

RAPHANUS SATIVUS JUICE ALLEVIATES MERCURY-INDUCED MULTISYSTEM TOXICITY IN RATS: AN INTEGRATED BIOCHEMICAL, HISTOPATHOLOGICAL AND REPRODUCTIVE STUDY

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Abstract

Mercury is a pervasive environmental toxin that disrupts cellular redox homeostasis and induces multisystem toxicity. This study evaluated the protective effects of *Raphanus sativus* juice against mercuric chloride (HgCl₂)-induced toxicity in adult male Wistar rats following 30 days of exposure. HgCl₂ caused marked biochemical and reproductive alterations, including increased plasma glucose, triglycerides, urea, creatinine, and hepatic enzyme activities, together with reductions in sperm concentration (-64.3%), motility (-51.7%), testosterone (-53.1%), and hepatic and renal reduced glutathione (GSH) levels (-62% and -63%, respectively). Qualitative histopathological assessment revealed tissue lesions in the liver, kidneys, testes, and epididymides. Co-administration of *R. sativus* juice substantially attenuated these alterations, improving biochemical and reproductive parameters, restoring GSH levels, and apparently preserving tissue architecture. Exploratory multivariate analyses identified coordinated associations among reproductive, metabolic, hepatorenal, hematological, and oxidative-stress variables; however, treatment adjustment markedly attenuated these relationships, indicating that group separation contributed substantially to the pooled multivariate structure. Thus, *R. sativus* juice showed protective potential against HgCl₂-induced multisystem toxicity, although the limited redox assessment and qualitative histopathology preclude attributing this to a demonstrated antioxidant mechanism.

Rezumat

Studiul a evaluat efectul protector al sucului de *Raphanus sativus* în toxicitatea indusă de clorura de mercur (HgCl₂) la șobolani masculi Wistar adulți, după 30 de zile de expunere. Administrarea HgCl₂ a determinat modificări biochimice și reproductive importante, caracterizate prin creșterea glicemiei, trigliceridelor, ureei, creatininei și activității enzimelor hepatice, asociată cu reducerea concentrației spermatozoizilor (-64,3%), a motilității acestora (-51,7%), a testosteronului (-53,1%) și a nivelurilor de glutatation redus (GSH) hepatic și renal (-62%, respectiv -63%). Examenul histopatologic calitativ a evidențiat leziuni la nivelul ficatului, rinichilor, testiculelor și epididimului. Administrarea concomitentă a sucului de *R. sativus* a atenuat considerabil aceste modificări, îmbunătățind parametrii biochimici și reproductivi, restabilind nivelurile de GSH și contribuind la menținerea integrității structurale a țesuturilor. Analizele multivariate exploratorii au evidențiat asocieri între parametrii reproductivi, metabolici, hepatorenali, hematologici și cei ai stresului oxidativ. Ajustarea tratamentului a redus semnificativ aceste asocieri, sugerând că diferențele dintre grupurile experimentale au influențat în mare măsură rezultatele analizei multivariate. În concluzie, sucul de *R. sativus* a demonstrat un potențial protector în toxicitatea sistemică indusă cu HgCl₂, însă evaluarea limitată a statusului redox și caracterul calitativ al examinării histopatologice nu permit confirmarea unui mecanism de acțiune antioxidant.

Keywords: mercury toxicity, radish juice, hepato-renal dysfunction, reproductive toxicity, histopathology

Introduction

Mercury (Hg) is a major environmental contaminant and an important public health concern. Its persistence and bioaccumulation increase exposure risk in humans and animals (1-3). Mercury can enter the

body through contaminated water and food. Industrial emissions are another important source of exposure (4). Once absorbed, Hg can affect several biological systems. Its toxicity is closely associated with oxidative stress. Mercury has a high affinity for

sulfhydryl groups. This interaction can deplete reduced glutathione (GSH) and other intracellular antioxidants (5). The resulting redox imbalance can promote lipid peroxidation, protein oxidation, mitochondrial dysfunction, and DNA damage. These processes may ultimately lead to tissue injury. The liver and kidneys are particularly vulnerable because of their roles in metabolism, detoxification, and excretion (6). Mercury exposure can also affect the male reproductive system. Sperm cells are highly sensitive to oxidative damage. Their membranes contain high levels of polyunsaturated fatty acids and have relatively limited antioxidant defenses. Oxidative imbalance can therefore impair spermatogenesis and sperm quality. It may also affect testosterone production (7). These effects indicate that mercury toxicity can involve several biological systems simultaneously. An integrated assessment is therefore useful for understanding its systemic effects. As oxidative stress underpins much of mercury toxicity, natural products rich in antioxidant phytochemicals have garnered increasing interest as protective interventions, with numerous polyphenols, flavonoids, vitamins, and sulfur-containing metabolites demonstrated to preserve redox homeostasis and mitigate toxicant-induced tissue injury (8). Among these natural products, *Raphanus sativus* L. (radish) is a promising candidate due to its diverse phytochemical composition and reported biological activities (9, 10). This popular cruciferous vegetable is a source of phenolic compounds, flavonoids, anthocyanins, vitamin C, and glucosinolate-derived isothiocyanates with antioxidant, anti-inflammatory, and detoxifying properties (9-12), and its roots exhibit high antioxidant capacity in DPPH, FRAP, and ORAC assays (10, 13). Further, hepatoprotective and nephroprotective effects of *R. sativus* preparations have been reported in models of chemically induced toxicity (14, 15), which suggest that it may be a promising dietary strategy against heavy metal-induced oxidative damage. Additionally, fresh radish juice has been found to protect against carbon tetrachloride-induced hepatotoxicity, supporting its wider hepatoprotective potential (16).

Despite these promising results, the protective effects of *R. sativus* against mercury-induced reproductive toxicity have not been investigated from an integrated multisystem view, as the previous studies have predominantly concentrated on specific endpoints, rather than the interrelations between metabolic disorders, hepatorenal dysfunction, oxidative stress, and reproductive impairment. Therefore, the objective of the current research was to assess the protective effects of freshly prepared *Raphanus sativus* juice against mercuric chloride-induced toxicity in male Wistar rats through an integrated hematological, biochemical, reproductive, histopathological, and tissue glutathione assessment, thereby providing a

wider understanding of the systemic consequences of mercury exposure and the potential protective role of *R. sativus* as a natural dietary intervention.

Materials and Methods

Reagent and chemicals

Analytical grade mercuric chloride (HgCl_2 , $\geq 99\%$) was purchased from a certified supplier (Sigma-Aldrich, St. Louis, MO, USA). All other chemicals and reagents used in the study were of analytical grade and purchased from commercial sources. All working solutions were freshly prepared just prior to use.

Experimental conditions and Wistar rats

Twenty-eight healthy adult male Wistar rats (*Rattus norvegicus*), 8 - 10 weeks old and weighing 170 - 230 g, were obtained from the accredited animal breeding facility of the Pasteur Institute (Algiers, Algeria). Animals were acclimated for 7 days under standard laboratory conditions before the experiment. Rats were housed in polypropylene cages (3 - 4 animals *per* cage) with clean wood-chip bedding in a temperature-controlled room ($23 \pm 1^\circ\text{C}$), relative humidity 50 - 60%, and a 12 h light/12 h dark photoperiod, with unrestricted access to standard laboratory chow and tap water. General health status was monitored daily throughout the study. All animal procedures were conducted according to internationally accepted guidelines for the care and use of laboratory animals and to institutional ethical standards.

Preparation of extract from *Raphanus sativus*

Fresh roots of red radish (*Raphanus sativus* L.) were obtained from a local market in Algeria. The plant material was authenticated by Dr. Lebtahy F. (INRF, Algiers, Algeria) based on its morphological characteristics, and a voucher specimen was deposited under reference number RS-2023-66. The washed roots were cut into small pieces and mechanically crushed using a domestic juice extractor. Approximately 100 g of fresh roots yielded 75 - 80 mL of fresh juice, corresponding to an estimated fresh-root equivalent of 1.25 - 1.33 g/mL of juice. The juice was filtered through sterile gauze and administered immediately after extraction without further dilution or storage. The freshly prepared juice was administered orally by gavage at 1 mL/rat/day for 30 consecutive days, corresponding to approximately 5 mL/kg body weight/day for a rat weighing 200 g. The juice administration volume was adjusted according to individual body weight. Immediate preparation and administration were used to minimize potential degradation of oxidation-sensitive constituents, including vitamin C, glucosinolates, isothiocyanates, anthocyanins, and phenolic compounds.

