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Investigating the synergistic effects of cadmium chloride and oxidative stress on male rats' immunity

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Abstract

This research investigates the bioaccumulation of cadmium (Cd) and hydrogen peroxide (H_2O_2) and their synergistic effects on the production of reactive oxygen species (ROS) in various tissues of male albino rats. The rats were exposed to 5 mg/kg body weight of cadmium chloride and 1 mM H_2O_2 for 7, 15, and 30 days. Levels of cadmium and hydrogen peroxide in blood, liver, spleen, kidney, testes, and adrenal gland tissues were measured using appropriate analytical methods. Additionally, ROS production in blood and liver cells was assessed using flow cytometry. Importantly, the combination of cadmium and hydrogen peroxide showed a significant synergistic effect ($P<0.001$) on the bioaccumulation of both agents. This synergistic effect was associated with a notable increase in ROS production in blood and liver cells. The mean fluorescence intensity (MFI) in the group receiving both cadmium and hydrogen peroxide was significantly higher than in groups receiving either cadmium or hydrogen peroxide alone (MFI: 1142 in blood cells and 805 in liver cells compared to MFI: 416 and 256 for cadmium, and 256 and 206 for hydrogen peroxide, in blood and liver respectively). These findings indicate heightened oxidative stress and cellular damage from simultaneous exposure to cadmium and hydrogen peroxide. Overall, the results of this study suggest that cadmium and hydrogen peroxide, both individually and particularly in combination, have significant toxic effects on rats. These effects include bioaccumulation in various tissues and increased ROS production. These findings highlight the importance of studying the combined effects of environmental toxins and the need to consider their synergistic impacts.

Keywords: Cadmium chloride, hydrogen peroxide, bioaccumulation, reactive oxygen species (ROS), oxidative stress, synergistic effect.

Introduction

Cadmium is a chemical element represented by the symbol Cd and atomic number 48 in the periodic table. This element was simultaneously discovered in 1817 by two German scientists, Friedrich Stromeyer and Hermann[1]. They identified this new element within impurities present in zinc carbonate, known as calamine. For a century, calamine was the sole source of cadmium production, and thus the metal was named after the Latin word calamine, as it was identified in combination with zinc. Like zinc, cadmium primarily exhibits an oxidation state of +2 in most of its compounds and, like mercury, has a lower melting point compared to transition metals in groups 3 to 11. The average concentration of cadmium in the Earth's crust ranges from 0.1 to 0.5 ppm[2].

Studies have shown that cadmium can impair immune cell function. This metal has the capability to suppress the body's immune responses, leading to a reduced effectiveness of the body's natural defenses against pathogens (such as viruses and bacteria)[3]. Cadmium stimulates the immune system, resulting in excessive production of inflammatory factors such as cytokines (e.g., interleukins and TNF-α). This persistent inflammation can cause damage to various tissues and organs[4].

The inflammatory response is a physiological condition that plays a critical role in the host's immune reactions against infection or injury. Inflammation initiates upon exposure to pathogens, foreign substances, damaging agents, the removal of necrotic cells, and the beginning of tissue repair[5].

Inflammation is characterized by a series of processes, including vasodilation, increased capillary permeability, enhanced blood flow, and leukocyte recruitment. Despite its beneficial effects, uncontrolled and inappropriate inflammation can damage surrounding healthy tissues and lead to inflammatory and autoimmune diseases. Recent evidence suggests that improper inflammatory responses are closely linked to many chronic diseases, including inflammatory bowel disease (IBD), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and diabetes. Both infectious and non-infectious triggers stimulate the inflammatory process by recruiting inflammatory cells and activating signaling pathways[6].

Bacteria, viruses, and other microorganisms are the main triggers of infectious inflammation. Several other factors, including physical (burns, frostbite, physical injury, foreign bodies, trauma, radiation), biological (damaged cells), psychological (emotions), and chemical stimuli (glucose, fatty acids, toxins, alcohol, and chemical irritants such as fluoride, nickel, cadmium, and other trace elements and transition metals) are non-infectious causes that play roles in inflammation onset[7].

Exposure to cadmium is associated with several disorders, including osteoporosis, diabetes, and cancer. Cadmium has been reported to activate multiple intracellular signaling pathways (particularly NF-κB and AP-1) in immune cells at micromolar concentrations (mM), upregulating inflammatory markers and mediators. Cadmium may induce acute and/or chronic inflammatory responses in tissues such as the heart, liver, kidneys, lungs, and reproductive system, potentially leading to tissue damage and/or even systemic inflammatory responses[8].

Innate immunity provides the first line of defense against infections through a non-specific mechanism. The innate immune system operates through various immune cells and mechanisms, facilitating the distinction between self and non-self. Innate immune responses are rapid and function through an antigen-independent pathway[9]. Exposure to cadmium has been shown to affect various aspects of innate immunity. Several studies indicate that cadmium, as an environmental pollutant, modulates innate immune responses by influencing chemokine release, gene expression, and susceptibility to microbial infections. Research has shown that cadmium can induce inflammation, though the precise mechanisms through which cadmium activates inflammatory responses are not fully understood[10].

