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Consequences of High Levels of Chlorine and Cadmium on the Health of Male Albino Mice

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ABSTRACT

Sufficient intake of protein-enriched food is essential for all to maintain a healthy lifestyle. The study targets to find out the Percentage of Protein in some available foods that are consumed daily by people of different incomes and ages. The chromatographic assay was done to explore the percentage of protein content in the food sources. Twenty-two locally available foods were selected for analysis and are prospective for people to consume to meet the protein for our bodies. All foods from animal sources contain a qualitative and quantitative percentage of protein (approximately one-fifth of each consumption), yet some plant-based food remainsa low protein content. But pulses can be substantial as an alternative source to animal-based foods due to their availability and digestibility. The study also pointed out the significance of RAS (Recirculating Aquaculture System) as potential fish farming technology in future. In the study, three in-house developed plant-origin foods were analyzed and contained approximately one-third of protein, which is the best option to prepare some food for nourishment. The study aims to recommend some available protein-enriched foods for our people, major selected foods in the study can be optioned based on age, sex, income and physical activity.

Keywords: Percentage of Protein, RAS, Fish, Meat, Pulses, Vegetables etc.

1. Introduction

Chlorine and Cadmium are two environmental pollutants that are of growing concern due to their widespread use and potential health impact (1,2). Chlorine is a highly reactive gas used in various industries, including water treatment, paper and textile production, and plastics manufacturing (Jordan et al., 2020;. However, the Cadmium is a toxic heavy metal in the Earth's crust and is released into the environment by mining, smelting, and manufacturing activities (3).

Exposure to chlorine and cadmium can lead to various health problems, including respiratory distress, liver and kidney damage, and cancer (2,4,5).and in The male reproductive system causes reductions in testosterone levels (6).
Exposure to chlorine and cadmium can occur through various sources. Occupational exposure to chlorine can occur in industries such as water treatment,chemical manufacturing since the workers in these industries may be exposed to high levels of chlorine gas or liquid and chlorine-containing compounds (7).Similarly,workers in industries such as battery manufacturing,welding, and mining (8).

Cadmium can enter the environment through mining, smelting, and using cadmium-containing fertilizers and pesticides (9).Consumer products can also be a source of chlorine and cadmium exposure,commonly used in household cleaning products, swimming pools, and drinking water treatment (10).Environmental toxins like chlorine and cadmium exposure can lead to severe histological changes in the testes and kidneys (11,12).
This study aims to investigate the effects of chlorine and cadmium on the *TNF*-α gene and identify any alterations or damage caused by the exposure.

2. Material and Method

Experimental Animals

In this study, 90 male mice were used as experimental animals, obtained from the Al-Razi Center in Baghdad. The mice were housed in controlled laboratory conditions with standard care and diet. For the experimental groups, some mice were exposed to water containing different concentrations of chlorine or cadmium for one month. After the exposure period, the mice were euthanized, and their kidneys were collected for histopathological examination. The researchers followed ethical guidelines and obtained approval from the institutional animal ethics committee to ensure the well-being and minimize suffering of the animals during the study.

Chemicals and Reagents

chemicals and reagents for the experimental procedures. These included chlorine powder or chloride, which was purchased from the Islamic Republic of Iran, and the cadmium compound is of Chinese origin. Chlorine Stock solutions were prepared at concentrations of 2.5 mg/l, 5 mg/l, and 10 mg/l (Taking into account the body weight of the animal). Water was used to prepare the stock solutions. Cadmium Stock solutions were prepared at concentrations of 3 ppm, 6 ppm, and 12 ppm (In B.W.). 10% neutral buffered formalin used for tissue fixation. Paraffin wax used for embedding tissue samples for histopathological examination. Hematoxylin and Eosin stains used for general tissue examination.

Experiment Design

The experimental design of the study involved dividing the 90 male mice into seven groups, including three subgroups for chlorine exposure (Cl_1, Cl_2, and Cl_3).exposed to concentrations of 2.5 mg/l, 5 mg/l, and 10 mg/l. Three subgroups for cadmium exposure (Cd_1, Cd_2, and Cd_3),exposed to concentrations of 3 ppm, 6 ppm, and 13 ppm. and one control group (Cg) that was not exposed to chlorine or cadmium The purpose of the study was to assess the effects of chlorine and cadmium exposure on the kidneys of the mice,as well as investigat the gene expression levels of *TNF-α* in their blood samples. The exposure period lasted for 30 days.The mice used in the study ranged in age from 2 months to 2.5 months.*TNF-α* is a pro-inflammatory cytokine involved in immune responses and inflammation.

