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EFFECT OF MERCURY ADMINISTRATION AS AN OXIDATIVE STRESS TRIGGER IN HEPATO-RENAL INJURIES

Ida Yuliana[1]*, Triawanti Triawanti[2], Muhammad Darwin Prenggono[3], Ika Kustiyah Oktaviyanti[4], Irfan Maulana[5], Fahrina Ulfah[6]

[1]Department of Biomedics, Faculty of Medicine and Health Sciences, Lambung Mangkurat University, Banjarmasin 70232, Indonesia
[2]Department of Biochemistry, Faculty of Medicine, Lambung Mangkurat University, Banjarmasin 70232, Indonesia
[3]Department of Internal Medicine, Faculty of Medicine and Health Sciences, Lambung Mangkurat University, Banjarmasin 70232, Indonesia
[4]Department of Anatomical Pathology, Faculty of Medicine and Health Sciences, Lambung Mangkurat University, Banjarmasin 70232, Indonesia
[5]Nursing Study Program, Faculty of Medicine, Lambung Mangkurat University, Banjarmasin 70232, Indonesia
[6]Medical Science Education Doctoral Programme, Faculty of Medicine and Health Sciences, Lambung Mangkurat University, Banjarmasin 70232, Indonesia

Corresponding Author:
*) iyuliana@ulm.ac.id

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Abstract

Introduction: *Mercury as the source of free radicals can trigger the activation of oxidative stress pathways. With its high toxicity, it can cause hepato-renal injuries. There have been many studies on mercury toxicity in various organs, but there are still few scientific studies that examine the hepato-renal injuries caused by mercury through the oxidative stress pathway. This study was conducted to investigate the triggering of the oxidative stress pathway due to mercury exposure in hepato-renal injuries.* ***Methods:*** *Research using randomized true laboratory experiment method with post-test control group design. The number of samples used was 28 Wistar rats. The research group consisted of 2 groups, control group was given aquadest ad libitum, and intervention group was given water contaminated with mercury per oral once a day (15 kg/WB). The treatment period was 14 consecutive days and on the 15th day, blood samples were taken. Oxidative stress marker was assessed by examining MDA and GPx levels and hepato-renal injuries were assessed by examining liver function (ALT and AST) and kidney function (ureum and creatinine). The collected data were analyzed by independent t-test with 95% confidence level; significant if p>0.05.* ***Results and Discussion:*** *The study found that mercury can trigger the activation of oxidative stress pathways and have an impact on hepato-renal function.* ***Conclusion:*** *Research still needs to be continued to prove that impaired hepato-renal injuries also occur at the cellular histomorphologic and discover other biomolecular mechanisms such as activation of inflammatory pathways that can also cause organ damage.*

INTRODUCTION

Mercury, a naturally occurring heavy metal found throughout the earth, has been utilized by humans in numerous activities throughout history. It has been utilized across multiple sectors, significantly increasing its atmospheric emissions and environmental accumulation (1–3). However, exposure to mercury, even in low concentrations, has been known to be toxic. The extent and duration of exposure of living organisms to mercury determine its level of toxicity. Mercury exposure may result in intoxication and multi-organ damage including hepato-renal injuries (4-5).

Mercury (Hg) is classified as a heavy metal and is recognized for its significant toxicity, particularly when evaluated against the chemical and physical properties of other heavy metals in its category. Mercury induces notable alterations in cellular biochemistry. Mercury can release oxygen radicals when it breaks down and releases ROS and induces significant cellular damage

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by initiating the lipid peroxidation cascade of the cell membrane. When mercury circulates in the body, it produces disulfides that strongly bind to the sulfide groups of other proteins, changing protein structure and enzyme function. Reduced antioxidant defense and mercury exposure that elevates reactive species levels leads to an imbalance in the pro-oxidant/antioxidant system, causing oxidative stress (6-7). $HgCl_2$, recognized for its high toxicity among mercury salts, undergoes metabolism mainly in the liver and accumulates in the kidneys. The decreased of epithelial cell osmotic water permeability brought on by mercury poisoning in the kidneys may hinder active water transport and osmotic water equilibration, leading to fluid buildup that may eventually cause kidney injury (8). The hepatotoxicity of mercury related to ROS production that actived oxidative stress (6). There have been many studies on mercury toxicity in various organs, but there are still few scientific studies that examine the hepato-renal injuries caused by mercury through the oxidative stress pathway. Therefore, this study was conducted to investigating the triggering of oxidative stress pathway due to mercury exposure in hepato-renal injuries. With this discovery, it will be the basis for finding the right antioxidant agent in preventing the occurrence of hepato-renal injuries due to mercury exposure.