Design of Experiments

After acclimatization, the rats were randomly divided into four groups ($n = 7$ *per* group). The Control group received the standard diet and tap water

throughout the experimental period. The HgCl₂ group received the standard diet containing HgCl₂ at 0.8 g/kg diet (0.08%, w/w) for 30 consecutive days. Because individual food consumption was not quantitatively recorded, the actual HgCl₂ intake in mg/kg body weight/day could not be determined for individual animals; therefore, no estimated daily body-weight-normalized dose is reported. The *R. sativus* (RS) group received 1 mL of freshly prepared juice/rat/day by oral gavage, whereas the combined HgCl₂ + RS group received both the HgCl₂-containing diet and RS juice. The juice was administered undiluted, while HgCl₂ was incorporated directly into the diet; therefore, no separate vehicle-control group was included. Treatments were administered once daily for 30 consecutive days. Thus, the *R. sativus*-treated groups underwent oral gavage, whereas the Control and HgCl₂-only groups did not; no sham-gavage or vehicle-gavage procedure was included in the original experimental design. No positive-control group receiving an established protective agent against HgCl₂-induced toxicity was included in the present study design. Animals were monitored daily throughout the experimental period, and no mortality or clinical-sign data were recorded in any group. Body weight was recorded before treatment and at the end of the study. At sacrifice, the liver and right kidney were weighed separately to obtain absolute organ weights. The left kidney was reserved for histological examination. Relative liver and right-kidney weights were calculated as organ weight (g) divided by final body weight (g) × 100. Histopathological examinations were performed by an investigator blinded to treatment allocation.

Sample collection and preparation

The animals were anesthetized by intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg) 24 h after the last treatment. Following anesthesia, blood was collected by cardiac puncture into EDTA-containing (purple-top) tubes for hematological analyses and heparin-containing (green-top) tubes for biochemical analyses. The animals were then euthanized by exsanguination. Animals were fasted for 12 h before blood collection. Blood collected in heparinized tubes was centrifuged at 4000 rpm for 15 min to obtain plasma, which was subsequently stored at -20°C until biochemical analysis. The liver, kidneys, testes, and epididymides were then dissected, trimmed, and rinsed in ice-cold saline solution. Portions of the liver and kidneys were homogenized for determination of glutathione (GSH), while representative samples of the liver, kidney, testis, and epididymis were fixed in 10% neutral buffered formalin and processed for blinded histological evaluation as described below.

Hematological and biochemistry assays

Hematological indices, including erythrocyte count (RBC), hemoglobin (HGB), hematocrit (HCT),

leukocyte count (WBC), lymphocyte count (LY), and platelet count (PLT), were determined by an automated hematology analyzer (Diatron ABACUS 4, Diatron, Hungary). Biochemical parameters, including glucose, triglycerides, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), were determined using an automated clinical chemistry analyzer (Diatron PICTUS 200, Diatron, Hungary) with commercial enzymatic reagent kits according to the manufacturers' instructions. Plasma testosterone concentrations were measured by an electrochemiluminescence immunoassay (ECLIA) using a Cobas e 411 automated immunoassay analyzer (Roche Diagnostics, Mannheim, Germany). The use of the Cobas e 411 ECLIA platform for testosterone determination in rat plasma has been previously validated and applied in experimental rat studies.

Evaluation of fertility parameters

Epididymal sperm concentration and motility were assessed using rat-specific procedures adapted from Adedara *et al.* (17), who described the evaluation of epididymal sperm concentration and progressive motility in rats, with additional reference to the general methodological principles described in the World Health Organization (WHO) (18), adapted specifically for the evaluation of epididymal sperm obtained from rats. The cauda epididymis was removed and minced with fine scissors in 5 mL of physiological saline (0.9% NaCl) to allow spermatozoa to diffuse into the medium. Approximately 1 µL of the resulting sperm suspension was then diluted with 49 µL of physiological saline (0.9% NaCl), corresponding to a 1:50 dilution (v/v). Approximately 10 µL of the diluted suspension was loaded into an improved Neubauer hemocytometer and allowed to stand for 5 min to permit uniform sedimentation of the spermatozoa. Spermatozoa were counted manually under a light microscope at 400× magnification in five 16-cell squares of the Neubauer chamber. Sperm concentration was calculated from the hemocytometer count and expressed as ×10⁶ spermatozoa/mL. Sperm motility was assessed immediately after preparation of the epididymal suspension by examining randomly selected microscopic fields and recording the percentage of progressively motile spermatozoa. The motility assessment was performed under controlled temperature conditions appropriate for rat epididymal sperm analysis. All assessments were performed by the same investigator, who was blinded to the experimental groups.

Estimation of reduced glutathione (GSH)

The liver and kidney tissues were homogenized in phosphate-buffered saline (PBS) at a ratio of 1 g tissue to 2 mL PBS and centrifuged at 10,000 × g for 15 min at 4°C. The resulting supernatant was collected and divided into separate aliquots. One aliquot was used for protein determination without

deproteinization, whereas a separate aliquot was deproteinized with sulfosalicylic acid before GSH determination. Reduced glutathione (GSH) was quantified using the colorimetric method of Ellman (19), as modified by Jollow *et al.* (20). Following deproteinization and removal of precipitated proteins, the clear supernatant was reacted with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), and the absorbance was measured at 412 nm. GSH concentration was calculated from a reduced-GSH standard curve, taking into account the relevant dilution and deproteinization factors, and was normalized to the protein concentration determined independently from the non-deproteinized tissue supernatant. GSH levels were expressed as nmol GSH/mg protein.

Protein quantification

Total protein of tissue homogenates was measured by the Bradford dye-binding method (21) using bovine serum albumin as a calibration standard.

Histopathological examination

Tissues of the liver, kidney, testis, and epididymis were fixed in 10% neutral buffered formalin for 24–48 h, processed according to standard histological procedures involving dehydration, clearing, and paraffin embedding, and sectioned at a thickness of 5 μ m. The sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope (Olympus, Japan) equipped with a digital camera. Representative photomicrographs were obtained at $\times 250$ magnification. The histological processing and staining procedure was adapted from Martoja and Martoja (22). All sections were evaluated qualitatively and descriptively by a single observer blinded to group allocation. No quantitative histomorphometry and no semi-quantitative lesion scoring were performed, and the histological material was therefore not subjected to statistical analysis. Accordingly, all histological findings reported below are descriptive observations on representative sections.

Statistical analyses

Values are expressed as mean $\pm$ standard deviation (SD) ($n = 7$ per group). Statistical analyses were performed using the Statistica software package (version 8.0, StatSoft Inc., USA). Prior to analysis, data normality was assessed using the Shapiro–Wilk test and homogeneity of variances using Levene's test.

Given the 2×2 factorial experimental design, with HgCl₂ exposure (absent/present) and *Raphanus sativus* supplementation (absent/present) as the two experimental factors, quantitative endpoints were analyzed using two-way factorial analysis of variance (ANOVA). The model included the main effects of HgCl₂, *R. sativus*, and the HgCl₂ $\times$ *R. sativus* interaction. The interaction term was specifically evaluated to determine whether the effect of *R. sativus* differed according to HgCl₂ exposure. Partial eta squared (η^2) was calculated as a measure of effect size for the main effects and interaction. When a significant interaction was detected, the corresponding group means were examined using Tukey's HSD post hoc/simple-effect comparisons to characterize the direction of the interaction. Statistical significance was set at $P < 0.05$.

Throughout the tables and figures, * denotes a significant difference versus the Control group and # denotes a significant difference versus the HgCl₂ group ($P < 0.05$). By definition, a symbol is never applied to the group serving as the reference for that comparison, so the HgCl₂ group does not carry the # symbol and the Control group does not carry the * symbol.

Pearson correlation analysis, principal component analysis (PCA), and hierarchical cluster analysis (HCA) were performed to explore relationships among the measured variables. Before hierarchical clustering, each variable was standardized to a Z-score across the animals to minimize the influence of differences in measurement scale. HCA was then performed using Euclidean distance and Ward's minimum-variance linkage method. Because these multivariate procedures involved a relatively large number of endpoints compared with the number of animals, they were considered exploratory, descriptive, and hypothesis-generating, and no causal or independent inferential conclusions were derived from the clustering structure.

Results and Discussion

Organ and body weight changes

ANOVA revealed a significant effect of treatment on final body weight. Initial body weights did not differ significantly among the four groups (Table I).

Table I

Effect of *R. sativus* juice on body weight changes in male Wistar rats exposed to HgCl₂ for 30 days

Group	Initial body weight (g)	Final body weight (g)	Body weight change (%)
Control	180.5 $\pm$ 21.3	210.3 $\pm$ 23.0	+16.5
HgCl ₂	182.2 $\pm$ 23.5	163.2 $\pm$ 15.3*	−10.4
<i>R. sativus</i>	185.3 $\pm$ 22.1	211.6 $\pm$ 18.9#	+14.2
HgCl ₂ + <i>R. sativus</i>	180.6 $\pm$ 19.6	195.2 $\pm$ 24.6*#	+8.1

Values are expressed as mean $\pm$ SD ($n = 7$ per group). * Significantly different from the control group ($P < 0.05$); # significantly different from the HgCl₂-treated group ($P < 0.05$). Statistical comparisons were made by ANOVA followed by Tukey's HSD post hoc test. A symbol is never applied to the group that serves as the reference for that comparison; the HgCl₂ group therefore carries * only, and the control group carries no symbol.