Razooli et al. used porcine epithelial cells to assess the effects of cadmium on pro-inflammatory gene expression, protein release, and infection in a Salmonella Typhimurium invasion model. They found that epithelial cells could absorb cadmium in a time- and concentration-dependent manner, leading to upregulation of important pro-inflammatory cytokines and chemokines (IL-8, IL-6, IL-18) and transcription factor genes (Nk-kB1, Nkfb-p65, MYD88). In vitro studies demonstrated that non-toxic cadmium concentrations could induce IL-6 and IL-8 production from astrocytes in a dose- and time-dependent manner via MAPK phosphorylation and NF-kB activation, without affecting cell morphology or viability[11]. Cytokines initiate inflammation and may be

associated with neurodegenerative disorders. Inhibiting IL-6 and IL-8 production may present new approaches to prevent cadmium-induced angiogenesis in gliomas and inflammation in the brain. Cadmium may also impair endoplasmic reticulum (ER) function and increase reactive oxygen species (ROS). Kim et al. suggested that cadmium exposure activates the CCAAT-enhancer-binding protein (C/EBP) signaling pathway. This activation alters gene expression across various mechanisms, including oxidative stress and inflammatory responses. Cadmium increases ROS production, leading to mitochondrial dysfunction and cellular apoptosis[12].

Studies have shown that cadmium can increase cyclooxygenase-2 (COX2), prostaglandin E2 (PGE2), and macrophage inflammatory protein (MIP-2) levels. It is suggested that cadmium elevates COX2 levels through the activation of the mitogen-activated protein kinase (MAPK) pathway. Elevated COX2 levels lead to increased inflammatory PGE2 production, endothelial damage, and cellular death. A study by Huang et al. demonstrated that cadmium activates phosphatidylinositol 3-kinase (PI3K)/Akt signaling, subsequently increasing macrophage inflammatory protein (MIP-2) and COX2 in macrophages[13].

Cormet-Boyaka et al. showed that cadmium induces Erk1/2 signaling in airway epithelial cells, where Erk activation leads to increased expression of pro-inflammatory mediators, including IL-8. They suggested that cadmium-induced airway inflammation occurs via an Erk1/2-dependent but NF-κB-independent mechanism[14].

Kendo et al. showed that temporary low-dose cadmium exposure significantly upregulates epidermal growth factor receptor (EGFR), which is crucial in regulating pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α. Moreover, EGFR overexpression may lead to cellular proliferation, survival, and oncogenesis[15].

While most studies indicate that cadmium induces inflammatory responses, there are reports suggesting immunosuppressive and anti-inflammatory aspects of cadmium exposure. It has been demonstrated that higher doses of cadmium exert suppressive effects on NO production by reducing iNOS in zebrafish models, while lower cadmium levels had no effect on iNOS expression and NO production. Furthermore, cadmium exposure increased IL-1, IL-6, and TNF-α expression in the liver while decreasing expression in the spleen, indicating that cadmium effects appear organ-specific[16].

Cadmium exposure has been observed to stimulate innate immune responses at a dose of 1 mg/kg, while inhibiting T-cell responses and reducing respiratory burst activation. However, a low dose (0.5 mg/kg) of cadmium enhances respiratory burst activation. This study suggests that higher cadmium levels may exert immunosuppressive effects on the mouse spleen. Ultimately, cadmium exhibits both pro-inflammatory and anti-inflammatory effects, depending on the exposure duration, dose, and cell type involved[17].

Another study showed that nuclear translocation and redox-active Trx1 accumulation in the nuclei play a critical role in cadmium-induced inflammation and cell death. Recent research has shown that cadmium exposure stimulates Trx1 nuclear translocation, activating the transcription factor NF-κB. Increased NF-κB activation leads to elevated inflammatory mediators, adhesion molecules, and cell death. This review suggests that these signaling pathways and inflammatory cascades are associated with low-dose cadmium toxicity[18].

Overall, cadmium can increase the risk of infectious diseases, inflammatory conditions, and even cancers by impairing immune mechanisms and disrupting the body's immune responses. Therefore, reducing cadmium exposure and thoroughly investigating its effects on public health are of critical importance.

Research method

Forty adult male Wistar rats, weighing between 200–250 grams and aged 8–10 weeks, were acquired from the Medical Sciences Laboratory Animal Breeding Center to serve as the animal model. The animals had unrestricted access to water and standard feed and were housed for a two-week acclimatization period in polypropylene cages under laboratory conditions: a constant temperature of 25 ± 2°C, relative humidity of 60 ± 5%, and a 12/12-hour light-dark cycle in the laboratory animal facility of the Veterinary Faculty at Urmia University.

This experimental study was conducted in accordance with the international guidelines for the ethical treatment of laboratory animals, within the Medical Sciences Laboratory Animal Facility at Urmia University.

Finally, the rats were anesthetized using ketamine and xylazine and euthanized via cervical dislocation. Tissues were promptly collected and stored in liquid nitrogen.

1. Control Group: The control group was not exposed to cadmium chloride or hydrogen peroxide. This group served as the baseline, receiving only water via oral gavage daily.

2. Cadmium Chloride Exposure Group: This group was treated with cadmium chloride at a dose of 5 mg per kg of body weight, administered via oral gavage daily for 14 days.

3. Hydrogen Peroxide Exposure Group: This group received hydrogen peroxide at a concentration of 1 mM, administered via oral gavage daily for 14 days.

4. Combined Exposure Group: This group was simultaneously treated with cadmium chloride (5 mg/kg) and hydrogen peroxide (1 mM), with both substances administered via oral gavage daily for a period of 14 days.

hus, each 250-gram rat required 1.25 mg of cadmium chloride. Accordingly, 12.5 mg of cadmium chloride was accurately weighed using a precision balance. The weighed cadmium chloride was then dissolved in 100 mL of double-distilled water. The solution was stirred gently to ensure complete dissolution of the cadmium chloride. For long-term storage, the solution was kept at 4°C (refrigerated).

Each 250-gram rat received an oral gavage of 2 mL of the cadmium chloride solution daily.