METHODS

Research using randomized true laboratory experiment method with post-test control group design.

Research Tools and Materials

The study subjects were 28 male Wistar strain Rattus Norvegicus rats, aged 8 to 12 weeks, and weigh between 200 and 250 grams. Determination number of samples using the Federer formula (commonly used for laboratory experimental research) and adult male rats were employed in this experiment for the homogenization of the research subject. Commercial enzyme-linked immunosorbent test (ELISA) kits were used to measure of MDA, GPx, ALT, AST, ureum, and creatinine levels (Elabscience TBARS/MDA Colorimetric Assay Kit (TBARS) cat. No E-BC-K025-S, Biovision Glutathione Peroxidase Activity Assay Kit cat. No K762-100, Medik Bio Alanine Transaminase Assay Kit cat No.MDE0753Ra, Medik Bio Aspartate Transaminase Assay Kit cat. No.MDE0761Ra., Medik Bio Ureum Assay Kit cat. No MDB0057 and Medik Bio Creatinine ELISA Kit cat. No MDB0058). The product's guidelines were carefully followed throughout the testing process. The reagents, standard solutions, and samples obtained underwent preparation and analysis at room temperature.

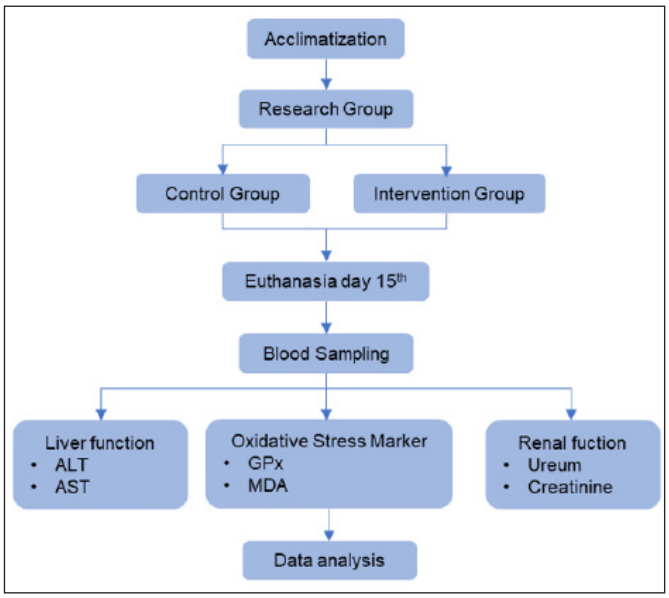


Figure 1. Research Flow

Research Procedure

Procedures, including the usage of rat, were conducted from the management and utilization of all laboratory animals adhered to the guidelines set forth by the guidelines of Indonesian Food and Drug Administration (BPOM) (9). The Ethical Committee of the Faculty of Medicine and Health Science Lambung Mangkurat University, South Kalimantan has granted ethical approval for this study (No: 042/KEPK-FKIK/EC/2024).

Acclimatization stage

The Wistar strain Rattus norvegicus underwent a one-week acclimatization period in cages of 40 cm x 60 cm x 60 cm within a controlled animal laboratory. The environment was maintained at a temperature of 20°C to 22°C, with humidity controlled between 50% and 60%, and a 12-hour light-dark cycle was implemented. Standard commercial rodent pellets and water were provided to the rats on an *ad libitum* basis. The experiment began with a one-week acclimation period, during which rats were housed individually to standardize physical and psychological conditions. Each rat was given a standard diet, and daily assessments of their behavior and physical health were conducted to identify any changes. Acclimatization was finalized on the eighth day. The exclusion criteria for this study included rats that died during the acclimatization phase and $HgCl_2$ induction process and those that appeared unwell after 3 days of induction (9).