After 30 days, the control animals showed a 16.5% increase in body weight, whereas the HgCl₂ group showed a 10.4% decrease relative to baseline. The *R. sativus*-treated group showed a 14.2% increase, while the HgCl₂ + *R. sativus* group showed an 8.1% positive change from baseline. Thus, although HgCl₂ exposure was associated with reduced body weight and a net loss relative to baseline, the combined HgCl₂ + *R. sativus* group maintained a positive change in body weight over the experimental period. However, because individual food consumption was not quantitatively monitored, the contribution of

differences in food intake to these body-weight changes cannot be excluded.

HgCl₂ exposure was associated with higher absolute liver weight (8.35 ± 1.01 g, Table II) and right-kidney weight (1.04 ± 0.09 g) compared with the Control group (6.60 ± 1.79 and 0.64 ± 0.03 g, respectively). The corresponding values in the HgCl₂ + *R. sativus* group were lower than those observed in the HgCl₂ group (7.51 ± 0.54 g for liver and 0.82 ± 0.09 g for the right kidney), whereas *R. sativus* alone showed values close to those of the Control group.

Table II

Effect of *R. sativus* juice on absolute liver and kidney weights of male Wistar rats exposed to HgCl₂ for 30 days

Group	Liver weight (g)	Relative liver weight (%)	Right kidney weight (g)	Relative right kidney weight (%)
Control	6.60 ± 1.79	3.14 ± 0.65	0.64 ± 0.03	0.304 ± 0.02
HgCl ₂	$8.35 \pm 1.01^*$	$5.12 \pm 0.78^*$	$1.04 \pm 0.09^*$	$0.637 \pm 0.05^*$
<i>R. sativus</i>	6.56 ± 1.01	3.10 ± 0.56	0.70 ± 0.16	0.331 ± 0.01
HgCl ₂ + <i>R. sativus</i>	$7.51 \pm 0.54^{* \#}$	$3.85 \pm 0.45^{\#}$	$0.82 \pm 0.09^{\#}$	$0.420 \pm 0.07^{\#}$

Values are expressed as mean $\pm$ SD ($n = 7$ per group). * Significantly different from the control group ($P < 0.05$); # significantly different from the HgCl₂-treated group ($P < 0.05$). Statistical comparisons were made by ANOVA followed by Tukey's HSD post hoc test. A symbol is never applied to the group that serves as the reference for that comparison; the HgCl₂ group therefore carries * only, and the control group carries no symbol.

These observations are consistent with reports that inorganic mercury decreases body weight gain by reducing food and water intake, disrupting nutrient utilization, and increasing metabolic stress (1), and by impairing mitochondrial energy metabolism and protein synthesis via its affinity for sulfhydryl groups (23), so the weight loss observed is likely indicative of overall systemic toxicity rather than a single organ-specific effect. The partial recovery of body weight with *R. sativus* co-treatment paralleled the normalization of metabolic parameters, hepatorenal function, tissue glutathione, and organ architecture observed in this study and may relate to the richness of radish in anthocyanins, flavonoids, phenolic acids, vitamin C, and glucosinolate-derived isothiocyanates (11, 24, 25). However, food intake was not quantitatively measured, precluding a definitive distinction between improved nutrition and direct cytoprotection. The increased liver and kidney weights in HgCl₂-treated rats are consistent with biochemical and histopathological evidence of organ injury, as organ enlargement is commonly associated with inflammation, vascular congestion, and degenerative changes (1, 26).

This trend of sustained body weight gain is in agreement with previous reports of enhanced growth performance and physiological adaptation after oral radish juice supplementation in other species (27)

Hematological responses

HgCl₂ exposure was associated with endpoint-specific hematological alterations rather than a generalized hematological disturbance. Compared with the Control group, RBC and hemoglobin concentrations

were numerically higher in the HgCl₂ group, by approximately 14.4% and 26.2%, respectively (Fig. 1E and 1B).

However, in the factorial analysis, the main effect of HgCl₂ was significant for HGB ($P = 0.0149$) but not for RBC ($P = 0.2696$) or HCT ($P = 0.7695$). The HgCl₂ $\times$ *R. sativus* interaction was significant for RBC ($P = 0.0009$; partial $\eta^2 = 0.434$) and HGB ($P = 0.0004$; partial $\eta^2 = 0.478$), but not for HCT ($P = 0.2278$; partial $\eta^2 = 0.072$). Thus, the numerical increase in RBC should not be interpreted as evidence of a HgCl₂-induced increase in erythrocyte production. Mercury toxicity is characterized by complex interactions with cellular thiol groups and redox homeostasis, and its biological effects can extend to blood cells and other systemic compartments (1, 3, 8).

WBC showed the clearest HgCl₂-associated leukocyte response, increasing by approximately 32% relative to the Control group (Fig. 1C), with a significant HgCl₂ main effect ($P = 0.0009$) and HgCl₂ $\times$ *R. sativus* interaction ($P = 0.0007$; partial $\eta^2 = 0.445$). LY counts increased by approximately 114.7% relative to the Control group (Fig. 1F) and showed also significant effects of HgCl₂ ($P = 0.0006$) and the HgCl₂ $\times$ *R. sativus* interaction ($P = 0.0003$; partial $\eta^2 = 0.494$). These findings are compatible with the broader systemic and oxidative-stress effects described for mercury exposure (1, 3, 8), although the present study did not measure specific inflammatory mediators or detailed leukocyte differentials. The LY values remained compatible with the corresponding total WBC counts, representing a substantial proportion of circulating leukocytes.

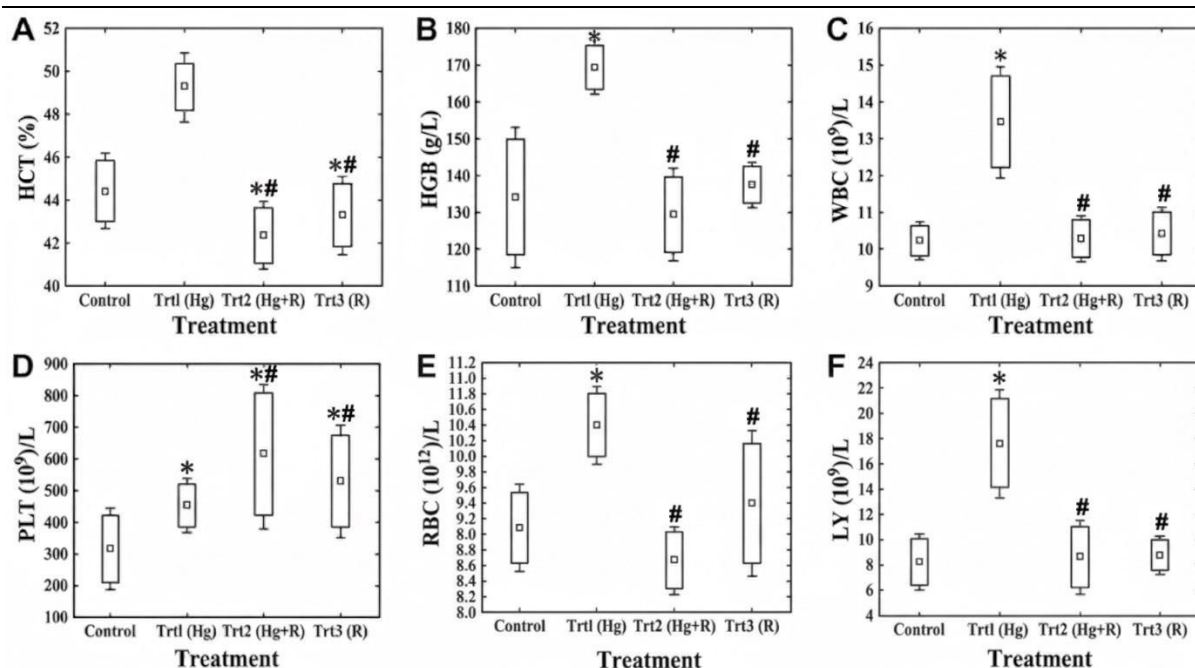


Figure 1.

