

Hepatoprotective Effect of Leaf Ethanolic Extract *Etlingera hemisphaerica* Blume to Recovery Mercuric Chloride Toxicity on Mice

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Abstract

The previous study revealed that leaf ethanolic extract *Etlingera hemisphaerica* Blume (LE3H) is potentially to decrease blood glucose and triglyceride levels on *Mus musculus* with hyperglycemia and hypertriglyceridemia respectively. This research was intended to investigate hepatoprotective effect of LE3H to recovery HgCl₂ toxicity in *M. musculus*. The total of experimental animals was 95 male *M. musculus*. Both treatment and control animals received drink and feed *ad libitum*, then the animals were killed by cervical dislocation (CD). Two test materials; LE3H (0.13, 0.26, 0.39 mg/g bw) were treated by gavage and HgCl₂ (5 mg/kg bw) was administrated by gavage or intraperitoneal injection. Livers were separated after CD, and their color, weight, and volume were observed. The livers were fixed, planted in paraffin, sliced, and stained to generate a liver histology. HgCl₂ appeared a "special red" liver difference from the "wine" control, this color could be recovered by 0.39 mg/g bw LE3H treatment. The triple dosage of 0.39 mg/g bw LE3H tend to restore the weights, meanwhile HgCl₂ and LE3H increased significantly liver volume. The dosage of 0.39 mg/g bw LE3H treatment was tend to reduce the liver weight caused-HgCl₂ as well as the control. Histological observation indicated that HgCl₂ in swelling of the tissue and a decrease in the extracellular space compared with the control, and then finally the symptoms was protected by 0.39 mg/g bw LE3H treatment. It should be concluded that LE3H indicates hepatoprotective effect to recovery HgCl₂ toxicity on *M. musculus*.

Key words: danau *E. hemisphaerica*; mercury; detoxification; *Mus musculus*; liver

Introduction

Gold mine activity by using mercury (Hg) in Rejang, Bengkulu province, Indonesia, has lasted one hundred and twenty-six years (1890-2016). The use of Hg in gold mining activities is a very difficult problem to solve. The presence of Hg in the body of the miners and his family were measured by atomic absorption spectroscopy (AAS) technique. Obtained data revealed that the miners and mothers are breastfeeding were contaminated by Hg (Ruyani *et al.*, 1997). Detected concentrations of Hg was still brought the threshold, so that the clinical symptoms of toxicity such as Acrodynia (pink disease; Bjorklund, 1995), Hunter-Russell syndrome, and Minamata disease (Clifton, 2007) has not been found in the

territory of people's gold mine. That facts led to the understanding that Hg is a safe ingredient used in everyday activities. Recent observation (October 2015) in the same location appeared that the miners were processing gold with Hg in each their house therefore the metal poisoning (<https://douglaslawton.files.wordpress.com/2015/11/dsc01037.jpg>) opportunities for whole members of their family will be much greater. Without risk consideration of Hg toxicity, up till now people gold mining activities in Bengkulu and some other areas in Indonesia still continue (Castilhos *et al.*, 2006).

Immediately chelation therapy is the standard of care for a patient showing symptoms of severe Hg poisoning or the laboratory evidence of a large total Hg load. Inorganic Hg ingestion such as mercuric chloride (HgCl_2) must be considered as the serious caustic. Chelation therapy for acute inorganic Hg poisoning can be performed with succinate or meso-2,3-dimercaptosuccinate (DMSA), 2,3-dimercapto-1-propanesulfonic acid (DMPS), D-penicillamine (DPCN), or dimercaprol (Risher and Amler, 2005). Epidemiological studies have found little evidence that selenium can help protect against the adverse effects of methylmercury, but experimental findings in the laboratory have successfully demonstrated an interaction between selenium and methylmercury (Watanabe, 2002). It was reported that an incorrect form of ethylene diaminetetraacetic acid (EDTA) used for chelation therapy, resulted in hypocalcaemia in August 2005, and then causing cardiac arrest that killed a five-year-old autistic boy (Baxter and Krenzelok, 2008). This case indicated that chelation therapy can be hazardous if administered incorrectly.