Intervention and termination stage

The research procedure included establishing the requisite equipment and techniques and identifying

research objects and subjects that fulfilled the inclusion requirements. Post acclimatization for 7-days, the rats were allocated into two groups (N = 19) as follows: control group rats received purified water, and the intervention group (Hg) received water contaminated with $HgCl_2$ per oral once a day (15 kg/WB) for 14 days. $HgCl_2$ was dissolved in 0.5% DMSO and given orally via oral gavage. The administration was carried out via the oral route. Feed and water were provided *ad libitium* (10).

Blood serum collection and ELISA examination

On the fifteenth day, the rats were anesthetized with lethal dose ketamine injection. After the rat died, the process of taking blood through intracardium was continued. After anesthesia and euthanasia, blood is taken and put in an EDTA tube, 10 ml of blood was obtained from the inferior vena cava to yield serum, which later was utilized to assess oxidative stress by measuring MDA and GPx levels, hepat0-renal injuries by evaluating ALT and AST levels for hepar and urea and creatinine levels for renal. The serum was permitted to clot for 10 to 20 minutes at room temperature, followed by centrifugation at 2000–3000 RPM for 20 minutes at 2–8℃. The supernatant was then collected, ensuring no sediment was included, to carry out the assay. Enzyme-linked immunosorbent assay (ELISA) kits, available commercially, were used to assess the levels of MDA, GPx, ALT, urea, and creatinine. The ELISA specimens and reagents were introduced to each well and incubated for one hour at 37°C, followed by five washes of the plates. After that, substrate solutions A and B were added, and the mixture was incubated at 37°C for 10 minutes. The stop solution was introduced once a color change was visible, and the optical density (OD) values were measured within 10 minutes. The mean OD findings were computed utilizing computer-based curve fitting software, and the optimal fit line was established by regression analysis.

Statistical analysis

The Shapiro-Wilk test was employed for data analysis to assess normality, and Levene's test was implemented to determine homogeneity. The data were homogeneous and normally distributed (p >0.05), and then independent t-tests were used. Statistical analysis was performed using SPSS software, v. 27 (IBM, Chicago, IL, USA). A p-value < 0.05 was considered statistically significant. The measured variables tested by the independent t-test were MDA (uM), GPx (uM), ALT (U/L), AST (U/L), ureum (mmol/L) and creatinine (ug/mL) levels.

RESULTS

Oxidative Stress Analysis by Examining MDA and GPx Levels

On day 14, after mercury treatment administration, a total of 10 mL of blood was drawn from the inferior vena cava to acquire blood serum for the assessment of lipid peroxidase and antioxidants from the measurement of MDA and GPx levels. In Table 1 and Figures 1, the mean MDA value increased significantly in the intervention group (r=4.77 ± 1.72) compared to the control group (r=2.82 ± 1.79). At the same time, the GPx level decreased significantly in the intervention group (r=37.22 ± 23.25) compared to the control group (r=72.28 ± 23.92).

Tabel 1. Effects of Mercury Administration on Markers of Oxidative Stress, Liver Cell Failure, and Kidney Function

Variables	Control (ctrl)	Intervention (Hg)	p-value
MDA (uM)	2.82±1.72	4.47±1.89	0.00*
GPx (uM)	72.28±23.92	37.22±23.25	0.00*
ALT (u/L)	51.77±14.07	70.54±28.60	0.02*
AST(u/L)	45.04±8.57	65.01±4.65	0.00*
Ureum (mmol/L)	18.59±4.72	27.58±7.13	0.01*
Kreatinin(ug/mL)	28.69±4.32	49.61±2.88	0.00*

