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PROTECTIVE EFFECTS OF SELENIUM AND ZINC AGAINST ENVIRONMENTAL HEXAVALENT CHROMIUM-INDUCED OXIDATIVE, MITOCHONDRIAL, AND ENDOCRINE TOXICITY IN PREGNANT RATS

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ABSTRACT

This investigation aimed to evaluate the toxic effects of potassium dichromate ($K_2Cr_2O_7$) on the adrenal glands of pregnant rats and the potential of selenium (Se) and zinc chloride ($ZnCl_2$) supplementation in ameliorating these deleterious impacts. Seventy pregnant Albino Wistar rats (180–250 g) were divided into five groups (n=14 per group) and received a single subcutaneous (s.c.) treatment on the third day of gestation: Group I (Control: 0.9 % NaCl); Group II ($K_2Cr_2O_7$: 10 mg/kg); Group III ($K_2Cr_2O_7$ + Se: 0.3 mg/kg); Group IV ($K_2Cr_2O_7$ + $ZnCl_2$: 20 mg/kg); and Group V ($K_2Cr_2O_7$ + Se + $ZnCl_2$). On the 20th day of gestation, hormonal profiles, oxidative stress biomarkers, mitochondrial dynamics, and histomorphometric parameters were assessed. Our results demonstrated that $K_2Cr_2O_7$ exposure significantly elevated plasma adrenocorticotropic hormone (ACTH) levels and increased both absolute and relative adrenal gland weights. In the adrenal mitochondria, chromium induced a sharp increase in mitochondrial swelling, mitochondrial permeability transition pore (mPTP) opening, and malondialdehyde (MDA) levels. Conversely, plasma cortisol levels and reduced glutathione (GSH) content were significantly diminished, while the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST) were markedly altered. Histopathological and histomorphometric examinations further confirmed significant structural remodeling of the adrenal architecture. Notably, supplementation with selenium and/or zinc demonstrated a robust protective action, modulating the harmful effects induced by $K_2Cr_2O_7$ through the restoration of antioxidant homeostasis and the preservation of mitochondrial and endocrine integrity.

Keywords: Hexavalent chromium, Selenium, Zinc, Adrenal gland, Oxidative stress, Mitochondria

INTRODUCTION

Among the endocrine organs, the adrenal gland is uniquely sensitive to xenobiotics and is frequently identified as the primary target for endocrine-related lesions **(Egalini *et al.*, 2022)**. This heightened vulnerability to environmental pollutants stems from several specialized physiological features. Adrenal cell membranes are rich in unsaturated fatty acids, rendering them highly susceptible to lipid peroxidation. Furthermore, the gland's robust vascularization, coupled with its lipophilic nature due to high concentrations of cholesterol and steroid hormones, significantly increases its exposure to and reactivity with lipophilic toxic agents **(Savici *et al.*, 2021).**

During pregnancy, adrenal hormones specifically glucocorticoids are indispensable **(St-Jean *et al.*, 2020)**. Maternal glucocorticoids regulate the hypothalamic-pituitary-adrenal (HPA) axis and support the metabolic and immunological adaptations necessary for a healthy pregnancy **(Clifton *et al.*, 2017)**. In the fetus, these steroids act as critical maturation signals that accelerate organ development, particularly the production of pulmonary surfactant and cardiovascular maturation, thereby preparing the neonate for extrauterine life **(Rog-Zielinska *et al.*, 2014; Pofi and Tomlinson, 2020).** Additionally, glucocorticoids contribute to the initiation of labor by modulating local endocrine activity at the fetoplacental junction **(Edwards, 2024)**.

Chromium (Cr) is a pervasive environmental pollutant and a major public health concern for both humans and animals **(Kapoor *et al.*, 2022)**. It is extensively utilized across diverse industrial sectors, including electroplating, leather tanning, steel production, and the manufacture of pigments and wood preservatives **(Mondal *et al.*, 2021)**. Exposure to hexavalent chromium [Cr (VI)] is associated with severe systemic pathologies, including hematotoxicity **(Ray, 2016)**, genotoxicity, immunotoxicity **(Hong *et al.*, 2024)**, nephrotoxicity **(Fedala *et al.*, 2021)**, neurotoxicity **(Zhang *et al.*, 2024)**, and reproductive toxicity **(Saouli *et al.*, 2023)**. Specifically, Cr (VI) is known to disrupt adrenal gland function **(Savici *et al.*, 2021)**. Hexavalent chromium enters the intracellular environment via anionic transport systems intended for sulfate and phosphate compounds **(DesMarais and Costa, 2019)**. Once inside the cell, Cr (VI) is rapidly reduced to Cr (III) by reducing agents such as ascorbate and glutathione. This reduction process generates significant quantities of reactive oxygen species (ROS) and unstable intermediates [Cr (V) and Cr (IV)], which trigger oxidative damage **(Sawicka *et al.*, 2021)**. Because mitochondria are the central hubs for ATP production, redox equilibrium, and the regulation of apoptosis **(González-Arzola and Díaz-Quintana, 2023)**, they are particularly vulnerable to this Cr-induced oxidative assault.

Endocrine disruptor chemicals, such as hexavalent chromium **(Philippat *et al.*, 2017)**, are exogenous environmental molecules which interfere with biosynthesis, secretion, transport, metabolism, binding, receptors, action, and catabolism of the natural hormones in the body **(Solecki *et al.*, 2017)**. Prenatal Cr (VI) exposure disrupts steroidogenesis in F1 progeny rats by repressing estrogen receptors, decreasing the specific activities of steroidogenic enzyme proteins **(Navin *et al.*, 2021)**. The dosages of cortisol were carried out on the 6th day during the start of implantation and on the 20th day of gestation, one day before parturition to understand a major mechanism underlying pathological in the endocrine system.

The maintenance of intracellular oxidative homeostasis relies on enzymatic defense systems including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) as well as non-enzymatic molecules like reduced glutathione (GSH) **(Amini *et al.*, 2023)**. Essential trace elements, notably Selenium (Se) and Zinc (Zn), are fundamental components of these antioxidant defenses **(Rahman *et al.*, 2019)**. Selenium is integral to the function of selenoproteins such as GPx, which works synergistically with CAT to neutralize ROS and protect against DNA damage **(Yildiz *et al.*, 2019; Shahidin *et al.*, 2025)**. Furthermore, Se influences the activity of the HPA axis and other endocrine glands **(Köhrle, 2016)**. Similarly, Zinc is a vital cofactor for numerous enzymes and regulates metallothionein levels. It protects thiol groups, inhibits mitochondrial lipid peroxidation, and preserves the structural integrity of cell membranes **(Maret, 2019)**. Zinc is also essential for HPA axis metabolism and has been shown to restore mitochondrial membrane potential while exerting anti-apoptotic effects against Cr-induced toxicity **(Takeda *et al.*, 2016; Zhang *et al.*, 2018; Fedala *et al.*, 2021)**.

A dietary level of 0.2–0.25 mg Se/kg diet was necessary to maintain tissue Se concentrations and GSHPx activities during pregnancy **(Sunde and Raines, 2011)**. also showed that when selenium is supplied as selenite, the minimal requirement during pregnancy and lactation is at least 0.4 mg Se/kg diet. If the dietary source of selenium is selenate or selenomethionine, the need could be reduced **(Wang *et al.*, 2020)**. Studies by **Su *et al.* (2015)** and **Li *et al.* (2022)** showed that in rats

receiving selenium as selenite or selenate supplemented at 0.5 mg/kg diet, liver toxicity was found.

Tupe *et al.* (2010) showed that 0.5-10 mg Zn/kg of diet was required to prevent nutritional liver injury in rats, but this does not represent a nutritional requirement. The requirement for optimal growth of the laboratory rat recommended by the National Research Council **(National Research Council, 1978)**, based on the studies of **Yu *et al.* (2019)**, and is approximately 12 mg Zn/kg in the basal purified diet. This level was found to be the minimum dietary zinc concentration necessary to achieve maximal activity of zinc-dependent enzymes such as glutathione reductase. This dose was not sufficient and does not provide optimally adequate Zn nutrition during reproduction; 20 mg/kg would be optimal **(Lim *et al.*, 1996).** This is the standard reference used in laboratory animal nutrition.

Although the antioxidant properties of zinc and selenium are well established, their comparative protective effects on adrenal gland structure and function in pregnant rats under toxic metal exposure remain insufficiently characterized. The present study addresses this gap by providing a comprehensive evaluation of zinc- and selenium-mediated protection against potassium dichromate–induced adrenal toxicity. By integrating histological and histomorphometric analyses with assessments of adrenal hormone production, oxidative stress biomarkers, and mitochondrial swelling and membrane permeability in pregnant Wistar albino rats, this work offers novel insights into trace element–mediated preservation of adrenal mitochondrial integrity and endocrine homeostasis.

MATERIALS AND METHODS

Chemical products

All chemicals utilized in this investigation were of high analytical grade. Potassium dichromate ($K_2Cr_2O_7$) (CAS No: 7778-50-9, 99% purity), zinc chloride ($ZnCl_2$) (CAS No: 7646-85-7, 99.99% purity), elemental selenium (Se) (CAS No: 7782-49-2, 99.99% purity), and organic solvents, including diethyl ether, were obtained from Sigma-Aldrich (Chemistry GmbH, Taufkirchen, Germany). Stock solutions of $K_2Cr_2O_7$, $ZnCl_2$, and Se were prepared in sterile saline (0.9% NaCl), with the pH adjusted to 7.5 as required, following the protocol described by **Adjroud (2013)**.

Biochemical reagents were procured from Sigma-Aldrich (Steinheim, Germany), including: thiobarbituric acid (TBA), trichloroacetic acid (TCA), phosphate-buffered saline (PBS), Tris-buffered saline (TBS), bovine serum albumin (BSA), 1-chloro-2,4-dinitrobenzene (CDNB), butylated hydroxytoluene (BHT), nitroblue tetrazolium (NBT), ethylenediaminetetraacetic acid (EDTA), methionine, riboflavin, 5,5-dithiobis-2-nitrobenzoic acid (DTNB), reduced glutathione (GSH), sulfosalicylic acid, hydrogen peroxide (H_2O_2), hydrochloric acid (HCl), and calcium (Ca^{2+}).

Histological Reagents for tissue processing and staining, including formalin, paraffin, ethanol, xylene, eosin, and hematoxylin, were purchased from Merck KGaA (Darmstadt, Germany).

Animals

All experimental procedures were conducted in strict accordance with institutional and international ethical guidelines for animal research **(NRC, 2011)**. Seventy healthy adult female Wistar Albino rats, weighing 180–250 g and aged 12 weeks, were obtained from the Pasteur Institute (Algiers, Algeria).

The animals were housed in a controlled laboratory environment characterized by a temperature of 23 ± 1°C, a relative humidity of 55 ± 5%, and a 12-hour light–dark cycle. Prior to the commencement of the experiment, the rats underwent a two-week adaptation period. Throughout the study, they were provided with distilled water and standard pelleted food (ONAB, Bejaia, Algeria) ad libitum.

