Etlingera hemisphaerica Blume attenuates male reproductive toxicity due to mercury chloride in Mus musculus

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Research Article

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Abstract

This study evaluated the effects of leaf ethanolic extract *Etlingera hemisphaerica* Blume (LE3H) on male reproductive toxicity due to mercury chloride ($HgCl_2$) in *Mus musculus* (mice). Those were three test materials; 5 mg/kg body weight (bw) $HgCl_2$, 0.2 mg/gbw Immunos®, and 0.13, 0.26, 0.39 mg/gbw LE3H. On day 1 (d-1), four groups of 5 mice were given $HgCl_2$ by gavage, and then on d-3, d-5, and d-7 days, three groups were administered 0.13, 0.26, and 0.39 mg/gbw of LE3H, another group as control. On d-16, the animals were killed using the cervical dislocation method for observing their testicular morphometrics. Three groups of 9 mice were given $HgCl_2$ by injected on d-1, and then on d-4 one group was administered Immunos® and one group was administered a determined LE3H, another group as control, and then were killed on d-7 for measuring glucose and malondialdehyde (MDA). Two groups of 15 mice were injected with $HgCl_2$ on d-1, and then on d-3, one group was administrated by the determined LE3H, another group as control, and then were killed on d-6 for investigating sperms quality. Results revealed that $HgCl_2$ increased the weight, length, and diameter of the testes compared to the control, while $HgCl_2$ + LE3H three times tended to restore testes similar to the control. A dose of LE3H (0.39 mg/gbw) was effective in testicular recovery conditions. $HgCl_2$ increased blood glucose dan MDA levels compared to the control. The blood glucose dan MDA level on $HgCl_2$ + Immunos® and $HgCl_2$ + LE3H did not differ from the control. $HgCl_2$ decreased sperm concentration compared to the control. $HgCl_2$ + LE3H was lower than $HgCl_2$ and approach the control. Sperm moves fast and straight in the control, $HgCl_2$, and $HgCl_2$ + LE3H were 30.63; 16.12; and 27.62% respectively. Thus, LE3H attenuates male reproductive toxicity duo to $HgCl_2$ in mice.

Introduction

The heavy metal known for its high toxicity and is of worldwide concern is mercury (Hg). One of the most harmful forms of mercury is mercury chloride ($HgCl_2$), which can handle form organomercury complexes with proteins [1]. $HgCl_2$ has an excellent affinity for the SH group of biomolecules, which may support its toxicity, such as sulfhydryl proteins and glutathione (GSH; [2]. Hg can flow in the blood or lymphatic system, after which Hg will keep in tissues in the form of the $HgCl_2$-GSH complex. $HgCl_2$ can induce oxidative stress because it is one of the pro-oxidants [3]. It happens when the body's defensive mechanisms are overwhelmed by reactive oxygen species (ROS) such as superoxide anions (NO2), hydroxyl radicals (NOH), and hydrogen peroxide (H_2O_2), causing damage to DNA macromolecules, lipids, and proteins [4] and then cause other pathological effects on the male reproductive system [5]. Male Swiss albino mice were given 5.0 mg/kg body weight (bw) of $HgCl_2$ showed the levels of alkaline phosphatase (ALP) and testicular acid phosphatase (ACP) were considerably greater than the control group [6]. In the liver, levels of malondialdehyde (MDA) in $HgCl_2$ group were higher compared to the control. MDA is a lipid oxidative damage marker. Decreasing the MDA level in rat liver with $HgCl_2$ can be treated with luteolin [7].

Phytopharmacology was an interesting field of research that attempted to detoxify several heavy metals and discovered that Pb and Hg might be detoxified by 21 and 10 plant species, respectively [8]. *Cipura paludosa* extract has also been shown to protect against neurological diseases caused by mercury toxicity [9]. Extracts from one variety of Etlingera, *Etlingera elatior*, were found to have the ability to reverse liver damage caused by Pb toxicity [10]. Many biochemical markers in rats have been altered due to $HgCl_2$ poisoning, including glutathione levels (GSH). The liver, kidneys, and testes all had histopathological changes. However, the consumption of *Urtica dioica*, a herbal ingredient widely distributed throughout the world, can prevent markers due to $HgCl_2$, especially motility and concentration of spermatozoa. The fresh nettle leaf is an inexpensive natural protective herb and plays an essential role in preventing $HgCl_2$ poisoning [11]. Seminal MDA concentration was previously found to be inversely linked with sperm concentration and motility [12].

Honje hutan or kunji or kecombrang [*Etlingera hemisphaerica* (Blume) R.M] was a traditional herb and spice used by several ethnic groups in Bengkulu, Indonesia. Previous research on herbal medicine found that $HgCl_2$ treatment at 5 mg/kg body weight (bw) raised the number of leukocytes significantly; however, $HgCl_2$ treatment followed by 0.39 mg/g bw leaf ethanolic extract *E. hemisphaerica* (LE3H) treatment may protect the number of leukocytes. $HgCl_2$ raised leukocytes while decreasing erythrocytes, and when $HgCl_2$ was followed by LE3H therapy, the number of both blood cells was protected. The LE3H treatment at 0.39 mg/kg bw diminished liver weight produced by $HgCl_2$ (5 mg/kg bw) and the control. Histological analysis revealed that $HgCl_2$ therapy resulted in tissue swelling and reduced extracellular space compared to the control, but the result showed the symptoms were prevented by

0.39 mg/g bw LE3H administration. According to the data, LE3H protects mice's livers from $HgCl_2$ toxicity [13]. $HgCl_2$ treatment resulted in the overexpression of 48 kDa proteins and the appearance of a new 125 kDa protein, which was recovered by LE3H treatment. LE3H protects mice against $HgCl_2$ toxicity by circulating in their blood [14].

There were no available reports describing the potential of LE3H in reducing the toxic effects of mercury on the male reproductive system. Therefore, it is deemed necessary to evaluate the effects of LE3H on testicular morphometrics, glucose-malondialdehyde, and sperm quality due to $HgCl_2$ in mice.

