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Cadmium chloride exposure induces inflammation and necroptosis in rat kidneys, liver, and testis by increasing oxidative stress

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Abstract

This study examines how hydrogen peroxide (H₂O₂) and cadmium (Cd) bioaccumulate and how they work together to produce reactive oxygen species (ROS) in different male albino rat tissues. For 7, 15, and 30 days, the rats were exposed to 1 mM H₂O₂ and 5 mg/kg body weight of cadmium chloride. Using the proper analytical techniques, the levels of hydrogen peroxide and cadmium in the tissues of the kidney, liver, spleen, testes, and adrenal glands were determined. Additionally, flow cytometry was used to measure the production of ROS in liver and blood cells. The results demonstrated significant bioaccumulation ($P < 0.001$) of cadmium and hydrogen peroxide across different tissues. The highest accumulation of cadmium was observed in blood (approximately 50 µg/L on day 30), followed by liver, spleen, and kidney. Hydrogen peroxide accumulation was lower than that of cadmium but exhibited a similar pattern. In all tissues, the accumulation levels significantly increased with longer exposure duration. One-way ANOVA and LSD post-hoc tests were applied to compare means among different groups. Overall, the study's findings imply that hydrogen peroxide and cadmium, both separately and especially together, have serious harmful effects on rats. These consequences include elevated ROS production and bioaccumulation in different tissues. These results emphasize the significance of researching the combined effects of environmental pollutants and the necessity of taking into account their synergistic effects.

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

Introduction

Oxidative stress occurs when there is a reduction in antioxidant defense activity or an increase in free radical production in the body, potentially leading to tissue damage. The generation of free radicals and reactive oxygen species (ROS) in biological systems is inevitable; however, nature has designed antioxidant defense mechanisms to mitigate their harmful effects[1]. Under oxidative stress, various tissues, including the kidneys, lungs, heart, skin, brain, spinal cord, joints, gastrointestinal tract, eyes, blood vessels, and red blood cells, are affected. These tissues increase antioxidant defenses to counter oxidative stress, but severe stress can lead to cellular damage and ultimately cell death. Oxidative stress impacts cellular components such as DNA, membrane lipids, and proteins, resulting in structural and functional changes that may lead to various diseases, including diabetes mellitus, rheumatoid arthritis, cardiovascular diseases, asthma, malaria, cancers, and aging[2].

One proposed mechanism for cadmium-induced free radical production involves disrupting cellular antioxidant systems, such as glutathione (GSH), which is primarily located in the liver and serves as the first line of defense against cadmium-induced hepatic toxicity. In kidney cells, particularly in the proximal tubule epithelium, cadmium exposure leads to damage and the onset of chronic kidney disease. The precise mechanism underlying oxidative stress-induced cellular damage in kidney disease remains unclear[3]. Additionally, mitochondrial damage, which is a primary intracellular source of ROS, has been identified as a key target of cadmium[4]. Normally, free radicals are neutralized by antioxidant enzymes within the cell, but when mitochondrial efficiency declines (e.g., upon exposure to toxins like cadmium), energy production decreases, and free radical generation increases, leading to oxidative stress. Cadmium contributes to oxidative stress by reducing the activity of antioxidant enzymes such as superoxide dismutase, glutathione reductase, and glutathione peroxidase and by increasing lipid peroxidation[5].

Compared to innate immunity, adaptive immunity differentiates self-antigens from non-self-antigens. The role of adaptive immunity involves interaction between antigen-presenting cells and lymphocytes, with memory cells being another key feature of adaptive immunity. Memory lymphocytes are generated upon first exposure to a foreign antigen. Upon re-exposure to the same antigen, these long-lived memory lymphocytes respond more rapidly and robustly[6].

Various studies have investigated cadmium's effects on adaptive immune responses, with Wang et al. conducting one of the most relevant studies. This study examined the effects of CdCl₂ and another environmental pollutant, chlorpyrifos (CPF), on immune responses. The findings revealed that both CdCl₂ and CPF alter immune function, reducing splenic proliferation and function and significantly affecting cytokine production by splenocytes[7]. The study also indicated that these elements significantly reduce INF- γ production, with cadmium and CPF showing a time- and dose-dependent synergistic effect on adaptive immune suppression. Another study demonstrated that cadmium exposure induces oxidative stress and subsequent immune suppression in the kidneys of common carp[8].

Cadmium is hypothesized to reduce adaptive immune responses by inhibiting lymphocyte proliferation and function. Studies show that high cadmium concentrations (100 mM) can suppress lymphocyte proliferation, whereas lower doses (50 mM) stimulate lymphocyte proliferation in animal models[9]. Additionally, cadmium pollution has been shown to induce apoptosis in T cells. In another study, cadmium exposure reduced serum complement activity in gilthead seabream (*Sparus aurata*) while increasing leukocyte phagocytosis over time. Cadmium exposure did not significantly affect IgM antibody production[10].

Cadmium may alter thymocyte populations, with prenatal exposure causing an increase in double-negative (DN1) thymocytes. In female offspring, CD4⁺ T and CD8⁺ T populations increased following cadmium exposure, while NK cell and granulocyte percentages decreased. Additionally, splenic Treg cell populations decreased in both male and female offspring[11].

While most studies suggest cadmium acts as an immunosuppressant, some reports indicate cadmium may exhibit immunostimulatory properties. It has been proposed that cadmium could act as an adjuvant, inducing T and B cell proliferation and activation. Cadmium stimulated adaptive immune responses to antigens and increased TH2 responses, which may result in allergic reactions and hypersensitivity[12].

Research method

For the animal model, forty adult male Wistar rats, ages 8–10 weeks, weighing 200–250 grams, were purchased from the Medical Sciences Laboratory Animal Breeding Center. The animals were housed in polypropylene cages in the laboratory animal facility of Urmia University's Veterinary Faculty for a two-week acclimatization period. They were given unrestricted access to water and standard feed, and they were kept in a 12-hour light-dark cycle with a constant temperature of $25 \pm 2^\circ\text{C}$ and a relative humidity of $60 \pm 5\%$.

This experimental study was carried out at Urmia University's Medical Sciences Laboratory Animal Facility in compliance with international standards for the humane treatment of laboratory animals.

Lastly, xylazine and ketamine were used to put the rats to sleep, and cervical dislocation was used to end their lives. As soon as possible, tissues were gathered and placed in liquid nitrogen storage.

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.