Sampling was conducted on day 8 (one day after the seventh treatment), day 15 (one day after the final treatment), and day 30 (15 days after the final treatment). Ketamine (200 mg/kg) combined with xylazine (20 mg/kg) was used for anesthesia and then sacrificed. Blood samples were collected by cardiac puncture using a 23-gauge needle. Collected blood was divided into two parts: one mixed with an anticoagulant (EDTA) for hematological parameters, and the second stored for analysis of toxic metals and bioelements after wet digestion. Blood with anticoagulant was centrifuged, plasma was separated, and the remaining blood was collected in tubes without anticoagulant for serum preparation. Plasma was frozen at -80°C and serum at -20°C for redox and biochemical analysis, respectively. The organs studied (liver, spleen, kidney, testes, and adrenal glands) were divided into three parts: one frozen immediately in liquid nitrogen at -80°C for antioxidant assessment, another stored at -20°C for toxic metal and bioelement analysis, and the third preserved in formalin for histopathological examination.

Tissue Homogenate Preparation:

Ten percent homogenized tissues (weight/volume) were prepared by mixing parts of these organs (of known weight) with chilled potassium phosphate buffer (50 mM, pH = 7.4; made with potassium hydrogen phosphate, potassium dihydrogen phosphate [POCh], and distilled water) and butylated hydroxytoluene (Sigma-Aldrich GmbH, Steinheim, Germany) using a glass/Teflon homogenizer (Schütt homogenplus, Schutt Labortechnik GmbH, Göttingen, Germany). Prepared homogenates for SOD, GPx, and GR assays were centrifuged (MPW-350R centrifuge, Medical Instruments, Warsaw, Poland) at 20,000 g for 30 minutes at 4°C. Other measurements were conducted on partially separated homogenates centrifuged for 20 minutes at 700 g (4°C). Samples were frozen at -70°C until analysis.

Serum Cytokine Measurement by ELISA:

Serum levels of pro-inflammatory cytokines (IL-8, IL-6, and TNF-α) were measured using an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Krishgen Biosystems, Mumbai, India). Briefly, samples were added to a pre-coated 96-well microplate with a monoclonal antibody. Biotinylated antibody solutions were added, and the plates were incubated at 37°C for the required duration. Plates were washed, streptavidin-HRP solution was added, and incubated for an hour. Plates were washed again, TMB substrate was added, and the reaction was allowed for 10-15 minutes before adding the stop solution. ELISA microplates were read on an Eon Biotek plate reader set at a 450 nm wavelength, and values were estimated using Gen5 software (version: 2.05.5) based on a five-point calibration curve. Results are expressed in pg/mL.

Catalase Activity Assay:

Catalase (CAT) activity in serum was measured following Aebi's method, which is based on the breakdown of hydrogen peroxide by catalase. One milliliter of H_2O_2 (50 mM concentration) was added to the reaction mixture to initiate the reaction. The decrease in absorbance due to hydrogen peroxide decomposition was measured at 240 nm at 1- and 3-minute intervals, with catalase activity calculated based on absorbance differences per minute. The extinction coefficient (13.6 mM) was used to calculate CAT activity, expressed in U/mL[19].

Results

Levels of pro-inflammatory cytokines using ELISA

Levels of pro-inflammatory cytokines (IL-8, IL-6, and TNF-α) in serum of Wistar rats, treated with cadmium chloride and H_2O_2, measured using ELISA. These compounds led to a significant increase in pro-inflammatory cytokines, due to oxidative stress and tissue damage which activate the immune response. In treatment groups, cytokine levels were notably higher compared to controls.

In the control group, cytokine levels remained at baseline (low). H_2O_2 exposure elevated cytokine levels due to free radicals and oxidative stress ($p < 0.05$). Cd exposure resulted in a substantial increase in cytokines, attributed to oxidative stress and cellular damage ($p < 0.01$). The combination of Cd and H_2O_2 produced the highest cytokine levels, indicating significant oxidative stress and inflammation ($p < 0.001$) (Chart 1).