Methods

a. Extract preparation

As research plant materials, the stem base of *E. hemisphaerica* leaves (Plant identification was verified with the aid of the Indonesian Institute of Sciences, Research Center for Plant Conservation and Botanic Garden, Bogor, Indonesia; http://lipi.go.id/; Nomor B-1750/IPH.3./KS/V/2019) were obtained in the Curup City, Rejang Lebong District, Bengkulu Province, Indonesia. The leaves were cleaned and then sliced into little pieces. 3000 g of fresh *E. hemisphaerica* leaves were wind-dried and crushed into a powder yielding 800 g of dried leaves. The powder was macerated for seven days in 2 L of 96 % ethanol, then using a rotary evaporator, the filtrate was condensed [15] to get a concentrated extract weighing 3 g. 2 g of the crude extract could be utilized as a test sample for this study once the ethanol included in the concentrated extract has evaporated [13; 14; 16].

b. Dosages

According to the report, the three doses of LE3H used in this study were 0.13, 0.26, and 0.39 mg/g bw [13;14;16;17]. The single dose of $HgCl_2$ (Merck, Germany; Product No. 104 417) was 5 mg/kg bw [18; 19; 20], and it was administered either by oral gavage or intraperitoneal (ip) injection. The positive control for LE3H treatment (POM SD 121542741) is 0.2 mg/g bw Immunos® (contains: 50 mg ascorbic acid, 15 mcg selenium, 10 mg zinc picolinate, 500 mg Echinacea) from PT Lapi Laboratories (Serang, Indonesia) [17]. This drug is a dietary supplement to boost the immune body through infection, both acute and chronic. It was acquired in a pharmacy in Bengkulu, Indonesia.

c. Group of experimental animals

The subject of this research was Swiss Webster *Mus musculus* (mice). The mice were allowed in a room with a temperature of 23–27°C and a humidity of 83%. Ad libitum food and drink were provided [22]. There were three stages of research that must be carried out sequentially in this study, namely; examining testicular morphometric (A), blood glucose and MDA (B), and then sperm quality (C; Table 1). Phase A is expected to produce the most effective dose of LE3H to restore testicular morphometric effects due to $HgCl_2$ and return to close to control conditions. The most effective dose of LE3H was used in stage B for blood glucose and MDA assays and was also coded for Immunos® positive control results. The selected dose of LE3H was then tested for its potential in restoring sperm quality due to $HgCl_2$ at stage C. A total of 109 male mice with 25-35 g bw, aged 6-8 weeks [17] was used to assess the curative efficacy of LE3H to protect male reproductive tissues from mercury-induced oxidative damage.

d. Testicular morphometric

A total of 25 male mice were used as experimental animals in the first stage of this research. On day 1 (d-1), A1, A2, A3, and A4 groups each consisting of 5 mice were given $HgCl_2$ by oral gavage, and then on d-3, d-5, and d-7 once day A2, A3, and A4 were administered 0.13, 0.26, and 0.39 mg/g bw LE3H by oral gavage, A0 as control. To observe testicular morphometrics, on d-16, A0-A4 groups were sacrificed by cervical dislocation (CD). Test animals were dissected, testes isolated, and morphometric male

reproductive organs (weight, length, and diameter) were measured, and each individual's data was the average of the measurement results on the left and right [23].

i. Blood glucose and malondialdehyde

The blood glucose concentration in mice was measured using a glucometer brand Accu-Chek® (https://www.accu-chek.com/meters) through a small amount of blood from mice from the tip of the tail dripped on a glucose strip that had been inserted into the glucometer. Previously, the glucometer was adjusted to the code indicated on the glucose strip packaging. After the blood drops on the strip, wait ±5 seconds to read the blood glucose concentration by the glucometer. The level indicated on the glucometer is the blood glucose concentration of mice in mg/dL units [24].

The twenty-four mice were formed into four groups (B0, B1, B2, and B3). Every group consists of nine male mice. B1, B2, and B3 were injected intraperitoneally with 5 mg/kg bw of $HgCl_2$ on d-1, then on d-4 B2 was administrated by oral gavage 0,2 mg/g bw of Immunos®, and B3 was administered by oral gavage 0.39 mg/g bw LE3H. The B0 group, who served as the study's controls, received just solvent in the same way. The experimental animals were sacrificed by CD on d-7, examined, and their livers were tested for malondialdehyde (MDA) levels. Using a Bioxytech MDA-586 Kit (Oxis Research, Portland, OR, USA), the level of MDA was evaluated as a lipid peroxidation indicator. To make a stable dye, the supernatants of liver homogenate were combined with N-methyl-2-phenylindole, hydrochloric acid, and probucol. After centrifugation, the absorbance of the clear supernatant was determined by spectrometry at 586 nm. The amount of MDA was measured in n moles per milligram of wet tissue and shown against a standard curve [25; 26].

e. Epididymis suspension

A total of 45 male mice were used as experimental animals in the third stage of this research. C1 and C2 each consisting of 15 mice were injected intraperitoneal with $HgCl_2$ on d-1, and then on d-3, C2 was administrated by oral gavage 0.39 mg/g bw LE3H, while B0 as control. C0, C1, and C2 were killed by CD on d-6 to determine sperm quality. Adult mice's fresh right epididymis was used to obtain spermatozoa [27]. The epididymis was disseminated in 3 mL of phosphate-buffered saline (pH 7.2) with moderate stirring after chopping into tiny pieces with a sharp blade. In an incubator, dispersed sperm samples were kept [28].

g. Sperm concentration and motility.

The suspension filter's aliquot (up to 0.5 mL) was placed on a leukocyte hemocytometer micropipette and dissolved in the phosphate-buffered solution to the 11 marks. To express the number of spermatozoa epididymis, the suspension was well diluted before being placed in Neubauer's counting chamber [11; 28].