Thus, 1.25 mg of cadmium chloride were needed for every 250-gram rat. Consequently, a precision balance was used to precisely weigh 12.5 mg of cadmium chloride. Next, 100 milliliters of double-distilled water were used to dissolve the weighed cadmium chloride. To guarantee that the cadmium chloride was completely dissolved, the mixture was gently swirled. The solution was refrigerated at 4°C for long-term storage.

Every day, two milliliters of the cadmium chloride solution were gavaged orally into each 250-gram rat.

On day 8, one day following the seventh treatment, on day 15, one day following the last treatment, and on day 30, fifteen days following the last treatment, sampling took place. After anesthesia with a 200 mg/kg dose of ketamine and 20 mg/kg of xylazine, the animal was sacrificed. A 23-gauge needle was used to puncture the heart and obtain blood samples. After wet digestion, the collected blood was separated into two portions: one was stored for analysis of toxic metals and bioelements, and the other was combined with an anticoagulant (EDTA) for hematological parameters. The anticoagulant-treated blood was centrifuged, the plasma was separated, and the other blood was collected in anticoagulant-free tubes for serum preparation; the plasma was frozen at -80°C and the serum at -20°C for redox and biochemical analysis, respectively. The organs under study (liver, spleen, kidney, testes, and adrenal glands) were separated into three sections: 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 analysis.

Tissue Homogenate Preparation:

Using a glass/Teflon homogenizer (Schütt homogenplus, Schütt Labortechnik GmbH, Göttingen, Germany), components of these organs (of known weight) were mixed 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) to create 10% homogenized tissues (weight/volume)[13]. The homogenates that were prepared for the SOD, GPx, and GR assays were centrifuged at 20,000 g for 30 minutes at 4°C using an MPW-350R centrifuge (Medical Instruments, Warsaw, Poland). Partially separated homogenates centrifuged for 20 minutes at 700 g (4°C) were used for additional measurements. Prior to analysis, the samples were frozen at -70°C .

Cadmium Bioaccumulation Analysis in Tissues:

Tissues (blood, liver, spleen, kidney, testes, and adrenal glands) were separated and stored at -20°C for cadmium bioaccumulation analysis. Tissue slices (0.02-0.15 g) were weighed, then digested with concentrated nitric acid (65% HNO_3) and hydrochloric acid (35% HCl) in a 9:1 ratio using microwave digestion. Samples were kept in an

oven at 80°C for 48 hours, and the dried materials were finely ground. The dried tissue powder was digested with nitric acid and hydrogen peroxide in a Teflon digestion vessel in the microwave. Excess acids were removed by gentle heating, then samples were diluted with distilled water and filtered through a 0.22 µm PTFE membrane filter.

The digestion solution was analyzed for metal accumulation by atomic absorption spectrophotometry (AAS) using a Hitachi Z-5000 atomic absorption spectrophotometer (Tokyo, Japan). The cadmium detection limit for tissue was 0.03 mg/kg, and the cadmium detection limit was 0.02 µg/L. Cadmium concentration was reported in µg per gram of wet tissue weight.

Oxidative Stress Assessment:

To assess oxidative stress levels, lipid peroxidation markers and cellular antioxidants were measured in serum and tissue samples using kits from Koushan Zist. Malondialdehyde (MDA) served as a lipid peroxidation indicator, and superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) were used as markers for antioxidant enzyme activity.

Results

Bioaccumulation of Cadmium and Hydrogen Peroxide in Tissues

The bioaccumulation of Cd and H₂O₂ in blood, liver, spleen, kidney, testes, and adrenal gland tissues of male albino rats was investigated over three time intervals: 7, 15, and 30 days post-treatment with 5 mg/kg body weight cadmium chloride and 1 mM H₂O₂. In the control group, the accumulation in all tissues was minimal, near zero. The mean cadmium levels increased significantly ($P < 0.001$) in treated rats compared to controls, with the highest cadmium concentration observed in blood (approximately 50 µg/L on day 30, $P < 0.001$). Liver, spleen, and kidney also showed notable accumulation ($P < 0.01$). Cadmium levels in most tissues significantly increased over time (from day 7 to day 30), with blood showing the highest accumulation. In rats treated with cadmium chloride for 15 days, cadmium concentration in the kidney (25.8 ± 0.3 µg) was higher than in the liver (20.3 ± 0.6 µg).

In the H₂O₂ group, the accumulation was comparatively lower than cadmium ($P < 0.05$), following a similar pattern but with less intensity across tissues. Time also influenced H₂O₂ accumulation, with levels rising over the exposure period. Generally, cadmium showed greater bioaccumulation in tissues than H₂O₂, with blood, liver, and kidney identified as primary accumulation sites for both substances, while tissues like the testes and adrenal glands had the lowest accumulation.

The synergistic effects of cadmium and hydrogen peroxide, indicative of their combined and intensified impact on bioaccumulation and cellular damage, revealed that cadmium and H₂O₂ interaction enhances tissue accumulation and exacerbates damage. This combination significantly elevated cadmium bioaccumulation across tissues and amplified harmful effects beyond those observed with each substance individually ($P < 0.001$) (Chart 1).