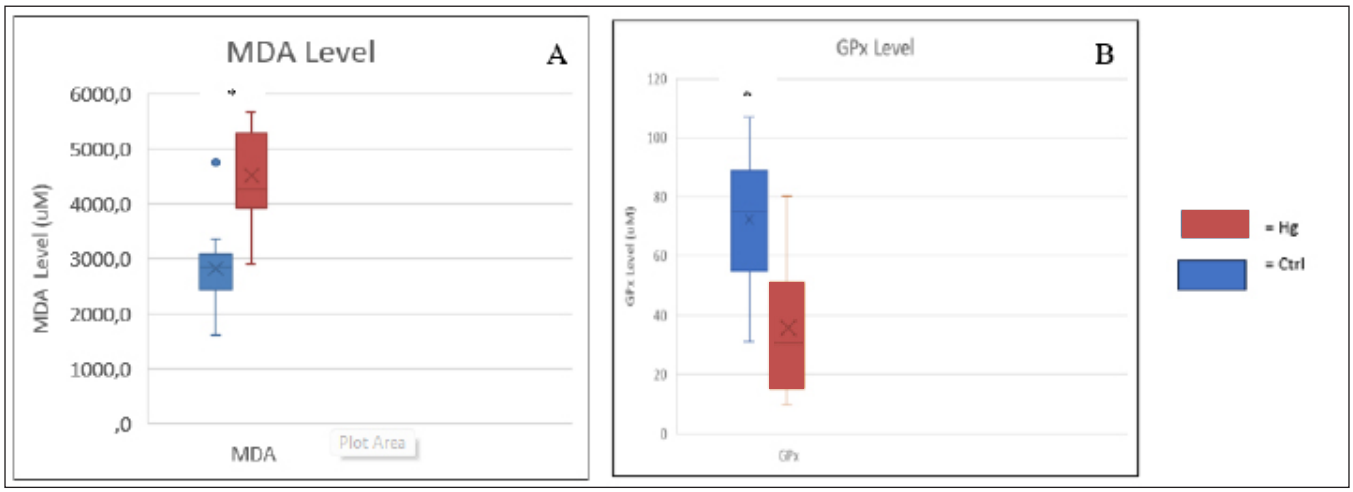
*$P < 0.05$ (significanty compared with the control group) ; independent t-tests

Hepato-Injuries Analysis by Examining AST and ALT Levels

Ten milliliter of blood was drawn from the inferior vena cava on day 14 after mercury treatment administration to obtain serum for determining liver cell faring ALT and AST levels. In Table levels 1 and Figure 2, the mean ALT value increased significantly in the intervention group (r=51.77 ± 14.07) compared to the control group (r=70.54 ± 28.60). The AST level also increased significantly in the intervention group (r=45.04 ± 8.57) compared to the control group (r= 65.01 ± 4.65).

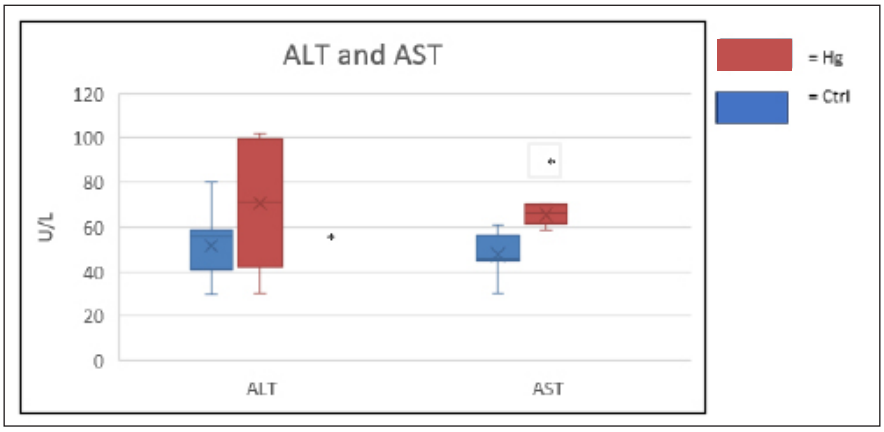
Renal Injuries Analysis by Examining Ureum and Creatinin Levels

On day 14, after mercury treatment administration, blood serum obtained form 10 ml of the inferior vena cava blood was used to determine renal function from the measurement of serum and creatinine levels. In Table 1 and Figure 3, the mean ureum value increased significantly (p=0.01) in the intervention group (r=51.77 ± 14.07) compared to the control group (r=27.58 ± 7.13). The creatinine level also increased significantly in the intervention group (r=49.61 ± 2.88) compared to the control group (r=28.69 ± 4.32).