For breeding, virgin female rats were housed overnight with healthy adult males at a ratio of five females to two males. Mating was confirmed the following morning by the presence of spermatozoa in the vaginal smear, which was designated as gestation day 0. In this breeding colony, the average gestation period was recorded as 21 days **(Adjroud and Mouffok, 2009).**

Study design

The study was conducted over a 20-day gestational period, representing subchronic exposure. A total of seventy pregnant rats were randomly assigned to five experimental groups (n = 14 per group).Since each animal has two adrenal glands, a total of 28 adrenal glands were obtained per group. For each type of analysis (biochemical, mitochondrial, oxidative, histological, and histomorphometric), six adrenal glands (n = 6) were selected and used as independent biological replicates. This selection was made to ensure technical feasibility, measurement accuracy, and reproducibility of results between the different trials. Statistical analyses were performed on the data from these six biological replicates per group (n = 6). This subset was selected to ensure technical precision and consistency across all quantitative and qualitative assays.

The preimplantation stage, which occurs during the first 4–5 days after fertilization and culminates in the formation of the blastocyst capable of uterine implantation **(Marikawa and Alarcón, 2012; Molè *et al.*, 2020)**, represents a critical window in early embryonic development. Therefore, in the present study, a single dose of the respective treatments was administered to pregnant rats on the third day of gestation, corresponding to the preimplantation phase, one day before implantation. All subsequent statistical analyses were performed using data obtained from six biological replicates per group.

The experimental groups were defined as follows:

- Group I (Control): Received sterile saline (0.9% NaCl) as a vehicle control.
- Group II ($K_2Cr_2O_7$): Administered 10 mg/kg bw of potassium dichromate. The dosage was selected based on established models of gestational chromium toxicity **(Adjroud, 2010)**.
- Group III (Se + $K_2Cr_2O_7$): Co-administered 0.3 mg/kg bw of elemental selenium and 10 mg/kg bw of $K_2Cr_2O_7$, This elemental Se dosage was selected based on our earlier research **(Adjroud 2010)**, and other earlier research has antagonized the reprotoxic effects of nickel in male and female rats and their progeny **(Käkelä *et al.*, 1999)**.
- Group IV (Zn + $K_2Cr_2O_7$): Co-administered 20 mg/kg bw of zinc chloride ($ZnCl_2$) and 10 mg/kg bw of $K_2Cr_2O_7$. This dose was selected for its proven efficacy in mitigating heavy metal-induced oxidative damage **(Paksy *et al.*, 1997; Salah *et al.*, 2022).** This dose was required to obtain adequate Zn nutrition during reproduction **(Lim et al 1996).**
- Group V (Zn + Se + $K_2Cr_2O_7$): Received a triple-combination dose of 20 mg/kg bw $ZnCl_2$, 0.3 mg/kg bw Se, and 10 mg/kg bw $K_2Cr_2O_7$.

The sample size was selected based on technical feasibility and the high degree of consistency observed across biological measurements.

Laboratory analyses and specimen collection

To mitigate distress and ensure immobilization, pregnant rats were briefly sedated with diethyl ether inhalation. On gestational days 6 and 20, blood samples were promptly collected via jugular vein puncture into heparinized tubes (for cortisol analysis) and EDTA-coated tubes (for ACTH analysis). Following centrifugation at 1500 × g for 15 minutes at 4°C, the plasma was aliquoted into Eppendorf tubes and stored at -20°C until hormonal quantification.

On gestational day 20, animals were euthanized via a diethyl ether overdose. The adrenal glands were rapidly excised and immediately immersed in ice-cold physiological saline (0.9% NaCl) to remove residual blood. The glands were meticulously cleared of periadrenal adipose tissue and weighed. One gland was fixed in 10% neutral buffered formalin for histological examination, while the contralateral gland was either processed immediately for mitochondrial swelling and membrane permeability assays to ensure organelle viability or cryopreserved at -80°C (or -20°C) for subsequent oxidative stress biomarker analysis.

Histological examination

Immediately following excision, adrenal glands were fixed in 10% neutral buffered formalin (NBF) for 24 hours to preserve structural integrity. The tissues underwent a standardized histological processing sequence, including automated dehydration in a graded series of ethanol, clearing in xylene, and infiltration with paraffin wax. The resulting tissue blocks were sectioned at a thickness of 5 µm using a rotary microtome.

The sections were subsequently stained with Hematoxylin and Eosin (H&E) to visualize general tissue morphology and cellular architecture, following the protocols described by **Bancroft and Gamble (2008)**. Histological assessment was conducted using an advanced light microscopy system (ZEISS Axioscope, Göttingen, Germany). Slides were systematically inspected at multiple magnifications (10×, 40×, and 100×) to evaluate both the overall zonal organization of the adrenal cortex and the specific cytological details of the medullary and cortical cells.

Histomorphometric study

A rigorous histomorphometric analysis was conducted to quantify the structural alterations within the adrenal architecture. For each animal, six representative sections per adrenal gland were evaluated. Within each section, 20 non-overlapping microscopic fields were randomly selected to ensure unbiased sampling. All observations were performed at a standardized ×40 magnification using an optical microscope coupled with an image acquisition system.

To minimize experimental variability, all digital images were captured under identical lighting and contrast parameters. The thicknesses of the adrenal cortical zones (zona glomerulosa,

zona fasciculata, and zona reticularis) and the medulla were measured using ImageJ software (Version 1.53, NIH, USA), following the standardized digital imaging protocols established by **Schneider *et al.* (2012)**. Measurements were calibrated and expressed in micrometers (µm) to provide a precise evaluation of chromium-induced tissue hypertrophy and the subsequent restorative effects of Se and Zn supplementation.

Weight of the body and adrenal glands

Maternal body weight was monitored and recorded at key gestational intervals (days 3, 6, and 20) to track the progression of pregnancy and the systemic impact of treatment. Immediately following euthanasia on day 20, the adrenal glands were excised, cleared of adherent adipose tissue, and weighed.
To evaluate adrenal changes, both absolute and relative weights were calculated. The absolute weight was expressed as the mean organ mass (g) per experimental group. The relative weight (also referred to as the adrenal-to-body weight ratio or adrenal somatic index) was determined using the following formula to account for variations in maternal growth:

Adrenal gland relative weight (g/100 g body weight) = (mean of the adrenal gland weight for each group/final body weight) × 100.

$$\text{Formula: } W_r = \left(\frac{\text{Actual Weight}}{\text{Standard Weight}} \right) \times 100.$$

Hormonal assay

On gestational days 6 and 20, blood samples were collected into heparinized and EDTA-coated tubes to prevent coagulation. The samples were immediately centrifuged at 1500 × g for 15 minutes at 4°C. The resulting plasma was aliquoted and stored at -20°C to ensure the stability of the hormonal analytes prior to analysis.

Plasma concentrations of cortisol and adrenocorticotropic hormone (ACTH) were quantified using a MAGLUMI™ 2000 analyzer (Snibe Diagnostics, Shenzhen, China) via commercially available Chemiluminescence Immunoassay (CLIA) kits. All procedures were performed in strict accordance with the manufacturer's specialized protocols. The sensitivity and specificity of the CLIA method provided high-resolution data, with results for cortisol and ACTH expressed in ng/mL and pg/mL, respectively.

Markers of oxidative stress assessment

Tissue and homogenate preparation

Adrenal oxidative stress markers were assessed following the experimental framework of Sahu *et al.*, (2014). For the quantification of SOD, MDA, CAT, GPx, GST, and GSH, approximately 0.4 g of adrenal tissue was minced and homogenized in ice-cold TSE buffer (10 mM Tris, 250 mM sucrose, 1 mM EDTA; pH 7.2) to generate a 10% (w/v) homogenate.
Subcellular fractionation was achieved through differential centrifugation at 4°C. Initially, the homogenate was centrifuged at 600 × g for 10 minutes to remove nuclear debris and intact cells. The resulting supernatant was subjected to further centrifugation at 10,000 × g for 10 minutes; the final supernatant was collected as the cytosolic fraction.
To isolate the mitochondrial fraction, the pellet was harvested, resuspended in TS buffer (10 mM Tris, 250 mM sucrose; pH 7.2), and washed via a second centrifugation cycle at 10,000 × g for 10 minutes. The purified mitochondria were then resuspended in the same buffer. To access the mitochondrial matrix and release intramitochondrial enzymes, the suspension underwent three consecutive freeze-thaw cycles to achieve complete membrane lysis.

Total protein

Total protein concentration in the adrenal mitochondrial fractions was determined using the method of **Bradford (1976)**. This assay is based on the complexation of Coomassie Brilliant Blue G-250 with basic and aromatic amino acid residues, causing a shift in the absorption maximum of the dye from red to blue.
Briefly, 5 µl of mitochondrial matrix and 20 µl of TS buffer were added; the reaction was then initiated by adding 1 ml of Bradford reagent. After incubating the mixture for 10 minutes, the spectrophotometer determined its optical density at 595 nmagainst a reagent blank. Protein concentrations were quantified using a calibration curve constructed with bovine serum albumin (BSA) as the standard. All enzymatic and biochemical activities were subsequently normalized to these protein values and expressed as specific activities per milligram of protein.

Malondialdehyde levels assessment

Lipid peroxidation was evaluated by quantifying malondialdehyde (MDA) levels according to the method of **Shagirtha and Pari (2011)**. This assay utilizes the reaction between MDA a secondary end-product of polyunsaturated fatty acid peroxidation and thiobarbituric acid (TBA) to form a stable chromophore.
Briefly, a 250 µL aliquot of the mitochondrial extract was combined with 1 mL of a reaction mixture containing 15% trichloroacetic acid (TCA), 0.37% TBA, and 0.25 N HCl. The mixture was heated in a boiling water bath (100°C) for 15 minutes to facilitate the formation of the MDA-TBA adduct. Following the incubation, the samples were rapidly cooled and centrifuged at 3000 rpm for 10 minutes to remove precipitates. The absorbance of the resulting supernatant was measured spectrophotometrically at 532 nm. MDA concentrations were calculated using the molar extinction coefficient (1.56 x 10^5 $M^{-1}cm^{-1}$) and expressed as nmol of MDA/mg of protein.

Estimation of Superoxide Dismutase Activity

Total superoxide dismutase (SOD) activity was determined using the photochemical method **of Beauchamp and Fridovich (1971)**. This assay measures the enzyme's ability to inhibit the photoreduction of nitroblue tetrazolium (NBT). In this system, superoxide radicals ($O^{\bullet-}_2$) are generated by the reaction of riboflavin and methionine under white light illumination. These radicals subsequently reduce NBT to form a blue formazan chromophore.
Briefly, the reaction mixture consisted of 50 µL of the mitochondrial fraction, 0.1 mL of methionine (10^{-2} M), and 2 mL of a reaction medium containing sodium cyanide (0.01 M), NBT (1.76 x 10^{-2} M), EDTA (66 mM), and riboflavin (2 x 10^{-3} M) in a phosphate buffer (pH 7.8). Following a 20 minute incubation period under 15 W of white light, the absorbance was measured at 560 nm against a reagent blank. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the NBT reduction rate. Results are expressed as UI /mg protein.