Up to 0.5 mL of an aliquot from the suspension was transferred to a histological slide and then obtained sperm motility was recorded using the *Dino Capture* device (http://www.dinolite.us/products/digital-microscopes/). The observation of the 100 sperm motility of sperm divides into four categories, namely; (a) moving fast and straight, (b) moving slowly, (c) moving in place, and (d) does not move [29;30].

h. Statistical analyses

Multiple comparisons were used to generalize the data from this study, followed by the least significant difference (Table 2-5), and then the χ^2 test of goodness of fit (Table 6) [31].

i. Ethical statement

This research focuses on the ethic of animals used, as well as aspects of human treatment, following the principle of five freedoms (F), which include (a) freedom from hunger and thirst; (b) freedom from discomfort; (c) freedom from pain, injury, and disease; (d) freedom from fear and long-term stress; and (e) freedom to express standard patterns of behavior [21]. This study followed the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. The protocol was accepted by Bengkulu University's Committee on the Ethics of Animal Experiments.

Results

a. Weight, length, and diameter of testis

Administration of 5 mg/kg bw $HgCl_2$ (A1) on day 1 (d-1) tends to increase the weight, length, and diameter of the testis compared to the control on d-16 (A0; Table 2). Meanwhile, administration of 5 mg/kg bw $HgCl_2$ (A2; A3; A3) on d-1 was followed by three doses of 0.13, 0.26, and 0.39 mg/g bw LE3H on d-3, d-5, and d-7 respectively. On d-16 general obtained data revealed that LE3H was able to recover the weight (Figure 1), length (Figure 2), and diameter (Figure 3) of the testes close to the control condition. The testicular weight and diameter on A4 are significantly closer to the control conditions (A1). Furthermore, LEH3 at a dose of 0.39 mg/g bw appears to be the most beneficial for the recovery of the testicular condition (Table 2). That dose will be selected as the working dose at the next research stage.

b. Glucose and Malondialdehyde (MDA)

Treatment with ip 5 mg/kg bw of $HgCl_2$ (B1) on d-1 significantly increased blood sugar levels (151.11 ± 18.031^b) compared to the control (B0; 121.22 ± 19.823^a) on d-7. Whereas treatment with ip 5 mg/kg bw of $HgCl_2$ on d-1 followed by oral gavage administration of 0.2 mg/g bw of Immunos® at d-4 (B2) revealed that blood glucose levels at d-7 (119.00 ± 22.085^a) were not different from the control (B0; 121.22 ± 19.823^a). Meanwhile, a similar incident was shown by giving LE3H. Treatment with ip 5 mg/kg bw of $HgCl_2$ on d-1 followed by oral gavage administration of 0.39 mg/g bw of LE3H on d-4 (B3) indicated that blood glucose levels on d-7 (121.00 ± 19.506^a) were not different from the control (B0; 121.22 ± 19.823^a). Immunos® and LE3H showed a similar phenomenon to the effect of $HgCl_2$ on blood glucose levels (Table 3).

Treatment with ip 5 mg/kg bw of $HgCl_2$ (B1) on d-1 significantly increased MDA levels (1.00 ± 0.29^b) compared to the control (B0; 0.48 ± 0.29^a) on d-7. Whereas treatment with ip 5 mg/kg bw of $HgCl_2$ on d-1 followed by oral gavage administration of 0.39 mg/g bw of LE3H on d-4 (B3) revealed that MDA levels on d-7 (0.48 ± 0.28^a) were not different from the control (B0; 0.48 ± 0.29^a). Meanwhile, a similar incident was shown by giving Immunos®. Treatment with ip 5 mg/kg bw of $HgCl_2$ on d-1 followed by oral gavage administration of 0.2 mg/g bw of Imunos® on d-4 (B2) indicated that MDA levels on d-7 (0.50 ± 0.28^a) were not different from the control (B0; 0.48 ± 0.29^a). LE3H and Immunos® showed a similar phenomenon to the effect of $HgCl_2$ on MDA levels (Table 4; Figure 4).

c. Sperm quality

$HgCl_2$ treatment reduced sperm concentration (C1; $7.93x10^6$) compared to the control (C0; $15.66x10^6$). Meanwhile, treatment with 5 mg/kg bw of $HgCl_2$ followed by 0.39 mg/g bw of LE3H administration on C2 ($11.16x10^6$) was smaller than on C1 and state approach C0 ($15.66x10^6$; Table 5; Figure 4). $HgCl_2$ treatment decreased percentage sperm moves fast and straight (C1; 16.12%) compared to the control (C0; 30.63%). Furthermore, 5 mg/kg bw $HgCl_2$ treatment was followed by 0.39 mg/g bw LE3H increased the percentage of sperm moving fast and straight (C2; 27.62%) closer to the control conditions (Table 6; Figure 5).

Discussion

Treatment with $HgCl_2$ on day 1 (d-1) increases the testis's weight, length, and diameter than the control group on d-16 (Table 2). Meanwhile, treatment with 5 mg/kg bw $HgCl_2$ on d-1 was followed by three doses of 0.13, 0.26, and 0.39 mg/g bw LE3H on d-3, d-5, and d-7 respectively. On d-16 general obtained data revealed that LE3H was able to recover the weight (Figure 1), length (Figure 2), and diameter (Figure 3) of the testes close to the control condition. A similar phenomenon, $HgCl_2$ treatment increases testicular weight in Sprague Dawley rats, and this condition can be recovered by giving *Chenopodium album* Linn and vitamin C. *C. album* Linn consumption can provide useful nutrients that protect the body from oxidative stress and reproductive damage caused by heavy Hg exposure [32]. It has also been reported that twenty-seven (27) phytochemicals produced from the plant have been discovered to alleviate mercury's impact, with four of these natural materials, vitamin C [34; 35], tocopherol [36], beta carotene [37], and the flavonoid quercetin [38], is recommended for more extensive clinical testing [33]. Quercetin is a flavonoid with a wide range of physiological and biochemical effects, including anti-inflammatory characteristics, estrogenic, and antiplatelet [39]. Vitamin C and *C. album* Linn appeared to have a similar effect to LE3H on reducing $HgCl_2$ toxicity [40]. Furthermore, 0.39 mg/g bw of LE3H appears most efficient for reversing testicular problems. (Table 2). That dose will be selected as the working dose at the next research stage.