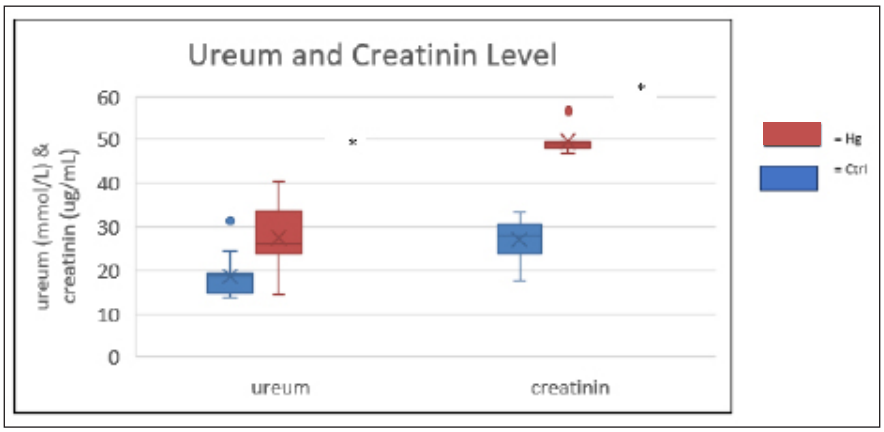
*(A) MDA levels in the control group and the intervention. (B) GPx levels in the control and intervention groups. Data were expressed in standard mean ± from 2 independent research groups. *P < 0.05 (compared with the control group)*

Figure 2. Mercury's Impact on Oxidative Stress Conditions



*ALT levels in the control group and the intervention. AST levels in the control and intervention groups. Data were expressed in standard mean ± from 2 independent research groups. *P < 0.05 (compared with the control group)*

Figure 3. Mercury's Impact on Cell Liver Failure



*Ureum levels in the control group and the intervention. Creatinin levels in the control and intervention groups. Data were expressed in standard mean ± standart from 2 independent research groups. *P < 0.05 (compared with the control group)*

Figure 4. Mercury's Impact on Renal Fuction

DISCUSSION

Our findings suggest that mercury administration, as an exogenous free radical, triggers a state of oxidative stress in rats treated with oral $HgCl_2$ 15/kgBB for 14 days, resulting in hepato-renal function disruption. To prove our research hypothesis, we used oxidative stress markers, namely the antioxidant class of glutathione (glutathione peroxidase/GPx) and the final product of oxidative stress from lipid peroxidation (malondialdehyde/MDA) to show that the administration of mercury may trigger oxidative stress conditions and this is proven in our research by decrease in GPx levels and increase in MDA levels.

The impact of damage to organs due to oxidative stress conditions, because the administration of mercury to the liver is characterized by increased ALT and AST levels, and disturbances in kidney function, are marked by increased urea and creatinine levels.