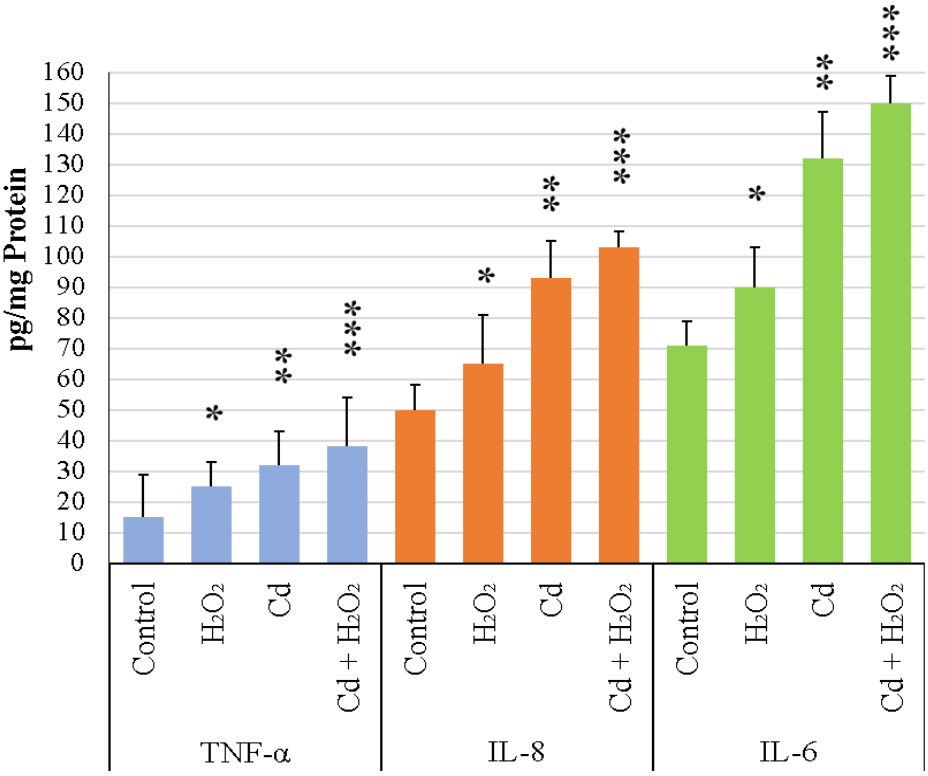

Chart 1. Effects of Cadmium Chloride and Hydrogen Peroxide on the Production of Pro-inflammatory Cytokines TNF-α, IL-6, and IL-8 in Rat Serum Measured by ELISA. A significant upward trend in cytokine levels is observed from the control group to the Cd+H2O2 combination. The effect of Cd alone is stronger than H2O2 alone. The Cd+H2O2 combination shows the highest cytokine levels. All changes are statistically significant compared to the control group. Error bars represent standard deviation (SD) or standard error of the mean (SEM).

Gene Expression Levels

Changes in the expression of genes related to oxidative stress (such as SOD1, CAT, and GPx1) and immune response (such as TNF-α, IL-6, and IL-8) were analyzed in Wistar rats following cadmium and hydrogen peroxide exposure using Real-Time PCR.

IL-6 Levels

The graph depicts mRNA expression changes for the cytokine IL-6 in various tissues (blood, liver, and kidney) of Wistar rats under different treatments (control, H_2O_2, Cd, and Cd + H_2O_2) at 7, 15, and 30 days. IL-6 levels in the control group across blood, liver, and kidneys remained relatively stable, showing no significant fluctuations over 7, 15, and 30 days, which suggests an absence of an inflammatory response in this group.

In the H_2O_2 group, IL-6 levels in blood, liver, and kidneys increased significantly, with the most notable rise observed on days 15 and 30 ($p < 0.05$). This increase was more prominent in the kidneys and blood than in the liver, indicating an inflammatory response to oxidative stress caused by H_2O_2. Similarly, in the Cd group, IL-6 levels in blood, liver, and kidneys rose significantly ($p < 0.01$), particularly in the liver and kidneys. The highest increases occurred on days 15 and 30, suggesting cadmium induces a strong inflammatory reaction.

The Cd + H_2O_2 group exhibited the most substantial increase in IL-6 levels across all three tissues (blood, liver, and kidney) ($p < 0.001$), reaching peak levels on days 15 and 30, which points to a synergistic effect of Cd and H_2O_2 in triggering an inflammatory response. The kidneys demonstrated the highest inflammation levels with this combination (Chart 2).

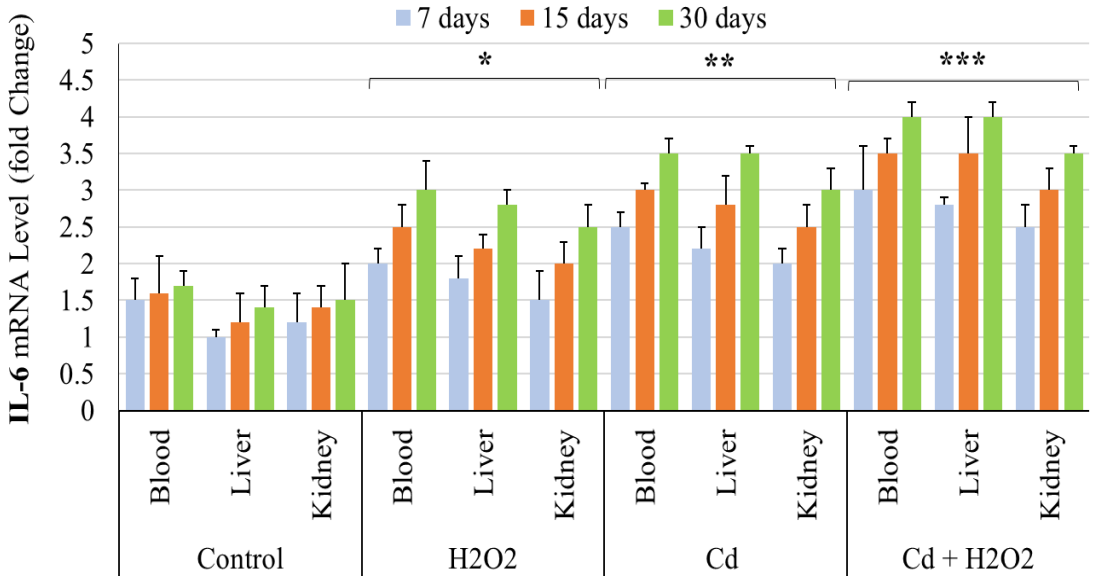

Chart 2. illustrates the mRNA levels of interleukin-6 (IL-6) in blood, liver, and kidney tissues of rats treated with cadmium chloride, hydrogen peroxide, and the Cd + H_2O_2 combination over three time points: 7, 15, and 30 days. The IL-6 levels are expressed as relative fold changes compared to the control group.

IL-8 Level

In the control group, IL-8 expression in all three tissues (blood, liver, and kidney) and at three measurement times (7, 15, 30 days) remained relatively stable, showing no significant change. This indicates a steady state without inflammatory stimulation in the control group. A notable increase in IL-8 expression was observed in the H_2O_2 group compared to the control group, particularly in the kidneys, where levels increased on day 30 relative to days 7

and 15. This change, in comparison to the control group, is statistically significant ($P < 0.05$), but its intensity is lower than in the other treatment groups.

In the Cd group, a substantial increase in IL-8 expression was observed compared to both the H_2O_2 and control groups. The increase in IL-8 expression is especially prominent in the liver and kidneys, particularly on day 30, indicating a statistically significant increase compared to the control and H_2O_2 groups ($P < 0.01$).