According to a previous study, mice treated for more than two weeks with 2 mg/kg of mercuric methyl chloride and 5 mg/kg of mercuric chloride ($HgCl_2$) had higher blood glucose levels and lower plasma insulin secretions [41]. It has also been published that treatment with $HgCl_2$ decreased plasma insulin secretions and increased blood glucose levels [42] [43]. The results of this study also show that treatment with 5 mg/kg bw of $HgCl_2$ significantly increased blood sugar levels than the control group. Whereas treatment with 5 mg/kg bw of $HgCl_2$ on d-1 followed by oral gavage administration of 0.39 mg/g bw LE3H on d-4 indicated that blood glucose levels on d-7 were not different from the control. Meanwhile, a similar phenomenon was shown by giving Immunos®. Furthermore, treatment with 5 mg/kg bw of $HgCl_2$ on d-1 followed by oral gavage administration of 0.2 mg/g bw of Immunos® at d-4 revealed that blood glucose levels at d-7 were not different from the control. The data show that Immunos® and LE3H revealed a similar phenomenon to the effect of $HgCl_2$ on blood glucose levels (Table 3).

The following are several previous studies that stated that treatment with $HgCl_2$ can significantly increase MDA compared to control. Mercury therapy reduced glutathione peroxidase (GPx) and aortic superoxide dismutase (SOD) activity while increasing plasma MDA levels. Apocynin treatment reduced plasma MDA levels to some extent [44]. Oral administration of 8 mg/kg bw $HgCl_2$ once a day for nine weeks caused renal interstitial fibrosis (RIF). RIF was triggered by $HgCl_2$, as evidenced by a considerable decrease in glutathione (GSH) content and a significant increase in MDA content in $HgCl_2$-treated rats compared to control rats. In the model rats, Fuzheng Huayu Formula (FZHY) dramatically raised GSH content while decreasing MDA content [45]. Male albino Wistar rats were given $HgCl_2$ and CO alone or in combination for 21 days showed that the level of MDA in the liver homogenates and kidney was significantly increased when compared to control [46]. $HgCl_2$ significantly raises liver MDA levels, decreases total sulfhydryl content, and increases serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity. The liver functions were enhanced by the *R. turkestanicum* extract, as evidenced by significant reductions in AST and ALT levels in serum, decreased MDA, and increased total sulfhydryl content [47].

MDA production increased in the heart and plasma of Hg-treated rats, which was utilized to measure oxidative stress indirectly. In the spontaneously hypertensive rats (SHR)+Hg group, MDA levels rose in the heart and aorta while decreasing in the lungs and brain [48]. The antioxidant indicators (catalase, CAT; superoxide dismutase, SOD; and GSH) were reduced, and the lipid peroxidation marker was raised by a low-dose heavy metal combination (LDHMM; $PbCl_2$+$CdCl_2$+$HgCl_2$). *Costus afer* treatment decreased LDHMM-mediated toxicity in rats in a dose-dependent manner [49]. The results of this study indicate that treatment with $HgCl_2$ (5 mg/kg bw) on d-1 significantly increased MDA levels compared to the control on d-7. Whereas treatment with $HgCl_2$ (5 mg/kg bw) on d-1 followed by oral gavage administration of LE3H (0.39 mg/g bw) on d-4 revealed that MDA levels on d-7 were not different from the control. Meanwhile, a similar incident was shown by giving Imunos®. Treatment with $HgCl_2$ (5 mg/kg bw) on d-1 followed by oral gavage administration of Imunos® (0.2 mg/g bw) on d-4 indicated that MDA levels on d-7 were not different from the control (Table 4; Figure 4). The facts show that Apocynin, FZHY, R. turkestanicum, *Costus afer*, Immunos®, and LE3H showed a similar phenomenon to the effect of $HgCl_2$ on MDA levels.

$HgCl_2$ treatment reduced sperm concentration (C1; $7.93x10^6$) than the control (C0; $15.66x10^6$). Meanwhile $HgCl_2$ (5 mg/kg bw) treatment was followed by LE3H (0.39 mg/g bw) administration on C2 ($11.16x10^6$) was smaller than on C1 and state approach C0 ($15.66x10^6$; Table 5; Figure 5). This result is consistent with previous research, which showed that Hg caused a significant decrease in spermatozoa concentration, an evident stabilization of organized seminiferous tubules, and an increase in sperm counts in Urtica dioica (UD) supplemented rats [11]. $HgCl_2$-treatment was also found to reduce sperm quantity, increase sperm transit time in the epididymis, and affect sperm morphology. In the testis and epididymis, $HgCl_2$ treatment enhanced lipid peroxidation, ROS levels, and antioxidant capacity, and promoted testicular inflammation also epididymis histological alterations. Because of its antioxidant properties, egg white hydrolysate (EWH) improved histological and immunohistochemical changes. The EWH may be a viable natural option for protecting the male reproductive system from Hg-induced sperm toxicity [50]. LE3H, UD, and EWH have the same effect on sperm concentration due to mercury toxicity.