Gold miner generally do not know that Hg is harmful to health. Meanwhile they are also economically very dependent on these activities. They require education about mercury, psychosocial assistance, and economic solutions in order to live more feasible. On a more micro scale, should also attempt Hg detoxification using natural ingredients that are traditionally be used by some ethnics in Java and Sumatra, including by Rejang people in Lebong, Bengkulu. Herbal therapy is an interesting study to attempt detoxifies heavy metals, and it revealed that 21 and 10 plant species have the potential to detoxify Pb and Hg (Sarma *et al.*, 2011). *Cipura paludosa* extract is also indicated to be able to prevent neurological disorders Hg poisoning (Lucena *et al.*, 2007). It was also reported that extracts one type honje, *Etlingera elatior*, proved to potentially restore liver damage due to the toxicity of Pb (Haleagrahara *et al.*, 2010).

The results of study on *Etlingera* (<http://www.theplantlist.org/tpl1.1/record/kew-243067>) revealed that leaf ethanolic extract *E. hemisphaerica* Blume (LE3H; 0.39 mg/g bw) is potentially to decrease blood glucose (36.2%) and triglycerides (21.19%) levels in *M. musculus* with hyperglycemia and hypertriglyceridemia respectively (Ruyani *et al.*, 2014). Bengkulu is one example of the many areas in Indonesia which have some center of people mining with risk of Hg toxicity, requiring effort detoxification using natural ingredients that are safe, easy, and inexpensive. According to the requirements, then it was really necessary to investigate hepatoprotective effect of LE3H to recovery HgCl_2 toxicity on Swiss Webster mice (*Mus musculus*).

Materials and Methods

Dosages of investigation

An investigation reported that the effective dosage of *Mulitinga calbura* extract which contains flavonoids in mice was 0.13 mg/g body weight (bw; Sunarso, 2011]. Based on the report it was determined in this study three dosage of LE3H were 0.13, 0.26, and 0.39 mg/g bw (Ruyani *et al.*, 2014). Meanwhile the dosage of HgCl_2 in this study was 5 mg/kg bw (Ghosh and Sil, 2008; El-Desoky *et al.*, 2013).

Experimental animals

In accordance with the principles of 5F (freedom), namely; (a) free from hunger and thirst, (b) free from discomfort, (c) free from pain, injury and disease, (d) free from fear and stress the long-term, (e) freely express behavior naturally, given the space and appropriate facilities (Ridwan, 2013). This study pay attention to the ethical use of animals includes aspects of the humane treatment of animals.

This research was conducted in three stages of work (Q, R, and S). *M. musculus* were used as experimental animals. The animals were reared in a room at 23-27°C, and 83 % humidity. Both food and water were given *ad libitum* (Ruyani *et al.*, 2005; Ruyani *et al.*, 2011). Total number of male *M. musculus* aged 6-8 weeks with 25-35 g bw (Ruyani *et al.*, 2014) were used in Hg detoxification on liver of *M. musculus* by LE3H as much as 95 male animals (Federer, 1967).

First stage of experiment (Q)

The twenty-five (25) of experimental animals were divided into five groups (Q0, Q1, Q2, Q3, and Q4), each consisting of five male mice. Q0 group as the controls of the research were administered solvent only in the same manner of Q1-Q4. In d-1 5 mg/kg bw HgCl₂ was given by gavage on Q1, Q2, Q3, Q4 group, then in d-3 0.13, 0.26, and 0.39 mg/g bw LE3H were given by gavage on Q2, Q3, and Q4 group respectively. Furthermore the dosage of 0.13, 0.26, and 0.39 mg/g bw LE3H were given by gavage on Q2, Q3, and Q4 group in d-4 and d-5 respectively.

