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Evaluation of Cortisol and Heat Shock Proteins in Rabbits Under Cadmium-Induced Stress and Chelation Therapy

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Abstract. Background**:** Cadmium (Cd) exposure poses a significant environmental and occupational health concern, as it induces oxidative stress and disrupts cellular homeostasis. This study investigated the cadmium-induced stress response by examining its effects on cortisol and heat shock protein expression in rabbits, as well as the potential use of chelation therapy**.** Methods**:** Thirty-six New Zealand White rabbits were randomly divided into four groups (n=9) - control, cadmium exposure group (5 mg/kg $CdCl_2$ for 28 days), chelation therapy group (cadmium exposure and DMSA treatment at 50 mg/kg for 14 days), and DMSA group only. Serum cortisol levels were measured using an enzyme-linked immunosorbent assay (ELISA) method. The expression levels of heat shock protein 70 (HSP70) and HSP90 were evaluated using Western blot on liver and kidney tissue. Results**:** Cadmium exposure increased serum cortisol (48.3 ± 6.2 ng/mL vs. 22.1 ± 3.4 ng/mL in controls, $p<0.001$). Cadmium exposure also increased HSP70 and HSP90 in liver (3-fold and 2.8-fold, respectively) and kidney (2.9-fold and 2.5-fold, respectively) tissues. Furthermore, chelation therapy with DMSA reduced serum cortisol to 31.7 ± 4.8 ng/mL ($p < 0.01$) and somewhat reversed heat shock protein levels. Conclusion**:** Cadmium exposure is a significant source of physiological stress in rabbits, as evidenced by increased serum cortisol levels and post-exposure expression of heat shock protein (HSP). DMSA chelation therapy has therapeutic effects for reducing cadmium-induced stress response, and shows potential value as a treatment plan for cadmium poisoning patients.

Highlights:

1. Cadmium exposure significantly elevates cortisol and heat shock proteins, indicating systemic and cellular stress in rabbits.
2. DMSA chelation therapy partially reduces these stress markers, showing potential as a treatment for cadmium poisoning.
3. The correlation between cortisol and HSP expression suggests integrated biomarkers can assess cadmium-induced stress effectively.

Keywords: Cadmium Toxicity, Cortisol, Heat Shock Proteins, Chelation Therapy, DMSA

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Introduction

Heavy metal contamination is one of the most urgent environmental issues of our time, and cadmium (Cd) is a pollutant of particular concern due to its use in industry and toxicity [1]. Cadmium exposure can occur through various pathways, including contaminated water sources, agricultural products grown on contaminated land, tobacco, and occupational environments such as battery manufacturing, electroplating, and mining [2]. The World Health Organization has classified cadmium as a Group 1 carcinogen, emphasizing the need for a comprehensive understanding of cadmium's biological effects and practical therapeutic considerations [3].

Cadmium has a biological half-life in humans of between 10 and 30 years. This primarily occurs because cadmium has a high affinity for metallothionein proteins and preferentially accumulates in liver and renal tissue [4]. The long half-life of cadmium in living organisms enables chronic, low-level exposure to accumulate a significant tissue burden, leading to subsequent toxicity. The mechanism of cadmium toxicity is multifactorial, disrupting the homeostasis of essential metals, generating free radicals, altering protein structure and function, and modulating gene expression [5].

Cortisol is the primary glucocorticoid hormone in mammals and is an established and reliable marker of physiological stress. In this respect, cortisol has been widely used to assess the impact of environmental contaminants and stressors on health and other conditions [6]. Activation of the hypothalamic-pituitary-adrenal (HPA) axis after exposure to cadmium has been reported in numerous species, with elevated cortisol concentrations serving as an early indicator of cadmium-induced stress [7]. The association between cadmium exposure and cortisol increase appears to be dependent on dose and duration of exposure. With chronic cadmium exposure, it is theoretically possible that cadmium continues to activate the HPA permanently, resulting in long-lasting health effects [8].

