the absorbance was promptly measured using an auto biochemistry analyzer (Toora & Rajagopal 2002; Thammitiyagodage *et al*. 2020).

Statistical analysis
Data were reported as mean ± SD, and the graphs were plotted using Graph Pad Prism 5.04 software. Data were statistically analyzed using analysis of variances (ANOVA) followed by a post-Tukey test, and a *p*-value less than 0.05 was considered a significant difference to compare the model group that received CPF without treatment. The asterisks replicate significant differences with *: $p < 0.05$, **: $p < 0.01$, and ***: $p < 0.001$.

RESULTS
Serum total oxidant status (TOS) and total antioxidant capacity (TAC)
Fig. 1A shows that the TAC concentration in the treatment group was significantly lower than in the CPF one ($p < 0.0001$). The TAC concentration in the prescribed vitamin D group showed no significant difference compared to the control group ($p = 0.084$). The data in Fig. 1B indicate a significant increase in TOS concentration in the treatment group with vitamin D compared to the CPF one ($p < 0.0001$). In contrast, rats exposed to vitamin D showed no significant differences from the control group ($p = 0.82$).

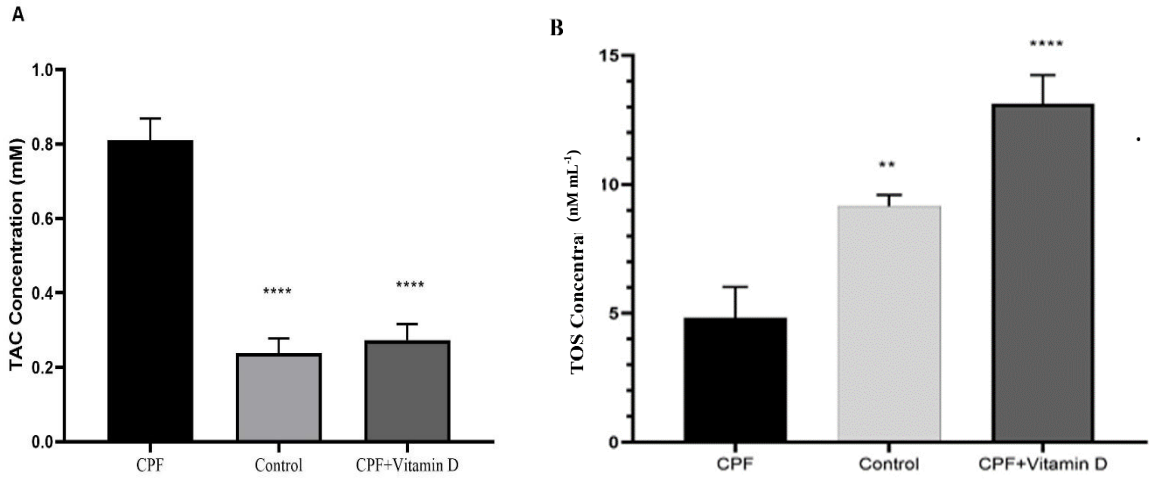

Fig. 1A. TAC activity in sera samples isolated from CPF, Control, and CPF + Vitamin D. **Fig. 2B**: TOS activity in sera samples isolated from CPF, Control, and CPF + Vitamin D. Data were statistically analyzed using analysis of variances (ANOVA) followed by a post-Tukey test, and a *p*-value less than 0.05 was considered a significant difference to compare the model group that received CPF without treatment. The asterisks replicate significant differences with *: $p < 0.05$, **: $p < 0.01$, and ***: $p < 0.001$.

Liver enzyme levels

Oral prescription of vitamin D significantly decreased the serum SGOT level compared to that in the CPF group ($p < 0.001$). The serum SGPT of rat in the treatment group was significantly lower than those in the CPF group ($p < 0.01$). Treatment with vitamin D had an overall decreasing effect on the ALP concentration compared to that in the CPF group ($p < 0.01$).