Previous studies have shown that 40 mg/kg of $HgCl_2$-treatment decreased sperm motility, while the 40 mg/kg of $HgCl_2$ and 500 mg/kg of *Solanum melongena* (SM) treatments were able to increase sperm motility again. Through an antioxidant system of actions, SM improves spermatogenesis and reduces $HgCl_2$-induced testicular toxicity [51]. Short-term exposure to $HgCl_2$, even at 0.031 g/mL, was found to have adverse effects on sperm functions, generate spontaneous acrosome response, and inhibit capacitation via blocking tyrosine-containing protein phosphorylation. Hg-induced ROS, as well as an increase in iCa^{2+} and cAMP levels, mediate these effects. As a result, animals exposed to Hg are more likely to have poor fertilizing ability, and their sperm should not be utilized for insemination [52]. Meanwhile, the results of this study indicate that $HgCl_2$ treatment decreased the percentage of sperm that move fast straight (C1; 16.12%) compared to the control (C0; 30.63%). Furthermore, $HgCl_2$ (5 mg/kg bw) treatment was followed by LE3H (0.39 mg/g bw) increased the percentage of sperm moving fast and straight (C2; 27.62%) closer to the control conditions (Table 6; Figure 6). $HgCl_2$ decreased 14.51% sperm motility compared to the control, while LE3H was able to increase 11.50% sperm motility closer to the control condition. $HgCl_2$ and LE3H act as antagonists to sperm motility, and LE3H is a motility-activating factor that in turn will have an effect on male fertility [53]. Thus, LE3H and SM have the same effect on sperm motility due to Hg toxicity.

The results of this discussion indicate that the negative effect of Hg on (1) testicular morphometrics was potentially reversed by *C. album* Linn, and vitamin C, on (2) glucose and malondialdehyde was potentially reversed by Apocynin, FZHY, *R. turkestanicum*, C. afer, and Immunos®, and (3) sperm quality has the potential to be recovered by UD, EWH, and SM. Meanwhile, LE3H is a natural ingredient from plants that is quite popular in Indonesia, this natural ingredient has real potential to recover the negative effects of Hg at all levels, namely testicular morphometric, glucose and malondialdehyde, and sperm quality. Furthermore, the molecular mechanism of the natural substance, LE3H, to recovery testes and sperms as the effects of $HgCl_2$ toxicity in mice still needs to be studied through suitable proteomic techniques [22].

Conclusion

LE3H has the potential to recover testicular morphometric, glucose, malondialdehyde, and sperm quality as a result of $HgCl_2$ toxicity in mice. Based on these facts, dietary supplementation with LE3H may be beneficial in populations that are occupationally exposed to $HgCl_2$.

Declarations

Conflict of interest

There are no conflicts of interest declared by the authors. The article's content and writing are solely the responsibility of the writers.

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Tables

Table 1 Research design to determine the effects of leaf ethanolic extract *Etlingera hemisphaerica* Blume (LE3H) on male reproductive toxicity due to mercury chloride ($HgCl_2$) in mice. Standard food and drink were given *ad libitum* by experimental animals.

Research stage	Experimental animals' group	N	Research activity, on day (d)					
			d-1 (T)	d-3 (T)	d-4 (O)	d-5 (T/O)	d-7 (T/O)	d-16 (O)
A. Testicular morphometric	A0: Controls (double-distilled (DD) water only)	5	DD water only	DD water only		DD water only	DD water only	Weight, Length, Diameter
	A1: $HgCl_2$ (5 mg/kg bw) on d-1	5	$HgCl_2$ [G]	DD water only		DD water only	DD water only	Weight, Length, Diameter
	A2: $HgCl_2$ (5 mg/kg bw) on d-1+ LE3H (0.13 mg/g bw) on d-3, d-5, and d-7.	5	$HgCl_2$ [G]	LE3H [G]		LE3H [G]	LE3H [G]	Weight, Length, Diameter
	A3: $HgCl_2$ (5 mg/kg bw) on d-1+ LE3H (0.26 mg/g bw) on d-3, d-5, and d-7.	5	$HgCl_2$ [G]	LE3H [G]		LE3H [G]	LE3H [G]	Weight, Length, Diameter
	A4: $HgCl_2$ (5 mg/kg bw) on d-1+ LE3H (0.39 mg/g bw) on d-3, d-5, and d-7.	5	$HgCl_2$ [G]	LE3H [G]		LE3H [G]	LE3H [G]	Weight, Length, Diameter
B. Glucose and Malondialdehyde (MDA)	B0: Controls (double-distilled (DD) water only)	9	DD water only		DD water only		Glucose, MDA	
	B1: $HgCl_2$ (5 mg/kg bw) on d-1	9	$HgCl_2$ [ip]				Glucose, MDA	
	B2: $HgCl_2$ (5 mg/kg bw) on d-1+ Immunos® (0.2 mg/g bw) on d-4	9	$HgCl_2$ [ip]		Immunos® [G]		Glucose, MDA	
	B3: $HgCl_2$ (5 mg/kg bw) on d-1+ LE3H (0.39 mg/g bw) on d-4	9	$HgCl_2$ [ip]		LE3H [G]		Glucose, MDA	
C. Sperm quality	C0: Controls (double-distilled (DD) water only)	15	DD water only.	DD water only.		Concentration, Motility		
	C1: $HgCl_2$ (5 mg/kg bw) on d-1	15	$HgCl_2$ [ip]			Concentration, Motility		
	C2: $HgCl_2$ (5 mg/kg bw) on d-1+ LE3H (0.39 mg/g bw) on d-3.	15	$HgCl_2$ [ip]	LE3H [G]		Concentration, Motility		

Note: bw= body weight; $HgCl_2$= mercuric chloride; LE3H= leaf ethanolic extract *E. hemisphaerica*; MDA=malondialdehyde; T=treatment; O=observation [G]= administrated by oral gavage; ip: intraperitoneal injection. DD=double-distilled (DD) water only.

Table 2 Average weight (g), length (mm), and diameter (mm) of mice testis on day-16 (d-16), which given by oral gavage; $HgCl_2$ on d-1 (A1); $HgCl_2$ on d-1 then 0.13 mg/g bw LE3H on d-3, d-5, and d-7 (A2); $HgCl_2$ on d-1 then 0.26 mg/g bw LE3H on d-3, d-5, and d-7 (A3); $HgCl_2$ on d-1 then 0.39 mg/g bw LE3H on d-3, d-5, and d-7 (A4). Controls of this study were only administered by double-distilled water (A0).