Tissue Collection

In this study, a proper anesthesia protocol was followed to ensure the humane handling of the mice during tissue collection. Ketamine and xylazine were used as anesthetic agents, administered via intraperitoneal injection based on the mice's weight, following standard veterinary guidelines to achieve effective anesthesia while minimizing discomfort.Blood was collected from the heart,and the kidneys were surgically removed using sterile instruments to minimize tissue damage.The collected tissues were promptly placed in containers with 10% neutral buffered formalin to preserve tissue morphology and prevent contamination during transportation to the laboratory.

Gene expression test

To assess the gene expression of TNF-α, blood samples were collected from each mouse in the study with proper measures taken to ensure their well-being and minimize distress . The heart was the selected site for collection. The chosen site was disinfected to prevent contamination. Blood was collected using a fine-gauge needle attached to a sterile syringe, with a slow collection process to avoid hemolysis and tissue damage .The collected blood samples were immediately transferred into tubes with appropriate anticoagulants(EDTA Tube),to prevent clotting and maintain blood cell integrity.Each tube was properly labeled with unique identifiers for accurate tracking.The labeled samples were stored at suitable temperatures to preserve RNA stability until further processing. Blood cell lysis was performed using Trizol to release RNA from the blood cells.

Real-Time PCR Setup

In Real-Time PCR, gene-specific primers and fluorescent probes are used to target the *TNF-α* gene of interest. By comparing the fluorescence signals to a standard curve or internal controls, the relative gene expression level of *TNF-α* can be determined. This information provides insights into the transcriptional activity of the *TNF-α* gene in the studied mice and its potential role in various biological processes or diseases.

Primer sequences:

in the Real-Time PCR analysis of gene expression, specific primer sequences are used to target the genes of interest. In this case, the genes analyzed are *TNF-α* and Gapdh, Here are the primer sequences for each gene table 1.

Table 1. primer sequences of Gene in Real-Time PCR.

Name	**Accession number**	**Forward primer (5'---3')**	**Reverse primer (5'---3')**
TNF-α	NM_012675.3	GGCTTTCGGAACTCACTGGA	CCCGTAGGGCGATTACAGTC

Gapdh control	NM_017008.4	AGTGCCAGCCTCGTCTCATA	GATGGTGATGGGTTTCCCGT

Statistical Analysis
The collected data from the histopathological analysis of the kidneys were subjected to statistical analysis to determine the significance of any observed histological changes between the different experimental groups.

Ethical approval:
The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 737 (including the number and the date in 12/10/2022) to get this approval.

3. The Result

Histopathological observations for the mice treated with Cadmium in kidney ;
The histopathological observations of renal tissue sections in the control group (Cg) showed normal architecture. The glomerular tuft of blood capillaries surrounded by Bowman's capsule appeared normal, with normal space in the Bowman's capsules. The proximal and distal convoluted tubules, lined by cuboidal cells, were of normal size with their nuclei intact (Figure 1). In the groups of mice treated with cadmium (Cd_1 and Cd_2) at doses of (1.56mg/Kg B.W) and (2.52mg/Kg B.W)respectively,mild to moderate histopathological focal interstitial inflammation was observed.Additionally, there was moderate dilatation and congestion in the renal blood vessels in the kidney tissue sections of mice in Cd_3 (Figure1 B and C).In Cd_3,the mice were treated with a high dose of cadmium (8.645mg/Kg B.W),and the renal tissue sections showed severe degeneration and damage of the glomeruli. These histopathological findings suggest that cadmium exposure at higher doses can cause progressive damage to the renal tissue,leading to severe pathological changes.The detrimental effects of cadmium on the kidneys (Figure 1).