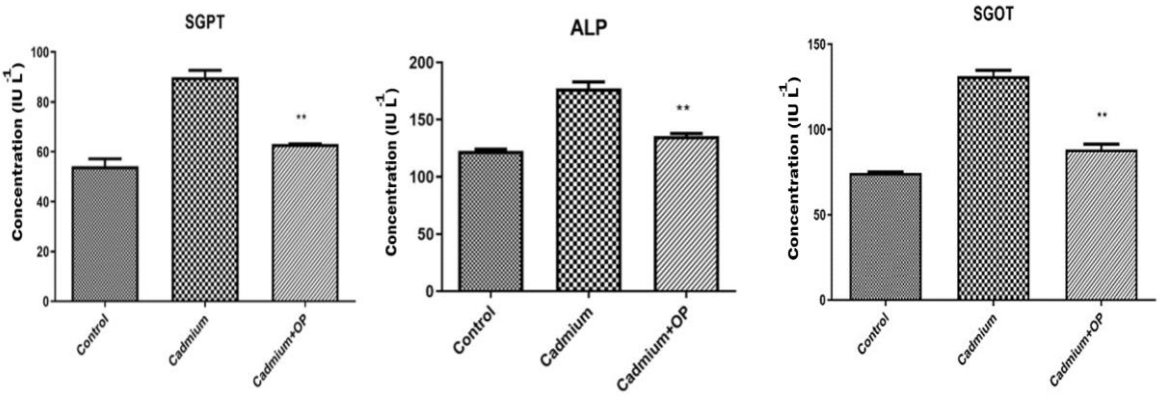

Fig .2. The effects of CPF administration and treatment with vitamin D on the serum level of SGOT, SGPT, and ALP in rats of experimental groups. Data are represented as Mean ± SD. One-Way ANOVA followed by a post hoc LSD test was used for comparison between different groups. The asterisks replicate significant differences with *: $p < 0.05$, **: $p < 0.01$, and ***: $p < 0.001$.

BUN and creatinine concentration

As shown in Fig. 3 A, the level of sera BUN increased following CPF injection, but after treatment with vitamin D, the serum concentration statistically declined ($p < 0.001$). The comparison of the control and CPF groups exhibited the statistical significant difference between them ($p < 0.001$).

DISCUSSION

This study is the first experimental examination of how vitamin D supplementation during pregnancy affects lung development in rats exposed to CPF. The results showed that vitamin D supplementation may mitigate CPF-induced liver and kidney damage by regulating inflammation and biomarkers while improving antioxidant rates. In humans, assessing specific immunotoxic effects linked to organophosphates (OPs) is complicated by repeated exposure to immunomodulatory contaminants. However, existing scientific literature indicates that OPs do exhibit immunotoxic properties. Specifically, OPs can influence neutrophil-mediated immune responses, as observed in

individuals with occupational exposure. Additionally, metabolites of the pesticide malathion have been shown to induce histamine release in human basophilic cells (Bernal-González *et al*. 2023). The complement system, an essential element of the immune response against pathogens, may also be influenced by OP exposure. Research has demonstrated that dimethoate and chlorpyrifos can alter the levels of pro-inflammatory cytokines (IL-1β and IL-8) while simultaneously downregulating the anti-inflammatory cytokine (IL-10), as well as affecting the Akt and ERK signaling pathways in dendritic cells exposed to OPs. Furthermore, exposure to OP has been reported to suppress the production of interferon-beta (IFN-β) by macrophages (Camacho-Pérez *et al*. 2022).