Both treated and control groups were killed by cervical dislocation (CD) in d-7, dissected, and then the livers were isolated for hepatotoxicity observation. Weight and volume of livers were observed shortly after the organs were immediately isolated. Liver tissue samples were separated shortly after the mice were killed, immediately immersed in 10% buffered formalin, and then put into paraffin. Thin section stained using hematoxylin and eosin with working techniques that have been standard (Bujanda *et al.*, 2006).

Second stage of experiment (R)

The twenty-five (25) of experimental animals were divided into five groups (R0, R1, R2, R3, and R4), each consisting of five male mice. R0 group as the controls of the research were administered solvent only in the same manner of R1-R4. In d-1 5 mg/kg bw HgCl₂ was given by gavage on R1, R2, R3, R4 group, then in d-3 0.13, 0.26, and 0.39 mg/g bw LE3H were given by gavage on R2, R3, and R4 group respectively. Furthermore the dosage of 0.13, 0.26, and 0.39 mg/g bw LE3H were given by gavage on R2, R3, and R4 group in d-5 and d-7 respectively.

Both treated and control group were killed by CD in d-16, dissected, and then the livers were isolated for hepatotoxicity observation. Weight and volume of livers were observed shortly after the organs were immediately isolated.

Third stage of experiment (S)

The forty-five (45) of experimental animals were divided into three groups (S0, S1, and S2), each consisting of 15 male mice. S0 group as the controls of the research were administered solvent only in the same manner of S1-S2. In d-1 5 mg/kg bw HgCl₂ was injected intra peritoneally on S1 and S2 group, and in d-2 0.39 mg/g bw LE3H was given by gavage on S2 group.

Both treated and controls were killed by CD in d-5, dissected, and then the livers were isolated for hepatotoxicity observation. Color, weight, and volume of livers were observed shortly after the organs were immediately isolated. Furthermore the liver colours were determined using the colour standard (<http://www.sentramultiwarna.com>).

Results

This HgCl₂ detoxification research on *M. musculus* liver as the effects of LE3H treatments were performed after killing the experimental animals, dissected, and then separated their liver for colour, weight, volume, histology observation. Results of the first stage of experiment (Table 1; Q) revealed that although liver weights in d-7 were not significant different statistically between the treatments (Table 6; Q1, Q2, Q3, and Q4) and the control (Q0), however the triple dosage of 0.39 mg/g bw LE3H which was administrated in d-3, d-4, and d-5 (Q4) tend to restore liver weights approach the control (Table 1; Q0; Figure 1). Meanwhile liver volume in d-7 indicated a different phenomena, 5 mg/kg bw HgCl₂ treatment increased significantly liver volume (Q1: 2.80±0.83 mL) comparing the control (Q0: 1.60±0.54 mL), and then administration the three dosages (0.13, 0.26, 0.39 mg/g bw) of LE3H further increased significantly liver volume (Q2: 2.20±0.44 mL; Q3: 3.00±0.70 mL; Q4: 3.50 ± 0.50 mL) consistent with the given LE3H concentrations (Table 1; Figure 2).

Table 1. Weight (g) and volume (mL) of *M. musculus* liver in days-7 (d-7) which previously in d-1 5 mg/kg body weight (bw) HgCl₂ was administrated by gavage on Q1, Q2, Q3 and Q4 group, and then in d-3, d-4, and d-5 the dosage of 0.13, 0.26, 0.39 mg/g bw LE3H were administrated respectively by gavage on Q2, Q3, and Q4 group.

Group of experimental animals	N	Day-7	
		Average of liver weight ±SD (g)	Average of liver volume ± SD (mL)
Q0: Control	5	1.83 ± 0.06	1.60 ± 0.54 ^a
Q1: 5 mg/kg body weight (bw) HgCl ₂	5	1.71 ± 0.20	2.80 ± 0.83 ^b
Q2: 5 mg/kg bw HgCl ₂ +0.13 mg/g bw LE3H	5	1.46 ± 0.43	2.20 ± 0.44 ^{abc}
Q3: 5 mg/kg bw HgCl ₂ +0.26 mg/g bw LE3H	5	1.76 ± 0.31	3.00 ± 0.70 ^{bcd}
Q4: 5 mg/kg bw HgCl ₂ +0.39 mg/g bw LE3H	5	1.81 ± 0.10	3.50 ± 0.50 ^{bd}

Note: Rates followed by the same letter are not significantly different data addressing the same column (Least Significant Difference test results; Steel and Torrie, 1981).