Oxidative Stress Activated by Mercury Administration

Reactive oxygen species (ROS) can arise from various external influences, including ionizing and ultraviolet radiation, environmental pollutants, dietary sources, pharmaceuticals, and recreational substances. The biological processes by which external factors contribute to ROS production involve mitochondrial injury, reduced activity of antioxidant enzymes, alterations in the glutathione (GSH)/glutathione disulfide (GSSG) ratio, heightened activity of enzymes like nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase (NOX) and nitric oxide synthase (NOS), activation of nuclear factor kappa-light-chain-enhancer of activated B (NF-kB), incomplete reduction of molecular oxygen (O_2), and the radiolysis of water (H_2O), and increased levels of redox-active metals such as iron (Fe^{2+}). The oxidation of lipids, such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), and modifications in deoxyribonucleic acid (DNA) are consequences reactive oxygen species (ROS) production. Mercury is one of the sources of ROS that is sourced from pollutants (11). The pathogenesis of ROS toxicity comes from heavy metal pollutants using the redox reaction principle. Based on their redox function, heavy metals can be grouped into two distinct groups: those that are redox-active (such as Fe, Cu, Cr, and Co) and those that are redox-inactive (including Cd, Zn, Ni, Al, and Hg). Redox-active metals play a crucial role in cellular redox reactions, engaging in the Haber–Weiss and Fenton reactions alongside H_2O_2 and O_2- to generate OH. Redox-inactive metals can generate reactive oxygen species via indirect pathways, including electron transport chain disturbance, defense mechanism disruption, or lipid peroxidation promotion due to elevated lipoxygenase activity (12). It is also recognized that certain enzymes utilize metal ions as cofactors; consequently, the replacement of one metal ion with another can lead to the inhibition or complete loss of enzymatic activity (13). The formation of free radicals mediated by heavy metals leads to various modifications of DNA bases, an increase in lipid peroxidation, and alterations in calcium homeostasis and sulfhydryl groups (14).

Free radicals cause changes in both enzymatic and nonenzymatic processes in cells. The alterations manifest in the respiratory chain within mitochondria, the cytochrome P450 system located in the endoplasmic reticulum, oxidative processes occurring in peroxisomes and during phagocytosis, as well as reactions catalyzed by transition metal ions, all serving as examples of enzymatic reactions that generate free radicals (15-16). The antioxidant defense system in healthy organisms roughly balances the creation of free radicals. This equilibrium is not flawless. Because free radicals are beneficial in moderation, cells cannot eradicate reactive oxygen and nitrogen species (ROS/RNS). An imbalance between oxidants and antioxidants brought on by either a rise in oxidants, a fall in antioxidants, or both is known as oxidative stress (17). Glutathione peroxidase, glutathione reductase, superoxide dismutase, and catalase are among the major enzymatic antioxidants directly involved in the neutralization of ROS. Cells experiencing oxidative stress are unable to neutralize the reactive intermediates or repair the consequent damage. Oxidative stress can lead to either oxidative damage or adaptability. Cells gradually lose their prior adaptability if the stress intensity continues (15-16,18). Cell damage may result from oxidative stress. Oxidative damage typically happens when vital biological components, such as proteins, DNA, or lipids in cell membranes, lose electrons and undergo chemical changes that can cause cellular malfunction. Every cell is continuously undergoing oxidative damage. Protein oxidation, lipid peroxidation, and DNA strand breaking are a few instances of oxidative damage. It is possible to replace or repair oxidatively damaged molecules. Severe oxidative stress results in cell death (apoptosis or necrosis) (19).

Malondialdehyde (MDA) is a dialdehyde compound that represents one of the final products resulting from the metabolic outcomes of reactive oxygen species (ROS), specifically as the end product of lipid peroxidation in the body. It is utilized more often as a marker of oxidative stress than other compounds. Numerous Research indicates that MDA serves as a reliable and precise metric for lipid peroxidation and can provide an overview of the metabolic results of lipid peroxidation from oxidative stress processes. Lipid peroxidation describes a process wherein free radicals target lipids containing carbon-carbon double bonds, particularly focusing on polyunsaturated fatty acids (PUFAs). PUFAs are integral constituents of lipid bilayer cellular membranes. The permeability properties of biological membranes largely depend upon the lipid bilayer's integrity. The permeability properties of the bilayer facilitate the maintenance of concentration gradients of metabolites and electrolytes between the intracellular and external environments. Damage to PUFAs results in a fast deterioration of membrane integrity, disruption