Experimental animals' group	N	Average of testis morphometric on day-16 (d-16)		
		Weight (g)	Length (mm)	Diameter (mm)
A0: Controls were administered double-distilled water only	5	0.12±0.02^{a}	7.99±0.56	4.54±0.70^{p}
A1: HgCl2 (5 mg/kg bw) on d-1.	5	0.13±0.03^{b}	8.43±0.62	5.47±0.37^{q}
A2: HgCl2 (5 mg/kg bw) on d-1; LE3H (0.13 mg/g bw) on d-3, d-5, and d-7.	5	0.11±0.01^{ab}	7.69±0.66	4.83±0.55^{pq}
A3: HgCl2 (5 mg/kg bw) on d-1; LE3H (0.26 mg/g bw) on d-3, d-5, and d-7.	5	0.10±0.01^{ab}	7.76±0.87	4.78±0.46^{rp}
A4: HgCl2 (5 mg/kg bw) on d-1; LE3H (0.39 mg/g bw) on d-3, d-5, and d-7.	5	0.12±0.03^{ab}	7.50±1.02	4.66±0.79^{pq}

Note: Data followed by the same letter, then the data is not significantly different [31].

Tabel 3 Average blood glucose (mg/dL) concentration of mice on day-7 (d-7); which injected on d-1 5 mg/kg bw $HgCl_2$ (B1); previously on d-1 injected 5 mg/kg bw $HgCl_2$ and on d-4 received 0.2 mg/g bw Immunos® by oral gavage (B2); previously on d-1 injected 5 mg/kg bw $HgCl_2$ and on d-4 received 0.39 mg/g bw LE3H by oral gavage (B3); Controls of this study were only administered by double-distilled water (B0).

Experimental animal group	N	Range glucose level (mg/dL) on d-7	Average glucose level (mg/dL) on d-7
B0: Controls were only administered by double-distilled water	9	77-149	121.22±19.823[a]
B1: $HgCl_2$ (5 mg/kg bw) on d-1.	9	111-174	151.11±18.031[b]
B2: $HgCl_2$ (5 mg/kg bw) on d-1+ Immunos® (0.2 mg/g bw) on d-4	9	86-149	119.00±22.085[a]
B3: $HgCl_2$ (5 mg/kg bw) on d-1+ LE3H (0.39 mg/g bw) on d-4	9	78-147	121.00±19.506[a]

Note: Data followed by the same letter, then the data is not significantly different [31].

Tabel 4 Average MDA (mol/L) concentration of mice liver on day-7 (d-7); which injected on d-1 5 mg/kg bw $HgCl_2$ (B1); previously on d-1 injected 5 mg/kg bw $HgCl_2$ and on d-4 received 0.2 mg/g bw Immunos® by oral gavage (B2); previously on d-1 injected 5 mg/kg bw $HgCl_2$ and on d-4 received 0.39 mg/g bw LE3H by oral gavage (B3); Only the solvent administered the controls in the same way (B0).

Experimental animal group	N	Range MDA concentration (mol/L) on d-7	Average MDA concentration (mol/L) on d-7
B0: Controls were only administered by double-distilled water	9	0.20-1.03	0.48±0.29[a]
B1: $HgCl_2$ (5 mg/kg bw) on d-1	9	0.68-1.45	1.00±0.29[b]
B2: $HgCl_2$ (5 mg/kg bw) on d-1+ Immunos® (0,2 mg/g bw) on d-4	9	0.20-1.00	0.50±0.28[a]
B3: $HgCl_2$ (5 mg/kg bw) on d-1+ LE3H (0.39 mg/g bw) on d-4	9	0.21-1.02	0.48±0.28[a]

Note: Data followed by the same letter, then the data is not significantly different [31].

Table 5 Average concentration (10^9/mL) of mice sperm on day-5 (d-5) which was injected intraperitoneally (ip) 5 mg/kg body weight (bw) $HgCl_2$ on d-1 (C1); injected ip 5 mg/kg bw $HgCl_2$ on d-1, and then given by oral gavage 0.39 mg/g bb LE3H on d-3 (C2). Only the solvent administered the controls in the same way (C0).

Experimental animal group	N	Average concentration (10^6/mL) of sperm on day-5 (d-5)
C0: Controls were administered double-distilled water only	15	15.66±6.11[a]
C1: $HgCl_2$ (5 mg/kg bw) on d-1	15	7.93±3.75[b]
C2: $HgCl_2$ (5 mg/kg bw) on d-1+ LE3H (0.39 mg/g bw) on d-3.	15	11.16±4.09[a]

Note: Data followed by the same letter, then the data is not significantly different [31].

Table 6 Four categories of mice sperm motility, namely; (a) moving fast straight, (b) moving slowly, (c) moving in place, and (d) not moving on day-5 (d-5) which was injected intraperitoneally (ip) 5 mg/kg body weight (bw) $HgCl_2$ on d-1 (C1); injected ip 5 mg/g bw $HgCl_2$ on d-1, and then given by oral gavage 0.39 mg/g bb LE3H on d-3 (C2). Only the solvent administered the controls in the same way (C0).

Experimental animal group	N	*M. musculus* sperm motility (%) on day-5 (d-5)				Amount
		Moving fast straight	Moving slowly	Moving in place	Not moving	
C0: Controls were administered double-distilled water only.	15	144 (30.63%)	139 (29.57%)	146 (31.06%)	41 (8.72%)	470 (100 %)
C1: $HgCl_2$ (5 mg/kg bw) on d-1	15	40 (16.12%)	72 (29.03)	85 (34.27%)	51 (20.56%)	248 (100%)
C2: $HgCl_2$ (5 mg/kg bw) on d-1+ LE3H (0.39 mg/g bw) on d-3.	15	92 (27.62%)	102 (30.63%)	101 (30.33%)	38 (11.41%)	333 (100%)

Note: χ^2= 33,03; There are significant differences between moving fast straight, moving slowly, moving in place, and not moving on C0, C1, and C2 ($p < 0.05$; [31]).