The Cd + H_2O_2 group showed the highest IL-8 expression in all three tissues, especially in the kidneys. On day 30, IL-8 levels peaked. This demonstrates a statistically significant difference between this group and the other groups, including Cd and H_2O_2. This increase suggests a synergistic effect between Cd and H_2O_2, inducing a stronger inflammatory response ($P < 0.001$) (Chart 3).

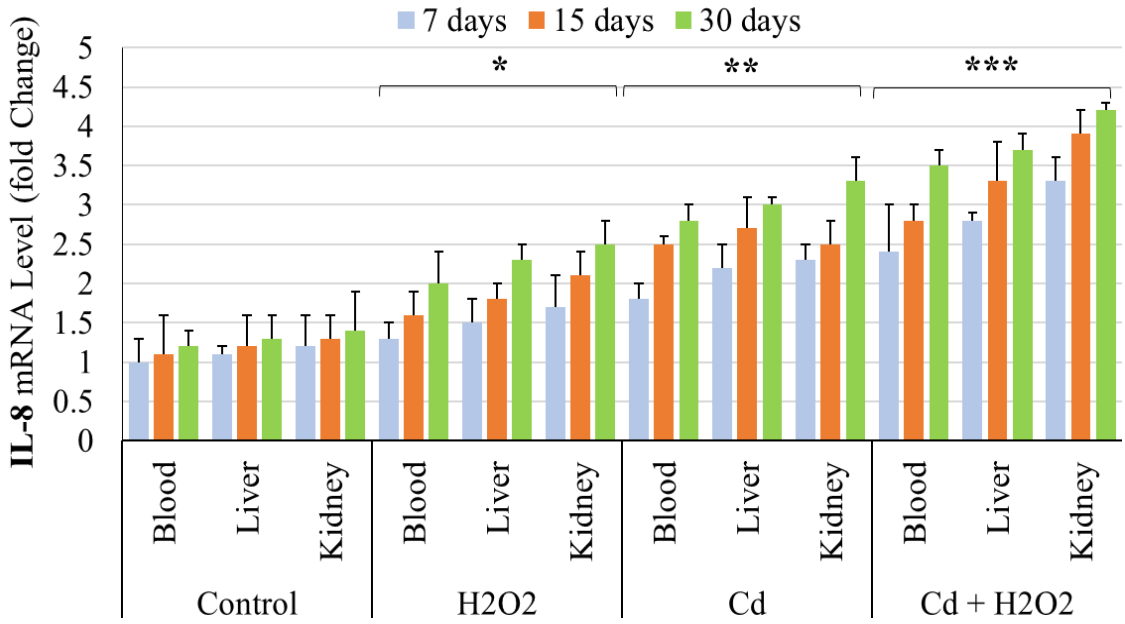

Chart 3. Changes in IL-8 gene mRNA levels in the blood, liver, and kidney tissues of rats across four groups: control, hydrogen peroxide treatment, cadmium chloride treatment, and the Cd + H_2O_2 combination over 7, 15, and 30 days. The results are expressed as relative fold changes, with various statistical significance levels indicated by different stars for P values.

TNF-α Levels

In the control group, TNF-α gene expression remains stable with no significant variation over time or between tissues, showing a consistent level close to 1 across all samples. In rats treated with H_2O_2, there is a mild increase in TNF-α expression levels across tissues, with more pronounced elevations on days 15 and 30. The kidney displays the highest expression relative to blood and liver ($P < 0.05$). Cadmium treatment results in a more substantial rise in TNF-α expression, especially notable in kidney tissues and surpassing the levels observed in the H_2O_2 group. The highest increase occurs on day 30 ($P < 0.01$). The Cd + H_2O_2 combination induces the most significant increase in TNF-α expression across all three tissues, with the highest values observed in the kidneys on day 30. This synergistic effect indicates that combined Cd and H_2O_2 exposure leads to a markedly intensified inflammatory response ($P < 0.001$) (Chart 4).

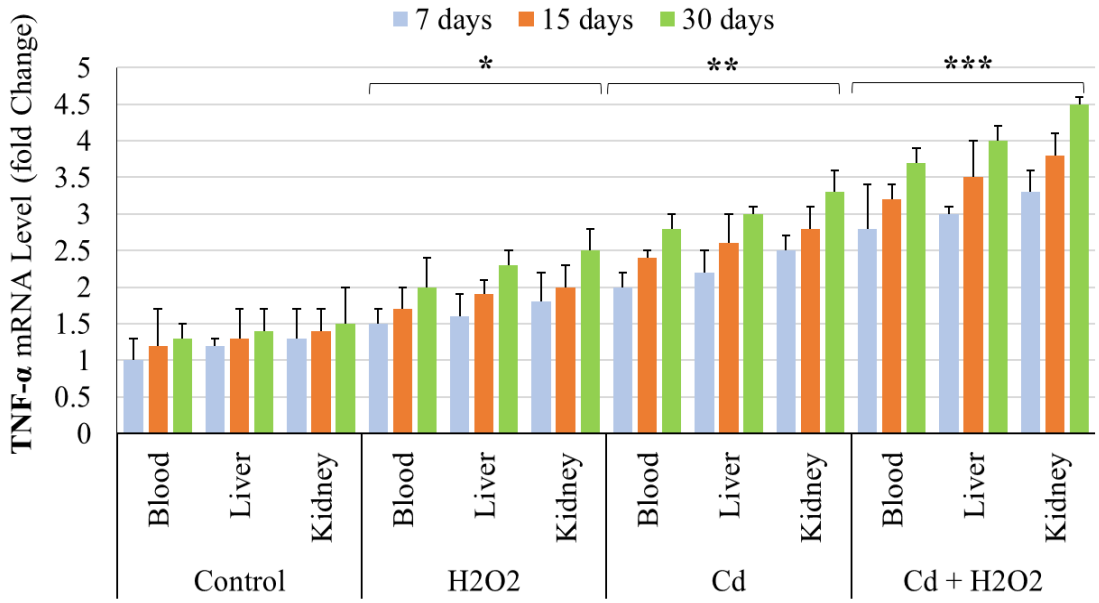

Chart 4. presents the changes in TNF-α gene expression levels in various tissues (blood, liver, kidney) of rats treated with H_2O_2, cadmium chloride (Cd), and the combined treatment of Cd + H_2O_2 over three time points: 7, 15, and 30 days.