Heat shock proteins, or HSPs, are a highly conserved group of molecular chaperones involved in protein folding, cellular and organismal responses to stress, and allowing cells to maintain homeostasis under extreme stress conditions [9]. HSP expression is rapidly upregulated in response to increased stress, like heavy metal exposure, making them ideal markers for cellular stress [10]. It is worth noting that among the families of HSPs, both HSP70 and HSP90 have generated a significant amount of research literature because of

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their central roles in protein quality control and their sensitivity to stress caused by heavy metals [11].

HSP70, a molecular chaperone of approximately 70 kDa, plays a crucial role in protein folding, the refolding of incorrectly folded proteins, and the prevention of protein aggregation under stress [12]. Cadmium exposure has been shown to upregulate the expression of HSP70 across multiple tissues, indicating that the cell's powerhouse is attempting to maintain protein homeostasis in response to cadmium's impact on protein damage [13]. HSP70 is upregulated through activation of heat shock factor 1 (HSF1), a transcription factor that activates and binds to heat shock elements in the promoter regions of HSPs [14].

HSP90, another member of the HSP family, is heavily involved in processes of protein maturation, steroid hormone receptor stability, and regulation of signaling pathways after cadmium exposure [15]. Similar to HSP70, cadmium exposure also impacts the expression of the HSP90 proteins. Similarly to HSP70, research has demonstrated tissue specificity and dose-dependent effects of HSP90 following cadmium exposure [16]. HSP90's dual role in both protective cellular responses and possible promotion of cadmium toxicity through protein misfolding makes it an attractive target for toxicology studies [17]. Only a few therapeutic options for cadmium poisoning have been developed, with anti-cadmium therapy primarily focusing on chelation therapy, which includes treatment with compounds that bind cadmium ions and ensure their elimination from the body [18]. Researchers have examined various chelating agents for cadmium detoxification, including ethylenediaminetetraacetic acid (EDTA), dimercaptosuccinic acid (DMSA), dimercaptopropanesulfonic acid (DMPS), and British Anti-Lewisite (BAL) [19]. Among the many agents, DMSA has received the most attention due to its excellent safety record, bioavailability, and effectiveness as a cadmium chelating agent, thereby reducing the cadmium burden on tissues [20]

DMSA (meso-2,3-dimercaptosuccinic acid) acts as a dithiol chelating agent that can readily form stable complexes with cadmium through its sulfur-containing functional groups [21]. DMSA chelates cadmium through a transient process, involving ring-like structures that encapsulate or enclose the cadmium ions [22]. DMSA reduces the bioavailability of cadmium to the cells and tissues, facilitating elimination through the urine. DMSA has shown efficacy administratively and therapeutically in the treatment of heavy metal

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poisonings and has undergone heavy investigation for protocols focusing on lead and mercury [23]

The rabbit has been commonly used in toxicological research due to its numerous benefits, including genetic similarity to humans, well-established physiological characteristics, and established protocols for most experimental procedures [24]. Rabbits exhibit cadmium accumulation and toxicity patterns similar to those of humans, making them suitable models for studying cadmium-induced stress responses and testing treatments [25]. As rabbits are much larger than rodent models, they also allow for much larger tissue samples and repeated blood draws, which are required for long-term studies [26]

Previous studies have shown that cadmium exposure in rabbits is associated with a variety of stress responses and cellular damage biomarkers [27]. We are aware of limited studies examining the combined cortisol and heat shock protein responses to cadmium exposure, as well as evaluating chelation therapy with endpoints that measure each stress response. The use of both endocrine and molecular biomarkers will provide a more holistic understanding of the mechanisms of cadmium toxicity and therapeutic responses [28].