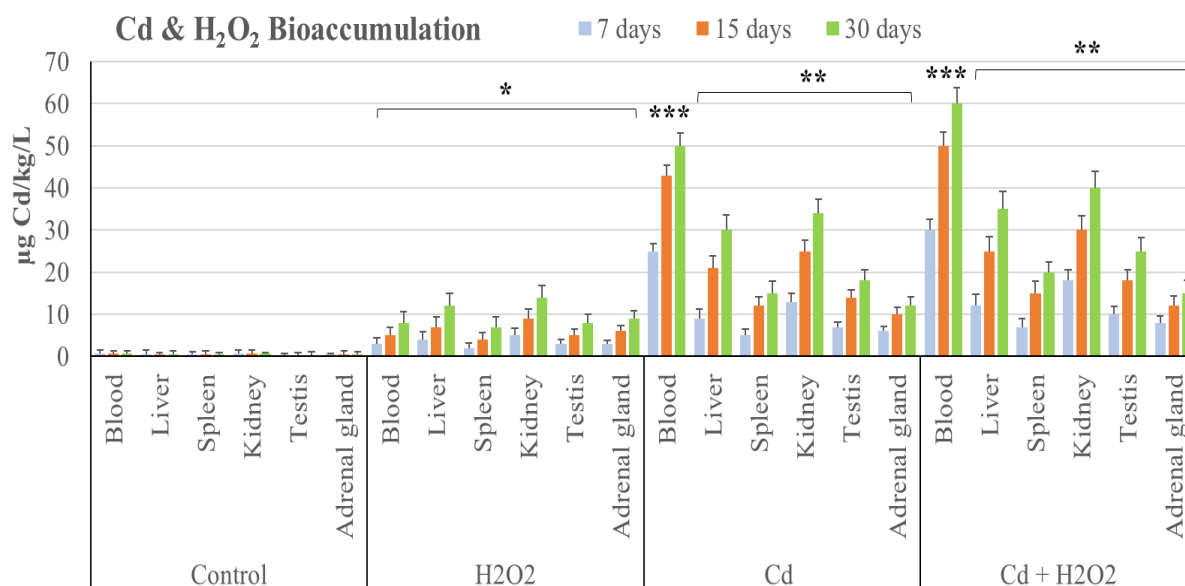


Chart 1. The bioaccumulation patterns of Cd and H₂O₂ in blood, liver, spleen, kidney, testes, and adrenal gland tissues of rats following exposure to 5 mg Cd/kg body weight and 1 mM H₂O₂ over 7, 15, and 30 days post-treatment. The chart highlights the varying levels of accumulation across these tissues, with blood displaying the highest cadmium concentration (up to 50 µg Cd/L by day 30) among all tissues assessed. In contrast, control groups maintained minimal baseline levels. The data reflect a time dependent accumulation, with overall concentrations rising from day 7 to day 30 after exposure. Statistically significant differences were observed compared to the control group, as analyzed by one-way ANOVA followed by the LSD post hoc test for paired comparisons.

ROS Level

The average fluorescence intensity (MFI) in untreated blood cells (MFI: 245), blood cells treated with cadmium chloride (MFI: 416), blood cells treated with hydrogen peroxide (MFI: 256), blood cells treated with both hydrogen peroxide and cadmium chloride (MFI: 1142), untreated liver cells (MFI: 303), liver cells treated with cadmium chloride (MFI: 856), liver cells treated with hydrogen peroxide (MFI: 206), and liver cells treated with both hydrogen peroxide and cadmium chloride (MFI: 805) is reported. The ROS assay results for blood and liver cells are shown in Figure 1.

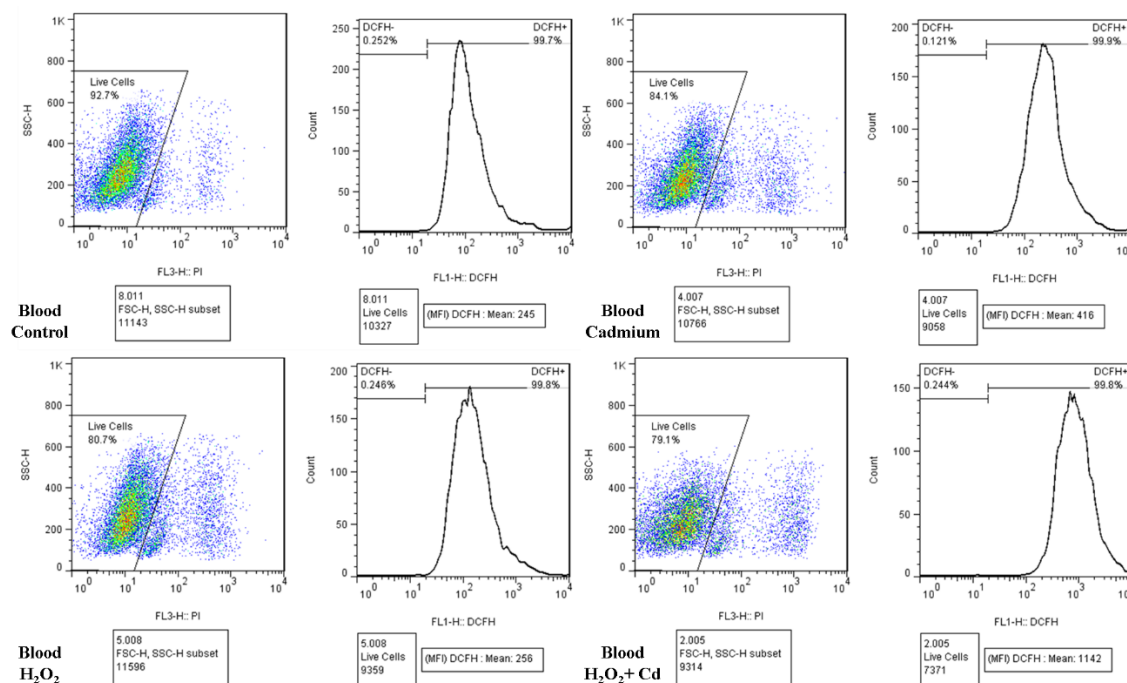


Figure 1. Flow Cytometry Analysis of ROS Production. MFI is reported in untreated blood cells (MFI: 245), blood cells treated with cadmium chloride (MFI: 416), blood cells treated with hydrogen peroxide (MFI: 256), blood cells treated with both hydrogen peroxide and cadmium chloride (MFI: 1142), untreated liver cells (MFI: 303), liver cells treated with cadmium chloride (MFI: 856), liver cells treated with hydrogen peroxide (MFI: 206), and liver cells treated with both hydrogen peroxide and cadmium chloride (MFI: 805).

In the control group, ROS production was at a baseline, natural level. In the H₂O₂ group, ROS production significantly increased, as hydrogen peroxide itself is a ROS that directly elevates oxidative stress. The cadmium group indirectly triggered ROS production, likely through mechanisms such as mitochondrial dysfunction and increased superoxide generation, though ROS levels in this group were lower than in the H₂O₂ group. In the Cd + H₂O₂ group, ROS production resulted from the combined effects of H₂O₂ and Cd, indicating a synergistic effect that exceeded the levels seen in either individual group. Consequently, ROS production followed the order: control < Cd < H₂O₂ < Cd + H₂O₂.

The ROS assay chart shows the percentage of live cells in blood and liver tissues under different treatments, including control, cadmium, hydrogen peroxide, and the combined Cd + H₂O₂ treatment. In the control group, a high percentage of cells remained viable, but the addition of cadmium and hydrogen peroxide sharply reduced cell viability. In particular, the Cd + H₂O₂ combination led to a further decline in live cells, underscoring the damaging, synergistic effects of these toxic substances, which increase active oxygen components and cause cellular damage (Chart 2).