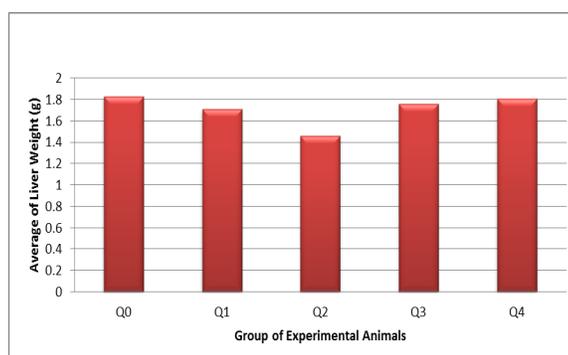


Figure 1. Weight (g) of *M. musculus* liver in days-7 (d-7) which previously in d-1 5 mg/kg body weight (bw) HgCl₂ was administrated by gavage on Q1, Q2, Q3 and Q4 group, and then in d-3, d-4, and d-5 the dosage of 0.13, 0.26, 0.39 mg/g bw LE3H were administrated respectively by gavage on Q2, Q3, and Q4 group.

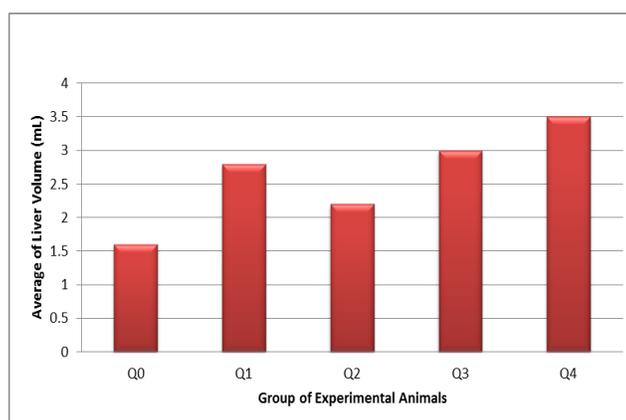


Figure 2. Volume (mL) of *M. musculus* liver in days-7 (d-7) which previously in d-1 5 mg/kg body weight (bw) HgCl₂ was administrated by gavage on Q1, Q2, Q3 and Q4 group, and then in d-3, d-4, and d-5 the dosage of 0.13, 0.26, 0.39 mg/g bw LE3H were administrated respectively by gavage on Q2, Q3, and Q4 group.

Table 2. Weight (g) and volume (mL) of *M. musculus* liver in day-16 (d-16) which previously in d-1 5 mg/kg bw HgCl₂ was given by gavage on R1, R2, R3, R4 group, then in d-3 0.13, 0.26, and 0.39 mg/g bw LE3H were given by gavage on R2, R3, and R4 group. Furthermore the dosage of 0.13, 0.26, and 0.39 mg/g bw LE3H were given by gavage on R2, R3, and R4 group in d-5 and d-7 respectively.

Group of experimental animals	N	Day-16	
		Average of liver weight ± SD (g)	Average of liver volume ± SD (mL)
R0: Control	5	2.46 ± 0.35	2.12 ± 0.41
R1: 5 mg/kg body weight (bw) HgCl ₂	5	2.39 ± 0.24	2.34 ± 0.38
R2: 5 mg/kg bw HgCl ₂ + 0.13 mg/g bw LE3H	5	1.87 ± 0.31	1.78 ± 0.42
R3: 5 mg/kg bw HgCl ₂ + 0.26 mg/g bw LE3H	5	2.16 ± 0.41	2.06 ± 0.47
R4: 5 mg/kg bw HgCl ₂ + 0.39 mg/g bw LE3H	5	2.27 ± 0.30	2.06 ± 0.47