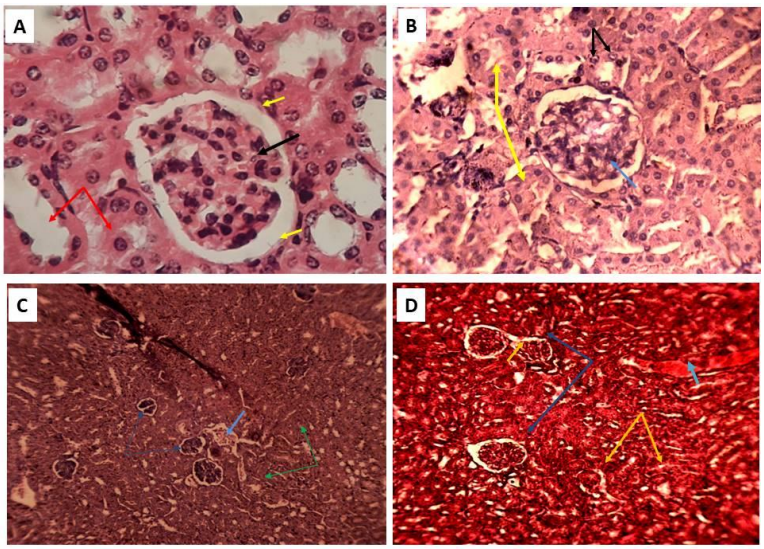

Figure1(A):Photomicrographs control group (Cg) for mice kidney tissue sections stained with Haematoxylin Eosin(HE.400):Observed normal tissue architecture,the proxiamal and distal convoluted tubules and lined by cuboidal cells appear in normal size with their nuclei.**(B)**: Photomicrographs control group(Cg)for mice kidney tissue sections stained with

Haematoxylin & Eosin(HE.400):Observed normal tissue architecture with normal glumerular tuft of blood capillaries.**(C)**: Photomicrographs (Cd1) for mice kidney tissue sections stained with Haematoxylin & Eosin(HE.400):Observed mild focal inflammation and atrophy in the glumerulous ,mild abnormal changes in renal tubules with decrease in lumen of cells in some of renal tubules.**(D)**:Photomicrographs(Cd2) for mice kidney tissue sections stained with Haematoxylin & Eosin(HE.100):Observed moderate abnormal changes in the architectures and coaggulative necrosis in the renal tubules,and congestion in the renal blood vessels .

Histopathological Observations For The Mice Treated With Chlorine In Kidney

The histopathological changes observed in the cortex of the kidney in the group Cl_1, which was treated with a dose of 0.0016 mg/kg body weight. And in the group Cl_2, treated with a higher dose of 0.0033 mg/kg body weight, the tubules in the kidney showed enlargement to a varying degree,indicating an abnormal expansion and focal interstitial inflammation. Additionally, Moderate dilatation and congestion in the renal blood vessels with infiltration of inflammatory cells in kidney tissues,indicating impaired blood flow.Furthermore,inflammatory cells infiltrated the kidney tissues, indicating an immune response to the chlorine exposure.These observations suggest that the higher dose of chlorine(0.0033mg/kg body weight) administered to the mice (group Cl_2) resulted in notable histopathological changes in the cortex of the kidney, affecting the renal tubules,glomeruli,blood vessels,and causing inflammatory responses (Figure 2 B). In group Cl_3,where mice were treated with chlorine at a dose of 0.0062mg/Kg B.W.,the kidney tissue sections exhibited shrinking and degeneration of the glomerular blood vessels tuft.There was necrosis observed in the proximal and distal convoluted renal tubules, Infiltration of inflammatory cells was also observed in the affected kidney tissues. These histopathological findings indicate that exposure to chlorine, particularly at higher doses,can induce pathological changes in the kidney.The presence of inflammatory cell infiltration further suggests an inflammatory response to chlorine exposure in the kidneys (Figure 2).

