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The effects of mercury chloride ($HgCl_2$) administration with different intervals on kidney damage in mice (*Mus Musculus*)

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Abstract

Introduction: Inorganic mercury could cause acute toxicity and absorbed through the digestive system and distributed throughout all tissues of the body, with a significant portion accumulating in kidneys and gallbladder.

Objective: This study aims to investigate the influence of mercury chloride ($HgCl_2$) administration at doses contained in rice clams (*Corbula faba*) from the Kenjeran coast in Surabaya, Indonesia, with varying administration intervals on kidney damage in mice. In this research, 24 male CBR strain mice, aged three months, were randomly divided into four groups, each consisting of six replicates. The groups included Control P0 (without mercury), Group P1 (mercury administration once a day), Group P2 (mercury administration twice a day), and Group P3 (mercury administration three times a day). Mercury administration was carried out orally for 52 days. Data analysis was conducted using non-parametric Kruskal-Wallis statistical test, followed by a Z-test with a significance level of 5%.

Results: Mercury chloride ($HgCl_2$) administration at a dose of 0.6418 ppm with varying administration intervals resulted in kidney damage characterized by interstitial hemorrhage, degeneration of proximal convoluted tubules, degeneration of distal convoluted tubules, and glomerulonephritis. Administration of mercury three times a day (P3) led to the highest kidney damage, which was not significantly different from the group receiving mercury twice a day (P2), while daily administration of mercury (P1) did not significantly differ from the control group (P0).

Conclusion: It can be concluded that administration of $HgCl_2$ could increase decrease kidney damage ($p<0.05$) and administration of mercury three times a day led to the highest kidney damage.

Keywords: Mercury chloride; Kidney damage; Mice; *Corbula faba*; Heavy metal

1. Introduction

The level of pollution has been increasing in recent times. In the past two decades, the quality of the environment in Indonesia has tended to decline to dangerous levels. Progress in the industrial sector has brought about advancements, but it has also resulted in negative environmental impacts, including pollution of air, water, and soil. Water pollution from industrial waste has reached a critical level, especially pollution caused by heavy metals such as Hg, Cd, and Pb. Most heavy metals accumulate biologically in aquatic organisms as cumulative toxins [1].

Heavy metal pollution in seawater and rivers can lead to contamination of aquatic animals such as fish, shrimp, squid, clams, and more. These metals are absorbed into the bodies of fish and are distributed throughout their muscle tissues, such as methylmercury, mercury salts, and others. The largest source of metal pollution is industrial waste disposal.

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Mercury is produced in various industries, including battery, thermometer, and barometer manufacturing. Mercury can also be found in pesticides used in agriculture. Before 1990, mercury was widely used as a fungicide in the paint industry. In the field of health, mercury is used as a preservative in vaccines. The content of substances in various organisms varies depending on various factors, such as the type of organism, age, availability of nutrients, and environmental conditions [2].

Kupang is a type of small clam known as rice clam (*Corbula faba*), which is a marine mollusk that lives in clusters. Kupang is highly favored by the people of East Java, Indonesia, especially in the northern coastal areas. Kupang is processed into various dishes such as pepes, petis, lontong kupang, and more. The waters of the Surabaya River have been found to contain heavy metals above normal levels, raising concerns about the safety of consuming kupang in that area [3]. Research on mercury pollution in rice clams (*Corbula faba*) from the Kenjeran Coast in Surabaya, conducted by Handajani and Budiono [4], revealed a mercury (Hg) content of 0.6418 ppm in the clams. This level exceeds the minimum mercury (Hg) content in food set by WHO/FAO, which was 0.5 ppm.

Water pollution, especially mercury (Hg) pollution, results in the accumulation of mercury (Hg) in marine biota through the food chain. When fish exposed to mercury are consumed by the population, it poses significant health risks. Detoxification mechanisms can lead to the storage of metals in inactive sites within living organisms temporarily or more permanently [5]. Mercury poisoning incidents have occurred in the past, such as in 1972 when 6,500 children in Iraq experienced nervous system development disorders and 459 people died due to methylmercury poisoning. Similarly, in Minamata, Japan, 121 people died, 9,000 people suffered paralysis and brain damage, and 50,000 people experienced mild health issues after consuming seafood contaminated with methylmercury [6]. In the United States, in 1996, mercury poisoning occurred due to the use of whitening cosmetic products [7].

Inorganic mercury could cause acute toxicity, resulting in bluish-gray mouth discoloration in test animals, accompanied by severe pain, vomiting, stomatitis, and gingival irritation [8]. Mercury is absorbed through the digestive system or the skin and is distributed throughout all tissues of the body, with a significant portion accumulating in the liver, kidneys, gallbladder, brain, and bones [9].