Determination of catalase activity

Catalase (CAT) activity was quantified according to the method of **Claiborne (1985)**, based on the spectrophotometric monitoring of hydrogen peroxide (H_2O_2) decomposition. Briefly, the reaction was conducted in a quartz cuvette containing 50 µL of the mitochondrial fraction and 1mL of phosphate buffer (0.1 M, pH 7.4). The reaction was initiated by the addition of the H_2O_2 substrate (0.019 M).
The decrease in absorbance, reflecting the enzymatic breakdown of H_2O_2, was recorded at 240 nm at 30 second intervals over a 3 minute period. Catalase activity was calculated based on the rate of disappearance of H_2O_2 using its molar extinction coefficient (43.6 $M^{-1}cm^{-1}$) and expressed in IU/mg protein.

Determination of glutathione S-transferase activity

The enzymatic activity of glutathione S-transferase (GST) was determined according to the method described by **Habig et al (1974)**. The assay is based on the enzyme-catalyzed conjugation of reduced glutathione (GSH) with the electrophilic substrate 1-chloro-2,4-dinitrobenzene (CDNB).
Briefly, a reaction mixture containing 850 µL of phosphate buffer (0.1 M, pH 6.5) and an aliquot of the mitochondrial fraction was pre-incubated in a water bath at 37°C for 10 minutes. The reaction was initiated by the addition of 50 µL of CDNB (20 mM). The formation of the dinitrophenylthioether conjugate (1-glutathione-2,4-dinitrobenzene) was monitored spectrophotometrically by recording the increase in absorbance at 340 nm at 30 second intervals over a 5-minute period. GST activity was calculated using the molar extinction coefficient of the conjugate and expressed in IU/mg protein.

Determination of glutathione peroxidase activity

The enzymatic activity of glutathione peroxidase (GPx) was determined according to the kinetic method of **Flohé and Günzler (1984)**. Briefly, a reaction mixture consisting of 200 µL of the mitochondrial fraction, 400 µL of reduced glutathione (GSH), and 200 µL of phosphate buffer (pH 7.8) was pre-incubated at 25°C for five minutes. The enzymatic reaction was initiated by the addition of 200 µL of hydrogen peroxide (H_2O_2, 1.3 mM). To terminate the reaction, 100 µL of 1% trichloroacetic acid (TCA) was added, and the samples were incubated in an ice bath for 30 minutes. Following centrifugation at 3000 rpm for 10 minutes, an aliquot of the supernatant (0.48 mL) was combined with 2.2 mL of Na_2HPO_4 (0.32 M) and 0.32 mL of Ellman's reagent (DTNB, 1 mM). The resulting chromophore, proportional to the remaining GSH, was measured spectrophotometrically at 412 nm at 30 second intervals over a 5 minute period. GPx activity was calculated based on the rate of GSH consumption and expressed as nmol/mg of proteins.

Determination of Reduced Glutathione Levels

Adrenal reduced glutathione (GSH) concentrations were quantified spectrophotometrically using the colorimetric method established by **Ellman (1959)**. To ensure accurate measurement of the non-protein thiol pool, a 50 µL aliquot of the mitochondrial fraction was deproteinized by adding an equal volume of 10% trichloroacetic acid (TCA). The mixture was centrifuged at 1400 g for two minutes to precipitate proteins.
Subsequently, 50 µL of the resulting supernatant was neutralized with 2 mL of phosphate buffer (pH 8.0) and reacted with 20 µL of Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid, DTNB). The reaction was incubated at room temperature for 15 minutes to allow for the stable formation of the yellow-colored 5-thio-2-nitrobenzoic acid (TNB) chromophore. The absorbance was then measured at 412 nm against a reagent blank prepared under identical conditions. GSH concentrations were calculated using a standard curve derived from known glutathione concentrations and are expressed in mmol/mL.

Mitochondrial swelling and permeability

Mitochondrial swelling was quantitatively assessed spectrophotometrically by monitoring the decline in absorbance at 540 nm, a parameter inversely correlated with mitochondrial volume. Freshly isolated mitochondria were suspended in TSE buffer and measured against a corresponding blank to ensure baseline stability. For each experimental cohort, mitochondrial fractions were derived from at least three independent preparations (n = 6), with each sample analyzed in triplicate to ensure technical reproducibility. Total protein concentration was determined via the Bradford method, and all results were normalized to a standard mitochondrial protein content ranging from 0.5 to 1 mg/mL.

The opening of the mitochondrial permeability transition pore (mPTP) was evaluated through Ca^{2+}-induced swelling kinetics. Following the protocol described by **Kristal *et al.*, (1996)**, Ca^{2+} ions were introduced to initiate pore opening, and subsequent changes in optical density were recorded at 30-second intervals over a 3 minute duration. An accelerated decrease in absorbance at 540 nm was utilized as a functional index of enhanced Ca^{2+} uptake and mPTP activation, reflecting a loss of mitochondrial membrane integrity.

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM) for six animals per group (n = 6). All statistical evaluations were conducted using GraphPad Prism 8.0. The normality of the distribution and homogeneity of variances were verified prior to analysis.

For parameters measured at a single time point, a **one-way analysis** of variance (ANOVA) was employed. For parameters assessed across multiple time points (gestational days 6 and 20), a **two-way ANOVA** was utilized to determine the main effects of treatment, gestational age, and their interaction (treatment× time). Upon detection of significant effects, Dunnett's post hoc test was applied for multiple comparisons As distinct, independent groups of animals were utilized for each gestational time point, a repeated measures design was not required. Statistical significance was pre-defined at $p \leq 0.05$. Results yielding $p \leq 0.01$ and $p \leq 0.001$ were designated as very significant and highly significant, respectively.

RESULTS

The impact of $K_2Cr_2O_7$, alone or in combination with Se and/or $ZnCl_2$, on body weight and on the absolute and relative weights of the adrenal glands in pregnant albino Wistar rats

The biometric data summarized in Table 1 details the changes in maternal body weight and adrenal organometry across all experimental cohorts. Subcutaneous administration of $K_2Cr_2O_7$ to pregnant rats induced a highly significant ($p \leq 0.001$) reduction in body weight gain on both gestational days 6 and 20 when compared to the control group.

Conversely, the simultaneous administration of Se and $K_2Cr_2O_7$ effectively mitigated this weight loss, demonstrating a notable increase in body weight on day 6 ($p \leq 0.05$) and a highly significant recovery by day 20 ($p \leq 0.01$) relative to the chromium-only group. $ZnCl_2$ co-administration yielded a modest initial improvement on day 6, which achieved statistical significance by day 20. Notably, the triple combination treatment (Se + $ZnCl_2$ + $K_2Cr_2O_7$) exerted the most potent protective effect, inducing a very significant ($p \leq 0.01$) elevation in body weight on both assessed gestational days compared to $K_2Cr_2O_7$ alone.

Regarding organ metrics, $K_2Cr_2O_7$ -treated rats exhibited a profound ($p \leq 0.001$) elevation in both absolute and relative adrenal gland weights by gestation day 20. In contrast, all three antioxidant interventions Se, $ZnCl_2$, and their combination significantly ($p \leq 0.01$) attenuated this hypertrophic response, resulting in a marked reduction in adrenal mass compared to the chromium-challenged group.

Table 1 Effect of $K_2Cr_2O_7$, administered alone or in combination with Selenium (Se) and/or Zinc Chloride $ZnCl_2$, on maternal body weight and absolute/relative adrenal gland weights in pregnant Wistar rats.

Parameters and treatments	Control (0.9% NaCl)	$K_2Cr_2O_7$ (10 mg/ kg)	$K_2Cr_2O_7$+Se (0.3 mg/kg)	$K_2Cr_2O_7$ + $ZnCl_2$ (20 mg/kg)	$K_2Cr_2O_7$ +Se+ $ZnCl_2$
Initial body weight (g) on 3rd day of gestation	208.67±4.06	207.5±2.75	208.83±3.68	205. 33±3. 3	209.33±4.33
Body weight on the 6th day of gestation	240.5±5.16	215.83±4.66^{+++}	230.33±5.25*	224.83±5.9	234.5±6.88**
Final body weight (g) on the 20th day of gestation	287.33±4.86	253.33±5.51^{+++}	274.17±8.83**	270.17±6.02*	276.83±8.82**
Absolute adrenal gland weight (g)	0.034±0.003	0.056±0.002^{+++}	0.044±0.002**	0.045±0.003**	0.039±0.001**
Relative adrenal gland weight (g/100g bw)	0.012±0.0009	0.022±0.0009^{+++}	0.016±0.0006***	0.015±0.0007**	0.017±0.0009**

Values are expressed as mean ± SEM (n = 6). Statistical significance is indicated as follows: $^{+++}$ $P \leq 0.001$ represents a highly significant difference compared to the control group. $^{*}P \leq 0.05$, ** $P \leq 0.01$, and *** $P \leq 0.001$ represent significant, very significant, and highly significant differences, respectively, when compared to the $K_2Cr_2O_7$-treated group.

Effects of $K_2Cr_2O_7$ on plasma ACTH and cortisol levels in pregnant albino Wistar rats, either alone or in conjunction with Se and/or $ZnCl_2$

Our results demonstrate that plasma Adrenocorticotropic Hormone (ACTH) levels in the K_2Cr_2O7 -treated group underwent a profound and highly significant ($p \leq 0.001$) elevation compared to control values on both gestational day 6 (control vs cr: 121.15 ± 36.6 vs 1310.73 ± 152.31) and day 20 (control vs Cr 70.64 ± 15.88 vs 1214.01 ± 121.12).

Conversely, on the sixth day of gestation, the co-administration of Se and/or $ZnCl_2$ significantly mitigated this ACTH surge. Specifically, concentrations were very significantly ($p \leq 0.01$) reduced in the Se and triple-combination (Cr+Se+$ZnCl_2$) groups, while $ZnCl_2$ co-administration alone yielded a significant ($p \leq 0.05$) decrease.By day 20 of pregnancy, a similar restorative trend was observed: co-treatment with Se ($p \leq 0.01$) or ZnCl2 ($p \leq 0.05$) significantly lowered ACTH levels, while the triple combination (Se+ZnCl2) exerted the most potent effect, resulting in a highly significant ($p \leq 0.001$) reduction in ACTH concentration compared to the group treated with K_2Cr_2O7 alone (Figure 1).

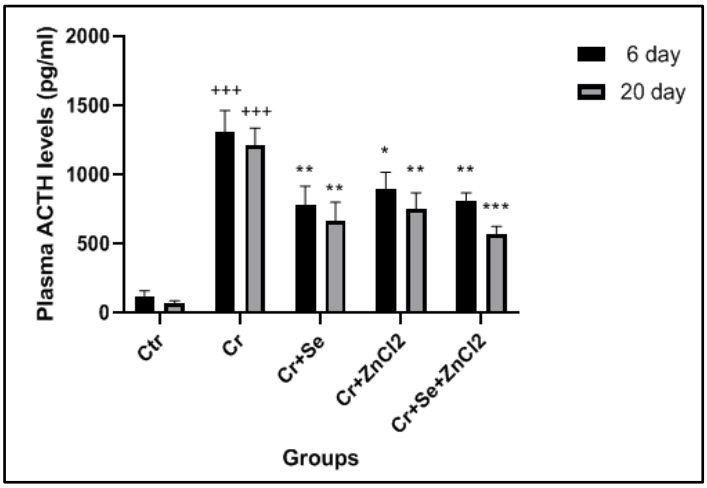


Figure 1 Effects of $K_2Cr_2O_7$ exposure, administered alone or in combination with Se and/or $ZnCl_2$, on plasma Adrenocorticotropic Hormone (ACTH) concentrations in pregnant Wistar rats on gestational days 6 and 20.