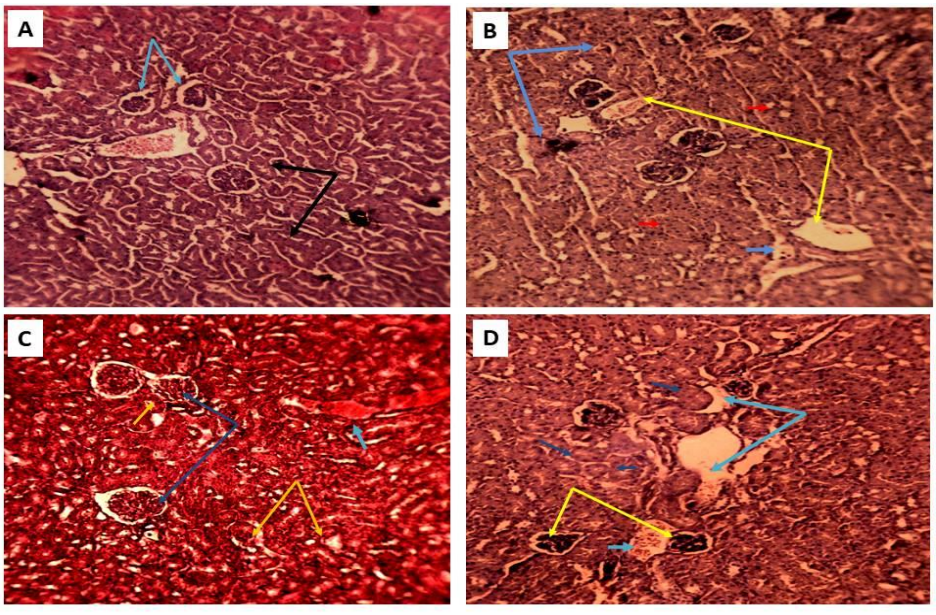

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Figure 2 : **(A).**Photomicrographs (Cl_1) for mice kidney tissue sections stained with Haematoxylin & Eosin(HE.100) : Observed mild focal inflammation and atrophy in the glumerulous mild abnormal changes in renal tubules with decrease in lumen . **(B)**: Photomicrographs (Cl_2)for mice kidney tissue sections stained with Haematoxylin &

Eosin(HE.100):Observed degeneration of cells in some of renal tubules moderate dilatation with infiltration of the inflammatory cells. **(C)**:Photomicrographs (Cl_3)for mice kidney tissue sections stained with Haematoxylin & Eosin (HE.100):Observed shrinking and degeneration of the glumerulous,and congestion of the blood vessels.**(D)**:Photomicrographs(Cl_3)for mice kidney tissue sections stained with Haematoxylin & Eosin (HE.100):observed sever degeneration and damage of the glumerulous coaggulative necrosis in the renal tubules,dilatation and congestion of the blood vessels .