It is essential to understand the consequences of consuming food containing mercury (Hg) that can cause permanent organ damage. Mercury poisoning may not always be acute but can accumulate in the body, causing gradual organ damage even without clear clinical signs. Given the danger posed by mercury (Hg) contained in food, research on its effects on laboratory animals is crucial to provide valuable information to the public. This research involves administering mercury doses contained in rice clams (*Corbula faba*) from the Kenjeran Coast in Surabaya, Indonesia, with varying administration intervals to mice (*Mus musculus*).

2. Material and methods

The research was conducted in the animal facility of the Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia. The preparation of histopathological specimens was performed in the Pathology Veteriner Division of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya Indonesia.

Experimental animals consisted of 24 male mice (*Mus musculus*) of the CBR strain, aged 3 months, sourced from Pusvetma Surabaya, Indonesia. The materials used included: kidneys, 70%, 80%, 95%, and 96% alcohol, absolute alcohol I, II, III, Xylene I, II, ether, 10% formalin, Hematoxylin Eosin (H.E) for staining specimens, mouse feed, tap water from the Surabaya City Government's PDAM, and mercury chloride ($HgCl_2$) at a dose of 0.6418 ppm (equivalent to 0.0128 mg of mercury in 0.5 ml of distilled water administered to the mice). The mouse cages were plastic bins fitted with wire, and they were provided with mouse feed and water, disposable syringes and needles, forceps, scalpels, surgical scissors, cotton, a microscope for histopathological examination.

This study involved 24 male mice aged 3 months, divided into four different groups, with each group consisting of 6 mice placed in cages according to their respective groups. The mice were acclimated for 7 days to adapt to their new environment. The four groups were as follows: Control P0: 6 mice received no mercury treatment. Group P1: 6 mice received mercury chloride once a day. Group P2: 6 mice received mercury chloride twice a day. Group P3: 6 mice received mercury chloride three times a day.

All four groups of mice were provided with pelleted food and tap water ad libitum. Mercury administration was carried out using disposable syringes with sonde needles orally. The treatment was given for 52 days, with the dosage determined based on previous research by Handajani and Budiono [4]. After 52 days, the mice were euthanized, and their kidneys were collected to create histopathological.

Mice were anesthetized by inhalation using ether, followed by abdominal surgery to extract the kidneys. The kidneys were then placed in containers filled with 10% formalin and underwent Hematoxylin Eosin (HE) staining. Microscopic examination was performed to observe the changes that occurred, and the degree of changes in the field of view of the kidney histopathological was calculated.

2.1. Observed Variables

The assessment of kidney damage was conducted by observing cellular changes that occurred in the kidneys. Observations were made in each field of view, starting from the left, right, upper, lower, and middle parts of the histopathological under a microscope at magnifications of 100 and 400 times. If cell damage exceeded 50% of normal cells, it was considered positive [10].

Table 1 Assessment of Kidney Damage Levels in Mice

Histopathological Change Level	Value
No change	0
A = Interstitial hemorrhage	1
B = Degeneration of proximal convoluted tubules	2
C = Degeneration of convoluted tubules	3
D = Glomerulonephritis	4

2.2. Data Analysis

This study used a completely randomized design, and the degree of changes observed in each field of view was given a score for each kidney [10]. Data were statistically analyzed using the Kruskal-Wallis test, followed by the Multiple Comparison Test or Z-test at a significance level of 5%.

3. Results and discussion

The results of the study using mice (*Mus musculus*) divided into 4 groups (P0, P1, P2, and P3) yielded the following results regarding kidney damage.

Table 2 Average Kidney Damage Scores in Mice Due to Mercury Chloride ($HgCl_2$) Administration for 52 Days

Group	Control	P1	P2	P3
Average Score	0.00 ± 0.00^a	1.37 ± 0.17^a	1.54 ± 0.12^b	1.71 ± 0.13^b

Note: Superscripts with the same letter indicate no significant difference ($p>0.05$), P0= control, P1= mercury administration once a day, P2= mercury administration twice a day, P3= mercury administration three times a day.

Macroscopic examination of the kidneys in all four treatment groups did not show any changes. Microscopic observations in the three groups (P1, P2, and P3) revealed kidney damage in the form of interstitial hemorrhage, degeneration of proximal convoluted tubules, distal convoluted tubules, and glomerulonephritis. Based on the collected data, a statistical analysis was conducted using the Kruskal-Wallis test with a significance level of one percent. The obtained result for H was 13.94, which is greater than the tabulated H value of 11.35. This result indicates that the administration of mercury chloride causes significantly different kidney damage ($P<0.01$). Subsequently, a Z-test was conducted, yielding a Z ($P>0.05$) value of 10.429, indicating a significant difference among the groups. Group P3 resulted in the highest kidney damage, which was not significantly different from Group 2 but significantly different from Group P1 and the control group.