SOD1 Level

In the control group, SOD1 gene expression remains normal (1 fold) across all tissues, with no significant differences observed between days 7, 15, and 30. In the H_2O_2 group, a mild increase in SOD1 expression is seen on day 7, with the highest level in the liver. Gradual reductions in SOD1 expression occur by days 15 and 30, particularly in the kidney, likely due to a decrease in oxidative stress and the body's efforts to return to baseline ($P < 0.05$). In the cadmium group, SOD1 expression increases across all tissues, especially in the liver, on day 7, likely due to oxidative stress induced by cadmium. By days 15 and 30, a decline in gene expression is noted, particularly in the kidney, potentially due to cellular damage and the exhaustion of the antioxidant system ($P < 0.01$). The Cd + H_2O_2 group exhibits the highest SOD1 gene expression on day 7, particularly in the liver, indicating a combined oxidative stress effect from both Cd and H_2O_2. On days 15 and 30, SOD1 gene expression decreases but remains elevated compared to the other groups, especially in the liver and blood ($P < 0.001$) (refer to Chart 5).

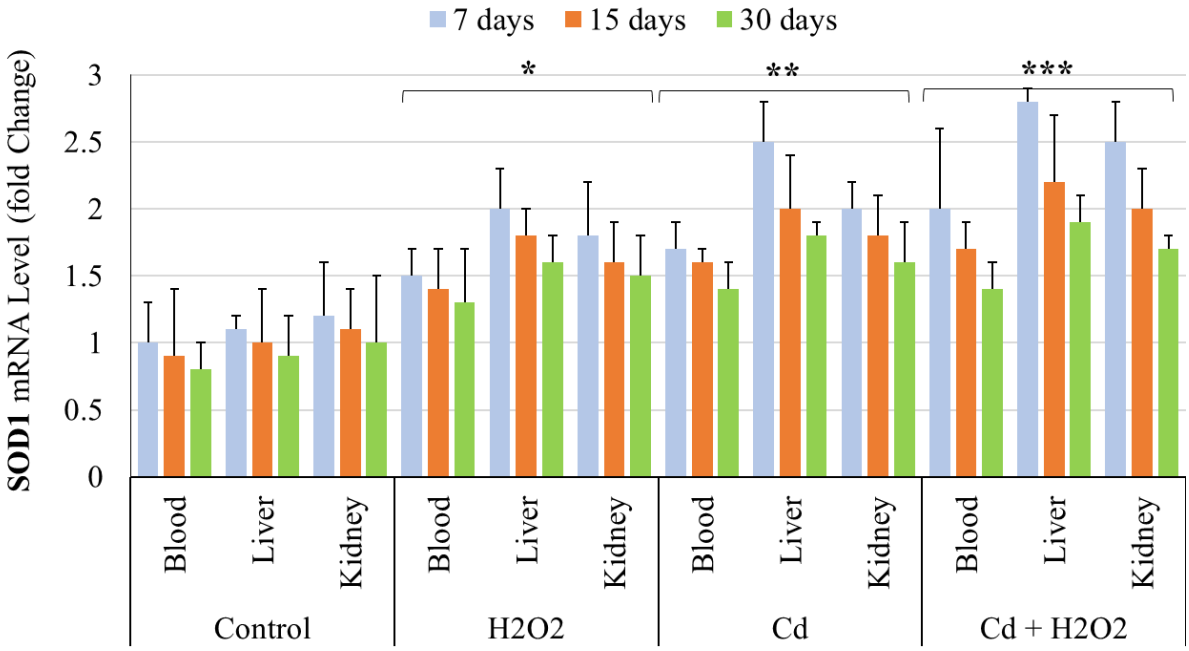

Chart 5. illustrates changes in SOD1 gene expression across various tissues (blood, liver, and kidney) at three different time points (7, 15, and 30 days) following exposure to H_2O_2, cadmium (Cd), and their combination (Cd + H_2O_2). SOD1 gene expression levels are measured as fold change (relative to the control) and are compared across the control, H_2O_2, Cd, and Cd + H_2O_2groups. The symbols *, **, and * indicate statistical significance levels of $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, denoting significant gene expression changes in these time points and experimental groups.

CAT Level

In the control group, CAT gene expression in all tissues remains close to baseline (1.0 fold), with no significant changes observed on days 7, 15, and 30. In the H_2O_2 group, a significant increase in CAT gene expression in blood and liver tissues is observed on day 7 ($P < 0.05$), indicating a response to oxidative stress caused by H_2O_2. On days 15 and 30, a gradual decrease in gene expression, especially in the kidney, is noted, potentially due to the adaptation of the body's antioxidant system. In the cadmium group, a marked increase in CAT gene expression in the liver and blood is observed on day 7 ($P < 0.01$), attributable to oxidative stress from cadmium exposure. Although gene expression decreases on days 15 and 30, it remains above control levels. In the Cd + H_2O_2 group, the highest CAT gene expression increase on day 7 is notably observed in the liver, reflecting the strong combined effect of oxidative stress from Cd and H_2O_2. A gradual decrease in gene expression is noted on days 15 and 30, but it remains higher than in the other groups. The most significant changes are seen in the combined Cd + H_2O_2 group in the liver and blood ($P < 0.001$), indicating a synergistic effect of these two factors (Chart 6).