Gene expression of TNF-α gene

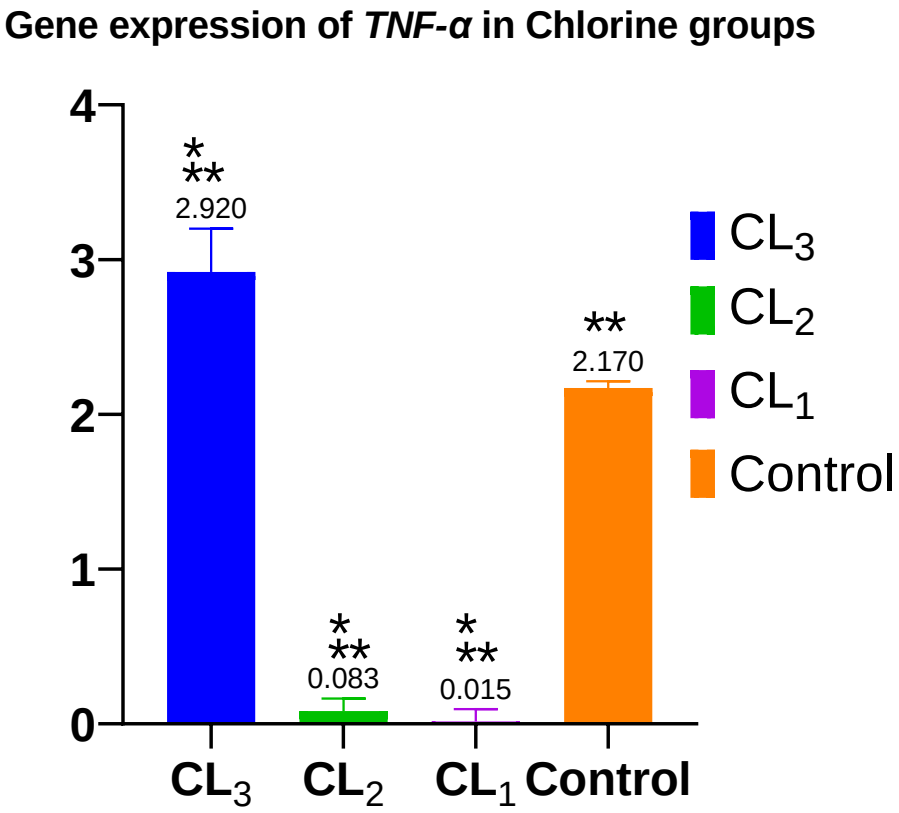

Figure 3; Gene expression levels of *TNF-α* in Cl group

The gene expression levels of *TNF-α* were assessed using Real-Time PCR analysis in different experimental groups. The results, as shown in **Figure 3**, indicate distinct variations in *TNF*-α expression among the groups. The Cl_3 group, which was exposed to a high chlorine concentration of 10 mg/l, exhibited the highest *TNF-α* expression (2.92 ± 0.28). This finding suggests a significant upregulation of *TNF-α* in response to high chlorine exposure. In contrast, the Cl_2 group, exposed to 5 mg/l of chlorine, showed relatively lower *TNF-α* expression (0.083 ± 0.08). Similarly, the Cl_1 group, exposed to 2.5 mg/l of chlorine, displayed minimal *TNF-α* expression (0.015 ± 0.08). Notably, the control group, which was not exposed to chlorine, demonstrated an intermediate level of *TNF-α* expression (2.17 ± 0.044). This suggests that even in the absence of chlorine exposure, there is some basal expression of *TNF-α.* These results indicate a dose-dependent effect of chlorine exposure on *TNF-α* expression, with higher concentrations of chlorine leading to increased expression

levels of *TNF-α*. The differential expression of *TNF-α* highlights its potential role in the immune response and inflammation processes triggered by chlorine exposure.

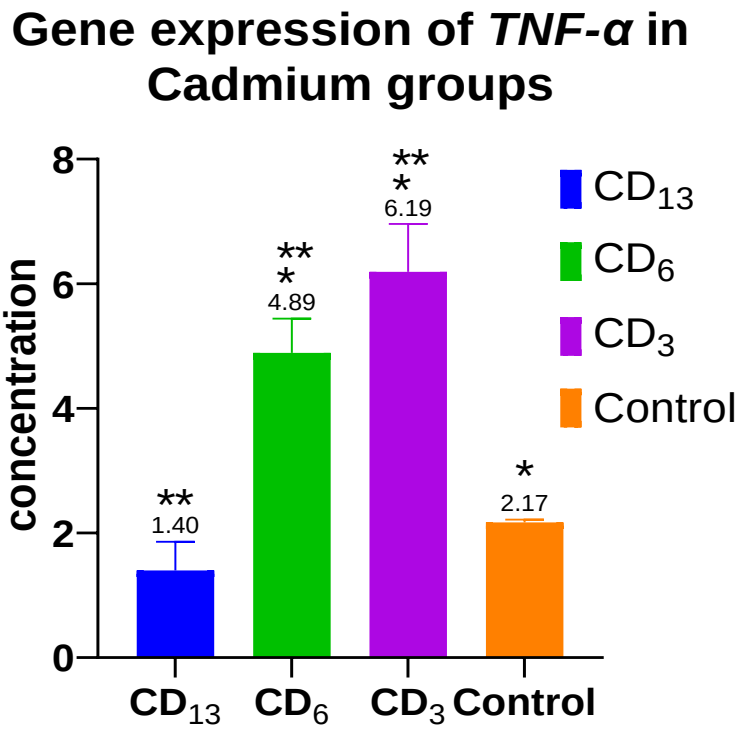

Figure 4; Gene expression levels of *TNF-α* in CD groups

The gene expression levels of *TNF-α* were analyzed in the cadmium-exposed groups using Real-Time PCR.The results in Figure 4 show distinct differences in *TNF-α* expression among the groups. The Cd_3 group, exposed to a cadmium concentration of 0.0062 mg/kg Bw, exhibited a significantly reduced *TNF-α* expression level(1.4 ± 0.46)compared to the control group. Similarly, the Cd_2 group, exposed to a cadmium concentration of 0.0033 mg/kg Bw, showed a further decrease in *TNF-α* expression (4.89 ± 0.55).The lowest *TNF-α* expression level was observed in the Cd_1 group,exposed to the lowest cadmium concentration of 0.0016 mg/kg Bw (6.19 ± 0.77).In comparison,the control group demonstrated an intermediate *TNF-α* expression level (2.17 ± 0.044). These findings suggest an inverse relationship between cadmium exposure and *TNF-α* expression, indicating that higher concentrations of cadmium lead to decreased *TNF-α* expression.The altered *TNF-α* expression in the cadmium-exposed groups highlights the potential role of *TNF-α* in the immune response and inflammatory processes associated with cadmium toxicity.