Data are expressed as mean ± SEM (n = 6). Statistical significance is indicated as: ***P ≤ 0.001 representing a highly significant difference compared to the control group; $^{+}$P ≤ 0.05, $^{++}$P ≤ 0.01, and $^{+++}$P≤ 0.001representing significant, very significant, and highly significant differences, respectively, compared to the $K_2Cr_2O_7$-treated group.

According to our findings, the administration of $K_2Cr_2O_7$ on the third day of pregnancy resulted in a profound and highly significant ($p \leq 0.001$) reduction in plasma cortisol levels compared to the control group. This suppression was observed on both gestational day 6 (Control vs Cr: 37.73 ± 3.88 vs 15.58 ± 1.83) and day 20 (Control vs Cr: 55.37 ± 9.63 vs 21.38 ± 6.47).
In contrast, cohorts co-treated with Se or the triple combination (Se + $ZnCl_2$) exhibited a highly significant ($p \leq 0.001$) elevation in plasma cortisol relative to the $K_2Cr_2O_7$-only group. Specifically, on day 6, levels recovered to 31.45 ± 1.67 (Se) and 29.88 ± 2.28 (Triple), with a similar robust recovery maintained through day 20. Furthermore, the group receiving $ZnCl_2$ co-administration showed a very significant ($p \leq 0.01$) increase on day 6 and a highly significant ($p \leq 0.001$) recovery by day 20 compared to the chromium-challenged rats (Figure 2).

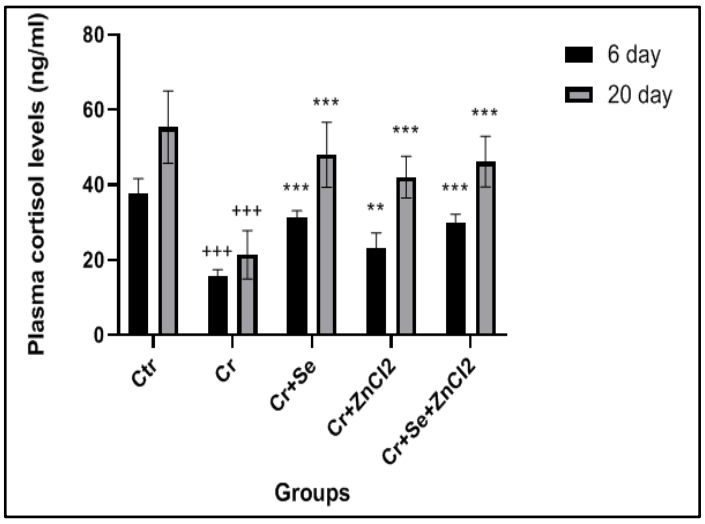


Figure 2 Effects of subcutaneous $K_2Cr_2O_7$ exposure, administered alone or in combination with Se and/or $ZnCl_2$, on plasma cortisol concentrations in pregnant Wistar rats on gestational days 6 and 20
Values are expressed as Mean ± SEM (n = 6). Statistical significance is indicated as follows: $^{***}p \leq 0.001$ denotes a highly significant difference compared to the control group. $^{+}p \leq 0.05$, $^{++}p \leq 0.01$, and $^{+++}p \leq 0.001$ denote significant, very significant, and highly significant differences, respectively, when compared to the $K_2Cr_2O_7$-treated group

Evaluation of oxidative stress markers

Evaluation of lipid peroxidation status in the adrenal gland

Malondialdehyde (MDA) serves as a critical biomarker for lipid peroxidation and a secondary indicator of impending cell death. In the present study, the administration of $K_2Cr_2O_7$ on the third gestational day triggered a profound and highly significant ($p \leq 0.001$) elevation in MDA levels within the adrenal tissue compared to the control cohort. Conversely, the co-administration of Se, $ZnCl_2$, or their combination significantly attenuated this oxidative damage. Specifically, all three antioxidant interventions induced a highly significant ($p \leq 0.001$) reduction in adrenal MDA concentrations relative to the group challenged with $K_2Cr_2O_7$ alone, suggesting a potent protective effect against chromium-induced membrane lipoperoxidation (Table 2).

Evaluation of SOD activities in the adrenal gland

Superoxide dismutase (SOD) represents a primary enzymatic defense line, facilitating the dismutation of the superoxide radical into hydrogen peroxide and molecular oxygen. Our findings revealed a highly significant ($p < 0.001$) elevation in adrenal SOD activity in pregnant rats exposed to $K_2Cr_2O_7$ relative to the control group. This increase likely reflects an adaptive compensatory response to the heightened production of reactive oxygen species (ROS) induced by chromium.
In contrast, antioxidant supplementation significantly modulated this enzymatic activity. The triple combination treatment (Se + $ZnCl_2$ + $K_2Cr_2O_7$) resulted in a highly significant ($p < 0.001$) reduction in SOD levels toward baseline, while the individual administration of Se or $ZnCl_2$ alongside $K_2Cr_2O_7$ yielded a significant ($p < 0.05$) decrease compared to the chromium-only cohort (Table 2). This restorative trend suggests that Se and $ZnCl_2$ alleviate the oxidative burden, thereby reducing the requirement for compensatory enzyme overproduction.

Evaluation of CAT activities in the adrenal gland

Catalase (CAT) constitutes a pivotal component of the cellular antioxidant defense hierarchy, specifically catalyzing the dismutation of hydrogen peroxide (H_2O_2) into water and molecular oxygen to prevent oxidative injury. Our data revealed that $K_2Cr_2O_7$ exposure induced a highly significant ($p<0.001$) surge in CAT activity within the adrenal glands of pregnant rats relative to the control group.
Conversely, the simultaneous administration of Se and $ZnCl_2$ with $K_2Cr_2O_7$ exerted a robust restorative effect, resulting in a highly significant ($p <0.001$) reduction in CAT levels back toward baseline. In comparison, individual supplementation with either Se or $ZnCl_2$ alongside the chromium challenge provoked only a marginal decrease in CAT activity (Table 2). This discrepancy underscores the superior efficacy of the combined antioxidant protocol in alleviating the oxidative burden, thereby stabilizing the enzymatic defense profile of the adrenal tissue.

Evaluation of GSH activities in the adrenal gland

Reduced glutathione (GSH) serves as an indispensable non-enzymatic antioxidant and an essential cofactor for several scavenging enzymes. Our analysis revealed a profound and highly significant ($p <0.001$) depletion of GSH levels within the adrenal glands of $K_2Cr_2O_7$-exposed pregnant rats compared to the control cohort. This marked reduction suggests an exhaustive consumption of the GSH pool in response to the massive influx of chromium-induced free radicals.
Conversely, antioxidant interventions effectively restored GSH homeostasis. We observed a highly significant ($p <0.001$) recovery of GSH levels in the Se-supplemented group and a very significant ($p<0.01$) increase in the triple combination group. Additionally, $ZnCl_2$ co-administration yielded a significant ($p<0.05$) elevation in GSH concentrations relative to the $K_2Cr_2O_7$-only group (Table 2). The superior recovery observed with Selenium likely reflects its role as a precursor in the synthesis of glutathione peroxidase, thereby reinforcing the endogenous GSH recycling pathway.

Evaluation of GPx activities in the adrenal gland

Glutathione peroxidase (GPx) is a critical enzymatic scavenger that neutralizes hydroperoxides, thereby playing a fundamental role in mitigating oxidative stress. Our results indicate that $K_2Cr_2O_7$ exposure induced a highly significant ($p <0.001$) elevation in adrenal GPx activity compared to the normal control group. This surge suggests a robust but overextended compensatory response by the adrenal tissue to combat the increased peroxide burden.
Conversely, concomitant treatment with Se and $ZnCl_2$ effectively alleviated this requirement, resulting in a highly significant ($p <0.001$) reduction in GPx activity toward physiological levels. Furthermore, the co-administration of $ZnCl_2$ with $K_2Cr_2O_7$ also yielded a significant ($p \leq 0.05$) decrease in GPx activity relative to the chromium-challenged group (Table 2). This restorative trend indicates that the exogenous antioxidants successfully reduced the systemic oxidative load, thereby sparing the endogenous enzymatic defense system.

Evaluation of GST activities in the adrenal gland

Glutathione S-transferases (GST) are a multigene family of enzymes that play a paramount role in cellular detoxification and the neutralization of electrophilic metabolites. Our findings indicate that $K_2Cr_2O_7$ exposure resulted in a highly significant ($p < 0.001$) reduction in GST activity within the adrenal glands of pregnant rats compared to the control group. This inhibition likely stems from the direct interaction of chromium with the enzyme's active site or the systemic depletion of its required substrate, GSH.Conversely, all three antioxidant co-treatments Se, $ZnCl_2$, and their combination significantly ($p < 0.01$) mitigated this inhibitory effect, resulting in a marked recovery of GST levels relative to the $K_2Cr_2O_7$ only group (Table 2). This recovery highlights the capacity of Se and $ZnCl_2$ to preserve the enzymatic detoxification machinery, thereby bolstering the adrenal tissue's defense against chromium-induced oxidative insult**.**

Assessment of swelling and mitochondrial permeability in the adrenal gland

This investigation revealed that $K_2Cr_2O_7$ exposure on the third gestational day triggered a profound and highly significant ($p<0.001$) increase in mitochondrial swelling and membrane permeability within the adrenal glands compared to the control group. This disruption of mitochondrial integrity is a hallmark of Cr (VI) induced cytotoxicity, often leading to the release of pro-apoptotic factors. Conversely, co-treatment with Se, $ZnCl_2$, or their combination significantly mitigated this damage. These antioxidant interventions resulted in a highly significant ($p<0.001$) reduction in both swelling and permeability relative to the $K_2Cr_2O_7$ only group (Table 3). The restoration of mitochondrial membrane stability suggests that Se and $ZnCl_2$ effectively preserve organelle architecture, thereby preventing the initiation of the apoptotic cascade in adrenal cells.