Effect of *R. sativus* juice on hematological parameters in male Wistar rats exposed to HgCl₂ for 30 days. Panels: (A) HCT, hematocrit; (B) HGB, hemoglobin; (C) WBC, white blood cell count; (D) PLT, platelet count; (E) RBC, red blood cell count; (F) LY, lymphocyte count. Groups: Control, untreated animals; Trt1 (Hg), HgCl₂-treated group; Trt2 (Hg+Rj), rats receiving both HgCl₂ and *R. sativus* juice; Trt3 (Rj), rats receiving *R. sativus* juice alone. * Significantly different from the control group ($P < 0.05$); # significantly different from the HgCl₂-treated group ($P < 0.05$). Statistical comparisons were made by Tukey's HSD post hoc test; the HgCl₂ group therefore carries * only, and the control group carries no symbol

The increases in RBC and HGB should nevertheless be interpreted cautiously. Although the HgCl₂ group showed numerically higher HCT (49.4%) and HGB concentration (169 g/L), HCT was not significantly affected by HgCl₂ exposure. Therefore, these findings do not provide sufficient evidence for a compensatory erythropoietic response to impaired oxygen transport. Hemoconcentration associated with altered hydration status or dehydration may represent an alternative explanation for the concurrent increase in RBC and HGB. However, hydration status, plasma volume, osmolality, and water intake were not quantitatively assessed in the present study; consequently, hemoconcentration cannot be distinguished from other possible mechanisms, including altered erythrocyte production or turnover. Mercury-induced oxidative stress and interactions with cellular sulfhydryl groups provide a biologically plausible context for hematological alterations, but the present data do not allow the specific mechanism responsible for the RBC and HGB changes to be established (1, 3, 8).

Platelet count also varied among the treatment groups; however, the factorial analysis showed no significant HgCl₂ $\times$ *R. sativus* interaction for PLT ($P = 0.7177$; partial $\eta^2 = 0.007$). Therefore, the higher platelet count observed in the HgCl₂ + *R. sativus* group should be

considered an observed group difference rather than evidence of a specific HgCl₂ $\times$ *R. sativus* protective interaction. Because platelet production, turnover, and activation were not investigated, the biological basis of this response remains uncertain.

R. sativus co-treatment was associated with movement of several hematological parameters toward the Control profile, particularly HGB, RBC, WBC, and LY. However, this response was not uniform across all endpoints, and the absence of significant HgCl₂ $\times$ *R. sativus* interactions for HCT and PLT indicates that a generalized normalization of hematological parameters cannot be concluded.

Biochemical and Metabolic Disorders

HgCl₂ exposure resulted in severe metabolic and organ dysfunction as evidenced by significant increments in glucose (+78.4%) (Fig. 2B), triglycerides (+90%), hepatic enzymes (ALT +158.5%, AST +163.6%, ALP +92.9%) (Fig. 2F, 2E and 2C, respectively) and renal biomarkers (urea +89.2%, creatinine +82.3%) (Fig. 2D and 2A, respectively), indicating a global disruption of metabolic homeostasis and impaired detoxification. Mechanistically, mercury binds thiol groups and causes oxidative stress, leading to compromised mitochondrial function and energy production (2, 3).

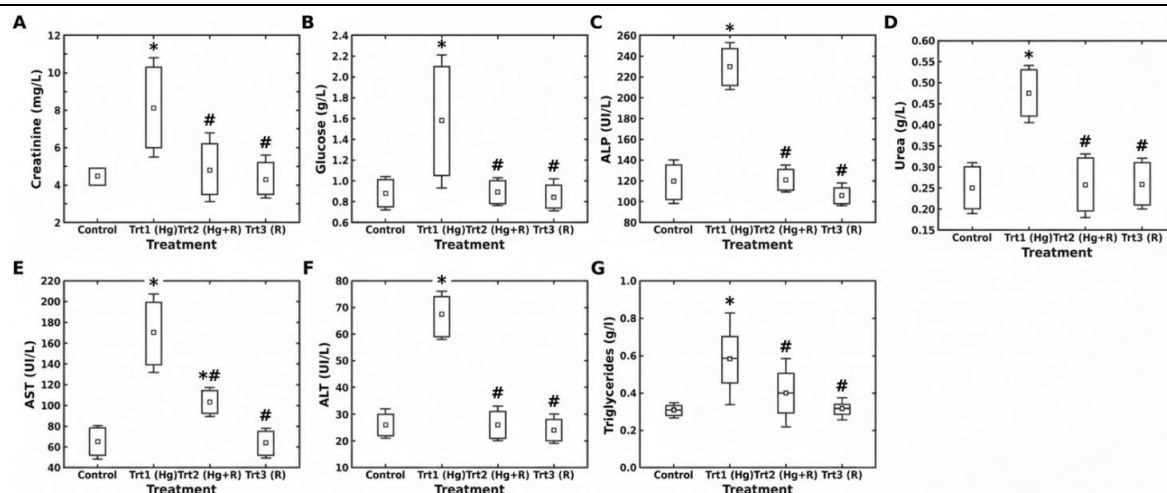


Figure 2.

Effect of *R. sativus* juice on plasma biochemical parameters in male Wistar rats exposed to HgCl_2 for 30 days. (A) Creatinine (mg/L); (B) Glucose (g/L); (C) ALP (UI/L): Alkaline Phosphatase; (D) Urea (g/L); (E) AST (UI/L); (F) ALT (UI/L); and (G) Triglycerides (g/L). Groups: Control, untreated animals; Trt1 (Hg), HgCl_2 -treated group; Trt2 (Hg+Rj), rats receiving both HgCl_2 and *R. sativus* juice; Trt3 (Rj), rats receiving *R. sativus* juice alone. * Significantly different from the control group ($P < 0.05$); # significantly different from the HgCl_2 -treated group ($P < 0.05$). Statistical comparisons were made by Tukey's HSD post hoc test; the HgCl_2 group therefore carries * only, and the control group carries no symbol

Raised hepatic enzymes are markers of hepatocellular injury, and raised urea and creatinine are markers of reduced renal filtration. The strong association between metabolic and hepatic parameters supports a central role for liver dysfunction in systemic metabolic imbalance (28). *R. sativus* treatment significantly improved all biochemical parameters toward normal values, suggesting a multi-target mechanism involving, in particular, strengthening of antioxidant

defenses, preservation of mitochondrial function, and stabilization of metabolic processes.

These findings are consistent with reports of radish juice, alone or in combination with leek juice, ameliorating hepatotoxicity and nephrotoxicity induced by other xenobiotics like dimethoate, indicating a broader protective role against chemically induced organ injury (29).

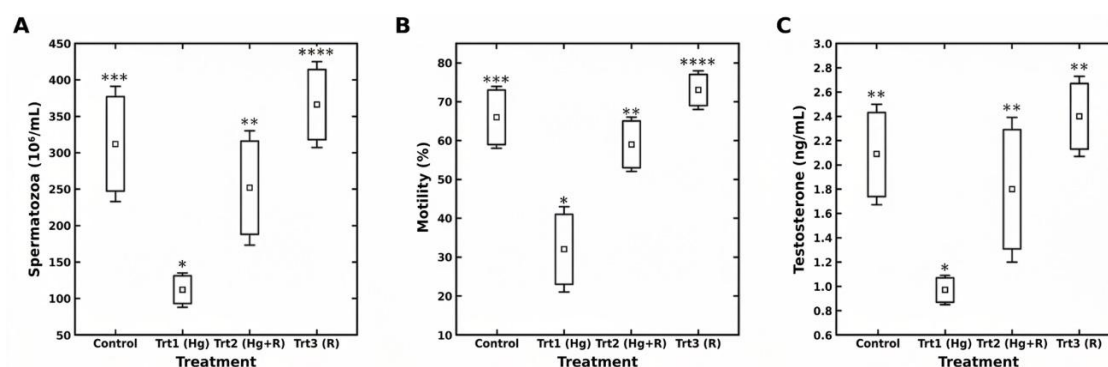


Figure 3.

Effect of *R. sativus* juice on spermogram parameters in male Wistar rats exposed to HgCl_2 for 30 days. Panels: (A) sperm concentration; (B) sperm motility; (C) plasma testosterone. Groups: Control, untreated animals; Trt1 (Hg), HgCl_2 -treated group; Trt2 (Hg+Rj), rats receiving both HgCl_2 and *R. sativus* juice; Trt3 (Rj), rats receiving *R. sativus* juice alone. * Significantly different from the control group ($P < 0.05$); # significantly different from the HgCl_2 -treated group ($P < 0.05$). Statistical comparisons were made by Tukey's HSD post hoc test; the HgCl_2 group therefore carries * only, and the control group carries no symbol

Glutathione (GSH) Status and Reproductive Disorders

The male reproductive system was highly vulnerable to HgCl_2 with a marked decline in sperm

concentration (-64.26%), sperm motility (-51.71%), and plasma testosterone (-53.14%) (Fig. 3A, 3B and 3C, respectively), indicating impaired spermatogenesis and endocrine function, which is consistent with

the well-known reproductive toxicity of inorganic mercury — oxidative damage, mitochondrial dysfunction, impaired steroidogenesis, and germinal epithelium degeneration (30, 31) — and the emerging view that natural antioxidants can alleviate heavy metal-induced reproductive toxicity (32). The exploratory multivariate analyses showed strong inverse correlations between the reproductive variables and biochemical indicators of metabolic and hepatorenal dysfunction, which is compatible with reproductive impairment forming part of a broader systemic toxic response rather than an isolated endpoint; these associations are descriptive and do not, by themselves, establish causality.