of gradients, and impairment of organelle or cellular function. The degradation of membrane lipids due to free radical damage and the resultant lipid peroxidation byproducts are detrimental to cellular survival (19-20). The glutathione system serves as a principal cellular antioxidant defense, consisting of the reduced form of glutathione (GSH) and the enzymes responsible for its production, renewal, or utilization as an electron donor to neutralize reactive species. Glutathione is a tripeptide that is present in a variety of tissues and is essential for regulating immune system function, mitigating oxidative stress by improving the metabolic detoxification processes for both enobiotic and endogenous substances, and maintaining redox balance. Its function involves the catalytic detoxification of lipid peroxides, peroxynitrites, and hydroperoxides, while also actively engaging with a range of oxidants, such as nitric oxide, carbon radicals, superoxide anion, and hydroxyl. Glutathione serves as a protective agent for cellular macromolecules against reactive oxygen species (ROS) and reactive nitrogen species (RNS) originating from both internal and external sources, addressing the root causes of oxidative stress, including harmful metals (21-22). The finding that the intervention (Hg) group exhibited reduced GPx levels compared to the control group indicates that GPx, as the main endogenous antioxidant enzyme, does not have sufficient capacity to protect the biological system against free radicals; in this case, mercury. The results of our study are consistent with the research which showed that The oral administration of $HgCl_2$ at a dose of 1 mg/kg body weight for 9 weeks ad libitum results in a reduction of GPx activity and an elevation of MDA levels in the brain, thymus, and lung (23). Other studies have also shown mercury to be a trigger for oxidative stress conditions and damage to hepato-renal organs (24-25). Organ damage including hepato-renal injuries as the target organ of the mercury also can be triggered by inflammation pathways that can caused defects in the intracellular calcium homeostasis, endoplasmic reticulum stress, mitochondrial dysfunction, cytoskeletal rearrangements, disturbances in cell adhesion and cell transporters, intracellular calcium, DNA methylation and reduced cellular viability with result in cell injury and cell death (26–28).

Hepato Injuries Due to Mercury Toxicity

The investigation focused on liver organs for integrating accumulation and toxicity data, considering their significance in the toxicokinetics and toxicodynamics of mercury (8,29-30). Transaminase enzymes in the liver include alanine transaminase (ALT) and aspartate transaminase (AST). Measuring their activity in serum can indicate the presence of certain liver cell abnormalities. ALT is present in liver, kidney, heart, and skeletal muscle and cells. Most are found in liver cells and the cytoplasm of liver cells. AST is found in the liver, kidney, pancreas, spleen, lung, brain, heart, and skeletal muscle cells. Heart cells exhibit the highest concentrations. AST is distributed with 30% located in liver cell cytoplasm and 70% found in the mitochondria of liver cells. Elevated AST levels correlate directly with the extent of cellular damage. Following cellular injury, AST levels rise within a 12-hour and persist in the bloodstream for 5 days. The liver contains transaminase enzymes such as alanine transaminase (ALT) and aspartate transaminase (AST). The AST/ALT ratio is a valuable tool in evaluating the extent of liver cell damage. During inflammation and the initial stages of hepatocellular injury, there is a cell membrane leakage, resulting in the release of cytoplasmic contents. This process leads to an elevation of ALT levels surpassing those of AST, with an AST/ALT ratio of 0.8 suggesting significant or chronic liver damage. Research by Nabil et al showed the impact of mercury exposure on changes in liver function through increased ALT and AST levels (31).

In this study, the AST/ALT ratio = 1.15, meaning that the rats' liver given mercury has severe liver damage. This study is in line with the research which found that the group exposed to Hg demonstrated the highest levels of transaminase enzymes (AST and ALT) compared to the other groups exposed to heavy metals like Cd and Pb (32). Increased AST or ALT is due to changes in permeability or damage to the liver cell wall, so it is used as a marker of impaired liver cell integrity (hepatocellular). The study also showed that $HgCl_2$ treatments in animal models demonstrated Hg-induced hepatotoxicity. The assessment of mercury's hepatotoxicity involved a histological investigation and the evaluation of increased liver enzyme levels, including alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) (31).

Renal Injuries due to Mercury Toxicity

The biochemical signs of renal damage, such as serum and urinary markers of renal functional integrity, can be used to evaluate kidney damage caused by mercury. The increased urea and creatinine levels are the markers for severe kidney oxidative damage (27,33-34). The antioxidative system in the rat kidney was impaired due to renal damage induced by $HgCl_2$. Reduced glutathione peroxidase activity, elevated levels of oxidative lipids and proteins, and morphological alterations in renal tissue (35–37). A research found that the administration of HgCl 4 mg/kgbw i.p. induced

renal damage (38). One of the parameters of renal function measured is increased plasma urea levels. Slightly different from the research conducted, another study found kidney function deteriorated by mercury associated with oxidative stress, glomerular damage, subsequent worsening of inflammation and cell death (18).