Table 2 Protective effects of Se and/or $ZnCl_2$ co-administration on adrenal oxidative stress biomarkers in $K_2Cr_2O_7$ exposed pregnant rats

oxidative stress marks and treatments	Groups				
	Control (0.9% NaCl)	**$K_2Cr_2O_7$** (10 mg/ Kg B w)	**$K_2Cr_2O_7$ +Se** (0.3mg/ Kg B w)	**$K_2Cr_2O_7$ + $ZnCl_2$** (20 mg/ Kg B w)	**$K_2Cr_2O_7$ + Se+ $ZnCl_2$**
MDA (nmol/mg of proteins)	0.21±0.01	$1.36±0.06^{+++}$	$0.42±0.03^{***}$	0.89±0.12**	0.33±0.03***
SOD (UI/mg of proteins)	19.82±0.5	$35.46±3.44^{+++}$	27.97±3.13*	28.75±1.88*	24.90±2.95**
CAT (UI/mg of proteins)	1.86±0.24	$6.95±3.1^{+++}$	4.16±1	4.36±1.08	$1.93±0.86^{***}$
GSH (mmol/mg of proteins)	0.82±0.05	$0.39±0.05^{+++}$	$0.61±0.03^{***}$	$0.52±0.04^{*}$	0.59±0.05**
GP_X (nmol/mg of proteins)	0.060±0.004	$0.012±0.002^{+++}$	$0.048±0.004^{***}$	$0.020±0.004^{**}$	$0.051±0.004^{***}$
GST (UI/mg of proteins)	49.40±4.47	$31.41±3.37^{++}$	43.96±3.71**	40.55±2.61**	42.41±3.32**

Values are expressed as mean ± SEM (n = 6). Statistical significance is indicated as follows: +++ $p \leq 0.001$ represents a highly significant difference compared to the control group. * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$ represent significant, very significant, and highly significant differences, respectively, when compared to the $K_2Cr_2O_7$ treated group.

Table 3 Assessment of adrenal mitochondrial integrity: impact of $K_2Cr_2O_7$ exposure and the protective role of Se and/or $ZnCl_2$ on swelling and membrane permeability in pregnant rats

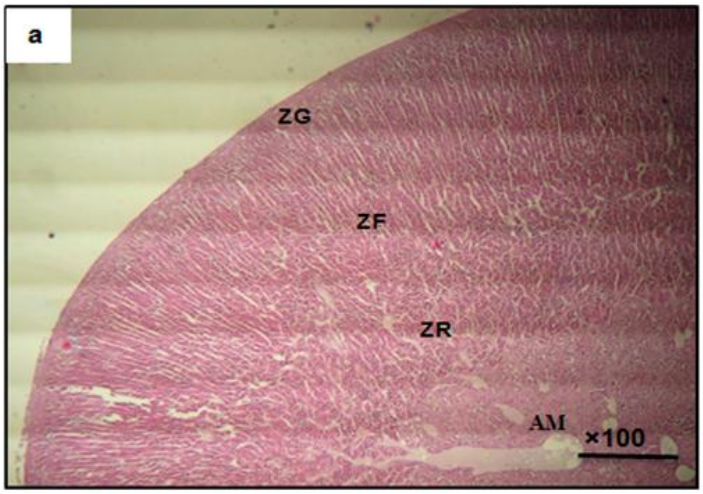
Groupes of rats	Mitochondrial swelling	Mitochondrial permeability ($\Delta DO/\Delta t$)
Control	0.316±0,011	0.018±0.001
$K_2Cr_2O_7$	$0.102± 0.004^{+++}$	$0.062±0.003^{++}$
$K_2Cr_2O_7$ + Se	$0.120±0.006^{**}$	$0.030±0.003^{***}$
$K_2Cr_2O_7$ + $ZnCl_2$	$0.263±0.021^{***}$	$0.023±0.002^{***}$
$K_2Cr_2O_7$ + Se+ $ZnCl_2$	$0.284±0.016^{***}$	$0.047±0.004^{**}$

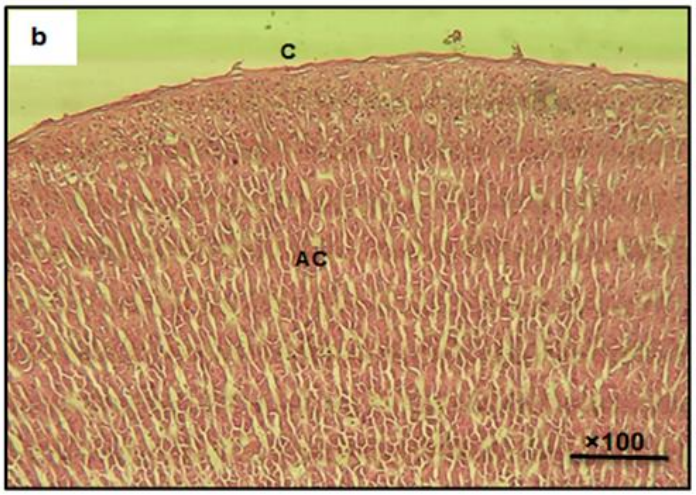
Values are expressed as mean ± SEM (n = 6). Statistical significance is indicated as follows $^{+++}$ $p \leq 0.001$ denotes a highly significant difference compared to the control group. $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, and $^{***}p \leq 0.001$ denote significant, very significant, and highly significant differences, respectively, compared to the $K_2Cr_2O_7$-treated group

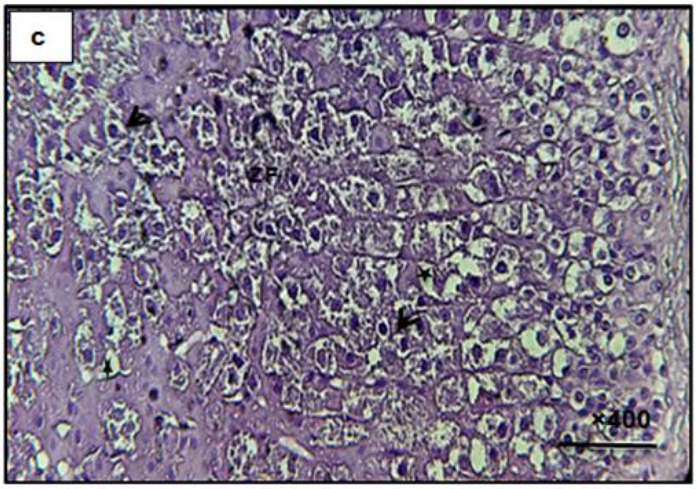
Effects of $K_2Cr_2O_7$ alone and in combination with Se and/or $ZnCl_2$ on adrenal gland histoarchitecture in the pregnant rats

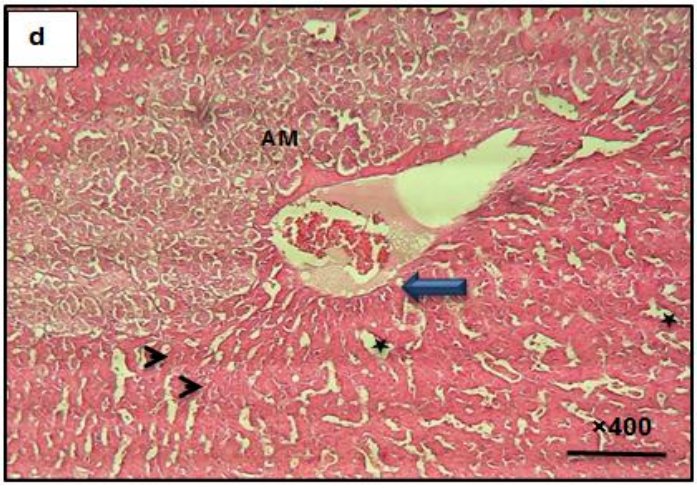
Histological examination of adrenal gland sections from the control cohort revealed a preserved architectural integrity. The gland was encapsulated by a thin layer of connective tissue, with a clearly demarcated cortex and medulla. The cortical region exhibited a systematic zonal organization comprising the zona glomerulosa, zona fasciculata, and zona reticularis all of which maintained normal cytoarchitecture. Cortical cells displayed typical morphology, characterized by well-preserved cytoplasm and prominent, centrally located nuclei. Similarly, the adrenal medulla appeared unremarkable, with chromaffin cells arranged in characteristic clusters and supported by a dense capillary network.

In stark contrast, the adrenal tissues of rats exposed to $K_2Cr_2O_7$ exhibited profound histopathological aberrations. Key findings included the disruption of the classical zonal architecture, widespread pycnotic necrosis of cortical cells, and significant cytoplasmic hypertrophy with extensive vacuolization. Furthermore, vascular distress was evident through the dilation of the central adrenal vein and marked congestion of blood vessels, accompanied by a prominent inflammatory infiltrate. Conversely, the simultaneous administration of Se, $ZnCl_2$, or their combination effectively preserved the histological framework of the gland. This co-treatment resulted in a substantial attenuation of Cr (VI)-induced structural damage, characterized by restored cortical organization and a reduction in both cytoplasmic vacuolization and cellular degeneration. These findings indicate that Se and $ZnCl_2$ exert a potent cytoprotective effect, maintaining adrenal morphology near physiological norms (Figure 3).