GSH concentrations were significantly affected in both liver and kidney tissues following HgCl₂ exposure and *R. sativus* treatment (Fig. 4). HgCl₂ exposure was associated with marked reductions in tissue GSH. Hepatic GSH decreased from 1.25 nmol/mg protein in the Control group to 0.47 nmol/mg protein in the HgCl₂ group, corresponding to an approximately 62% reduction. Renal GSH similarly decreased from 1.11 to 0.41 nmol/mg protein, corresponding to an approximately 63% reduction. Co-administration of *R. sativus* with HgCl₂ increased GSH concentrations to 1.58 nmol/mg protein in the liver and 1.15 nmol/mg protein in the kidney, whereas *R. sativus* alone produced values of 1.22 and 1.04 nmol/mg protein, respectively.

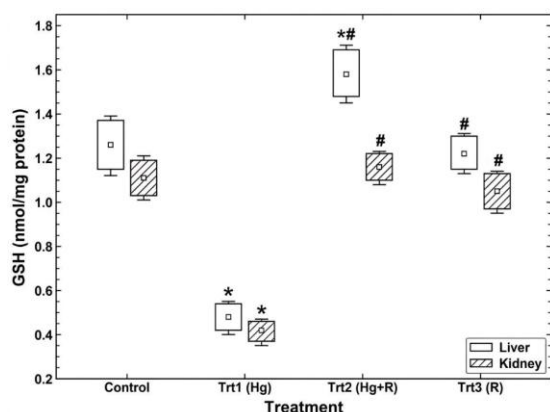


Figure 4.

Effect of *R. sativus* juice on hepatic and renal reduced glutathione (GSH) concentrations in male Wistar rats exposed to HgCl₂ for 30 days. Groups:

Control, untreated animals; Trt1 (Hg), HgCl₂-treated group; Trt2 (Hg+Rj), rats receiving both HgCl₂ and *R. sativus* juice; Trt3 (Rj), rats receiving *R. sativus* juice alone. * Significantly different from the control group ($P < 0.05$); # significantly different from the HgCl₂-treated group ($P < 0.05$). Statistical comparisons were made by Tukey's HSD post hoc test; the HgCl₂ group therefore carries * only, and the control group carries no symbol

The factorial analysis indicated significant effects of HgCl₂ exposure, *R. sativus* treatment, and their interaction on both hepatic and renal GSH. For hepatic GSH, the HgCl₂ main effect was significant ($P = 0.0001$), as were the *R. sativus* main effect and HgCl₂ $\times$ *R. sativus* interaction (both $P < 0.0001$). Similarly, for renal GSH, significant effects were observed for HgCl₂ exposure, *R. sativus* treatment, and their interaction (all $P < 0.0001$). These results indicate that the effect of *R. sativus* on GSH depended on the HgCl₂ exposure context rather than representing a uniform increase in basal GSH.

Inorganic mercury has a high affinity for sulfhydryl groups and can interact with GSH, thereby disturbing cellular redox homeostasis and increasing susceptibility to oxidative injury (1, 23). The marked GSH depletion observed after HgCl₂ exposure is therefore biologically compatible with mercury-associated oxidative stress. The increase in GSH following combined HgCl₂ and *R. sativus* administration is also compatible with a restoration of redox homeostasis. However, the pattern requires cautious interpretation. The hepatic GSH concentration in the HgCl₂ + *R. sativus* group (1.58 nmol/mg protein) was higher than that of the control group (1.25 nmol/mg protein), whereas *R. sativus* alone did not produce a comparable increase (1.22 nmol/mg protein). This finding argues against a simple explanation in which *R. sativus* uniformly elevates basal GSH concentrations. Instead, the response may reflect a context-dependent adaptation to HgCl₂-induced redox disturbance, in which the presence of the toxicant alters the biological response to supplementation. Nevertheless, the present study measured only tissue GSH and did not assess GSH synthesis, turnover, glutathione-related enzymes, oxidized glutathione (GSSG), or other redox biomarkers. Consequently, the mechanism responsible for the hepatic value exceeding the control level cannot be established from the present data and should not be interpreted as evidence of a direct GSH-stimulating effect of *R. sativus*.

The association between *R. sativus* administration and higher tissue GSH under HgCl₂ exposure is biologically compatible with the antioxidant and cytoprotective properties attributed to its phenolic compounds, flavonoids, anthocyanins, vitamin C, glucosinolates, and isothiocyanates (11, 24, 25, 33). Red radish roots have been reported to contain substantial phenolic and flavonoid contents and to exhibit DPPH, FRAP, and ORAC antioxidant activity (13). Glucoraphasatin can also be converted into bioactive isothiocyanates, including raphasatin and sulforaphene, following tissue disruption (12). In addition, radish supplementation has been reported to attenuate toxin-induced oxidative stress and hepatorenal injury (33). These observations provide biological plausibility for the present GSH findings, but

they do not establish that a specific phytochemical constituent was responsible for the observed changes, because the freshly prepared juice was not chemically standardized and its phenolic, flavonoid, glucosinolate, isothiocyanate, or antioxidant content was not quantified before administration.

The redox interpretation is further limited by the fact that GSH was the only oxidative-stress-related biomarker measured. Lipid peroxidation products, antioxidant enzyme activities, ROS generation, inflammatory mediators, and tissue mercury concentrations were not determined. Thus, the present findings support an association between *R. sativus* administration and restoration of tissue GSH under HgCl_2 exposure, but they do not demonstrate a complete antioxidant mechanism. The absence of a positive-control group receiving an established protective agent, such as N-acetylcysteine (NAC), also prevents direct comparison of the magnitude of the observed response with that produced by a reference intervention. Future studies should therefore incorporate a reference antioxidant arm together with measurements of lipid peroxidation, antioxidant enzymes, GSH/GSSG balance, oxidative and inflammatory markers, and tissue mercury concentrations.

The absence of direct mercury measurements is also important when interpreting the apparent GSH restoration. Tissue Hg concentrations were not quantified; therefore, the internal Hg burden cannot be established, and it cannot be determined whether *R. sativus* altered gastrointestinal Hg absorption or elimination or instead attenuated downstream biological effects after Hg absorption. These possibilities are not mutually exclusive and require direct measurement in future experiments. Accordingly, the present GSH findings should be interpreted as evidence of an association with altered redox status rather than proof that *R. sativus* directly counteracted tissue Hg accumulation.

It is also worth noting that fresh *R. sativus* juice has been reported to have antifertility effects in other experimental settings, indicating that its effects on reproductive function may depend on dose and preparation (34). This observation further supports the need for dose-response studies before extrapolating the present reproductive findings beyond the tested preparation and dose.

Histopathology findings

The histopathological evaluation was qualitative and descriptive; the observations reported below therefore describe the appearance of representative sections and are not supported by statistical testing. Histopathological examination was concordant with the biochemical evidence of HgCl_2 -induced injury and the apparent protective effect of *R. sativus* juice (Figs. 5-8). Histopathological examination of the testicular sections showed normal seminiferous tubules with intact germinal epithelium and an

abundance of mature spermatozoa in the control and RS-treated rats (Fig. 5A and 5C), whereas HgCl_2 caused degeneration and disorganization of the seminiferous epithelium, depletion of spermatogenic cells, and a marked reduction in mature spermatozoa (Fig. 5B). Co-administration of RS was associated with better preserved tubular integrity and more evident spermatogenic activity (Fig. 5D).

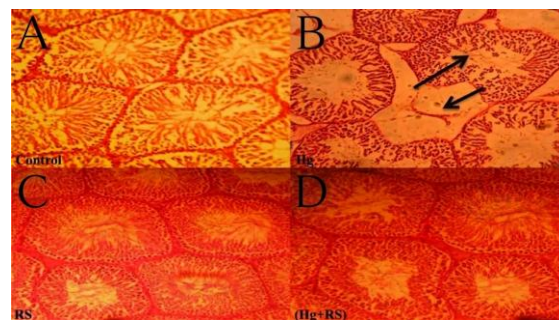


Figure 5.

Histopathological alterations in the testes of male Wistar rats following HgCl_2 exposure and *Raphanus sativus* treatment. Representative H&E-stained sections ($\times 250$). Panels: (A) Control; (B) HgCl_2 (Trt1); (C) *R. sativus* alone (Trt3); (D) HgCl_2 + *R. sativus* (Trt2). Groups: Control, untreated animals; Trt1 (Hg), HgCl_2 -treated group; Trt2 (Hg+Rj), rats receiving both HgCl_2 and *R. sativus* juice; Trt3 (Rj), rats receiving *R. sativus* juice alone

A similar pattern was observed in epididymal sections (Fig. 6A-D). HgCl_2 reduced luminal sperm density and disorganized the epithelium, and these changes appeared less marked in the HgCl_2 + RS group.