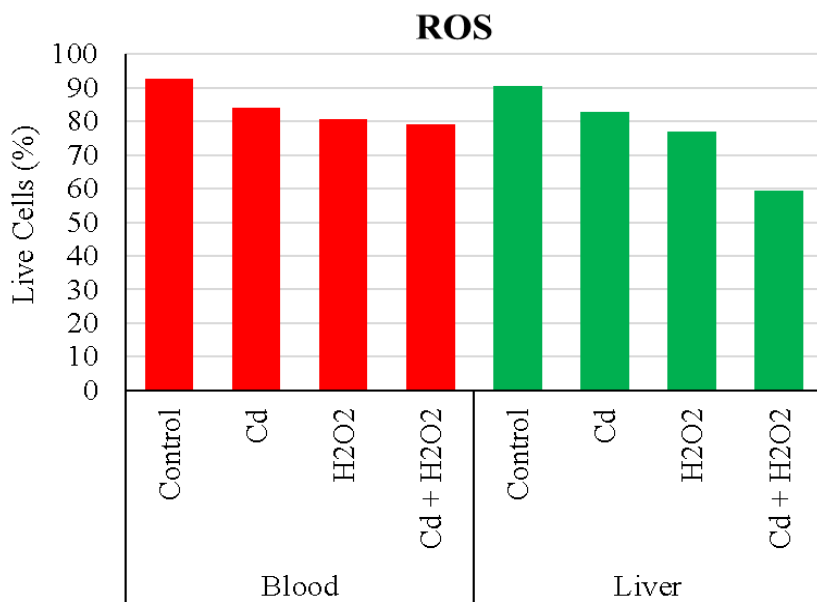


Chart 2: ROS assay depicting the percentage of viable cells in blood and liver tissues under control, Cd, H₂O₂, and combined Cd + H₂O₂ treatments. Results indicate a significant decrease in cell viability in groups treated with cadmium and hydrogen peroxide, particularly in the combined treatment group.

Effect of Cadmium and Hydrogen Peroxide on Serum Enzymatic Antioxidants

SOD Activity Levels

The activity of SOD enzyme in various tissues (blood, liver, spleen, kidney, testes, and adrenal glands) was examined under different conditions, including treatments with H₂O₂, cadmium, and a combination of Cd and H₂O₂. In the control group, SOD activity was highest across all tissues, with minimal variation over the time intervals of 7, 15, and 30 days. This stability indicates that, in normal conditions without oxidative stress, SOD activity remains relatively constant across tissues with no significant decline observed. In the H₂O₂ group, SOD activity decreased compared to the control, indicating oxidative stress induced by hydrogen peroxide. With increased exposure time (from 7 to 30 days), further reductions in SOD activity were noted in most tissues, reflecting the cumulative impact of oxidative stress (Chart 3).

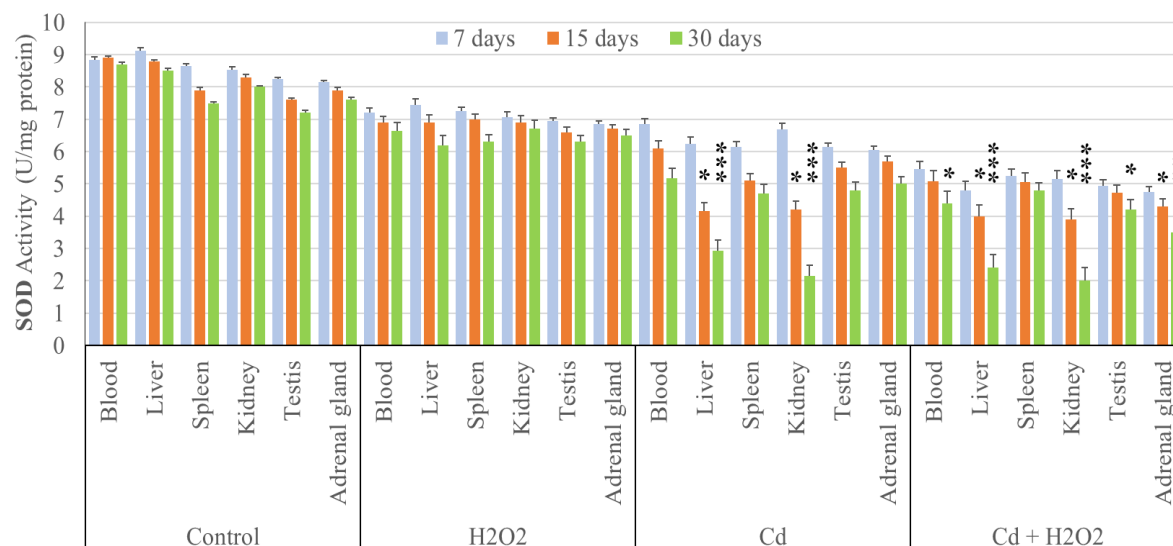


Chart 3. The activity levels of SOD enzyme in various tissues under different conditions (control, H₂O₂, Cd, and the Cd + H₂O₂ combination) over three time intervals: 7 days, 15 days, and 30 days. In the control condition, SOD activity is generally maintained across tissues. In contrast, treatments with H₂O₂, Cd, and the Cd + H₂O₂ combination show a marked decrease in SOD activity, especially in sensitive tissues like the liver and kidneys. Data is presented as mean $\pm$ standard error of the mean. Statistical analysis was conducted using one-way ANOVA, followed by Tukey's test, with n=3 per group and significance level ($p < 0.05$).

In the cadmium group, a significant reduction in SOD activity is observed, indicating cadmium's strong effect as a heavy metal and oxidative stress inducer. The time intervals (7, 15, and 30 days) clearly show that the longer the exposure to cadmium, the more the SOD activity in tissues significantly decreases, with this reduction being most pronounced in the liver and kidneys ($p < 0.001$). The group treated with the combination of Cd and H₂O₂ shows the greatest decline in SOD activity across all tissues, highlighting the synergistic oxidative effect of both agents. A substantial decrease in tissues, including the liver, kidneys, testes, and adrenal glands, is especially evident in the 30-day interval, where SOD activity reaches its lowest level ($p < 0.001$).

Catalase Activity Levels

Catalase activity varies across tissues, with the highest levels observed in the adrenal glands and the lowest in the testes. Over time, catalase activity decreases in all groups. H₂O₂ treatment increases catalase activity, while cadmium treatment decreases it. The combination of Cd and H₂O₂ results in a balanced effect but still leads to reduced catalase activity compared to the control group (Chart 4).