4. Discussion

Histopathological Observations In The Mice Treated With Cadmium In Kidney ;
In the kidney,The cortex contains the Malpighian corpuscles and both the proximal and distal convoluted tubules.The Malpighian corpuscles consist of a tuft of blood capillaries, the glomerulus,and Bowman's capsule.(13,14). The results of this study observed the treated with cadmium in doses (1.56mg/Kg B.W.),(2.52mg/Kg B.W.)respectively exhibited mild to moderate histopathological focal interstitial inflammation and atrophy in the glumerulous,the renal tubules observe mild to moderate abnormal changes in their architectures, while the

mice treated with high dose of cadmium(8.645 mg/kg Bw) observed sever degeneration and damage of the glumerulous with sever coaggulative necrosis in the cells of the renal tubules, dilatation and congestion of the blood vessels In group (Cd_3)the mice treated with chlorine in high dose (13.099ppm)observed sever degeneration and damage of the glumerulous with sever coaggulative necrosis in the cells of the renal tubules, multiple foci of hemorrhage, and infiltration of the inflammatory cells. Toxicity to chlorine gas depends on the dose and duration of exposure, where at concentrations of 1 to 3 ppm, chlorine gas acts as an eye and oral mucous membrane irritant, while at 15 ppm, there is an onset of pulmonary symptoms, and it can be fatal at 430 ppm within 30 minutes (15,16). Cadmium (Cd) is an industrial and environmental pollutant that can cause a variety of health problems, including kidney damage. The study by (17) showed that mice treated with different doses of cadmium exhibited histopathological changes in the kidney. Mice treated with lower to moderate doses of cadmium(1.56 mg/kg B.W. and 2.52 mg/kg B.W.) showed mild to moderate focal interstitial inflammation with mild abnormalities and atrophy in the glomeruli.These findings are consistent with previous studies that have shown that low to moderate doses of cadmium can cause mild kidney damage while thw mice treated with a high dose of cadmium (8.645 mg/kg B.W.)exhibited severe degeneration and damage of the glomeruli, along with severe coagulative necrosis in the cells of the renal tubules.These findings are consistent with previous studies that have shown that high doses of cadmium can cause severe kidney damage (18,19).Mice treated with a high dose of chlorine(13.099 ppm)showed similar severe degeneration and damage of the glomeruli,coagulative necrosis in the cells of the renal tubules,and congestion of the blood vessels. Additionally,infiltration of inflammatory cells was observed.These findings suggest that chlorine can also cause severe kidney damage (20).When cadmium acetate is administered to rats at different concentrations, it interacts with enzyme molecules and inhibits the activity of superoxide dismutase (SOD).Superoxide dismutase is an important antioxidant enzyme that helps protect cells from damage caused by reactive oxygen species.The inhibition of SOD activity by Cd2+ leads to an increase in lipid peroxidation in the liver and kidney (21).It has been suggested that the elevation of lipid peroxidation caused by cadmium is not solely due to the inhibition of SOD activity but also due to the direct action of Cd2+ on the peroxidation reaction itself (20,22). The study by Chen (23) reported that exposure to a higher dose of cadmium(1.5 mg Cd/kg) resulted in several kidney abnormalities in rats.The glomeruli showed shrinkage and irregular nuclei.In the study conducted by Capaldo(24,25) the administration of a high dose of cadmium (178 nM/L of Cd)for three months affected the kidneys.The observed effects included glomerular expansion,reduction of Bowman's space,with karyolysis(nucleus dissolution)and karyorrhexis(nuclear fragmentation).

While the study regarding the association between cadmium exposure and renal dysfunction or kidney damage(26,27),which,cadmium exposure has been strongly linked to renal dysfunction and kidney damage.It can lead to symptoms such as polyuria and proteinuria. Cadmium exposure in humans can lead to various adverse effects,including renal and hepatic dysfunction, pulmonary edema,testicular damage, osteomalacia,and damage to the adrenals and hematopoietic system(2,28).