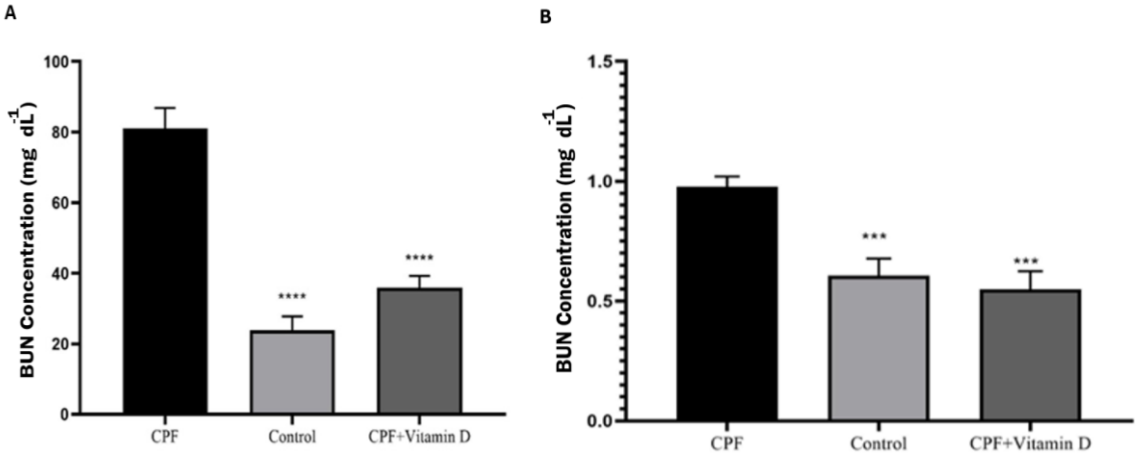

Fig. 3A. The effects of CPF administration and treatment with vitamin D on the serum level of BUN in rats of experimental groups. **Fig. 3B.** The effects of CPF administration and treatment with vitamin D on the serum level of creatinine in rats of experimental groups. Data are represented as Mean ± SD. One-Way ANOVA followed by a post hoc LSD test was used for comparison between different groups. The asterisks replicate significant differences with *: $p < 0.05$, **: $p < 0.01$, and ***: $p < 0.001$.

Chlorpyrifos, a widely utilized organophosphate insecticide, plays a significant role in pest management across global agricultural practices. Nonetheless, its repeated and indiscriminate application during crop cultivation can adversely impact non-target species, including aquatic and terrestrial wildlife, by contaminating water bodies. This contamination poses potential risks to human health through the food chain. Numerous studies have documented elevated residual concentrations of chlorpyrifos in water, soil, and various agricultural products, including fruits and vegetables, across multiple countries (Gheibi *et al*. 2020). Consequently, extensive research has been undertaken to assess the hazards linked to the ongoing consumption of these contaminated products. Previous investigations have indicated that environmental contaminants from pesticides may play a role in the onset of various neurodegenerative diseases. Additionally, it has been demonstrated that heavy metals, such as cadmium sulfate present in chlorpyrifos, exhibit teratogenic effects on the developmental processes of chick embryos and zebrafish. Recent findings have highlighted that residues of chlorpyrifos ethyl in food represent a considerable risk to vulnerable populations, including infants, children, and pregnant women. Therefore, it is crucial to explore the implications of chlorpyrifos exposure on vital organs (Barzi *et al*. 2020; Vahabi Barzi *et al*. 2022). Previous studies have described the protective effect of vitamin D in reducing oxidative stress. Vitamin D inhibits the generation of ROS and protects cell membranes from damage induced by ROS. Researchers have ascribed the protective role of vitamin D to the prevention of anemia induced by ribavirin in Wistar rats by promoting erythropoietin production as well (Alatawi *et al.* 2018). The results of previous experimental studies indicated that administering vitamin D, magnesium, or a synergistic combination of both reduced the toxicity index and the expression levels of VEGF and BMP-4 proteins and p53 mRNA levels. Additionally, histopathological analyses revealed that cadmium-induced lung tissue damage was significantly reduced following treatment. These findings suggest that administering vitamin D and magnesium could counteract the cadmium-induced decrease in p53 gene expression. The results emphasize the potential therapeutic benefits of vitamin D and magnesium in alleviating the negative consequences of cadmium exposure, especially regarding respiratory health. Targeting the BMP-4, VEGF, and p53 signaling pathways may be feasible to prevent airway pathologies associated with cadmium exposure (Amanpour *et al*. 2024). Elmubarak & Özsoy in 2016 reported