An important difference between the third stage of experiment (R) and the second stage of experiment (Q) was nine days maintenance after the administration of LE3H three times for each dosage of 0.13, 0.26, and 0.39 mg/g bw LE3H. Therefore, observations of HgCl₂ detoxification were performed in d-16. Hg toxification induction by gavage of 5 mg/kg bw HgCl₂ and detoxification efforts using three times administration for each dosage of 0.13, 0.26, and 0.39 mg/g bw LE3H in d-16 did not influence statistically to both the weight (Table 2; Figure 3) and volume (Table 2; Figure 4) of liver on treatments (R1, R2, R3, and R4) comparing the control (R0). The nine days maintenance after the administration of LE3H three times for each dosage of 0.13, 0.26, and 0.39 mg/g bw was predicted allegedly one of the factors why the symptoms of HgCl₂ detoxification did not look through the simple observation. Nevertheless it could be determined from the phenomena that the dosage of 0.39 mg/g bw LE3H was most potential to recovery due to the toxicity of HgCl₂.

Furthermore the number of repeat test animals in each experimental group needs to be added to make the symptoms of HgCl₂ detoxification by LE3H would be easily observed.

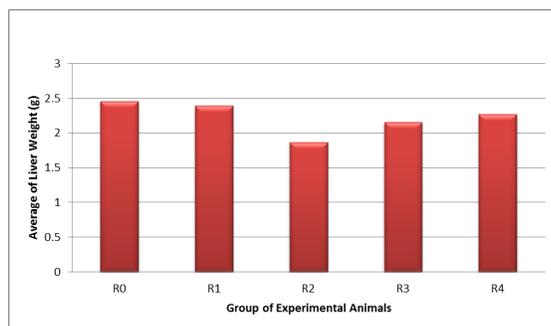


Figure 3. Weight (g) of *M. musculus* liver in day-16 (d-16) which previously in d-1 5 mg/kg bw HgCl₂ was given by gavage on R1, R2, R3, R4 group, then in d-3 0.13, 0.26, and 0.39 mg/g bw LE3H were given by gavage on R2, R3, and R4 group. Furthermore the dosage of 0.13, 0.26, and 0.39 mg/g bw LE3H were given by gavage on R2, R3, and R4 group in d-5 and d-7 respectively.

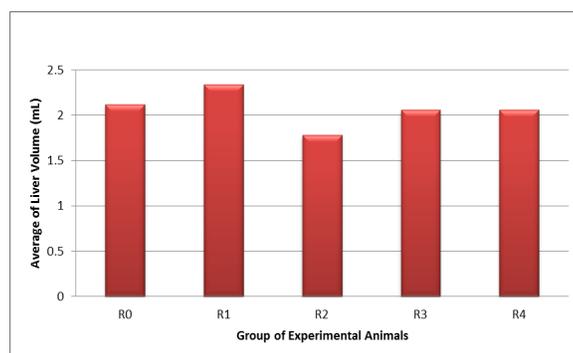


Figure 4. Volume (mL) of *M. musculus* liver in day-16 (d-16) which previously in d-1 5 mg/kg bw HgCl₂ was given by gavage on R1, R2, R3, R4 group, then in d-3 0.13, 0.26, and 0.39 mg/g bw LE3H were given by gavage on R2, R3, and R4 group. Furthermore the dosage of 0.13, 0.26, and 0.39 mg/g bw LE3H were given by gavage on R2, R3, and R4 group in d-5 and d-7 respectively.