When selecting suitable biomarkers to assess cadmium toxicity and treatment efficacy, several key attributes beyond the choice of the biomarker and its significance must be considered, including sensitivity, specificity, temporal responses, and the feasibility of quantification [29]. Undoubtedly, cortisol is a stress biomarker with a wealth of knowledge and standardization in measurement and interpretation [30]. Heat shock proteins also provide valuable insights into the stress response from a cellular perspective, or information about the biologically acute protein damage thought to occur with cadmium exposure [31]

The time course of biomarkers related to tobacco and treatment was considered in essential ways about study design and interpretation [32]. When stress is acute, elevated cortisol can shift quickly, but with chronic tobacco exposure, it can remain elevated for more extended periods [33]. Heat shock protein expression occurs shortly after exposure to stress, but whether it remains expressed will vary widely depending on the dose and duration of exposure to the stressor [34]

Cadmium-induced biomarker dose responses should be considered when designing

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studies that establish thresholds and treatment protocols [35]. Critical clinical information includes knowing the minimum effective dosage for a given treatment, understanding the daily dose-response relationship, and the duration of therapy before biological normalization of the biomarker can be ascertained [36-38]. The people trained in this profile should know about the business, computational tools, and statistical analysis and interpretation. Among then objectives of Information Science is to provide a means for making relevant information available to individuals, groups, and organizations involved with science and technology [39].

Research gaps remain, including the need for better-designed studies that evaluate multiple biomarker systems simultaneously, inadequate studies investigating optimal chelation treatment protocols, and a lack of knowledge on the time course of recovery measures after ceasing cadmium exposure and initiating treatment. Our study plan is to measure and report cortisol and heat stress protein responses to cadmium exposure and chelation treatment in a suitable rabbit model, advancing knowledge in this field.

Methodology

A. Experimental Design and Animal Selection

This study employed a randomized controlled experimental design conducted over 12 weeks. All experimental procedures were performed by the Guide for the Care and Use of Laboratory Animals [40]. Thirty-six healthy New Zealand White rabbits (Oryctolagus cuniculus), aged 3-4 months and weighing 2.0-2.5 kg, were obtained from a certified laboratory animal supplier and subjected to a 14-day acclimatization period before experimental procedures commenced.

Animals were housed individually in stainless steel cages (60 × 40 × 35 cm) within a temperature-controlled environment (22 ± 2°C) with 12-hour light/dark cycles and relative humidity maintained at 55 ± 10%. Standard laboratory rabbit pellets (Purina LabDiet 5321) and filtered water were provided ad libitum throughout the study period. Daily health monitoring included assessment of body weight, food consumption, behavioral observations, and clinical examination for signs of distress or illness.

B. Experimental Groups and Treatment Protocols

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Animals were randomly assigned to four experimental groups using a computer-generated randomization sequence, with nine animals per group to ensure adequate statistical power based on preliminary pilot studies. Group 1 (Control) received daily oral administration of distilled water (vehicle control) for 42 days. Group 2 (Cadmium exposure) received cadmium chloride ($CdCl_2 \cdot 2H_2O$, Sigma-Aldrich, >99% purity) dissolved in distilled water at a dose of 5 mg/kg body weight daily for 28 days, followed by distilled water for 14 days. Group 3 (Chelation therapy) received the same cadmium exposure protocol as Group 2, followed by meso-2,3-dimercaptosuccinic acid (DMSA, Sigma-Aldrich, >95% purity) at a dose of 50 mg/kg body weight daily for 14 days. Group 4 (DMSA-only) received distilled water for 28 days, followed by DMSA treatment for 14 days to assess potential direct effects of the chelating agent.

The cadmium dose was selected based on previous studies demonstrating significant toxicological effects without causing severe morbidity or mortality, representing a chronic low-level exposure scenario relevant to environmental and occupational exposure conditions [41]. The DMSA dose and treatment duration were determined based on established protocols for heavy metal chelation therapy in laboratory animals and scaled appropriately for rabbit physiology [36].

C. Sample Collection and Processing

Blood samples (3 mL) were collected from the central ear artery using 23-gauge needles and sterile syringes at baseline (day 0), day 14, day 28, and day 42 of the experimental period. All blood collections were performed between 8:00 and 10:00 AM to minimize the effects of circadian variation on cortisol measurements. Blood samples were allowed to clot at room temperature for 30 minutes, then centrifuged at 3,000 rpm for 15 minutes at 4°C. Serum was separated and stored in sterile microcentrifuge tubes at -80°C until analysis.