Heavy metals have a wide range of toxicity due to their various toxic properties. The primary function of heavy metals is to inhibit enzymes when there is an interaction between metal sulfhydryl groups on cell membranes [11]. Many heavy metals such as Hg, Cd, Pb have the potential to cause nephrotoxicity [12]. The kidneys are one of the primary targets when toxic substances enter the body because they accumulate during the processes of filtration, resorption, and excretion [13]. Therefore, the entry of toxic substances into the kidneys can result in a toxic response from the kidneys

[14]. Mercury in the kidneys binds to sulfhydryl groups on the kidney membrane and inhibits mitochondria, which are sensitive to mercury and have an inhibitory effect on oxidative processes [15]. The cell membrane functions to selectively regulate the entry and exit of substances between the cell and the external environment, so the presence of inorganic substances inhibits the permeability of the cell membrane to the transport of K^+ and Na^+ ions within the cell [16]. If there is inhibition of Na^+ ion transport, it will result in an imbalance of ion composition within the cell.

The sodium-potassium pump functions to maintain the balance of fluids and electrolytes within kidney cells. If more ions are inhibited from leaving the cell, the energy required to operate the potassium pump increases. If the cell is unable to contain the substances entering the cell, it will result in the death of that cell [16]. Kidney damage in this study showed interstitial hemorrhage, degeneration of proximal convoluted tubules, degeneration of distal convoluted tubules, and glomerulonephritis in mice after mercury chloride administration for 52 days, while the control group did not show such damage. Kidneys are organs with a high capacity to bind chemical substances due to their metabolic and excretory functions [11]. When a toxic chemical enters the bloodstream, it is distributed to various organs in the body. Heavy metals enter the body's cells by binding to sulfhydryl groups that are associated with metallothionein, an important protein for metal ion binding [17]. The binding of a substance can quickly increase its concentration in the body organs [11].

Glomerulonephritis is inflammation of the glomeruli, which can be diffuse or focal, acute or chronic, and can be caused by various factors, including infections, poisoning, ischemia, metabolic disorders, and immunological disorders [18]. In pathological conditions, there is penetration of proteins through the Bowman's space into the renal glomeruli, leading to impaired glomerular function, disrupting the glomerular filtration process. This results in glomerular dysfunction characterized by increased glomerular permeability, marked by the swelling of endothelial cells of glomerular capillaries and loss of Bowman's space [19]. Due to glomerular dysfunction, infiltrated mercury will enter the tubular lumen and come into direct contact with mercury, thus binding to sulfhydryl groups on the tubular brush border membrane [17].

The binding between metal ions and sulfhydryl groups reduces enzyme activity or completely inhibits it, leading to damage to the cell's metabolic system [11]. Disturbances in cell metabolism and enzyme activity will result in structural cell damage characterized by degeneration and ultimately cell death [20]. Damage to proximal tubular cells is marked by the loss of the brush border, which continues into the distal tubules. Hemorrhage can be caused by various factors, including foreign chemicals (toxins) in the body that directly or indirectly affect the blood vessel endothelium. The impact of these foreign chemicals causes blood vessels to rupture, leading to the leakage of blood from the blood vessels into the interstitial area of the kidney [14].

All toxic chemicals accumulate in the kidney tubules, especially the proximal tubules. Consequently, these cells undergo changes from degeneration to necrosis when exposure occurs over an extended period or at high doses [12]. The toxic effects of mercury in an organism's body depend on the quantity of the substance in the body, the route of entry, and the duration of contact between mercury and the surface of the organism that is susceptible to mercury [21]. The more frequent the contact between mercury and the susceptible organism's surface, the higher the toxic effects [22]. The accumulation of mercury in the body occurs because mercury that enters the organism's body tends to form complex compounds with organic substances present in the organism's body [23].

4. Conclusion

The administration of mercury chloride ($HgCl_2$) at a dose of 0.6418 ppm with different intervals of administration resulted in kidney damage, including interstitial hemorrhage, degeneration of proximal convoluted tubules, degeneration of distal convoluted tubules, and glomerulonephritis. Administration of mercury three a day produced the highest changes, which were not significantly different from administration twice a day, while administration three times a day was not significantly different from the control group.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The study was approved by the Faculty of Veterinary Medicine Animal Ethics Committee of Universitas Airlangga. All variables were considered in accordance with the Ethics Committee related to the animal handling to ensure no discomfort or pain was caused to the animals during sampling(certificate registration number: 2012/112-KE).

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Author's short biography

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Nurdita Dwi Handayani, DVM, whose nickname is Dita, was born in Madiun on December 20, 1977. She is a veterinarian who graduated from the Faculty of Veterinary Medicine at Universitas Airlangga in 2003. Work experience as a veterinarian and as a civil servant at the city government Ngawi at the Fisheries and Livestock Department since 2005, East Java Province, Indonesia.

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Prof. Widjiati, a professor at Universitas Airlangga (UNAIR) delivered a embryo transfer program (TE) as a solution for breeding cattle in Indonesia. Prof. Widjiati said that it is time to think of other assisted reproductive technologies besides artificial insemination to overcome the slow growth of the cattle population. Prof. Widjiati studied Bachelor at Universitas Airlangga, Master at IPB-Bogor, and Doctoral at Universitas Brawijaya Indonesia.

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