Figures

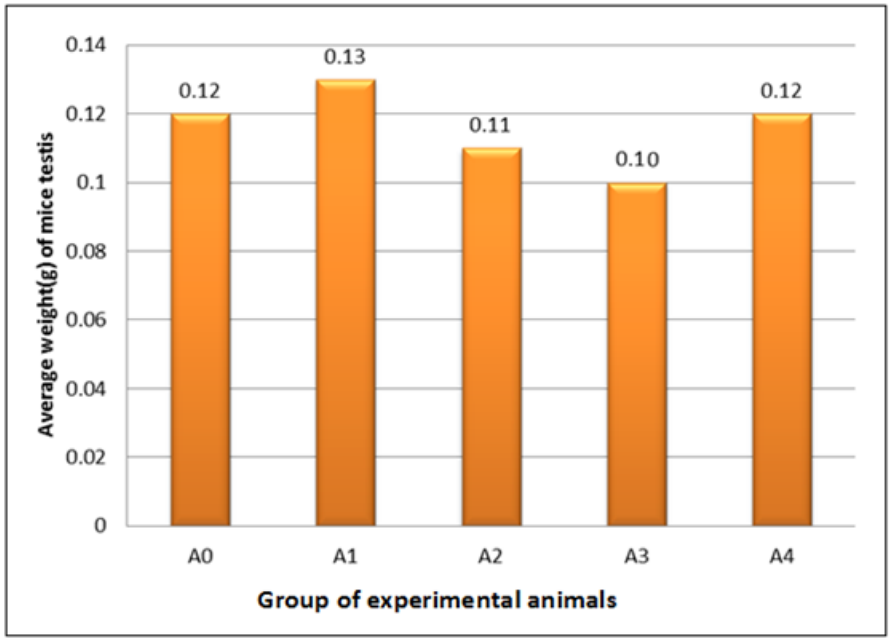

Figure 1

Average weight (g) of mice on day-16 (d-16) which given by oral gavage; HgCl2 on d-1 (A1); HgCl2 on d-1 then 0.13 mg/g bw LE3H on d-3, d-5, and d-7 (A2); HgCl2 on d-1 then 0.26 mg/g bw LE3H on d-3, d-5, and d-7 (A3); HgCl2 on d-1 then 0.39 mg/g bw LE3H on d-3, d-5, and d-7 (A4). Controls of this study were only administered by double-distilled water (A0).

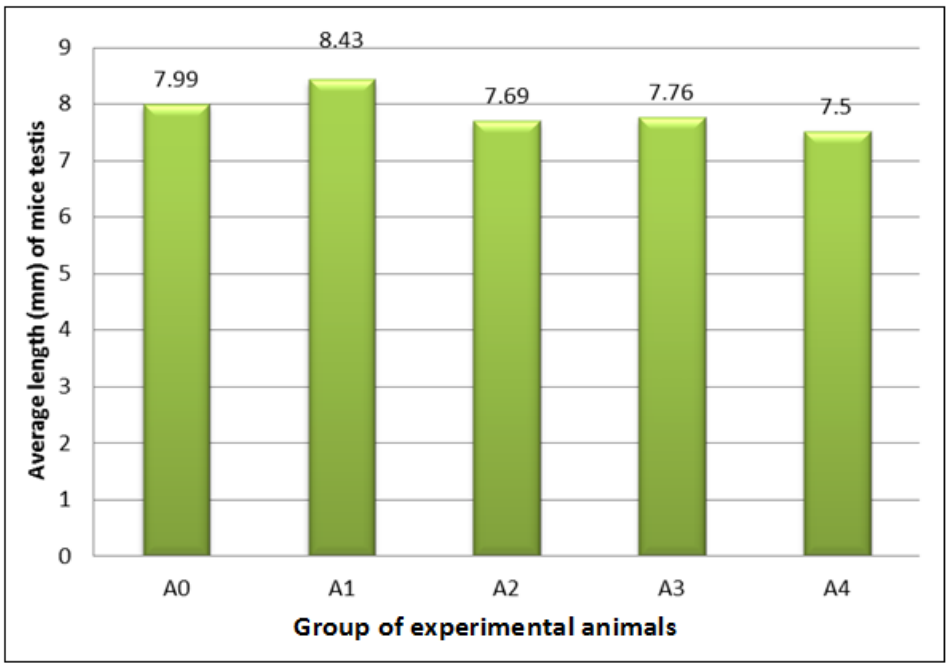

Figure 2

Average length (mm) of mice testis on day 16 (d-16) which given by oral gavage; HgCl2on d-1 (A1); HgCl2 on d-1 then 0.13 mg/g bw LE3H on d-3, d-5, and d-7 (A2); HgCl2 on d-1 then 0.26 mg/g bw LE3H on d-3, d-5, and d-7 (A3); HgCl2 on d-1 then 0.39 mg/g bw LE3H on d-3, d-5, and d-7 (A4). Controls of this study were only administered by double-distilled water (A0).