At study termination (day 42), animals were euthanized using intravenous pentobarbital sodium (100 mg/kg) following a 12-hour fasting period. Liver and kidney tissues were rapidly excised, weighed, and divided into portions for different analytical procedures. Samples for protein extraction were immediately frozen in liquid nitrogen and stored at -80°C.

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D. Cortisol Analysis

Serum cortisol concentrations were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit specifically validated for rabbit specimens (Rabbit Cortisol ELISA Kit, MyBioSource, San Diego, CA). The assay utilizes a competitive immunoassay principle with a detection range of 0.1-100 ng/mL and reported intra-assay and inter-assay coefficients of variation of <8% and <12%, respectively.

Serum samples were thawed at room temperature and vortexed gently before analysis. Duplicate measurements were performed for each sample according to the manufacturer's protocol. Briefly, 50 µL of standards, controls, and diluted samples (prepared at a 1:10 dilution in sample diluent) were added to antibody-coated microtiter plates, followed by 50 µL of cortisol-horseradish peroxidase conjugate. Plates were incubated at room temperature for 60 minutes with gentle shaking, followed by five washes with wash buffer. A substrate solution (100 µL) was added to each well and incubated in the dark for 15 minutes before the addition of the stop solution. Optical density was measured at 450 nm using a microplate reader (BioTek ELx800, Winooski, VT), and cortisol concentrations were calculated using a four-parameter logistic curve fitting model.

E. Protein Extraction and Western Blot Analysis

Total protein extraction from liver and kidney tissues was performed using RIPA lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with protease inhibitor cocktail (Sigma-Aldrich). Approximately 100 mg of tissue was homogenized in 1 mL of lysis buffer using a tissue homogenizer (Polytron PT-MR 2100, Kinematica AG) on ice. Homogenates were centrifuged at 12,000 rpm for 20 minutes at 4°C, and the supernatant was collected for protein quantification using the Bradford assay (Bio-Rad Protein Assay Kit).

Western blot analysis was performed using standard protocols with modifications for rabbit tissue analysis. Equal amounts of protein (30 µg per lane) were separated by SDS-polyacrylamide gel electrophoresis (10% acrylamide gels) and transferred to polyvinylidene fluoride membranes using a semi-dry transfer system. Membranes were

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blocked with 5% non-fat dry milk in Tris-buffered saline containing 0.1% Tween-20 (TBST) for 1 hour at room temperature.

The primary antibodies used were mouse monoclonal anti-HSP70 (1:1000, Cell Signaling Technology), rabbit polyclonal anti-HSP90 (1:1000, Abcam), and mouse monoclonal anti-β-actin (1:5000, Sigma-Aldrich), which served as a loading control. Membranes were incubated with primary antibodies overnight at 4°C, followed by three washes with TBST and incubation with appropriate horseradish peroxidase-conjugated secondary antibodies (1:5000) for 1 hour at room temperature. Protein bands were visualized using enhanced chemiluminescence detection reagent and quantified using ImageJ software (National Institutes of Health).

F. Statistical Analysis

Statistical analyses were performed using SPSS software version 26.0 (IBM Corporation, Armonk, NY). Data normality was assessed using the Shapiro-Wilk test, and homogeneity of variance was evaluated using Levene's test. Continuous variables are presented as the mean ± standard deviation (SD) for normally distributed data or as the median (interquartile range) for non-normally distributed data.

Between-group comparisons were performed using one-way analysis of variance (ANOVA), followed by the Tukey post-hoc test for multiple comparisons, when the data met the parametric assumptions. For non-parametric data, the Kruskal-Wallis test was used, followed by Dunn's post-hoc test. Within-group temporal changes were analyzed using repeated-measures ANOVA or Friedman's test, as appropriate.

Correlation analyses between cortisol levels and heat shock protein expression were performed using Pearson or Spearman correlation coefficients, depending on data distribution. Statistical significance was set at $p < 0.05$ for all analyses, and trends were noted for p-values between 0.05 and 0.10. Power analysis indicated that a sample size of nine animals per group provided 80% power to detect differences of 25% or greater in primary outcome measures with an α level of 0.05.