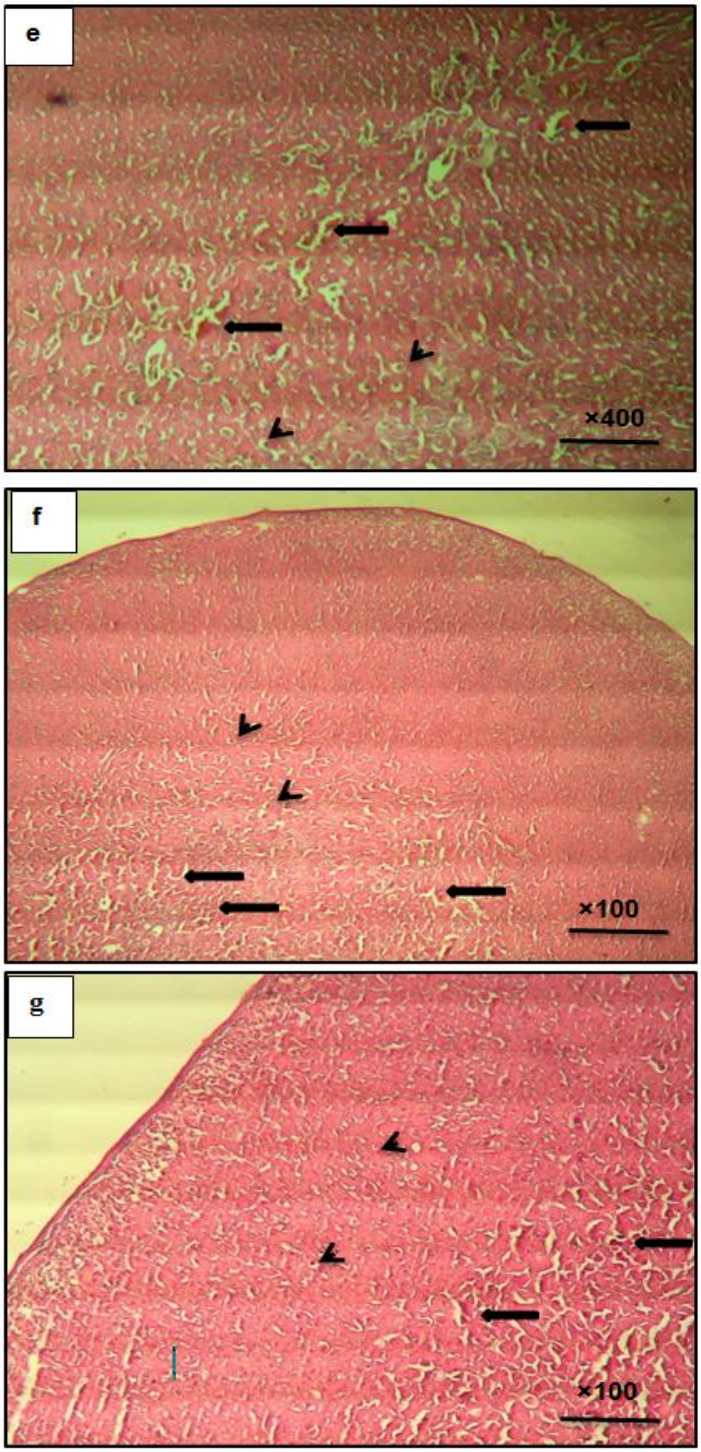


Figure 3 Representative photomicrographs of adrenal gland sections from pregnant Wistar Albino rats (H&E staining). (a, b) Control group displaying normal architectural integrity: the outer adrenal cortex subdivided into the zona glomerulosa (ZG), zona fasciculata (ZF), and zona reticularis (ZR) and the centrally located adrenal medulla (AM), all enclosed by a fibrous capsule. (c, d, e) $K_2Cr_2O_7$-treated group revealing severe histopathological damage: widespread pycnotic necrosis (arrowheads), dilation of the central adrenal vein (blue arrow), extensive cytoplasmic hypertrophy and vacuolization (stars), and pronounced vascular congestion (black arrows) with associated inflammatory infiltrate. (f, g, h) Sections from the Se, $Zncl_2$, and triple combination co-treated groups, respectively, showing a remarkable restoration of histological architecture and a significant reduction in chromium-induced lesions. These observations are in strong agreement with the adrenal histopathological profiles reported by **Savici *et al.*, (2021)** in male rat models of chromium toxicity.

Histomorphometric parameters of the adrenal gland

Histomorphometric analysis of the adrenal gland revealed significant variations in cortical and medullary dimensions between the control and experimental groups (Table 4). In the control group, the gland exhibited a physiological zonal distribution, with the zona fasciculata representing the predominant layer, followed by the zona glomerulosa and zona reticularis. The adrenal medulla was clearly demarcated within the central region, characterized by typical chromaffin cell clusters.

Administration of K2Cr2O7 induced a highly significant ($p \leq 0.001$) increase in total cortical thickness, primarily driven by a profound expansion of the zona fasciculata. Furthermore, the glomerular and reticular zones displayed very significant ($p \leq 0.01$) hypertrophy, coupled with severe architectural disorganization and extensive lipid vacuolization. The adrenal medulla exhibited mild hypertrophy accompanied by marked vascular congestion.

In contrast, supplementation with Se, $ZnCl_2$, or their combination facilitated a partial restoration of cortical dimensions and improved the demarcation between the histological zones. Adrenal cortical cells in these groups displayed near-normal morphology, with a significant attenuation of vacuolization and enhanced preservation of the overall cortico-medullary architecture. These results suggest that antioxidant intervention limits the pathological hypertrophy typically triggered by chromium-induced stress.

Table 4 Influence of treatment with $K_2Cr_2O_7$ alone or in combination with Se and/or $ZnCl_2$ on histomorphometric parameters of the adrenal gland in pregnant rats

Groups of rats	adrenocortical thickness (µm)			adrenal medullary thickness (µm)
	Zona glomerulosa	Zona fasciculata	Zona Reticularis	
Control (0.9% NaCl)	88.73±9.41	433.33±51.06	62.20±11.32	710.42±161.6
$K_2Cr_2O_7$ (10 mg/ kg)	125,66±17,75^{++}	801,15±6,27***	88,66±5,39**	953,90±156,61*
$K_2Cr_2O_7$ +Se (0.3mg/kg)	94,63±7,7^{++}	663,43±6,52**	79,72±5,73*	738,00±75,7*
$K_2Cr_2O_7$ + $ZnCl_2$ (20mg/kg)	90,387±6,75^{++}	524,599±19,51***	66,657±6,4***	823,972±79,57
$K_2Cr_2O_7$ + Se+ $ZnCl_2$	81,545±8,46^{++}	542,187±28,36***	74,476±5,93**	778,43±68,95

Values are expressed as mean ± SEM (n = 6). Statistical significance is indicated as follows: $^{+++}$ $p \leq 0.001$ denotes a highly significant difference compared to the control group. $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, and $^{***}p \leq 0.001$ denote significant, very significant, and highly significant differences, respectively, compared to the $K_2Cr_2O_7$-treated group

DISCUSSION

Previous reports have suggested that $K_2Cr_2O_7$ may affect the adrenal gland. In line with these findings, our results demonstrate that this is the first investigation to assess the impact of $K_2Cr_2O_7$ exposure during pregnancy on the adrenal gland. This evaluation was conducted through measurements of body and adrenal weights, endocrine function, histopathological and histomorphological alterations, and oxidative stress markers, as well as mitochondrial swelling and permeability in Wistar rats. Accordingly, the aim of the present study was to determine whether the antioxidants selenium (Se) and zinc chloride ($ZnCl_2$) could mitigate or reverse the adrenal damage induced by $K_2Cr_2O_7$.

At present, heavy metal research remains a critical focal point in environmental toxicology, largely driven by the pervasive use of these elements across diverse industrial sectors. The pathological impact of heavy metal exposure is multifaceted, governed by the specific chemical nature of the element, the dosage, and the duration of exposure **(Jomova *et al.*, 2025)**. Within this context, the adrenal gland emerges as a uniquely vulnerable site for metallic insult. This susceptibility is dictated by its intrinsic physiological properties, including high lipophilicity, which facilitates the sequestration of fat-soluble contaminants, and dense vascularization, which ensures a high-volume delivery of systemic toxins. Furthermore, the presence of specialized metabolic enzymes may inadvertently catalyze the biotransformation of metals like chromium into highly reactive intermediates, exacerbating localized structural and functional damage **(Harvey *et al.*, 2010)**.

Hexavalent chromium is a ubiquitous environmental contaminant recognized for its extensive capacity to induce systemic pathophysiology **(Shin *et al.*, 2023)**. Within the endocrine system, the adrenal glands are regarded as premier target organs for chemical insult, exhibiting a heightened sensitivity to xenobiotic-induced damage compared to other secretory tissues **(Egalini *et al.*, 2022)**. This vulnerability is primarily attributed to the gland's high lipid content and its specialized cytochrome P450 enzymatic machinery, which can inadvertently facilitate the metabolic activation of heavy metals into reactive intermediates.

Our findings indicate that the subcutaneous administration of $K_2Cr_2O_7$ produces a dual-pronged physiological impact: a substantial attenuation in maternal body weight gain alongside a significant elevation in both the absolute and relative weights of the adrenal glands compared to the control group. This divergence where overall somatic growth is suppressed while a specific endocrine organ expands is a hallmark of systemic stress. The increase in relative adrenal weight, in particular, underscores a disproportionate allocation of biological resources toward the adrenal cortex, likely as a compensatory mechanism to counteract the chromium-induced disruption of the hypothalamic-pituitary-adrenal (HPA) axis.

Maternal body weight serves as a sensitive and reliable biometric index for characterizing systemic toxicological risks in developmental studies **(EFSA et ECETOC 2013)**. The present findings align with a substantial body of evidence demonstrating that heavy metal exposure specifically hexavalent chromium markedly attenuates body weight gain in pregnant rats **(Fedala *et al.*, 2021; Saouli *et al.*, 2023)**. This reduction in gestational weight gain is typically regarded as a macroscopic indicator of overt maternal toxicity, likely stemming from the interplay between diminished caloric intake, impaired nutrient assimilation, and the metabolic cost of mounting an antioxidant defense against systemic oxidative stress.

Thompson *et al.* (2020) reported a significant reduction in both water and food consumption among exposed cohorts, an effect partially attributed to the poor palatability of chromium-contaminated intake. Beyond sensory aversion, the gastrointestinal (GI) tract undergoes severe histopathological damage upon Cr (VI) ingestion, which impairs nutrient bioavailability and absorption kinetics. These local disruptions in the GI architecture characterized by mucosal erosion and compromised barrier integrity directly diminish feed conversion efficiency and overall metabolic vigor **(Perosa *et al.*, 2024)**. Consequently, the observed decline in nutritional intake likely acts as a secondary stressor, compounding the systemic toxicity and contributing to the growth retardation frequently observed in metal-exposed subjects.

The results of the present investigation demonstrate that $K_2Cr_2O_7$ treatment significantly increased both the absolute and relative adrenal gland weights in pregnant rats by gestational day 20, as compared to the control group. This hypertrophic expansion appears to specifically target the zona fasciculata and zona reticularis of the adrenal cortex **(King *et al.*, 2016)**. The selective remodeling of these regions is highly significant, as they represent the primary sites of glucocorticoid and adrenal androgen biosynthesis, respectively, suggesting that the observed increase in organ mass is a direct response to the functional demands placed on the steroidogenic machinery.

Within the endocrine system, the adrenal glands are regarded as premier target organs for chemical insult, exhibiting a heightened sensitivity to xenobiotic-induced damage compared to other secretory tissues **(Egalini *et al.*, 2022)**

Prior investigations have established that exposure to heavy metals specifically cadmium (Cd), lead (Pb), and chromium (Cr) can trigger significant adrenal hypertrophy **(De Falco *et al.*, 2010; Gay *et al.*, 2013; Al-Derawi 2018; Savici *et al.*, 2017)**. The observed increase in adrenal weight in Cr (VI)-intoxicated rats likely reflects a combination of organ-level edema and compensatory cellular hypertrophy. This phenomenon may be further exacerbated by the cumulative and bio-absorptive capacity of the metal within glandular tissues, a mechanism previously documented in the renal architecture of pregnant rats **(Fedala *et al.*, 2021)**. Consequently, the increased organ mass serves as a macroscopic marker of the underlying inflammatory and stress-induced remodeling precipitated by chronic metal accumulation.

Conversely, the disruption of adrenocortical endocrine function in this study is characterized by a marked dissociation between elevated ACTH levels and diminished cortisol concentrations

Within the endocrine system, the adrenal glands are regarded as premier target organs for chemical insult, exhibiting a heightened sensitivity to xenobiotic-induced damage compared to other secretory tissues **(Egalini *et al.*, 2022)**

The accumulation of heavy metals, such as cadmium (Cd) and lead (Pb), has been shown to severely compromise adrenal function, resulting in attenuated cortisol secretion and a concomitant rise in catecholamine levels. These hormonal imbalances are often accompanied by pronounced morphological alterations and fluctuations in organ weight, reflecting the cumulative structural and functional damage induced by metallic toxicity **(Lopotych *et al.*, 2020)**. These observations parallel the findings in our study, suggesting that Cr(VI), like Cd and Pb, targets the adrenal cortex to induce a state of endocrine insufficiency characterized by cellular attrition and disrupted secretory dynamics.