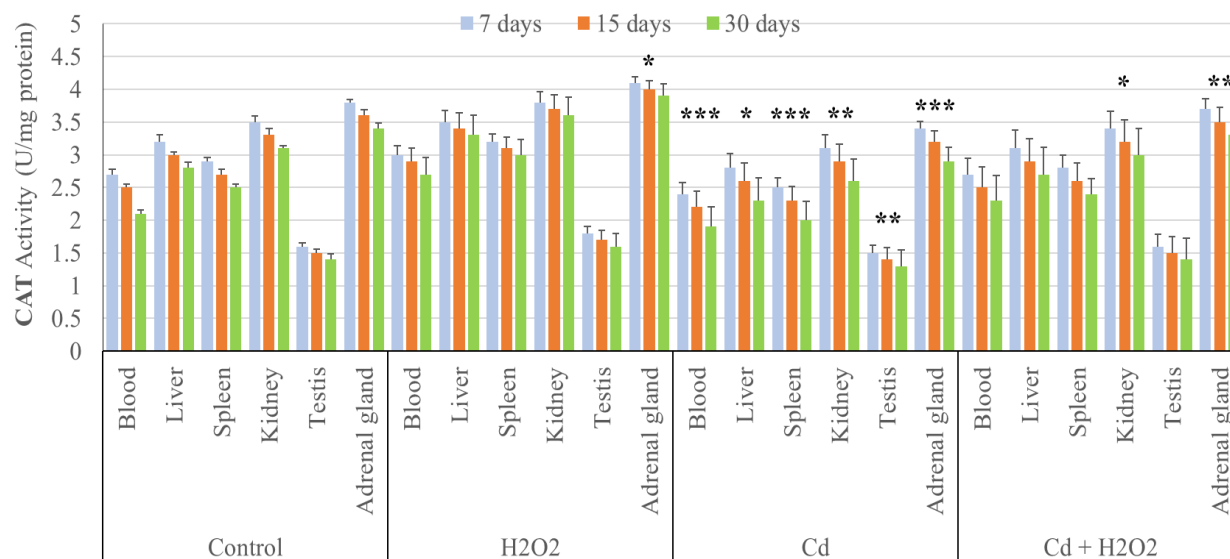


Chart 4. CAT enzyme activity in various tissues (blood, liver, spleen, kidney, testes, and adrenal glands) under control conditions and following exposure to H₂O₂, Cd, and the combined treatment (Cd + H₂O₂) across three time intervals (7, 15, and 30 days). Measurements are presented in units per milligram of protein (U/mg protein). Statistical markers (* and **) indicate significant differences at the 0.05 and 0.01 levels, respectively. Data are expressed as mean $\pm$ standard error of the mean (SEM). One-way ANOVA followed by Tukey's post hoc test was applied, with n=3 for each group and a significance threshold of $p < 0.05$.

As expected, catalase activity levels in the H₂O₂ group were higher than those in the control group since H₂O₂ promotes the production of antioxidant enzymes. Over time, there was a slight decrease compared to the control group. In the Cd group, catalase activity levels decreased in the blood and testes. This decline was also observed in other tissues, as cadmium is a toxic heavy metal that induces oxidative stress. Catalase activity levels in the combined treatment group fell between those of the Cd and H₂O₂ groups. The combined effect of these two agents was complex and varied depending on the tissue.

GPx Activity Levels

GPx activity was assessed in different tissues of rats under four experimental conditions (control, H₂O₂, Cd, and Cd + H₂O₂) across three time intervals (7, 15, and 30 days). In the control group, GPx activity remained relatively stable. In the H₂O₂ group, however, there was a significant increase in GPx activity, likely indicating a cellular antioxidant response to oxidative stress ($P < 0.01$). In contrast, cadmium treatment caused a substantial decrease in GPx activity, particularly in the liver and kidneys, possibly due to the toxic effects of cadmium on enzyme function ($P < 0.01$) (Chart 5).

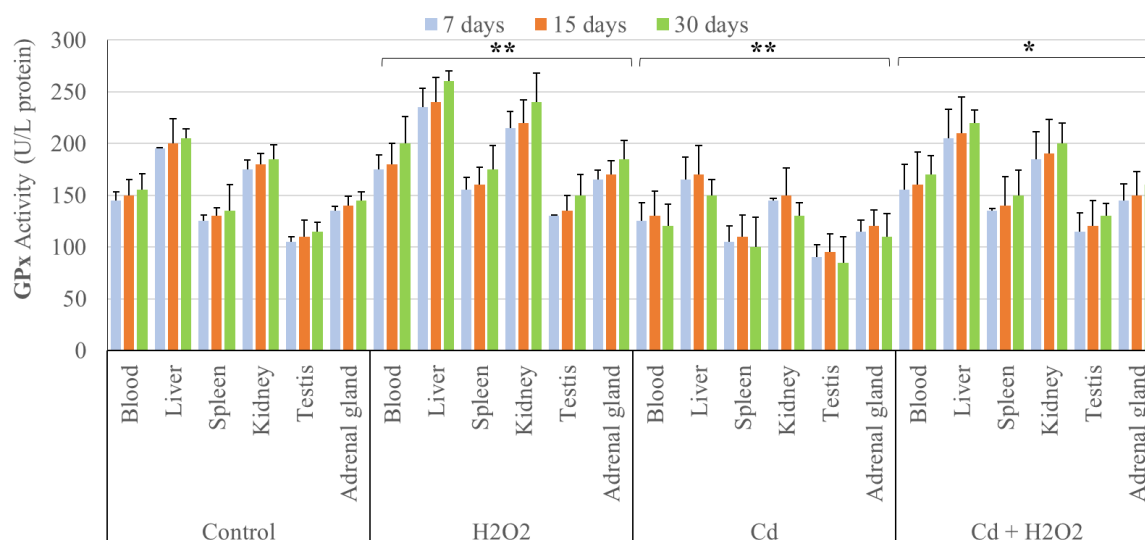


Chart 5. GPx activity in various tissues of rats treated with hydrogen peroxide, cadmium, and the combined treatment (Cd + H₂O₂), compared to the control group. Data are presented for three time intervals (7, 15, and 30 days), with error bars indicating standard deviation. Statistically significant differences between treated groups and the control are marked with * (P < 0.05) and ** (P < 0.01). GPx activity is expressed in units per liter per gram of protein (U/L protein). A one-way ANOVA followed by Tukey's post hoc test was conducted, with n=3 for each group and a significance level of p<0.05.