Results

A. Serum Cortisol Levels

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Cadmium exposure resulted in a significant elevation of serum cortisol levels throughout the treatment period compared to control animals. The temporal progression of cortisol changes is presented in Table 1. At baseline (day 0), all groups demonstrated comparable cortisol levels with no significant differences between groups ($p > 0.05$). Following 14 days of cadmium exposure, the cadmium group exhibited a notable increase in cortisol levels (35.2 ± 5.1 ng/mL) compared to the control group (23.4 ± 3.8 ng/mL, $p < 0.01$).

Table 1: Temporal Changes in Serum Cortisol Levels (ng/mL)

Group	Day 0	Day 14	Day 28	Day 42
Control	22.3 ± 3.2	23.4 ± 3.8	22.1 ± 3.4	21.8 ± 3.6
Cadmium	22.7 ± 3.5	35.2 ± 5.1**	48.3 ± 6.2***	46.9 ± 5.8***
Chelation	22.5 ± 3.1	34.8 ± 4.9**	47.1 ± 6.4***	31.7 ± 4.8*#
DMSA-only	22.1 ± 3.4	23.1 ± 3.9	22.8 ± 3.7	23.5 ± 4.1

*p < 0.05, **p < 0.01, ***p < 0.001 vs. control; #p < 0.01 vs. cadmium group at day 42

The peak cortisol elevation occurred at day 28, with the cadmium group reaching 48.3 ± 6.2 ng/mL, representing a 118% increase over control levels ($p < 0.001$). The chelation therapy group followed a similar pattern during the cadmium exposure phase, with comparable elevations at days 14 and 28. However, following the initiation of DMSA treatment, cortisol levels in the chelation group showed a significant reduction by day 42 (31.7 ± 4.8 ng/mL), although they remained elevated compared to controls ($p < 0.05$). The DMSA-only group-maintained cortisol levels comparable to those of the controls throughout the study period, indicating no direct stress effects from the chelating agent.

B. Heat Shock Protein Expression - Western Blot Analysis

HSP70 and HSP90 protein expression levels in liver and kidney tissues demonstrated significant upregulation following cadmium exposure, with partial normalization observed following chelation therapy. Quantitative Western blot results are summarized in Table 2.

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Table 2: Heat Shock Protein Expression Relative to β-actin (Fold Change vs. Control)

Tissue	Protein	Control	Cadmium	Chelation	DMSA-only
Liver	HSP70	1.00 ± 0.12	3.21 ± 0.45***	2.02 ± 0.31*#	1.08 ± 0.15
Liver	HSP90	1.00 ± 0.09	2.84 ± 0.38***	1.78 ± 0.24*#	0.96 ± 0.13
Kidney	HSP70	1.00 ± 0.14	2.91 ± 0.41***	1.89 ± 0.28*#	1.05 ± 0.16
Kidney	HSP90	1.00 ± 0.11	2.53 ± 0.35***	1.65 ± 0.22*#	0.98 ± 0.14

*p < 0.05, ***p < 0.001 vs. control; #p < 0.01 vs. cadmium group

In liver tissue, cadmium exposure induced a 3.21-fold increase in HSP70 expression and a 2.84-fold increase in HSP90 expression compared to controls (both $p < 0.001$). Chelation therapy resulted in a significant reduction of both proteins, with HSP70 decreasing to 2.02-fold that of the control ($p < 0.01$ vs. cadmium group) and HSP90 to 1.78-fold ($p < 0.01$ vs. cadmium group). Kidney tissue showed similar patterns, with cadmium-induced increases of 2.91-fold for HSP70 and 2.53-fold for HSP90, both of which showed a significant reduction following DMSA treatment.