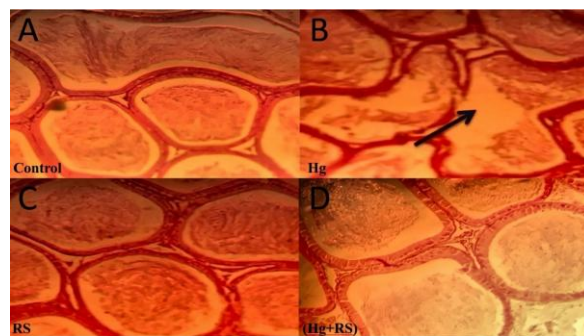


Figure 6.

Histopathological alterations in the epididymides of male Wistar rats following HgCl_2 exposure and *Raphanus sativus* treatment. Representative H&E-stained sections ($\times 250$). Panels: (A) Control; (B) HgCl_2 (Trt1); (C) *R. sativus* alone (Trt3); (D) HgCl_2 + *R. sativus* (Trt2). Groups: Control, untreated animals; Trt1 (Hg), HgCl_2 -treated group; Trt2 (Hg+Rj), rats receiving both HgCl_2 and *R. sativus* juice; Trt3 (Rj), rats receiving *R. sativus* juice alone

HgCl₂ induced centrilobular congestion, sinusoidal dilatation, hepatocellular degeneration, and focal necrosis in the liver (Fig. 7B), which were largely absent in the HgCl₂ + RS group, in which hepatic cords and hepatocyte morphology were better maintained (Fig. 7A, 7C and 7D).

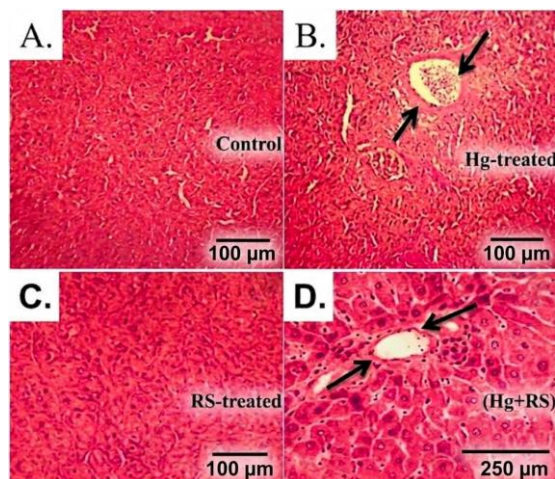


Figure 7.

Histopathological alterations in the livers of male Wistar rats following HgCl₂ exposure and *Raphanus sativus* treatment. Panels: (A) Control; (B) HgCl₂ (Trt1); (C) *R. sativus* alone (Trt3); (D) HgCl₂ + *R. sativus* (Trt2). Groups: Control, untreated animals; Trt1 (Hg), HgCl₂-treated group; Trt2 (Hg+Rj), rats receiving both HgCl₂ and *R. sativus* juice; Trt3 (Rj), rats receiving *R. sativus* juice alone. All photomicrographs were obtained from liver tissue sections of the male Wistar rats included in the present study

In the kidney, HgCl₂ caused glomerular degeneration, tubular swelling, cytoplasmic vacuolization, tubular necrosis, and vascular congestion (Fig. 8B). These changes were qualitatively less severe in the HgCl₂ + RS group, in which glomerular integrity and tubular organization appeared better preserved (Fig. 8A, 8C and 8D).

These findings are consistent with reports of mercury-induced hepatotoxicity by oxidative stress, lipid peroxidation, mitochondrial dysfunction, and membrane disruption (1, 23, 26, 35, 36) as well as similar hepatoprotective effects of *R. sativus* in other models of chemically induced liver injury (15, 33). Mercury preferentially concentrates in the renal cortex, binds sulfhydryl-containing proteins, and induces oxidative injury (1, 2). The near-normal renal architecture in RS-treated rats is consistent with the nephroprotective activity of *R. sativus* reported in experimental nephrotoxicity models (37). The agreement between the qualitative histological observations and the functional (biochemical, reproductive) findings suggests that the protective effect of *R.*

sativus may also extend to the preservation of tissue organization. Because the histological evaluation was purely qualitative, however, the apparent protection described here remains descriptive; semi-quantitative lesion scoring or quantitative histomorphometry, performed on coded slides, will be required to establish its magnitude and to support any claim of statistical significance. However, the involvement of antioxidant mechanisms is considered biologically plausible but not mechanistically proven due to the lack of mercury quantification in tissues and complementary oxidative-stress evaluations.

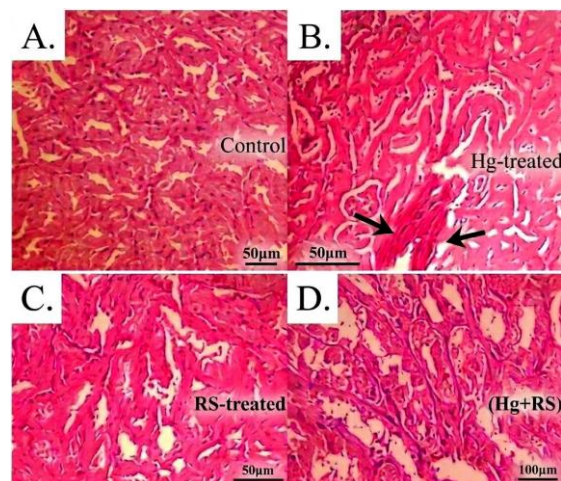


Figure 8.

Histopathological alterations in the kidneys of male Wistar rats following HgCl₂ exposure and *Raphanus sativus* treatment. Panels: (A) Control; (B) HgCl₂ (Trt1); (C) *R. sativus* alone (Trt3); (D) HgCl₂ + *R. sativus* (Trt2). Groups: Control, untreated animals; Trt1 (Hg), HgCl₂-treated group; Trt2 (Hg+Rj), rats receiving both HgCl₂ and *R. sativus* juice; Trt3 (Rj), rats receiving *R. sativus* juice alone. All photomicrographs were obtained from liver tissue sections of the male Wistar rats included in the present study

Effects of HgCl₂ Exposure and Raphanus sativus Supplementation: Two-Way Factorial ANOVA

To provide a more appropriate assessment of the experimental design, the effects of HgCl₂ exposure, *Raphanus sativus* supplementation, and their combined effect were evaluated using a two-way factorial ANOVA (Table III). This approach allowed the independent main effects of the two factors to be distinguished from their interaction, which specifically tests whether the response to *R. sativus* supplementation differed according to the presence or absence of HgCl₂ exposure. Such an interaction is particularly relevant to the interpretation of a potential protective response because a significant interaction indicates that the effect of one factor is conditional on the level of the other factor.

Table III

Two-way factorial ANOVA assessing the effects of HgCl₂ exposure, *Raphanus sativus* supplementation, and their interaction on physiological, biochemical, hematological, and antioxidant parameters in male Wistar rats

Variable	Hg effect P	RS effect P	Hg × RS interaction P	Partial η^2 interaction
Motility	<0.0001	<0.0001	0.0051	0.331
Testosterone	<0.0001	0.0023	0.1409	0.105
Sperm concentration	<0.0001	0.0012	0.1117	0.122
Glucose	0.0166	0.0189	0.0335	0.207
Triglycerides	<0.0001	0.0038	0.0033	0.357
Creatinine	0.0050	0.0175	0.0316	0.211
Urea	0.0006	0.0009	0.0005	0.460
AST/TGO	<0.0001	0.0015	0.0018	0.394
ALT/TGP	<0.0001	<0.0001	<0.0001	0.744
ALP/PAL	<0.0001	<0.0001	<0.0001	0.719
RBC	0.2696	0.0131	0.0009	0.434
HGB	0.0149	0.0017	0.0004	0.478
HCT	0.7695	0.0128	0.2278	0.072
PLT	0.1190	0.0125	0.7177	0.007
WBC	0.0009	0.0021	0.0007	0.445
LY	0.0006	0.0009	0.0003	0.494
GSH—liver	0.0001	<0.0001	<0.0001	0.892
GSH—kidney	<0.0001	<0.0001	<0.0001	0.882

The factorial analysis revealed a heterogeneous response across the investigated physiological, biochemical, hematological, and antioxidant endpoints. Among the physiological and reproductive parameters, motility was significantly affected by both HgCl₂ and *R. sativus* (both $P < 0.0001$), with a significant HgCl₂ × *R. sativus* interaction ($P = 0.0051$; partial $\eta^2 = 0.331$), indicating that the effect of supplementation on motility differed according to HgCl₂ exposure. In contrast, testosterone and sperm concentration showed significant main effects of both factors (testosterone: HgCl₂, $P < 0.0001$; *R. sativus*, $P = 0.0023$; sperm concentration: HgCl₂, $P < 0.0001$; *R. sativus*, $P = 0.0012$), but their interactions were not statistically significant ($P = 0.1409$ and 0.1117 , respectively). These findings suggest that HgCl₂ exposure and *R. sativus* supplementation were independently associated with reproductive alterations, whereas a statistically demonstrable HgCl₂-dependent modification of the supplementation effect was not established for testosterone or sperm concentration.