Histopathological observations for the mice treated with Chlorine in kidney

In Cl_1 and Cl_2 groups, the cortex of the kidney showed abnormal histopathological changes, including mild to moderate dilation of the renal tubules.Additionally, mild abnormalities and atrophy were observed in the glomeruli, which are the tiny blood vessels responsible for filtration in the kidneys.The degeneration of cells in some renal tubules' cytoplasm further indicates damage to the tubular structures.Moreover,infiltration of inflammatory cells

suggests an inflammatory response in the kidney tissue. In contrast, the kidney tissue sections from the Cl_3 group, treated with a higher dose of chlorine (0.0062mg/Kg B.W.), showed more severe histopathological changes. The glomerular blood vessels exhibited shrinking and degeneration, and necrosis (cell death) was observed in the proximal and distal convoluted renal tubules.These findings indicate significant damage to the renal structures and increased inflammation compared to the Cl_1 and Cl_2 groups. Overall, these results suggest that exposure to chlorine, especially at higher doses, can induce kidney damage in mice,and it's important to note that these findings are specific to mice and may not directly translate to the effects of chlorine on human kidneys. Further research would be needed to understand the implications for human health (29).The effects of chlorine dioxide and sodium chlorite exposure in animal studies,In a study by Daniel (30) involving Sprague-Dawley rats, exposure to chlorine dioxide in drinking water for 90 days at concentrations of 0, 25, 50, 100, or 200 mg/L resulted in significant reductions in body weights and body weight gain. The exposed groups showed body weight gains 26% to 29% lower than the control group. The Harrington et al. (31,32) reported the administration of sodium chlorite doses to rats orally via gavage at 80 mg/kg-day for 13 weeks.The rats exhibited salivation and showed significant decreases in erythrocyte counts. Additionally, there were decreases in hematocrit and hemoglobin levels,which can have oxidative effects and lead to morphological changes in erythrocytes (33). Overall, these studies demonstrate the toxic effects of chlorine and its derivatives on various physiological systems, including the respiratory system, growth and development, hematological parameters, and organ weights. It's important to note that these studies were conducted on rats, and the effects on humans may differ. Further research is necessary to understand the potential risks and implications for human health. The Nabil et al. (34,35) highlights the toxicity of mercury chloride (HgCl2), which is considered the most toxic form of mercury salts,because HgCl2 is primarily metabolized in the liver and accumulates in the kidneys. Administration of HgCl2 initiates the formation of highly reactive substances, such as reactive oxygen species,leading to the activities of antioxidant enzymes decrease, further impairing the body's defense against oxidative damage (36,37).

Gene Expression

the expression of *TNF-α* (tumor necrosis factor-alpha) in response to chlorine and cadmium exposure are consistent with previous research in the field. Studies have shown that exposure to chlorine leads to an upregulation of *TNF-α* expression, indicating its involvement in the inflammatory response to chlorine-induced toxicity. Furthermore, the dose-dependent effect of chlorine on *TNF-α* expression, with lower expression levels observed at lower concentrations, aligns with the dose-response relationship reported in other studies. This suggests that higher chlorine concentrations induce higher *TNF-α* expression, further supporting the involvement of *TNF-α* in chlorine-induced inflammation. In the case of cadmium exposure, your results showed a significant decrease in *TNF-α* expression with increasing cadmium concentrations. This is in line with previous studies demonstrating a downregulation of *TNF-α* expression in response to cadmium exposure. Cadmium is known to have immunosuppressive effects, and the observed reduction in *TNF-α* expression suggests a potential role of *TNF-α* in the immune response to cadmium toxicity. The inverse relationship between cadmium concentration and *TNF-α* expression levels further supports the idea that higher cadmium concentrations lead to greater immunosuppression and inhibition of *TNF-α* production. These findings are consistent with previous studies highlighting the immunosuppressive effects of cadmium and the modulation of *TNF-α* expression. The higher expression of *TNF-α* in the group exposed to the highest chlorine concentration (Cl_3) suggests an increased inflammatory response due to the higher chlorine concentration. In the case of cadmium exposure, the lower *TNF-α* expression levels observed

in the cadmium-exposed groups compared to the control group can be attributed to the immunosuppressive and oxidative effects of cadmium.

These studies can help unravel the specific pathways through which chlorine and cadmium exert their effects on *TNF-α* expression and provide more insight into the implications for immune response and inflammation-related diseases.

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