Under homeostatic conditions, ACTH serves as the primary trophic stimulus for cortisol biosynthesis within the adrenal cortex, which in turn exerts negative feedback inhibition on the hypothalamic-pituitary-adrenal (HPA) axis. The observed hormonal profile a reciprocal rise in ACTH alongside a significant decline in cortisol strongly corroborates a diagnosis of primary adrenal dysfunction rather than central dysregulation **(King *et al.*, 2016)**. This pattern indicates that while the pituitary remains responsive to the lack of circulating glucocorticoids, the adrenal target organ is biochemically or structurally incapable of fulfilling the secretory demand.

Exposure to hexavalent chromium appears to induce a physiological state resembling primary adrenal insufficiency. Although the hypothalamic-pituitary-adrenal (HPA) axis is activated in response to Cr (VI)-induced stress triggering the increased secretion of corticotropin-releasing hormone (CRH) and subsequent adrenocorticotropic hormone (ACTH) the adrenal cortex fails to mount an adequate steroidogenic response. This blunted sensitivity can be attributed to several interrelated mechanisms, primarily the structural degradation of the zona fasciculata and the biochemical disruption of the enzymatic pathways required for cortisol synthesis. Consequently, the observed neuroendocrine profile suggests a state of "adrenal resistance," where the compensatory drive of the HPA axis is unable to overcome the underlying organ-level pathology.

The observed adrenal insufficiency likely results from two interconnected pathogenic mechanisms centered on mitochondrial dysfunction and impaired steroidogenesis. Mitochondria play a pivotal role in steroid hormone biosynthesis by mediating the initial and rate-limiting steps of steroidogenesis. This process begins with the transport of cholesterol from the outer to the inner mitochondrial membrane through the Steroidogenic Acute Regulatory (StAR) protein, which is considered the principal regulator of steroid hormone production. Once inside the mitochondria, cholesterol is converted into pregnenolone by the cholesterol side-chain cleavage enzyme P450, constituting the common precursor for glucocorticoids and sex hormones. Pregnenolone is subsequently transformed into different steroid hormones through enzymatic reactions involving close cooperation between mitochondria and the endoplasmic reticulum. In the present context, Cr (VI)-induced oxidative stress appears to directly damage adrenocortical mitochondria, thereby impairing StAR-mediated cholesterol transport and suppressing the activity of key steroidogenic enzymes, including CYP11A1 and CYP11B1.**(Midzak and Papadopoulos 2016)** In parallel, chromium may disrupt intracellular signaling pathways, particularly the cAMP/PKA cascade responsible for ACTH-stimulated steroidogenesis, leading to reduced StAR phosphorylation and diminished steroidogenic efficiency **(Beob *et al.* 2010)**.Consequently, cortisol synthesis becomes markedly compromised despite elevated ACTH concentrations, reflecting a state of primary adrenal dysfunction or resistance. Such impairment of mitochondrial steroidogenic machinery may also promote intracellular cholesterol accumulation and severe hormonal insufficiency affecting metabolic homeostasis, electrolyte balance, and endocrine function.Furthermore, the observed imbalance in apoptotic regulatory proteins characterized by the upregulation of Bax and the concomitant downregulation of Bcl_2 indicates a shift toward programmed cell death within the adrenal cortex. This alteration in the Bax/Bcl_2 ratio serves as a molecular trigger for mitochondrial membrane permeabilization, leading to accelerated adrenal cell loss. Such attrition likely results in a diminished functional cortical mass, ultimately compromising the gland's biosynthetic capacity and contributing to the significant decline in cortisol production observed in Cr (VI) exposed subjects **(Savici *et al.* 2021).**

Taken together, these findings suggest that hexavalent chromium toxicity precipitates adrenal dysfunction through a multi-pronged mechanism involving localized oxidative damage, mitochondrial bioenergetic failure, and the disruption of key steroidogenic signaling pathways. The resulting morphological disruptions and cellular attrition via apoptosis culminate in a significant attenuation of cortisol biosynthesis. This hypocortisolemia, in turn, weakens the negative feedback inhibition typically exerted on the hypothalamic-pituitary-adrenal (HPA) axis. Consequently, the observed elevation in ACTH levels likely represents a compensatory neuroendocrine effort to restore glucocorticoid homeostasis, driving the secondary hypertrophy observed within the zona fasciculata.

To elucidate the underlying mechanisms by which $K_2Cr_2O_7$ orchestrates the observed deleterious effects, we systematically evaluated a panel of oxidative stress biomarkers within the adrenal tissue.

Our investigation demonstrated that the subcutaneous administration of potassium dichromate significantly increased malondialdehyde (MDA) levels in the adrenal glands of pregnant rats, signaling enhanced lipid peroxidation. This outcome aligns with the well-established susceptibility of polyunsaturated fatty acids (PUFAs) to oxidative degradation by reactive oxygen species (ROS), including superoxide anions, hydrogen peroxide, and hydroxyl radicals generated during the

intracellular reduction of Cr (VI) **(Singh *et al.*, 2022).** The elevated MDA concentrations observed here likely reflect widespread oxidative damage to membrane lipids, which may be functionally linked to mitochondrial dysfunction and the toxic accumulation of peroxidized lipid byproducts **(Chappell *et al.*, 2020).** In parallel, a significant depletion of reduced glutathione (GSH) was observed in the adrenal tissue of $K_2O_2Cr_2$-treated animals. As a thiol-based tripeptide, GSH maintains cellular redox homeostasis by directly scavenging free radicals, serving as an essential cofactor for antioxidant enzymes such as glutathione peroxidase (GPx) and glutathione S-transferase (GST), and facilitating detoxification. This reduction in GSH content likely stems from its accelerated consumption during ROS neutralization and its direct involvement in the intracellular reduction of Cr (VI) intermediates **(Yen *et al.*, 2017; Kurutas 2016**). These results corroborate previous findings of GSH depletion across various tissues following chromium challenge **(Soudani *et al.*, 2013; El-Demerdash *et al.*, 2021; Zhang *et al.*, 2024).** Intriguingly, the activities of key antioxidant enzymes specifically superoxide dismutase (SOD), catalase (CAT), GPx, and GST were significantly upregulated in the adrenal glands of treated rats. While such an increase might superficially suggest enhanced antioxidant capacity, it must be interpreted with caution. This enzymatic upregulation more likely represents a compensatory adaptive response to chronic ROS overproduction rather than a hallmark of successful oxidative stress regulation. Such induction is frequently reported under pro-oxidant conditions and typically results from the transcriptional activation of stress-responsive pathways aimed at restoring redox equilibrium **(Espinosa-Diez *et al.*, 2015).** Consequently, the concomitant increase in enzymatic activity alongside GSH depletion and elevated MDA confirms that chromium exposure induces a profound state of oxidative stress. The observed enzymatic shift appears to be an abortive attempt by the organism to counteract ROS generation, which ultimately remains insufficient to prevent systemic oxidative damage.

This analysis focused on the interplay between pro-oxidant generation and the exhaustion of endogenous antioxidant defenses, providing a comprehensive view of the redox imbalance driving chromium-induced adrenal toxicity.

Hexavalent chromium is characterized by its potent oxidizing potential, which facilitates extensive cytotoxicity across diverse cellular lineages. Upon intracellular uptake, Cr (VI) undergoes reductive metabolism, generating highly reactive intermediates that drive the overproduction of reactive oxygen species (ROS). This reductive process is a critical determinant of toxicity, as the resulting ROS disrupt cellular homeostasis through the oxidative modification of DNA, proteins, and membrane lipids. Ultimately, these molecular lesions trigger cell cycle arrest and initiate the apoptotic cascade **(Lin *et al.*, 2024)**.

Cells possess an intrinsic capacity to mitigate the deleterious effects of reactive oxygen species through a sophisticated antioxidant defense network. This protective apparatus encompasses both enzymatic and non-enzymatic systems, which function synergistically to maintain redox equilibrium **(Neamati *et al.*, 2011)**. In the face of exogenous chemical challenge, the efficiency of this defensive hierarchy determines the extent of oxidative damage and the ultimate fate of the cell.

The present research demonstrated pronounced mitochondrial swelling and altered membrane permeability within the adrenal glands of $K_2O_2Cr_2$-treated rats. Under conditions of acute oxidative stress, mitochondria serve as primary targets for ROS-mediated damage. Such oxidative insults can induce mutations in mitochondrial DNA, thereby compromising endogenous antioxidant defense mechanisms and impairing the mitochondrial respiratory chain. Furthermore, these disruptions destabilize membrane permeability and dysregulate calcium homeostasis, collectively leading to a total collapse of mitochondrial function **(Guo *et al.*, 2013)**.

The reactive end-products derived from intensive lipid peroxidation and an accumulation of free radicals interact with the phospholipids and proteins of the mitochondrial membrane, severely compromising its structural architecture. This localized oxidative degradation may facilitate the opening of mitochondrial permeability transition pores (MPTP), thereby augmenting membrane permeability to potassium ions (K^+). The subsequent osmotic influx of water into the mitochondrial matrix induces organelle swelling and the dissipation of the mitochondrial membrane potential **(Bonora *et al.*, 2016; Li *et al.*, 2024)**. These events mark a critical failure in bioenergetic stability, ultimately committing the cell to the apoptotic pathway.

Exposure to Cr (VI) stimulates the expression of key mitophagy markers, such as Light Chain 3 (LC3), Beclin-1and promotes the mitochondrial accumulation of Phosphatase and Tensin homolog-induced kinase 1 (PTEN-induced kinase 1, (PINK1)/Parkin in various cellular models **(Guo *et al.*, 2022)**. This recruitment of the PINK1/Parkin complex serves as a critical signaling event for the selective degradation of damaged mitochondria, suggesting that hexavalent chromium triggers a compensatory autophagic response to clear dysfunctional organelles.

Hexavalent chromium primarily orchestrates apoptosis by inducing mitochondrial dysfunction, which subsequently triggers the intrinsic mitochondrial apoptotic pathway **(Mi *et al.*, 2017; Li *et al.*, 2022)**. This activation typically involves the loss of mitochondrial membrane potential and the release of pro-apoptotic factors into the cytosol, marking a critical transition from chemical stress to programmed cell death within the adrenal cortex.

In the present study, histopathological and histomorphometric assessments revealed pronounced deleterious effects of $K_2Cr_2O_7$ on the adrenal architecture of pregnant rats. Microscopic examination identified severe lesions, characterized by the disruption of the cyto-adrenal framework and the presence of pyknotic nuclei indicative of cortical cell necrosis. Significant vascular changes were also evident, including the marked dilation of the central adrenal vein and vascular engorgement, which were further associated with prominent inflammatory infiltration. At the cellular level, these structural alterations were accompanied by extensive cytoplasmic hypertrophy and widespread vacuolization, highlighting a profound loss of cellular integrity within the cortical layers.

These histological modifications align with the findings of **Savici *et al.* (2021)**, who reported cellular hypertrophy and the accumulation of lipid droplets in adrenal tissue following Cr (VI) exposure, attributing these phenomena to oxidative stress-mediated lipid peroxidation. According to **Kotyzová *et al.* (2015)**, Cr (VI) facilitates the generation of reactive oxygen species (ROS) that directly compromise the structural integrity of cell membranes. This mechanism likely account for the extensive vacuolization and diminished cellular cohesion observed specifically within the zona fasciculata.