C. Correlation Analysis

Significant positive correlations were observed between serum cortisol levels and the expression of tissue heat shock proteins. Cortisol showed strong correlation with liver HSP70 ($r = 0.782$, $p < 0.001$), liver HSP90 ($r = 0.745$, $p < 0.001$), kidney HSP70 ($r = 0.758$, $p < 0.001$), and kidney HSP90 ($r = 0.731$, $p < 0.001$). These correlations remained significant even when controlling for treatment group assignment, suggesting a fundamental relationship between systemic stress response and cellular protection mechanisms.

Discusion

The current investigation is strong evidence that DMSA chelation therapy reduces cadmium-induced stress responses in rabbits, evidenced by both systemic endocrine indicators and more localized cellular stress protein expression. Increases in serum cortisol following cadmium exposure confirm an increased level of hypothalamic-pituitary-adrenal (HPA) axis activation, a significant physiological response to heavy metal toxicity, as

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previously reported in various animal studies [42]. The progressive increases in cortisol levels throughout the exposure period indicated that cadmium-induced physiological stress is not subject to normal physiological adaptations, and as a result, likely means continued activation of the HPA axis, which has potentially harmful health implications both acutely and chronically.

The 118% increase in cortisol levels at peak exposure can be considered a significant physiological stress response in rabbits, comparable to that observed in severe or prolonged environmental stress and chronic disease states [43]. Such high levels of cortisol have been linked to immunosuppression, metabolic dysfunction, and an increased likelihood of secondary health complications in both laboratory animal settings and clinical populations [44]. The chronic elevation of cortisol throughout the exposure period without apparent physiological adaptation indicates the severity of as a result of cadmium-induced stress, and provides strong justification for therapeutic intervention.

The significant upregulation of liver and kidney heat shock proteins provides further strong evidence of cellular stress and activation of cellular cytoprotective mechanisms induced by cadmium exposure. The 3.2-fold and 2.8-fold increases in hepatic HSP70 and HSP90 expression, respectively, are all significant cellular-level responses to protein damage and misfolding resulting from cadmium exposure in rabbits [45]. These results are consistent with the general mechanisms of cadmium toxicity, namely, breaking protein structures through interactions with sulfhydryl groups and replacing essential metals from metalloproteins [46]. These results support the tendency for cadmium toxicity to preferentially impact liver and kidney tissues, which is consistent with existing knowledge regarding cadmium bioaccumulation and toxicity. The liver is the primary site of cadmium detoxification and excretion, facilitated by the synthesis of metallothionein. At the same time, the kidneys are the primary pathway for cadmium excretion and are also susceptible to cadmium toxicity [47]. The ongoing expression of heat shock proteins indicates an ongoing cellular stress response and a need for facilitation of protein quality control in cells with ongoing cadmium stress.

The established distribution or regional upregulation of heat shock proteins suggests a direct response to cadmium-mediated cellular damage, rather than a secondary response to more generalized systemic toxicity (48).

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The positive and protective effects shown by DMSA chelation therapy suggest possible clinical utility in cases of cadmium poisoning. The measurable positive changes in circulating serum cortisol concentrations following DMSA acute treatment represented a meaningful 34% reduction from peak (48.3 ng/mL to 31.7 ng/mL), which suggests a positive recovery of HPA axis function [48]. While cortisol levels showed persistently elevated levels compared to controls, the partial normalization demonstrates that chelation therapy can provide considerable benefit even after significant cadmium exposure has occurred .

The partial normalization of the stress protein response, specifically concerning heat shock proteins (HSP), following the administration of DMSA, suggests that there may be some level of cellular recovery due to ongoing and reduced molecular stress. The reduction in HSP70 response levels, from 3.21-fold to 2.02-fold over controls in liver tissue, represented a 37% decrease in response. Similarly, HSP90 showed a similar trend, with a 37% reduction from its peak.

These reductions suggest that DMSA treatment may have reduced the cadmium burden sufficiently to allow at least partial recovery of cellular homeostasis and a reduced reliance on stress response systems.

The strong correlations between serum cortisol and tissue HSP expression ($r = 0.731$-0.782) show evidence of coordinated responses among biological levels and support the concept of integrated stress response systems. The relationship strongly supports the potential of serum cortisol as a non-invasive biomarker for assessing stress on the cellular level due to cadmium exposure, particularly in both aspects of monitoring (decreased reliance on invasive tissue sampling) [49].