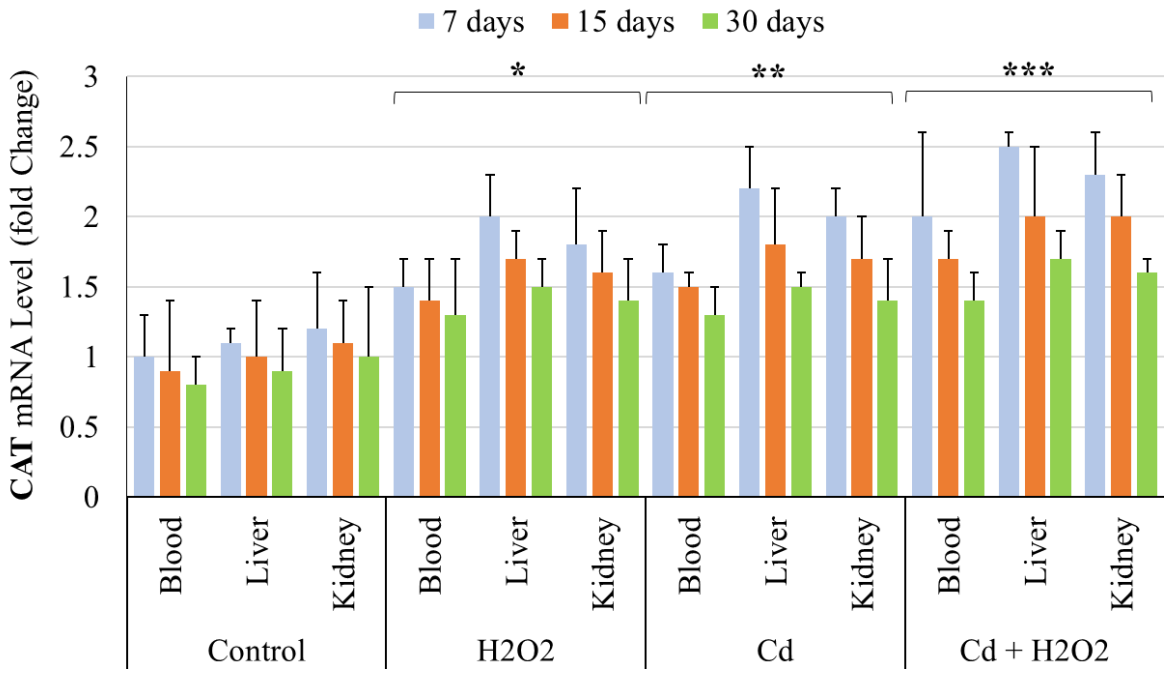

Chart 6. The changes in CAT gene expression in various tissues (blood, liver, and kidney) at three different times (7, 15, and 30 days) after exposure to H_2O_2, cadmium, and the combination of both (Cd + H_2O_2). The level of CAT gene expression is measured as fold change relative to the control group, and values are presented with standard deviation. The symbols *, **, and * indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

GPx1 Level Analysis

In the control group, GPx1 gene expression remained relatively stable across all tissues, indicating that GPx1 levels are naturally maintained under non-stress conditions without significant variation. This baseline suggests that, under normal circumstances, GPx1 remains at a steady level throughout the body.

For the H_2O_2-treated group, a notable increase in GPx1 expression was observed in the blood and liver on day 7, but minimal changes were seen in the kidneys. By days 15 and 30, gene expression gradually decreased, nearing control levels, suggesting an adaptive response aimed at rebalancing GPx1 levels. Differences in GPx1 expression between the control and H_2O_2 groups were statistically significant ($P < 0.05$), supporting the idea that H_2O_2 exposure leads to an initial rise in GPx1.

In the cadmium group, a significant increase in GPx1 expression was observed across the blood, liver, and kidney on day 7, with a gradual decrease by days 15 and 30, although levels remained above control values, particularly in the liver. This prolonged elevation highlights oxidative stress due to cadmium exposure, with a notable statistical significance ($P < 0.01$) compared to the control, reinforcing cadmium's role in driving oxidative stress.

In the Cd + H_2O_2 group, the highest GPx1 gene expression increase was observed on day 7, especially in the blood and liver, reflecting the cumulative oxidative stress from both agents. While GPx1 levels gradually decreased by days 15 and 30, they remained significantly higher than in other groups, particularly on day 15. This significant variation ($P < 0.001$) underscores the synergistic impact of Cd and H_2O_2 in inducing oxidative stress and elevating GPx1 expression (as illustrated in Chart 7).