In the third stage of experiment (S), the number of experimental animals for each groups were added three times more than the previous experimental stages, at this stage the dosage of 5 mg/kg bw HgCl₂ was injected intraperitoneally (ip), and both HgCl₂ and LE3H were administered throught the single dosage. Administration of 5 mg/kg bw HgCl₂ through ip injection caused the liver colour to change from "wine" (Table 3; S0) into "special red" (Table 3; S1), meanwhile the administration of 5 mg/kg bw HgCl₂ via ip at d-1 which was followed by administration of 0.39 mg/g bw LE3H by gavage in d-3 was able to restore the liver color into "wine" (Table 3; S2) as well as the control (Table 3; S0). The facts indicated that LE3H was potentially restoring color HgCl₂ induced liver toxicity. Furthermore it was appeared that the dosage of 0.39 mg/g bw LE3H was tend potentially to reduce the liver weight caused-HgCl₂ (Table 4; S1; 1.60±0.73 g) becomes lower weight (S2; 1.51±0.63 g) approached the liver weight of control (Table 4; S0; 1.56 ± 0.66 g; Figure 5).

Table 3. Score of *M. musculus* liver color day-5 (d-5) which previously in d-1 5 mg/kg body weight (bw) HgCl₂ was injected intraperitoneally (ip) on S1 and S2 group, and then in d-3 0.39 mg/g bw LE3H was administrated by gavage on S2 group.

Group of experimental animals	N	Day-5	
		Average score of color	Liver color
S0: Control	15	8.0	8 (wine)
S1: 5 mg/kg body weight (bw) HgCl ₂	15	8.9	9 (special red)
S2: 5 mg/kg bw HgCl ₂ + 0.39 mg/g bw LE3H	15	7.8	8 (wine)



Table 4. Weight (g) of *M. musculus* liver in day-5 (d-5) which previously in d-1 5 mg/kg body weight (bw) HgCl₂ was injected intraperitoneally (ip) on S1 and S2 group, and then in d-2 0.39 mg/g bw LE3H was administrated by gavage on S2 group.

Group of experimental animals	N	Day-5, average weight of liver ± SD (g)
S0: Control	15	1.56 ± 0.66
S1: 5 mg/kg body weight (bw) HgCl ₂	15	1.60 ± 0.73
S2: 5 mg/kg bw HgCl ₂ + 0.39 mg/g bw LE3H	15	1.51 ± 0.63

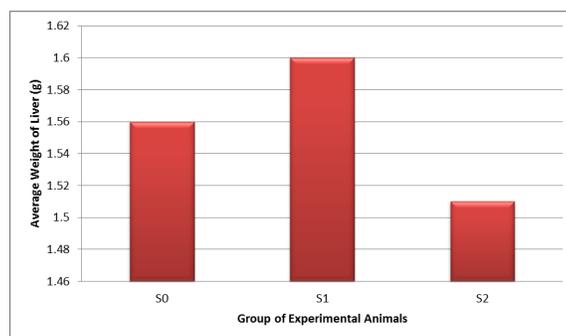


Figure 5. Weight (g) of *M. musculus* liver in day-5 (d-5) which previously in d-1 5 mg/kg body weight (bw) HgCl₂ was injected intraperitoneally (ip) on S1 and S2 group, and then in d-2 0.39 mg/g bw LE3H was administrated by gavage on S2 group.

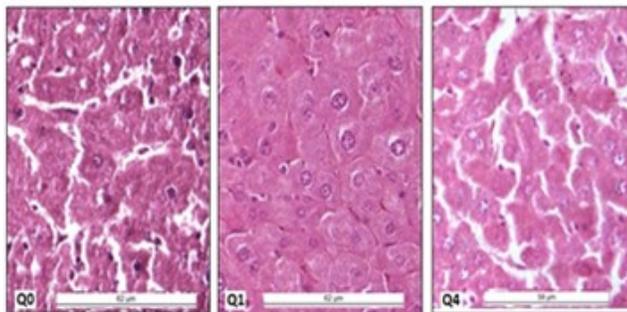


Figure 6. Thin liver section of *M. musculus* were stained by hematoxylin and eosin with the standard working techniques. Oral administration of 5 mg/kg bw HgCl_2 resulted in swelling of the tissue and a decrease in the extracellular space (Q1) compared with the control (Q0), and then the symptoms were reduced by the triple dosage of 0.39 mg/g bw (Q4) LE3H to reach nearly the control condition (Q0).