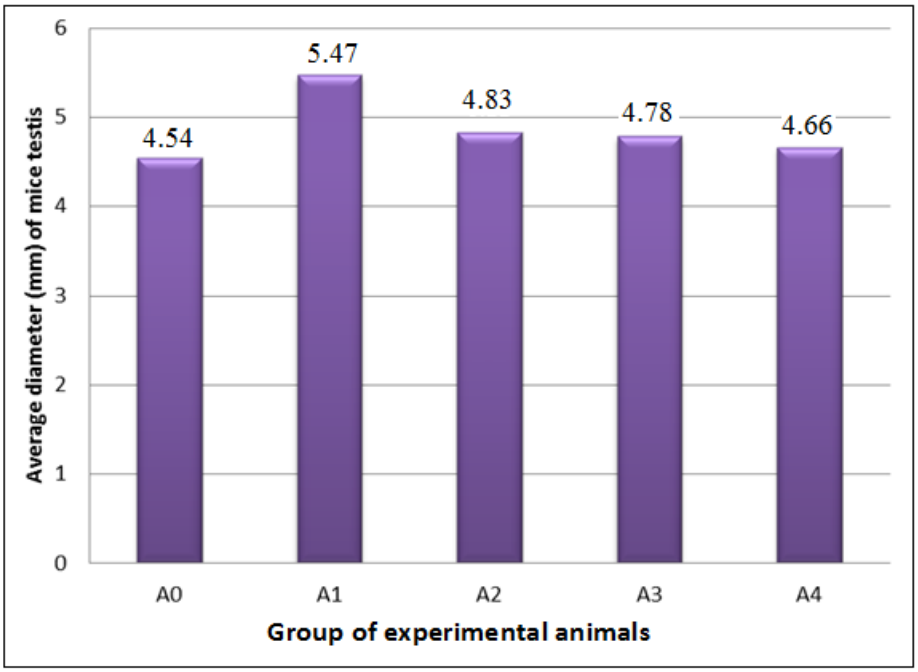

Figure 3

Average diameter (mm) of mice testis on day 16 (d-16) which given by oral gavage; HgCl2on d-1 (A1); HgCl2 on d-1 then 0.13 mg/g bw LE3H on d-3, d-5, and d-7 (A2); HgCl2 on d-1 then 0.26 mg/g bw LE3H on d-3, d-5, and d-7 (A3); HgCl2 on d-1 then 0.39 mg/g bw LE3H on d-3, d-5, and d-7 (A4). Controls of this study were only administered by double-distilled water (A0).

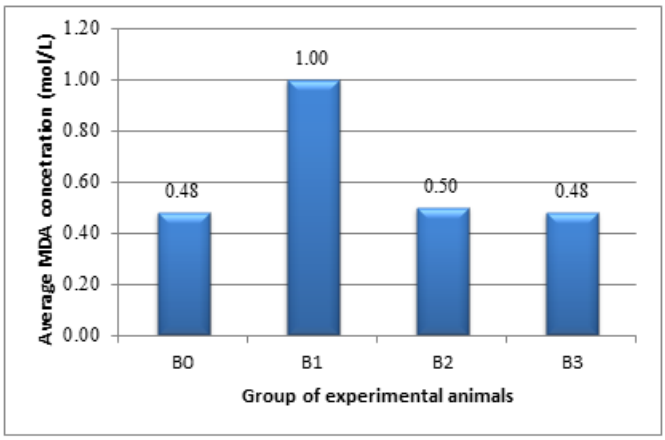

Figure 4

Average MDA (mol/L) concentration of mice liver on day-7 (d-7); which injected on d-1 5 mg/g bw HgCl2 (B1); previously on d-1 injected 5 mg/g bw HgCl2 and on d-4 received 0.2 mg/g bw Immunos® by oral gavage (B2); previously on h1 injected 5 mg/g bw HgCl2 and on d-4 received 0.39 mg/g bw LE3H by oral gavage (B3); Only the solvent administered the controls in the same way (B0).

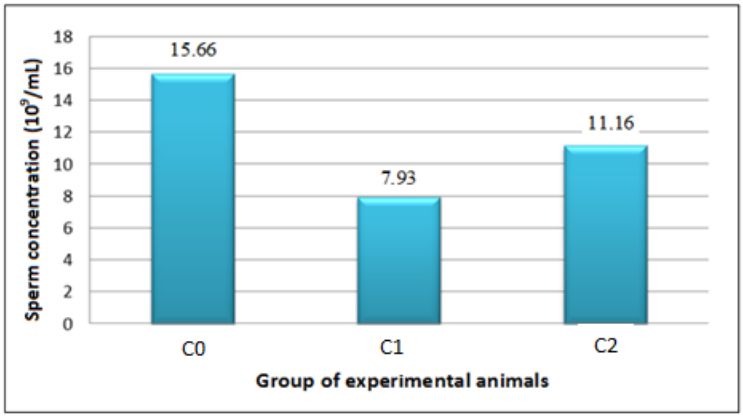

Figure 5

Average concentration (106/mL) of mice sperm on day-5 (d-5) which was injected intraperitoneally (ip) 5 mg/kg body weight (bw) HgCl2 on d-1 (C1); injected ip 5 mg/kg bw HgCl2on d-1, and then given by oral gavage 0.39 mg/g bb LE3H on d-3 (C2). Only the solvent administered the controls in the same way (C0).

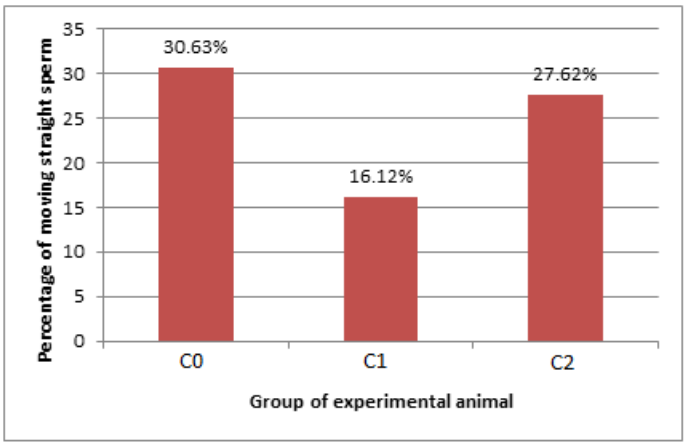

Figure 6

Percentage of moving fast straight mice sperm on day-5 (d-5) which was injected intraperitoneally (ip) by 5 mg/kg body weight (bw) HgCl2 on d-1 (C1); injected ip 5 mg/kg bw HgCl2 on d-1, and then given by oral gavage 0.39 mg/g bb LE3H on d-3 (C2). Only the solvent administered the controls in the same way (C0).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- RuyaniGraficalAbstractAug292022.jpg