It is noteworthy that the combined treatment of cadmium and H₂O₂ exhibits a moderating effect, maintaining GPx activity at an intermediate level between the cadmium-only and H₂O₂-only groups, suggesting a complex interaction between these two agents. Over time, these patterns intensify, indicating cumulative effects of the treatments. Observed differences across tissues, especially the higher GPx activity in the liver and kidneys, likely stem from the significant role of these organs in detoxification and combating oxidative stress. Based on the chart data, most of these changes were statistically significant (P < 0.05).

Effect of Cadmium and Hydrogen Peroxide on Non-Enzymatic Antioxidant Status

Reduced Glutathione Levels

Changes in the levels of reduced glutathione (GSH) were assessed in Wistar rat tissues and measured in $\mu\text{mol/mg}$ protein. In the control group, GSH levels remained relatively stable. However, in the H₂O₂ group, while GSH levels decreased compared to the control (P < 0.05), a significant increase in GSH levels over time was observed in each tissue, likely reflecting a cellular defensive response to oxidative stress. In contrast, cadmium treatment resulted in a marked decrease in GSH levels, particularly in the liver and kidneys, likely due to GSH consumption for mitigating cadmium's toxic effects (P < 0.01) (Chart 6).

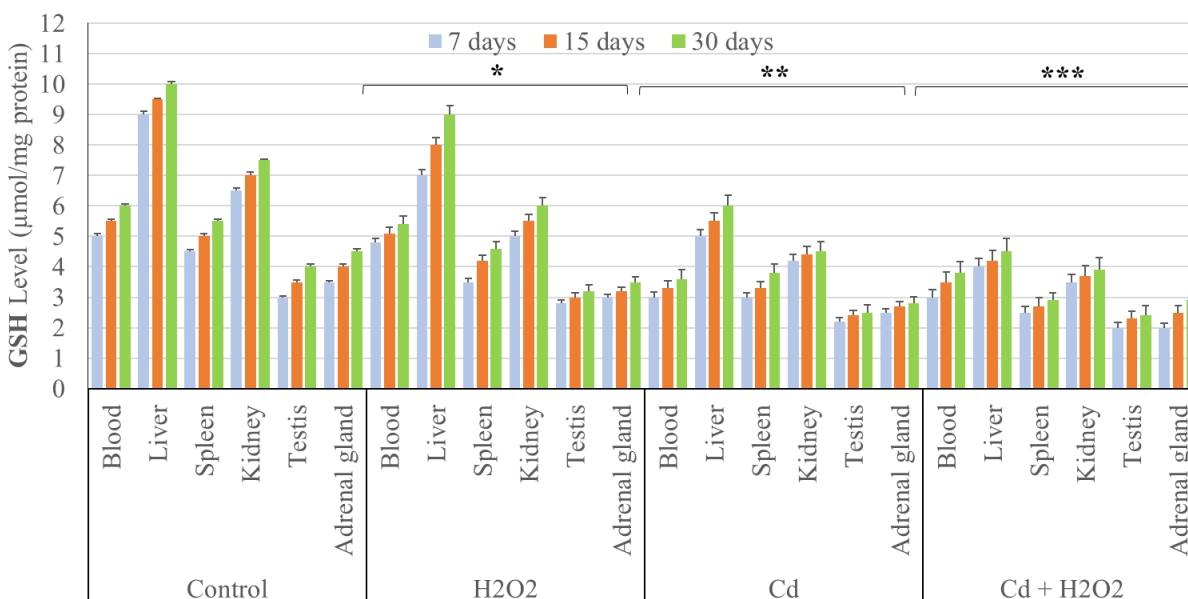


Chart 6. Glutathione (GSH) levels in various tissues of rats under four different conditions (control, H₂O₂, Cd, and Cd + H₂O₂) over three time intervals (7, 15, and 30 days). The H₂O₂ group exhibits a significant decrease in GSH levels across most tissues compared to the control. Over time, GSH levels increase in each tissue, likely indicating an adaptive response to oxidative stress ($P < 0.05$). Cadmium treatment results in a marked decrease in GSH levels, particularly in the liver and kidneys ($P < 0.01$). The combined Cd + H₂O₂ treatment further reduces GSH levels significantly across most tissues compared to the control ($P < 0.001$). Statistical indicators (*, **, and ***) represent significant differences at the 0.05, 0.01, and 0.001 levels, respectively. Data are presented as mean $\pm$ SEM, analyzed using one-way ANOVA followed by Tukey's test, with $n=3$ per group.

Interestingly, the combination of cadmium and H₂O₂ exhibits a moderating effect, maintaining GSH levels at an intermediate range between the cadmium-only and H₂O₂-only groups, indicating a complex interaction between these two agents. Over time, these patterns become more pronounced, suggesting cumulative effects of these treatments. The observed differences among tissues, particularly the higher GSH levels in the liver and kidneys, are likely due to the crucial role these organs play in detoxification. Most of these changes, as shown in the chart, were statistically significant ($P < 0.001$).

MDA Levels

The levels of MDA were measured in various tissues of Wistar rats following treatment with cadmium chloride (5 mg/kg) and hydrogen peroxide (1 mM), as well as the combined treatment. The data is presented as mean $\pm$ standard deviation ($n=3$). An increasing trend was observed across all tissues over time, with the highest MDA concentration found in the liver and the lowest in the adrenal gland. Liver and kidney tissues showed the most significant increases compared to other tissues. Measurements are reported in nanomoles per milligram of protein. A synergistic effect was observed in the combined treatment, where MDA levels exceeded the sum of the individual treatments. The highest increase was found in the liver, showing a similar tissue distribution pattern to the separate treatments but with higher intensity. A time-dependent increase was observed in all tissues, with the adrenal gland consistently displaying the lowest accumulation. Figure 4-6 illustrates the MDA levels in various tissues under different treatments.