The observation of pycnotic nuclei defined by marked chromatin condensation and nuclear shrinkage is indicative of an underlying apoptotic process. This programmed cell death is mediated by the activation of caspase-3, which orchestrates the systematic proteolysis of nuclear proteins and structural scaffolding **(McLaughlin *et al.*, 2003)**. Such morphological hallmarks suggest that the cellular damage induced by chromium exposure progresses toward irreversible apoptotic signaling within the adrenal tissue.

Histomorphometric analysis further revealed a significant increase in adrenal cortical thickness, localized primarily within the zona fasciculata. This expansion was accompanied by marked cellular disorganization and widespread cytoplasmic vacuolization. Such hypertrophy likely represents a compensatory adaptation to Cr (VI) induced oxidative stress, which compromises adrenal homeostasis and exhausts endogenous antioxidant defenses. Furthermore, the chronic elevation of adrenocorticotropic hormone ACTH may serve as a primary driver of this fascicular expansion; it is well-established that prolonged ACTH stimulation triggers cortical hypertrophy in response to systemic stressors or endocrine dysfunction **(Harvey and Sutcliffe 2010).**

Trace elements, specifically zinc and selenium, serve as fundamental regulators of redox homeostasis through their diverse antioxidant mechanisms. Emerging research indicates that the exogenous supplementation of these elements effectively mitigates oxidative damage, thereby reinforcing their multifaceted protective roles within biological systems **(Branca *et al.*, 2025)**. By bolstering the endogenous enzymatic repertoire, these micronutrients offer a robust defense against the molecular disruptions induced by heavy metal toxicity.

In the present study, the administration of zinc chloride, selenium, or their combination to pregnant rats exposed to $K_2Cr_2O_7$ exerted significant protective effects. Specifically, we observed a substantial increase in maternal body weight, accompanied by marked improvements in both the absolute and relative mass of the adrenal glands. Furthermore, this supplementation facilitated a partial normalization of disrupted hormonal parameters and attenuated the severity of histopathological lesions and histomorphometric aberrations within the adrenal tissue. Concurrently, these trace elements contributed to the mitigation of oxidative stress and cellular swelling while promoting the stabilization of mitochondrial membrane permeability.

The observed increases in total body weight, as well as the absolute and relative mass of the adrenal glands in rats treated with Se and/or $ZnCl_2$, likely reflect the modulatory influence of these trace elements on systemic energy metabolism. Clinical evidence suggests that selenium and zinc supplementation can optimize energy efficiency and promote weight gain, underscoring their potential to facilitate the restoration of tissue integrity under conditions of chemical stress **(Jotty *et al.*, 2009; Zavros *et al.*, 2023)**. These findings indicate that Se and Zn may counteract the catabolic effects typically associated with hexavalent chromium exposure.

As reported by **Saouli *et al.* (2023)**, the pretreatment of pregnant rats with selenium and/or zinc significantly attenuates the developmental toxicity induced by $K_2Cr_2O_7$. This protective intervention is evidenced by a marked increase in both maternal and fetal body weights, alongside a higher incidence of viable fetuses. Furthermore, the administration of these micronutrients was found to reduce the frequency of gestational loss and abortions, suggesting that Se and Zn bolster reproductive resilience against hexavalent chromium challenge.

Furthermore, the combined administration of selenium and/or zinc chloride significantly improved plasma ACTH and cortisol concentrations in pregnant rats subjected to hexavalent chromium exposure. This hormonal restoration is likely attributable to the protective influence of these trace elements on the hypothalamic-pituitary-adrenal (HPA) axis, which effectively limits the structural and functional aberrations induced by $K_2Cr_2O_7$. By mitigating damage at the regulatory level, Se and Zn facilitate the recovery of the endocrine signaling pathway essential for gestational health.

Selenium, in particular, mitigates heavy metal toxicity by facilitating the formation of insoluble complexes. This sequestration significantly reduces the bioavailability of toxic ions, thereby preserving the functional integrity of the adrenal gland and ensuring the maintenance of appropriate cortisol secretion levels **(Battin and Brumaghim 2009)**.

Furthermore, the antioxidant and anti-inflammatory properties of selenium inhibit the NF-κB (nuclear factor-κB) pathway. Under conditions of oxidative stress, this pathway typically triggers the production of pro-inflammatory cytokines, such as IL-6 and TNF-α, which interfere with the central secretion of CRH and ACTH. By suppressing this inflammatory cascade, Se facilitates the restoration of hormonal equilibrium within the hypothalamic-pituitary-adrenal (HPA) axis **(Rayman 2012; Huang *et al.*, 2012)**.

In the present study, the administration of Se and/or $ZnCl_2$ to pregnant rats exerted a substantial protective effect against $K_2Cr_2O_7$ induced fluctuations in oxidative stress markers within the adrenal gland. These findings highlight the efficacy of selenium and zinc in preserving adrenal redox integrity under hexavalent chromium challenge.

The substantial attenuation of Cr (VI) induced oxidative stress is evidenced by the marked reduction in lipid peroxidation among pregnant rats treated with Se and/or $ZnCl_2$. This improvement aligns with the established roles of these two trace elements in modulating redox homeostasis and reinforcing cellular defense mechanisms. The data suggest that the synergistic or individual application of these micronutrients effectively counteracts the pro-oxidant burden imposed by hexavalent chromium during gestation.

Zinc exerts a cellular antioxidant effect primarily as an essential cofactor for CuZnSOD and by inducing metallothionein synthesis. Metallothioneins are particularly effective at sequestering free radicals and chelating transition metals involved in ROS generation **(Prasad 2014)**. Furthermore, zinc promotes GSH synthesis and inhibits NADPH oxidases, an induction that minimizes Fenton-type reactions and subsequent lipid peroxidation. Extensive research indicates that zinc supplementation during gestation significantly attenuates MDA levels, reinforcing its protective role against heavy metal-induced lipid damage **(Oteiza 2012; Boulila and Adjroud 2020)**. Complementing this, selenium functions as a vital antioxidant through its integration into selenoproteins, most notably glutathione peroxidase (GPx), which is essential for the reduction of lipid hydroperoxides **(Rayman 2012)**. Through this enzymatic activity, Se limits the propagation of lipid peroxidation chains. Additionally, Se participates in the direct neutralization of free radicals and forms inert complexes with specific toxic.

Indeed, cumulative evidence indicates that selenium can sequester or neutralize the toxic effects of heavy metals. For instance, Se has been shown to bolster endogenous antioxidant defenses, thereby inhibiting lipid peroxidation and preventing GSH depletion in male rats subjected to lead-induced oxidative stress **(Atteia *et al.*, 2018).**

However, the co-administration of selenium and/or zinc chloride successfully restored enzymatic antioxidant equilibrium in pregnant rats exposed to potassium dichromate. This recovery is likely driven by the intrinsic antioxidant properties of these two essential micronutrients, which appear to neutralize the oxidative burden induced by chromium exposure.

Zinc functions as a critical structural cofactor for numerous enzymes involved in DNA synthesis and transcription. Furthermore, it is integral to the architecture of zinc-binding proteins and transcription factors, providing a stable platform for essential interactions with nucleic acids and regulatory proteins **(Narasimhaiah *et al.*, 2018)**. Beyond its structural utility, zinc exhibits a competitive inhibitory effect against chromium (VI) for membrane transporters; this competition effectively reduces the intracellular uptake and subsequent cytotoxic impact of the heavy metal **(Rahman and Thomas 2021).**

Beyond its antigenotoxic and anticancer properties **(Wang *et al.*, 2017)**, zinc is fundamental to maintaining genomic stability. This is achieved primarily through the modulation of DNA repair gene expression via zinc finger transcription factors **(Ho 2004)**. The capacity of zinc to mitigate heavy metal toxicity is well-documented **(Bhattacharya 2022)**, largely due to its efficacy in neutralizing free radicals and maintaining redox homeostasis. For instance, zinc supplementation has been shown to prevent nickel-induced neurotoxicity by inhibiting lipid peroxidation and preserving glutathione levels (Šulinskienė *et al.*, 2019). Similarly, elevated zinc intake has been found to restore the oxidant/antioxidant equilibrium, thereby shielding neural macromolecules from the deleterious effects of cadmium exposure **(Brzóska *et al.*, 2021)**.

Selenium nanoparticles (SeNPs) play a pivotal role in modulating inflammatory signaling pathways, specifically targeting Toll-Like Receptor 4 (TLR4), which significantly bolsters their cytoprotective profile **(Al-Dossari *et al.*, 2020)**. Furthermore, dietary SeNPs have been shown to counteract oxidative stress by upregulating the transcription of essential heat shock proteins, including Hsp30, Hsp70b, and Hsp90α. This protective mechanism is further supported by the induction of selenoproteins and growth factors such as TGF-β, Gpx1a, Gpx1b1, and Trx **(Li *et al.*, 2023)**.

Recent studies have increasingly highlighted the protective potential of combined selenium and zinc supplementation against heavy metal-induced toxicity, particularly through the modulation of oxidative stress, inflammation, mitochondrial dysfunction, and apoptosis.

Importantly, several studies suggest that the simultaneous administration of selenium and zinc may exert synergistic rather than merely additive protective effects against metal-induced cellular damage. In this context, **Fedala *et al.* (2021)** demonstrated that selenium and zinc co-supplementation significantly attenuated potassium dichromate-induced oxidative stress, thyroid dysfunction, and DNA damage in pregnant rats. Similarly, **Antia *et al.* (2023)** reported that combined selenium and zinc treatment reduced cardiopulmonary toxicity induced by metal mixtures through antioxidant, anti-inflammatory, and antiapoptotic mechanisms. Furthermore, previous reviews emphasized that these micronutrients cooperate in preserving mitochondrial integrity, reducing lipid peroxidation, limiting ROS overproduction, and improving endogenous antioxidant defenses during exposure to toxic metals **(Orisakwe *et al.*, 2019)**

Compared with previous investigations that mainly focused on selenium or zinc administered individually, the present study provides additional evidence supporting the beneficial effects of their combined administration against chromium-induced alterations.

Therefore, the novelty of the current work resides not only in evaluating the protective role of selenium and zinc during chromium exposure, but also in demonstrating the potential synergistic interactions between these two essential trace elements in mitigating endocrine, mitochondrial, biochemical, and histopathological disturbances.

CONCLUSION

In conclusion, our findings demonstrate that subcutaneous exposure to $K_2Cr_2O_7$ induces significant adrenal toxicity in pregnant Wistar Albino rats. This toxicity is characterized by disrupted plasma hormone concentrations, oxidative lesions, and mitochondrial dysfunction, alongside marked alterations in adrenal histoarchitecture. Conversely, the co-administration of Se and/or $ZnCl_2$ exerted protective effects through antioxidant and mitochondrial stabilization. While histological and histomorphometric damage was only partially mitigated, the results suggest that Se and Zn may serve as viable adrenoprotective agents against $K_2Cr_2O_7$ -induced adrenal disruption.

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