A significant interaction pattern was observed for the metabolic and renal-related parameters. Glucose was significantly influenced by HgCl₂ ($P = 0.0166$) and *R. sativus* ($P = 0.0189$), with a significant interaction ($P = 0.0335$; partial $\eta^2 = 0.207$). Triglycerides showed highly significant main effects of HgCl₂ ($P < 0.0001$) and *R. sativus* ($P = 0.0038$), together with a significant interaction ($P = 0.0033$; partial $\eta^2 = 0.357$). Similarly, creatinine showed significant main effects of HgCl₂ ($P = 0.0050$) and *R. sativus* ($P = 0.0175$), accompanied by a significant interaction ($P = 0.0316$; partial $\eta^2 = 0.211$). Urea displayed an even stronger interaction pattern, with significant effects of HgCl₂ ($P = 0.0006$) and *R. sativus* ($P = 0.0009$) and a highly significant HgCl₂ × *R. sativus* interaction ($P = 0.0005$;

partial $\eta^2 = 0.460$). Thus, the response of these metabolic and renal-related endpoints was not adequately described by independent main effects alone, as the magnitude of the supplementation-associated response depended on HgCl₂ exposure.

The hepatic enzyme parameters exhibited particularly pronounced interaction effects. AST/TGO showed significant main effects of HgCl₂ ($P < 0.0001$) and *R. sativus* ($P = 0.0015$), together with a significant interaction ($P = 0.0018$; partial $\eta^2 = 0.394$). More pronounced interactions were observed for ALT/TGP and ALP/PAL, for which both main effects were highly significant ($P < 0.0001$ for each factor), and the corresponding interactions were also highly significant ($P < 0.0001$ for both), with partial η^2 values of 0.744 and 0.719, respectively. These large interaction effect sizes indicate that the responses of hepatic enzyme activities were strongly dependent on the combined experimental conditions. Accordingly, the effects of *R. sativus* on hepatic biochemical indices should be interpreted in relation to HgCl₂ exposure rather than as an unconditional supplementation effect.

The hematological variables also demonstrated endpoint-specific responses. RBC showed no significant main effect of HgCl₂ ($P = 0.2696$), whereas the effect of *R. sativus* was significant ($P = 0.0131$); nevertheless, the HgCl₂ × *R. sativus* interaction was significant ($P = 0.0009$; partial $\eta^2 = 0.434$). HGB showed significant effects of HgCl₂ ($P = 0.0149$) and *R. sativus* ($P = 0.0017$), as well as a significant interaction ($P = 0.0004$; partial $\eta^2 = 0.478$). In contrast, HCT showed a significant effect of *R. sativus* ($P = 0.0128$) but neither a significant HgCl₂ effect ($P = 0.7695$) nor a significant interaction ($P = 0.2278$; partial $\eta^2 = 0.072$). Likewise, PLT was significantly affected by *R. sativus* ($P = 0.0125$), but

neither the HgCl₂ main effect ($P = 0.1190$) nor the interaction ($P = 0.7177$; partial $\eta^2 = 0.007$) was significant. For WBC and LY, both factors exerted significant main effects (WBC: HgCl₂, $P = 0.0009$; *R. sativus*, $P = 0.0021$; LY: HgCl₂, $P = 0.0006$; *R. sativus*, $P = 0.0009$), with significant interactions for WBC ($P = 0.0007$; partial $\eta^2 = 0.445$) and LY ($P = 0.0003$; partial $\eta^2 = 0.494$). Thus, the hematological response was not uniform across all measured indices, with significant factor interactions occurring for RBC, HGB, WBC, and LY but not for HCT or PLT. The strongest interaction effects were observed for tissue glutathione. Liver GSH was significantly affected by HgCl₂ ($P = 0.0001$) and *R. sativus* ($P < 0.0001$), with a highly significant interaction ($P < 0.0001$; partial $\eta^2 = 0.892$). Kidney GSH showed a closely comparable pattern, with significant main effects of both HgCl₂ and *R. sativus* (both $P < 0.0001$) and a highly significant interaction ($P < 0.0001$; partial $\eta^2 = 0.882$). These were the largest interaction effect sizes observed among all endpoints, indicating that tissue GSH responses were particularly strongly dependent on the combination of HgCl₂ exposure and *R. sativus* supplementation. Given the central role of glutathione in cellular redox homeostasis, the marked interaction observed in both liver and kidney is consistent with a treatment-dependent modification of the antioxidant response; however, the direction of the interaction should be interpreted together

with the corresponding group means rather than from the interaction statistic alone.

Correlation, clustering, and principal component analysis

Exploratory Pearson correlation analysis revealed numerous strong associations among reproductive, metabolic, hepatorenal, hematological, and oxidative-stress variables. In the pooled dataset, sperm motility was positively correlated with sperm concentration and testosterone, while showing strong inverse associations with several indicators of metabolic and hepatic dysfunction, including PAL, TGP, triglycerides, and urea. Triglycerides were positively correlated with TGO, TGP, and PAL, whereas glucose was positively associated with creatinine and urea. The hepatic enzymes were also strongly inter-correlated, particularly TGP with PAL and TGO. Hepatic and renal GSH showed a strong positive intercorrelation ($r = 0.930$) and were inversely associated with TGP and PAL, whereas lymphocyte counts were inversely associated with sperm motility and positively associated with PAL. The two-way factorial ANOVA further demonstrated significant main effects of HgCl₂ and/or *R. sativus* for most endpoints, with significant HgCl₂ $\times$ *R. sativus* interactions observed for the evaluated variables, including sperm motility, glucose, triglycerides, creatinine, urea, hepatic enzymes, selected hematological indices, and hepatic and renal GSH (Table IV).

Table IV

Two-way factorial ANOVA P values and interaction effect sizes

Variable pair	Pearson r	P-value	BH-FDR q
GSH Liver – GSH Kidney	0.930	9.01×10^{-13}	1.38×10^{-10}
TGP – GSH Kidney	−0.912	1.37×10^{-11}	1.04×10^{-9}
PAL – GSH Kidney	−0.902	5.88×10^{-11}	2.25×10^{-9}
TGP – PAL	0.902	5.34×10^{-11}	2.25×10^{-9}
Motility – PAL	−0.899	8.37×10^{-11}	2.56×10^{-9}
PAL – LY	0.875	1.11×10^{-9}	2.82×10^{-8}
Triglycerides – TGO	0.872	1.51×10^{-9}	3.30×10^{-8}
Motility – LY	−0.868	2.13×10^{-9}	4.08×10^{-8}
Urea – GSH Kidney	−0.867	2.47×10^{-9}	4.19×10^{-8}
TGO – TGP	0.866	2.74×10^{-9}	4.19×10^{-8}
PAL – GSH Liver	−0.862	3.77×10^{-9}	5.25×10^{-8}
TGP – GSH Liver	−0.857	5.81×10^{-9}	7.41×10^{-8}
LY – GSH Kidney	−0.854	7.64×10^{-9}	8.34×10^{-8}
Triglycerides – PAL	0.854	7.33×10^{-9}	8.34×10^{-8}
PAL – WBC	0.843	1.76×10^{-8}	1.80×10^{-7}
TGO – PAL	0.835	3.19×10^{-8}	3.05×10^{-7}
Urea – PAL	0.833	3.73×10^{-8}	3.28×10^{-7}
Urea – TGP	0.832	4.07×10^{-8}	3.28×10^{-7}
Motility – TGP	−0.833	3.93×10^{-8}	3.28×10^{-7}
Motility – Concentration	0.822	8.17×10^{-8}	6.25×10^{-7}

In contrast, testosterone, sperm concentration, HCT, and PLT showed no significant HgCl₂ $\times$ *R. sativus* interaction. Overall, 125 of the 153 pairwise correlations were significant before correction and remained significant after Benjamini–Hochberg false-discovery-rate (FDR) correction ($q < 0.05$).

However, because the treatment groups showed marked separation across several endpoints, pooled correlations may partly reflect treatment-related group structure. Accordingly, partial Pearson correlations controlling for treatment group were also examined. After adjustment, 17 of the 153 associations

remained nominally significant ($P < 0.05$), but only two remained significant after FDR correction: sperm motility with lymphocyte count ($r_{\text{partial}} = -0.632$, $q = 0.026$) and sperm concentration with urea ($r_{\text{partial}} = 0.629$, $q = 0.026$). These findings indicate that much of the strong pooled correlation structure was influenced by treatment-related

separation; therefore, the remaining associations should be considered exploratory and hypothesis-generating rather than evidence of direct mechanistic relationships or causality.