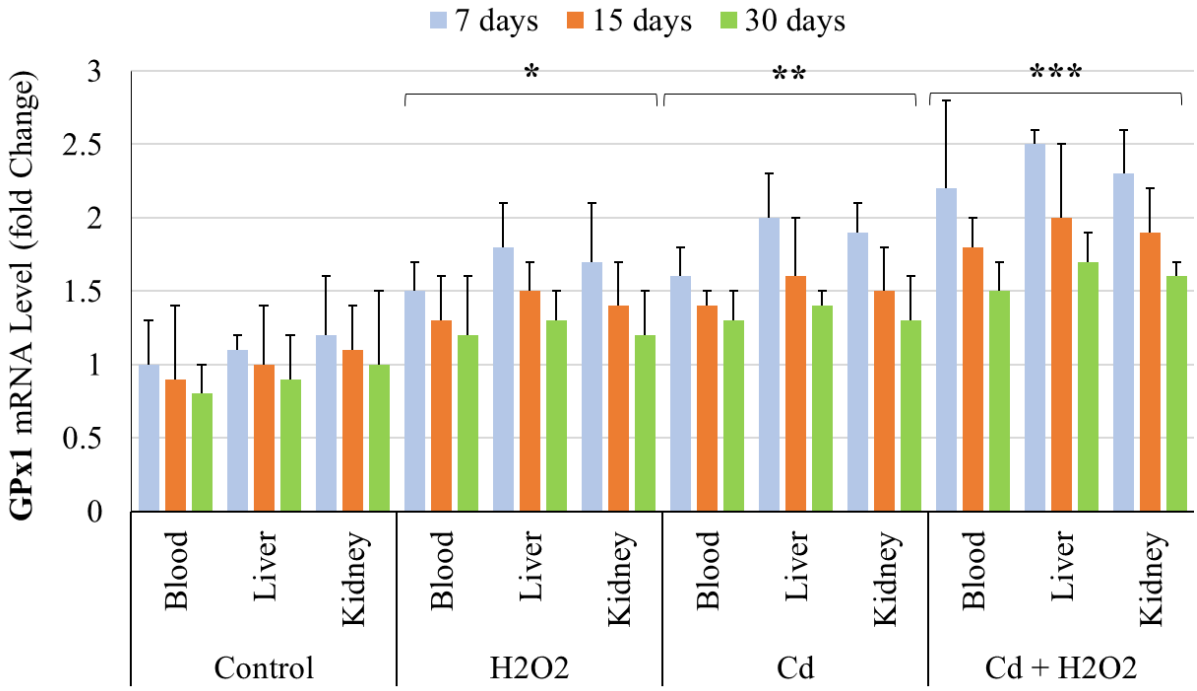

Chart 7. Changes in GPx1 gene expression in various tissues (blood, liver, and kidney) of rats exposed to hydrogen peroxide (H_2O_2), cadmium (Cd), and their combination. The chart is plotted based on different days (7, 15, and 30), and the level of GPx1 gene expression changes is displayed as fold change. The symbols *, , and * indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

discussion

Exposure to cadmium as an environmental pollutant has significant implications for human health. Cadmium contamination greatly impacts the function of the immune system, causing both acute and chronic inflammation, which leads to the production of inflammatory cytokines and chemokines. Although the inflammatory mechanisms triggered by cadmium toxicity vary between the gastrointestinal and respiratory systems, the overall result is tissue damage and impaired organ function.

Several reports have suggested that cadmium induces inflammation and necroptosis (a cell death process between necrosis and apoptosis) in various tissues. For instance, Hesaraki et al. (2010) demonstrated that high cadmium concentrations in the diet (50 and 100 ppm) lead to necrosis, apoptosis, and degeneration in the kidneys and liver of eight-week-old broiler chickens [20]. Gharagozlou et al. (2011) showed similar apoptotic and degenerative effects in the bursa of Fabricius of broiler chickens exposed to high cadmium levels [21]. Xin et al. (2020) concluded that cadmium exposure activates the ALK4/5 signaling pathway, inducing necroptosis in renal tubular epithelial cells. Moreover, cadmium exposure activated the PI3K/AKT signaling pathway in peripheral blood lymphocytes of chickens, resulting in necroptosis [22].

The current study confirmed that cadmium exposure activates the NF-kB pathway, leading to increased expression of inflammatory factors. Furthermore, cadmium exposure activated the MAPK pathway, as indicated by elevated P38 expression, which contributed to adrenal necroptosis. Discrepancies such as ubiquitination in protein translation processes were noted, resulting in partial protein degradation, showing a mismatch between mRNA expression and protein expression levels. Lv et al. (2021) stated that inflammation and necroptosis are inextricably linked, with necroptosis observed in non-alcoholic steatohepatitis (NASH) as TNF receptor 1 expression increases in liver tissue [23].

Additionally, Nicolè et al. (2021) highlighted that the NF-kB signaling pathway, often regarded as a classical inflammatory pathway, also induces necroptosis by activating RIPK1/RIPK3 [24]. Zhang et al. (2021) reported that lead contamination in chicken lymphocytes leads to necroptosis via the MAPK/NF-kB pathway [25]. This study showed that elevated NF-kB expression in cadmium-exposed groups contributes to the necroptosis process. Similarly, Ramesh et al. (2001) found that lead exposure activates genes in the MAPK/NF-kB pathway (ERK, JNK, P38, NF-kB, TNF-α), driving necroptosis [26]. Liu et al. (2022) further reported that cadmium contamination in chicken liver enhances the expression of genes like MLKL, RIPK1, RIPK3, ERK, JNK, and P38, leading to necroptosis [27].

Experimental results from this study on cadmium exposure revealed significant increases in P38 protein expression in the adrenal glands, indicating MAPK signaling pathway activation. Elevated levels of cytokines and chemokines are commonly observed in various liver diseases, particularly in severe liver failure. Activated macrophages may contribute to the production of these immune modulators. CD163, a member of the receptor family primarily expressed on activated macrophages, has a soluble form (sCD163) released by activated macrophages. Hiraoka et al. (2005) and Kazankov et al. (2022) identified elevated serum sCD163 levels in patients with severe liver failure and acute hepatitis, with levels exceeding those of healthy individuals. Serum sCD163 levels were highest in patients with severe liver failure, and a strong correlation between serum sCD163 levels and prothrombin time was observed ($r = -0.677$; $P<0.0001$). Previous studies indicated that products of activated macrophages might contribute to the pathogenesis of severe liver failure, and sCD163 may serve as a prognostic marker in the fibrosis associated with severe liver failure [28].

This study's findings on cadmium-induced necroptosis align with numerous observations of necroptosis in H&E staining results.

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