The dose and duration of DMSA treatment described in this study (50 mg/kg daily for 14 days) appeared to be well tolerated and effective, as indicated by the improvement of the previous biomarkers. The lack of a stress response in the DMSA-only group suggests that the observed therapeutic outcomes were due to cadmium chelation rather than the chelator's direct pharmacological effects, supporting the safety profile of DMSA for potential clinical use and indicating that the observed benefits were primarily due to cadmium elimination, rather than nonspecific effects. The presence of elevated levels of both cortisol and heat shock proteins following chelation therapy indicates that a full

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recovery may require longer treatment durations or combined treatments. The nature of cadmium elimination kinetics and tissue repair may require a longer intervention strategy to achieve normalization of the stress indicators [50]. Further exploration is warranted to determine the best duration of treatment and the potential merit of repeating the chelation cycle or adding antioxidants or other adjunctive agents.

The experimental model used in this study has provided significant insights into cadmium toxicity and the mechanisms of therapeutic response. However, there are limitations to acknowledge in the current study. Although a short acute exposure is the ethical standard for most current biomedical research, it may not reflect the chronic low-level cadmium exposure and absorption patterns that occur in human environmental and occupational exposures [51]. Similarly, although we attempted to monitor the animals once a week after recovery, the short follow-up period may have missed longer-term recovery or even delayed effects of cadmium exposure and treatment interventions.

The stress response biomarkers selected for use in this study were widely accepted measures of stress response. However, future work could explore the use of other stress-related factors or biomarkers, such as oxidative stress biomarkers, inflammatory cytokines, or tissue injury markers. The use of a combination of biomarkers would provide a richer picture of cadmium toxicity and treatment [52].

To facilitate the clinical translation of these findings, we should focus on the differences between species regarding cadmium metabolism, chelation pharmacokinetics, and stress response patterns. Clinical examinations will be required to determine effective dosages, treatment durations, and monitoring for DMSA chelation therapy applied to cases of cadmium poisoning [53]. Approval for the regulatory use of DMSA for chelation therapy will require multifaceted and continuous evidence of safety and efficacy for DMSA treatment, delivered through controlled clinical trials.

The implications of this research for environmental and public health go beyond individual treatment protocols and treatment occurrence to provide a foundation for prevention and exposure limit recommendations. The sensitivity of the biomarkers measured in response to cadmium implies they may also have utility in environmental monitoring programs and occupational health tracking systems [54]. Early indications of stress response induced by cadmium provide opportunities for early prevention and monitoring of responses,

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potentially before identifiable toxic symptoms.

Conclusion

This study highlighted how cadmium exposure can induce considerable physiological stress in rabbits, as evidenced by elevated serum cortisol and significantly elevated heat shock proteins (HSP70 and HSP90) in liver and kidney tissues. Overall, the strong relationships between systemic stress markers and systemic cellular responses indicate that protective portals were activated and stress response was coordinated across biological levels. Evidence also suggested that DMSA chelation therapy could successfully mitigate the cadmium stress response in part, as evidenced by serum cortisol and heat shock protein levels following treatment. The therapeutic effectiveness of DMSA treatment in exercising some degree of efficacy supports future clinical studies regarding the use of DMSA in clinical scenarios involving heavy metal poisoning; however, there is a possibility that patients may require several extension treatment protocols or combined interventions with other modalities for full normalization to occur .

This work has provided foundational knowledge regarding cadmium's mechanisms of toxicity and therapeutic intervention approaches, laying the groundwork for future clinical efficacy studies. Specifically, this study aims to establish evidence-based treatment protocols for heavy metal exposure. Moreover, this study has demonstrated the utility of using both endocrine and cellular/molecular biomarkers, as well as stress responses, to take an integrated approach to establishing a comprehensive assessment for cadmium toxicity and therapeutic interventions. This represents a valuable framework for various contexts, including clinical and environmental health.

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