that Vitamin D treatment in rats exposed to CCl_4 resulted in improved kidney function, as indicated by normalized serum urea and creatinine levels, which were elevated in untreated groups. Histological examinations showed that Vitamin D mitigated tubular and glomerular degeneration, restoring tissue integrity and reducing inflammation (Elmubarak & Özsoy 2016). In a study involving CP, vitamin D supplementation led to significant reductions in malondialdehyde (MDA) and total oxidant status (TOS), while increasing total antioxidant status (TAS) and asprosin levels, which are crucial for liver health. The histopathological analysis revealed that vitamin D administration alleviated liver damage, suggesting its role in protecting against CP-induced hepatotoxicity. Conversely, while vitamin D shows promise in mitigating toxicity, the potential for over-supplementation and its effects on calcium metabolism warrants further investigation, as excessive vitamin D can lead to hypercalcemia and associated complications (Turk *et al*. 2023). Sharifi *et al.,* found that taking vitamin D_3 supplements significantly improved the levels of serum $25(OH)D_3$ in patients with non-alcoholic fatty liver disease (NAFLD). This indicates that the supplementation was effective in raising vitamin D levels in the body. Vitamin D supplementation led to a significant reduction in serum malondialdehyde (MDA) levels, which is a marker of oxidative stress. This suggests that vitamin D may help in reducing oxidative damage in patients with NAFLD. Despite these positive effects on oxidative stress and inflammation, the study did not find significant changes in liver enzymes (like ALT) or insulin resistance (measured by HOMA-IR) between the vitamin D and placebo groups. This suggests that while vitamin D may help with inflammation and oxidative stress, it does not directly improve liver function or insulin sensitivity in NAFLD patients. The authors concluded that vitamin D supplementation could be considered as an additional therapy to help manage systemic inflammation and oxidative stress in patients with NAFLD. However, it should not replace other treatments that are specifically aimed at improving liver health. Overall, the findings suggest that while vitamin D has beneficial effects on certain biomarkers in NAFLD patients, more comprehensive studies are needed to fully understand its role and effectiveness in treating this condition (Sharifi *et al*. 2014; Effati *et al*. 2018). The investigations assessed the impact of vitamin D supplementation on the molecular mechanisms underlying renal and testicular damage caused by lead (Pb) toxicity. Exposure to Pb significantly decreased serum levels of vitamin D (VD) and calcium (Ca^{2+}), leading to considerable renal and testicular damage, alongside notable changes in the expression of VD metabolizing enzymes, its receptor, binding protein, and the calcium-sensing receptor. Additionally, Pb exposure resulted in increased lipid peroxidation and elevated levels of pro-inflammatory cytokines (IL-4 and TNF-α) in the affected organs, while simultaneously decreasing several antioxidant markers (glutathione, glutathione peroxidase, and catalase) and the anti-inflammatory cytokine IL-10. In summary, this study demonstrated the potential protective role of VD against Pb-induced renal and testicular damage through mechanisms involving anti-inflammatory and antioxidant responses (BaSalamah *et al*. 2018; Rajsekhar *et al*. 2024). This study supports published findings that prescribed vitamin D reduces the liver enzyme levels and TAC concentration while significantly increases TOS concentration, indicating its protective effect. Vitamin D, particularly in the form of calcitriol, may be used in the treatment of CPF (chlorpyrifos) toxicity, as it has shown potential benefits in reducing oxidative stress and improving kidney function in patients with low vitamin D levels. While there are studies indicating this connection, it is essential to consult healthcare professionals for personalized treatment plans and to confirm the appropriateness of vitamin D supplementation in individual cases. The study highlights that vitamin D supplementation led to decreased levels of malondialdehyde (MDA) and total oxidant status (TOS) while increasing total antioxidant status (TAS). This suggests that vitamin D may help in managing oxidative stress, which is crucial for maintaining liver health and preventing further organ damage. Our findings suggest that vitamin D can significantly reduce liver and kidney damage caused by CPF exposure. This indicates that vitamin D supplementation could be a viable strategy for individuals at risk of exposure to organophosphate pesticides, especially agricultural workers and their families