Results indicated that exposure to cadmium (5 mg/kg) and hydrogen peroxide (1 mM) significantly elevated MDA levels in all examined tissues compared to the control. The combined treatment (Cd + H₂O₂) exhibited a synergistic effect, resulting in a more pronounced increase in MDA levels in all tissues than the individual treatments ($p < 0.001$). After 30 days of combined exposure, the liver exhibited the highest MDA level (8.94 ± 0.42 nmol/mg).

protein), followed by the kidney (8.15 ± 0.4 nmol/mg protein) and blood (7.25 ± 0.38 nmol/mg protein), while the adrenal gland showed the least increase (4.35 ± 0.3 nmol/mg protein) ($p < 0.05$ across tissues). A time-dependent analysis revealed a progressive increase in MDA levels in all tissues, with the most significant changes observed between days 7 and 30 ($p < 0.001$). Individual treatments with cadmium and hydrogen peroxide also showed significant time-dependent increases ($p < 0.01$), though lower than the combined treatment, with cadmium inducing a stronger oxidative stress than hydrogen peroxide alone ($p < 0.01$) (Chart 7).

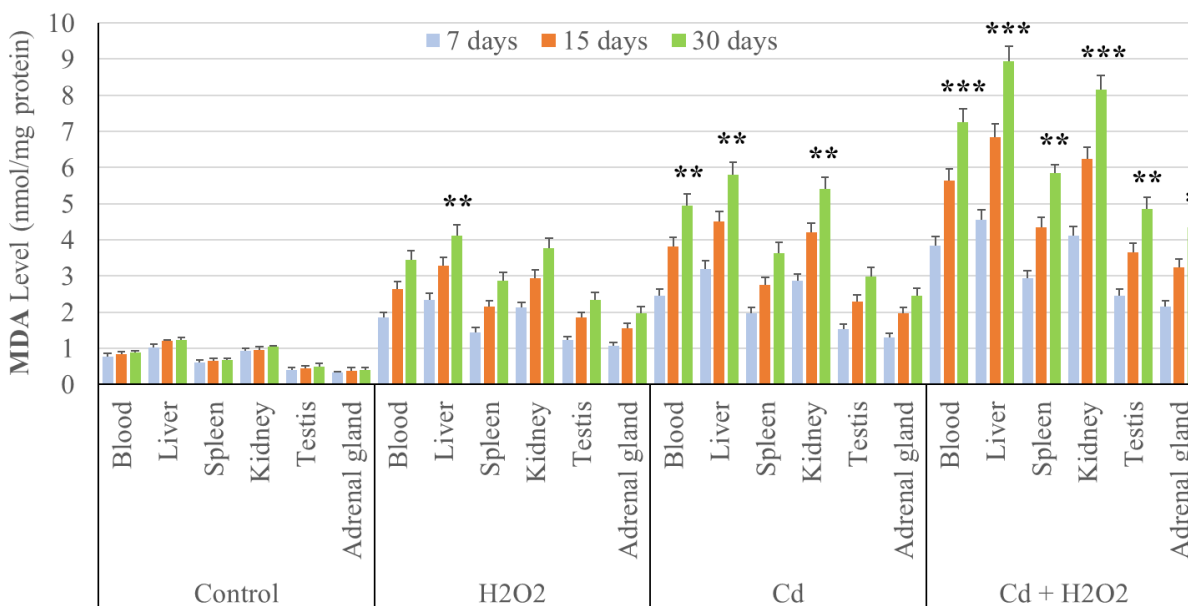


Chart 7. Tissue- and time-dependent changes in malondialdehyde (MDA) levels following exposure to cadmium (5 mg/kg), hydrogen peroxide (1 mM), and their combination in Wistar rats. MDA levels were measured in blood, liver, spleen, kidney, testis, and adrenal gland tissues at 7, 15, and 30 days post-treatment. The data shows varying oxidative stress responses across different tissues, with the liver and kidney exhibiting the highest sensitivity. The combined treatment (Cd + H₂O₂) demonstrated a synergistic effect, leading to significantly higher MDA levels compared to individual treatments. Data is presented as mean $\pm$ standard error of the mean. One-way ANOVA, Tukey's post hoc test, $n=3$ per group, with significance at $p < 0.05$.

Kidney and liver tissues from the rats were photographed at 400x magnification using light microscopy after H&E staining. The kidneys of the control rats displayed no abnormalities, with the proximal and distal convoluted tubules, renal cells, collecting ducts, and Henle's loop arranged normally. The tubular epithelial cells, nuclei, and glomeruli had a typical appearance. The glomerular capillary loops were thin and delicate, with endothelial and mesangial cells appearing morphologically normal, and the urinary spaces were clear.

In the cadmium-treated group (5 mg/kg), the kidneys appeared pale, showing tissue lesions in the medulla and cortex along with necrosis, dilation, and calcification in the tubules. Severe localized hemorrhage and degradation of the basement membrane occurred within the renal tissue. In the H₂O₂-treated group (1 mM), kidney structure was abnormal with a thin epithelial layer, narrowed lumen, and distorted brush borders in the microvilli. Renal parenchyma showed cellular infiltration and a few pyknotic nuclei in the tubular epithelial cells, indicating cellular necrosis. In the Cd + H₂O₂ group, severe necrosis and degeneration in the tubules were observed, with considerable distortion in Bowman's capsule glomeruli. There was significant loss of cellular integrity in the proximal and distal convoluted tubules, with disrupted nuclei, pale cytoplasm, and sparse chromatin.

The liver of the control rats appeared normal. However, in the cadmium-treated group (5 mg/kg), the liver showed vacuolation and degeneration in hepatocytes along with focal necrosis. In the H₂O₂-treated group (1 mM), the liver presented cytoplasmic infiltration, cellular inflammation, and damage to the central vein. Rats treated with Cd + H₂O₂ exhibited severe lesions, with a highly constricted central vein (Figure 2).