Mercury Toxicity Pathomechanism

Mercury (Hg), as a heavy metal, ranks first in the order of toxicity to tissues based on physical and chemical properties (Hg^{2+} > Cd^{2+} >Ag^{2+} > Ni^{2+} > Pb^{2+} > As^{2+} > Cr^{2+} Sn^{2+} >Zn^{2+}) (29). Because of their exceptional binding for proteins (containing sulfur, thiol, and selenium groups), ease of accumulation, and difficulty of biodegradation, elemental mercury, and organic and inorganic mercury compounds pose a significant danger to the ecological environment and human well-being. These unique characteristics of mercury cause physiological malfunction and irreversible physiological harm once they enter organisms. The damage caused by mercury stress is intricately connected to the onset of oxidative stress and the disturbance of redox balance, stemming from two mechanisms. The initial mechanism involves mercury as an external toxin that can directly disrupt the structure of biological molecules, leading to cellular damage. An additional process involves mercury binding to intracellular sulfhydryl groups found in antioxidants such as thiols and proteins, so diminishing or nullifying their antioxidant capabilities. This mechanism leads to a redox imbalance and increased reactive oxygen species (ROS) levels (39-40). With a typical mechanism of toxicity, mercury ($HgCl_2$) interacts through its active sites on enzymes involved in the biological metabolism of cells. The presence of such metals can interact with and replace the native metal in proteins or metalloenzymes, leading to cellular impairment and toxicity. These metals primarily engage with the -SH and -NH_2 groups of proteins, modifying their structure and leading to enzyme inactivation (18). The formation of free radicals within cells caused by ionic heavy metals results in damage through oxidation. ROS are produced and used during regular metabolic processes. Nonetheless, disruption of homeostasis and loss of regulatory control contribute to the etiology and progression of various human diseases, including hepatic and renal disorders (18,41).

Based on this study, it was found that the pathomechanism of mercury triggers the activation of oxidative stress pathways that have an impact on hepato-renal injuries through disturbances in liver and kidney function. This study has limitations, it cannot prove that hepato-renal injuries occur at the cellular level, so next research is needed to observe damage in liver cells and kidney cells through examination with a microscope. Also, this study did not investigate other biomelocellular mechanisms that can cause hepato-renal injuries such as inflammatory pathways that can trigger as well defects in the intracellular calcium homeostasis, endoplasmic reticulum stress, mitochondrial dysfunction, cytoskeletal rearrangements, disturbances in cell adhesion and cell transporters, intracellular calcium, DNA methylation and reduced cellular viability with result in cell injury and cell death. However, the results of this study, it will be the basis for finding the right antioxidant agent in preventing the occurrence of hepato-renal injuries due to mercury exposure.

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AUTHORS' CONTRIBUTION

IY, T: Conceptualization and methodology. IY, IM: Formal analysis. IY: Investigation. IY, FU: Data curation. MDP, IKO: Writing and original draft preparation. T: writing—review and editing. T: Supervision. T:Funding acquisition. All authors have read and agreed to the published version of the manuscript.

CONCLUSION

Our findings indicate that the mercury exposure results in hepato-renal injury through liver (increases levels of ALT and AST) and kidney function disruption (increases levels of ureum and creatinine). Our data also suggest that the the antioxidant functions of GPx are crucial in protecting against ROS induced by mercury (increases level of MDA). This study still has limitations but also gived potential to conduct further research on how the pathomechanism of involvement mercury in oxidative stress and inflammation causes toxicity in other organs such as endoplasmic reticulum stress, mitochondrial dysfunction, cytoskeletal rearrangements, disturbances in cell adhesion and cell transporters, intracellular calcium, and reduced cellular viability with result in cell injury and cell death. Also from this study, it will be the basis for finding the right antioxidant agent in preventing the occurrence of hepato-renal injuries due to mercury exposure.

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