Hierarchical clustering after Z-score standardization revealed two broad branches with several nested associations (Fig. 9).

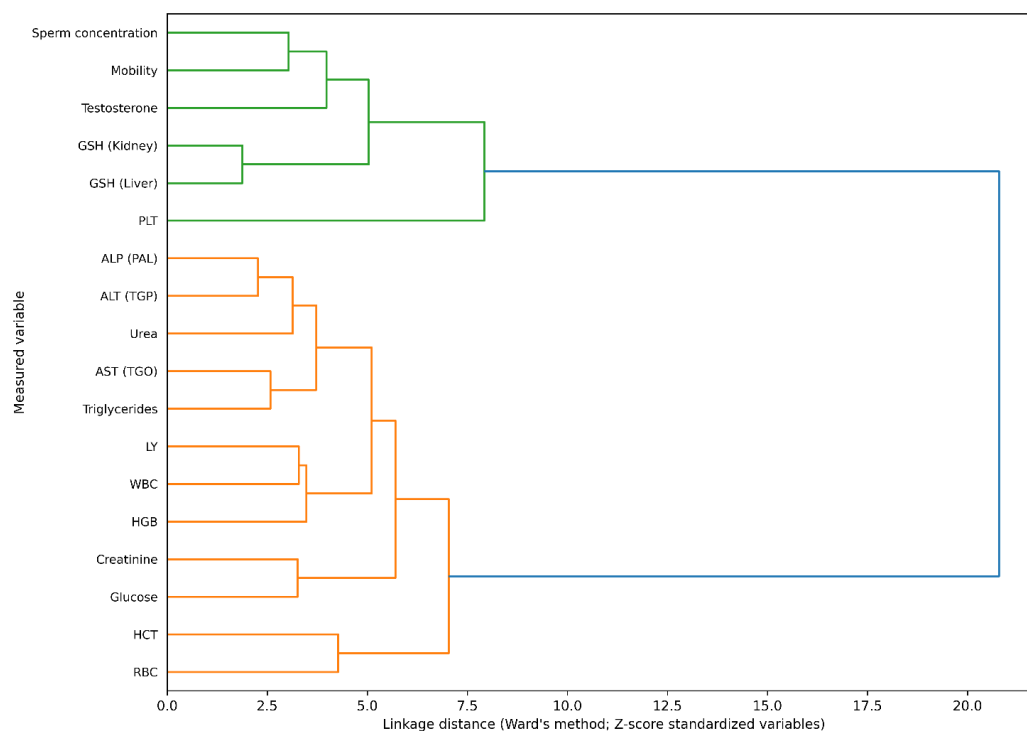


Figure 9.

Hierarchical clustering of measured variables after Z-score standardization using Euclidean distance and Ward's minimum-variance linkage ($n = 28$ animals, 7 rats *per* group)

A relatively compact reproductive/antioxidant branch comprised sperm concentration and motility, followed by testosterone and hepatic and renal GSH, indicating coordinated variation among these endpoints in the present dataset. In a separate branch, hepatic and metabolic variables showed close associations, including ALT and ALP, triglycerides and AST, and glucose and creatinine, with urea subsequently joining the hepatic/metabolic subgroup. Hematological variables were incorporated into this broader branch, with WBC and lymphocytes forming a close pair, HGB joining this subgroup, and RBC and HCT forming a separate closely associated pair. Platelet count remained relatively distinct and joined the reproductive/antioxidant branch only at a substantially greater linkage distance. The two broad branches converged only at a relatively large standardized linkage distance. These relationships describe statistical similarity among variables and should not be interpreted as evidence of discrete biological pathways or causality.

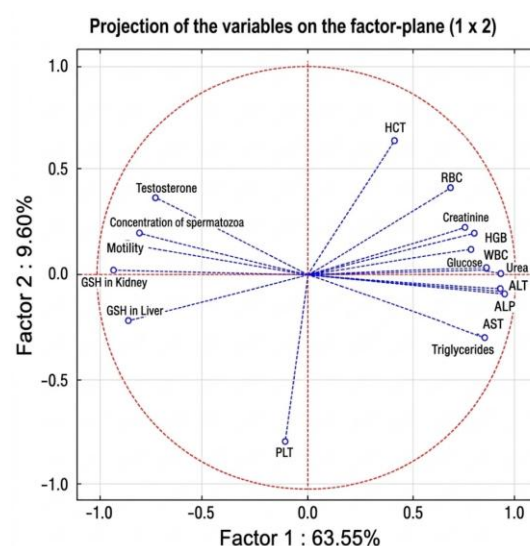


Figure 10.

Principal component analysis of measured variables (28 animals, all groups pooled; PC1 63.55% and PC2 9.60% of total variance)

Principal component analysis (Fig. 10) revealed PC1 and PC2 accounted for 63.5% and 9.6% of total variance, respectively, with a cumulative explained variance of 73.1%. PC1 was characterized by a strong positive loadings for variables associated with hepatic, renal, metabolic and inflammatory alterations, particularly PAL ($r = 0.96$), TGP ($r = 0.95$), TGO ($r = 0.88$), urea ($r = 0.87$), WBC ($r = 0.87$), lymphocytes ($r = 0.88$), triglycerides ($r = 0.84$), HGB ($r = 0.82$), creatinine ($r = 0.78$), glucose ($r = 0.76$), and RBC ($r = 0.72$). In the opposite direction, PC1 showed negative loadings for renal GSH ($r = -0.95$), hepatic GSH ($r = -0.88$), sperm motility ($r = -0.88$), sperm concentration ($r = -0.81$), and testosterone ($r = -0.78$). PC2 was characterized primarily by negative loadings for platelet count (PLT; $r = -0.79$) and positive loadings for hematocrit (HCT; $r = 0.64$), with weaker contributions from RBC ($r = 0.41$) and testosterone ($r = 0.36$).

These multivariate analyses were interpreted strictly as exploratory and descriptive, particularly because the number of variables was high relative to the number of experimental observations. Moreover, the marked separation among the four treatment groups means that the PCA structure can substantially reflect between-group treatment effects rather than treatment-independent biological relationships. Therefore, the opposing loading patterns on PC1 should not be interpreted as evidence of distinct “toxicity” and “protective” axes or as proof of mechanistic relationships among the variables. Rather, they summarize the coordinated differences observed across the experimental groups. This interpretation is consistent with the correlation analysis, in which the strong pooled correlation structure was substantially attenuated after adjustment for treatment group. Accordingly, PCA and other multivariate analyses were used only to identify broad patterns and generate hypotheses regarding the relationships among systemic toxicity, reproductive endpoints and tissue redox status. These hypotheses require confirmation in larger, independently replicated studies incorporating comprehensive oxidative-stress biomarkers, tissue mercury measurements and molecular investigations.

Conclusions

The present study demonstrates that chronic mercuric chloride exposure induces significant multisystem toxicity in adult male rats as shown by metabolic derangements, hepatorenal dysfunction, depletion of tissue glutathione, histopathological changes, and impaired reproductive function. The combination of biochemical, reproductive, qualitative histopathological, and exploratory multivariate analyses provided an integrated view of the systemic effects of mercury exposure and revealed consistent associations

among the measured variables. Coadministration of *R. sativus* juice markedly attenuated these changes, improving biochemical and reproductive parameters, restoring tissue glutathione levels, and, on qualitative examination, better preserving hepatic, renal, testicular, and epididymal histoarchitecture. These findings suggest that *R. sativus* juice's protective potential against mercury-induced toxicity and its role in preserving cellular redox homeostasis may contribute to the observed benefits. However, the redox assessment was limited to glutathione, the histopathological evaluation was qualitative, no positive-control group receiving a reference protective agent such as N-acetylcysteine was included, and a single dose was evaluated; the precise protective mechanisms, their magnitude relative to an established antioxidant, and dose-response relationships therefore remain to be determined. Future studies should include dose-response designs, a positive-control arm, full oxidative-stress profiling, quantitative histopathological scoring, mercury bioaccumulation assessment, larger cohorts, and molecular approaches.

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Funding

Not applicable.

Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All experimental procedures involving animals were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (8th edn) and reported in accordance with the ARRIVE 2.0 guidelines, with the principles of Replacement, Reduction, and Refinement (3Rs) applied to minimize animal use and animal distress (38). The study protocol was reviewed and approved by the Institutional Ethics Committee of Mohamed Boudiaf Hospital, Médéa, Algeria, under Approval/Certificate No. MBHM/IEC/2026/02. The approval number identifies the ethics certificate and does not represent an approval period. The certificate was issued before the commencement of the animal experiment. The study was conducted only after the ethical approval had been obtained.

AI use disclosure

During the preparation of this manuscript, the author(s) used AI tools for editing assistance and

language improvement, specifically to improve spelling, grammar, word choice, readability, and clarity. The authors reviewed and revised all outputs from these tools and take full responsibility for the final content of the work.

Conflict of interest

The authors declare no conflict of interest.

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