CONCLUSION

Vitamin D supplementation exhibited a significant protective effect against liver and kidney damage induced by CPF exposure. The treatment resulted in lower levels of liver enzymes and kidney biomarkers, indicating reduced organ damage compared to the CPF-only group. The study found that vitamin D treatment led to significant reductions in malondialdehyde (MDA) and total oxidant status (TOS), while increasing total antioxidant status (TAS). This suggests that vitamin D may help combat oxidative stress, which is a critical factor in the toxicity associated with CPF. In summary, the study concludes that Vitamin D has significant protective effects against

CPF-induced toxicity, particularly in the liver and kidneys, while also highlighting the need for further research to explore its mechanisms and potential clinical applications.

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REFERENCES

Abod, KS, Mohammed, MT & Taay, YM 2021, 'Evaluation of total oxidant status and antioxidant capacity in sera of acute-and chronic-renal failure patients. *Journal of Physics: Conference Series*, IOP Publishing, p. 12038.

Adamantidi, T, Maris, G, Altantsidou, P & Tsoupras 2024, Anti-Inflammatory Benefits of Vitamin D and Its Analogues against Glomerulosclerosis and Kidney Diseases. *Sclerosis*, 2(3): 217–265.

Alatawi, FS, Faridi, UA & Alatawi, MS 2018, Effect of treatment with vitamin D plus calcium on oxidative stress in streptozotocin-induced diabetic rats. *Saudi Pharmaceutical Journal*, 26(8): 1208–1213.

Amanpour, P, Eftekhari, Z, Eidi, A & Khodarahmi, P 2024, Ameliorative mechanism of dietary vitamin d and magnesium on newborn's pulmonary toxicity induced by cadmium. *Journal of trace elements in medicine and biology: organ of the Society for Minerals and Trace Elements (GMS)*, 84: 127469.

Bargagli, M, Ferraro, PM, Vittori, M, Lombardi, G, Gambaro, G & Somani, B 2021, Calcium and vitamin D supplementation and their association with kidney stone disease: A narrative review. *Nutrients*, 13(12): 4363.

Barzi, NV, Eftekhari, Z, Doroud, D & Eidi 2020, DNA methylation changes of apoptotic genes in organogenesis stage of mice embryos by maternal chlorpyrifos induction. *Environmental Toxicology*, 35(7): 794-803.

BaSalamah, MA, Abdelghany, AH, El-Boshy, M, Ahmad, J, Idris, S & Refaat, B 2018, Vitamin D alleviates lead-induced renal and testicular injuries by immunomodulatory and antioxidant mechanisms in rats. *Scientific Reports* 8(1): 4853.

Bernal-González, KG, Covantes-Rosales, CE, Camacho-Pérez, MR, Mercado-Salgado, U, Barajas-Carrillo, VW, Girón-Pérez, DA, Montoya-Hidalgo, AC, Díaz-Resendiz, KJG, Barcelos-García, RG, Toledo-Ibarra, GA & Girón-Pérez, MI 2023, Organophosphate-Pesticide-Mediated Immune Response Modulation in Invertebrates and Vertebrates. *International Journal of Molecular Sciences*, 24(6).

Blain, PG 2011, Organophosphorus poisoning (acute). *BMJ Clinical Evidence*, 2011, England.

Camacho-Pérez, MR, Covantes-Rosales, CE, Toledo-Ibarra, GA, Mercado-Salgado, U, Ponce-Regalado, MD, Díaz-Resendiz, KJG & Girón-Pérez, MI 2022, Organophosphorus pesticides as modulating substances of inflammation through the cholinergic pathway. *International Journal of Molecular Sciences*, 23(9).

Effati, N., Mohammadi, M., Nazifi, S. and Rahimabadi, E 2018, Serum profiles of calcium, phosphorus, magnesium, vitamin D and parathyroid hormone in Caspian horses during different seasons.*Caspian Journal of Environmental Sciences* , 16: 85-92.

Elmubarak, S & Özsoy, N 2016, Histoprotective effect of vitamin D against carbon tetrachloride nephrotoxicity in rats. *Human & Experimental Toxicology*, 35(7): 713-723.