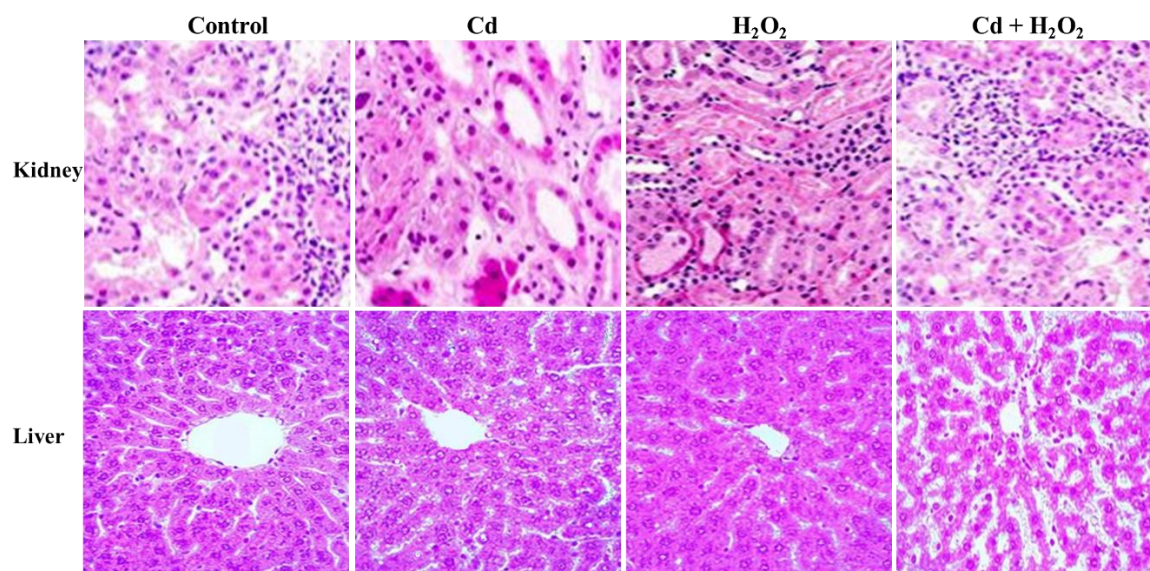


Figure 2. Photomicrographs of rat kidney and liver tissues stained with H&E, captured at 400x magnification using light microscopy. The kidney and liver in the control group exhibit normal structures. In the cadmium-treated group (5 mg/kg), kidney tissue displays lesions, including necrosis, tubular dilation, calcification, hemorrhage, and basement membrane damage, while the liver shows vacuolation and focal necrosis. In the H₂O₂-treated group (1 mM), the kidneys exhibit abnormal structure with cellular necrosis and disrupted brush borders in the microvilli, and the liver presents cellular inflammation, cytoplasmic infiltration, and central vein damage. In the Cd + H₂O₂ group, the kidneys undergo severe tubular necrosis and degeneration, with disrupted cellular integrity, while the liver exhibits more severe lesions and a constricted central vein.

discussion

Bioaccumulation Pattern

Cadmium shows a strong tendency for bioaccumulation across various tissues compared to H₂O₂, consistent with findings by Järup et al. (2008). After 30 days, the highest cadmium concentration was observed in the blood (around 50 µg/L), aligning with the results of Wang et al. (2019). The bioaccumulation order was as follows: blood > liver > spleen > kidney > testes and adrenal glands[14].

A significant increase in cadmium concentration over time, from day 7 to day 30, aligns with Liu et al. (2020), who reported time-dependent cadmium accumulation[15].

The combination of Cd and H₂O₂ resulted in heightened bioaccumulation with synergistic effects ($P < 0.001$), supporting findings by Zhang et al. (2021) on the enhancing effects of oxidative stress on heavy metal accumulation[16].

Selective accumulation was noted in specific tissues (blood, liver, and kidneys). By day 15, cadmium concentration in the kidneys (25.8 ± 0.3 µg) surpassed that in the liver (20.3 ± 0.6 µg), a pattern that corresponds with Chen et al. (2018), who highlighted the pivotal role of these organs in cadmium metabolism and excretion[17].

Potential mechanisms include increased blood accumulation due to its role in transporting these substances, while liver and kidney accumulation is likely attributed to their metabolic and excretory functions, consistent with Rodriguez et al. (2022) on cadmium toxicity mechanisms[18].

Gradual, sustained accumulation may lead to chronic damage. The observed synergistic effect underscores the clinical relevance of multiple exposures. These results align with clinical studies by Kim et al. (2023) regarding the chronic effects of cadmium exposure[19].

Limitations and recommendations include examining various doses to establish effect thresholds, conducting longer studies to assess chronic impacts, exploring molecular mechanisms of bioaccumulation and toxicity, and studying interactions with other environmental pollutants. Overall, this study highlights that cadmium and H₂O₂ bioaccumulation shows a time- and tissue-dependent pattern with notable synergistic effects, offering insights for health risk assessment and preventive strategies.

5.3. ROS Production

The ROS production in blood cells showed a 70% increase in the cadmium chloride group compared to the control, while the H₂O₂ group experienced a minor rise. However, in the Cd+H₂O₂ combined group, there was a striking 366% increase. These findings are consistent with Wang et al. (2021), who reported heightened ROS production in blood cells under cadmium exposure[20].

In liver cells, ROS production was at baseline in the control group. The cadmium chloride-treated group saw an ROS increase of 182%, whereas the H₂O₂ group experienced a decrease relative to the control. In the Cd+H₂O₂ group, ROS production rose by 166%, aligning with Liu et al. (2020) on liver cells' vulnerability to cadmium-induced oxidative stress[21].

The mechanisms likely involve cadmium's indirect ROS generation through mitochondrial disruption, superoxide production, and interference with antioxidant systems, as noted by Zheng et al. (2022)[22]. Conversely, H₂O₂ acts directly as a ROS, causing immediate oxidative stress, membrane impact, and apoptosis induction (Chen et al., 2019)[23].

The Cd+H₂O₂ group's substantial ROS increase and synergistic effects notably intensified cellular damage and reduced cell viability, paralleling Rodriguez et al. (2023)[24]. on the synergistic impacts of environmental pollutants. Tissue sensitivity variations may be due to inherent differences in blood and liver cells' susceptibility to H₂O₂ and unique patterns of cellular damage, consistent with Kim et al. 2021.[25]

Clinically, elevated ROS production under multi-exposure increases oxidative damage risk, highlighting the need for cellular protection against oxidative stress and addressing pollutants' cumulative effects. Park et al. (2023) emphasize the clinical importance of these findings[26].

Future research should explore ROS production in other tissues, examine a broader dose spectrum, and assess long-term impacts and molecular mechanisms.

Conclusion

This study showed that Cd and H₂O₂, separately and especially in combination, accumulate significantly in various tissues of male albino rats. The highest accumulation occurred in the blood, liver, and kidneys, with Cd showing greater accumulation than H₂O₂, and levels increased with prolonged exposure. Additionally, the Cd and H₂O₂ combination had a synergistic effect on bioaccumulation and cellular damage, significantly raising tissue levels of both substances. This synergy was associated with a notable increase in ROS production and decreased SOD enzyme activity, with a marked reduction in viable cell percentages in the blood and liver of the Cd+ H₂O₂ group, reflecting the severe toxicity of this combination.

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