Gheibi, P, Eftekhari, Z, Doroud, D & Parivar, K 2023, Association between uterine toxicity induced by chlorpyrifos and downregulation of heparin-binding epidermal growth factor and L-selectin genes. *Veterinary Research Forum: an International Quarterly Journal*, 14(1); 45–52.

Gheibi, P, Eftekhari, Z, Doroud, D & Parivar, K 2020, Chlorpyrifos effects on integrin alpha v and beta 3 in implantation window phase. *Environmental Science and Pollution Research*, 27: 29530-29538.

Kermani, M, Dowlati, M, Gholami, M, Sobhi, HR, Azari, A, Esrafili, A, Yeganeh, M & Ghaffari, HR 2021, A global systematic review, meta-analysis and health risk assessment on the quantity of Malathion, Diazinon, and Chlorpyrifos in Vegetables. *Chemosphere*, 270: 129382.

Lips, P 2006, Vitamin D physiology. *Progress in Biophysics and Molecular Biology*, 92: 4–8.

Mansukhani, M, Roy, P, Ganguli, N, Majumdar, S & Sharma, S 2024, Organophosphate pesticide chlorpyrifos and its metabolite 3,5,6-trichloropyridinol downregulate the expression of genes essential for spermatogenesis in caprine testes. *Pesticide Biochemistry and Physiology*,204; 106065.

Mizobuchi, M, Morrissey, J, Finch, JL, Martin, DR, Liapis, H, Akizawa, T & Slatopolsky, E 2007, Combination therapy with an angiotensin-converting enzyme inhibitor and a vitamin D analog suppresses the progression of renal insufficiency in uremic rats. *Journal of the American Society of Nephrology*, 18(6): 1796-1806.

Nandi, NK, Vyas, A, Akhtar, MJ & Kumar, B 2022, The growing concern of chlorpyrifos exposures on human and environmental health. *Pesticide Biochemistry and Physiology*, 185: 105138.

Rajsekhar, S, Singh, TA & Deep, KC, 2024, Cadmium and Arsenic: A Deadly Duo for Diabetic Rats. *Journal of Chemical Health Risks*, 14(4): 685-694.

Sharifi, N, Amani, R, Hajiani, E & Cheraghian, B 2014, Does vitamin D improve liver enzymes, oxidative stress, and inflammatory biomarkers in adults with non-alcoholic fatty liver disease? A randomized clinical trial. *Endocrine*, 74: 70–80.

Sharifnia, M, Eftekhari, Z & Mortazavi, P 2024, Niosomal hesperidin attenuates the M1/M2-macrophage polarization-based hepatotoxicity followed by chlorpyrifos-induced toxicities in mice. *Pesticide Biochemistry and Physiology*, 198: 105724.

Thammitiyagodage, MG, de Silva, NR, Rathnayake, C, Karunakaran, R, WGSS, K, Gunatillka, MM, Ekanayaka, N, Galhena, BP & Thabrew, MI 2020, Biochemical and histopathological changes in Wistar rats after consumption of boiled and un-boiled water from high and low disease prevalent areas for chronic kidney disease of unknown etiology (CKDu) in North Central Province (NCP) and its comparison with low disease prevalent Colombo, Sri Lanka. *BMC Nephrology*, 21(1): 38.

Toora, BD & Rajagopal, G 2002, Measurement of creatinine by Jaffe's reaction--determination of concentration of sodium hydroxide required for maximum color development in standard, urine and protein free filtrate of serum. *Indian Journal of Experimental Biology*, 40: 352-354.

Turk, A, Kuloglu, T, Karadag, A & Metin, TO 2023, Effects of vitamin D on asprosin immunoreactivity against cyclophosphamide-induced liver injury in rats. *Cureus*, 15(10).

Vahabi Barzi, N, Eftekhari, Z, Doroud, D & Eidi, A 2022, Maternal exposure during organogenesis to chlorpyrifos insecticide induce apoptosis process. *The Journal of Maternal-Fetal & Neonatal Medicine*, 35(20): 3845-3852.