Incision liver histology which were stained by hematoxylin and eosin in d-7 revealed that oral administration of 5 mg/kg bw HgCl_2 resulted in swelling of the tissue and a decrease in the extracellular space (Q1) compared with the control (Q0; Figure 6), and then the symptoms were reduced by the triple dosage of 0.39 mg/g bw (Q4; Figure 6) LE3H to reach nearly the control condition (Q0).

Discussion

Previous investigations reported that HgCl_2 is a widespread environmental toxin that affects mainly liver and kidney (Ghosh and Sil, 2008) and it causes also oxidative damage in several tissues (Brandão and Nogueira, 2011). Furthermore it was revealed that HgCl_2 lead to more pronounced oxidative stress and hepatotoxicity (Agrawal *et al.*, 2014). Exposure (ip) to the single dosage of 5 mg/kg bw HgCl_2 induced oxidative stress in mice and substantially increased lipid peroxidation (LPO) and oxidized glutathione (GSSG) levels, decreased the level of reduced glutathione (GSH) and various antioxidant enzymes in liver and also increased the activities of liver marker enzymes in serum (Zhao *et al.*, 2009). Several studies have tried to find potential compounds as hepatoprotective or hepatocurative agent induced by HgCl_2 , among others; oral administration of either selenium or garlic produces a significant protection against liver damage induced by the HgCl_2 injection, but garlic appears to be more protective (El-Shenawy and Hassan, 2008). Furthermore, it is known that *spirulina* treatment augments the antioxidants defense mechanism in HgCl_2 induced toxicity and provides evidence that it may have a therapeutic role in free radical mediated diseases (Sharma *et al.*, 2007) and propolis augments the antioxidants defense against HgCl_2 induced toxicity and provides evidence that it has therapeutic potential as hepatoprotective agent (Zhao *et al.*, 2009). In this study a question arised; whether LE3H has potential as a therapeutic for the hepatocurative agent?

The administration of 5 mg/kg bw HgCl_2 through ip injection in d-1 which was followed by administration of 0.39 mg/g bw LE3H by gavage in d-3 was able to restore the liver color from "special red" (Table 3; S2) into "wine" as well as the control (Table 3; S0). Because of color changes of the liver has become one of the indicators for cases hepatotoxicity (Xu *et al.*, 2010; Kurniawan *et al.*, 2014), then the color recovery can be as proof that LE3H has potential as a therapeutic potential for the hepatocurative agent. While the weight of the liver can also commonly used as one indicator of hepatotoxicity cases (Lei *et al.*, 2015). Liver weight in this study indicated that the triple dosages of 0.39 mg/g bw LE3H which was administrated in d-3, d-4, and d-5 (Q4) tend to restore liver weights approach the control

(Table 1; Q0; Figure.2). Hg toxification induction by gavage of 5 mg/kg bw HgCl₂ and detoxification efforts using three times administration for each dosage of 0.13, 0.26, and 0.39 mg/g bw LE3H in d-16 did not influence statistically to the weight (Table 1; Figure 4) of liver. This fact allegedly associated with the mechanism of liver regeneration in mice (Noschinski *et al.*, 2015). The dosage of 0.39 mg/g bw LE3H was tend potentially to reduce the liver weight caused-HgCl₂ (Table 3; S1; 1.60 ±0.73 g) becomes lower weight (S2; 1.51±0.63 g) approached the liver weight of control (Table 3; S0; 1.56 ± 0.66 g; Figure 6). Based on the data liver weight could be determined that the liver weight changes which was induced by HgCl₂ can be restored by LE3H administration.

Volume determination of the liver is important in the study of hepatotoxicity which can be performed by manual measurement or semiautomated liver volumetry measurement (Ghate *et al.*, 2015). Results of liver manual measurement on this research revealed that the three dosages (0.13, 0.26, 0.39 mg/g bw) of LE3H increased significantly liver volume (Q2: 2.20±0.44 mL; Q3: 3.00±0.70 mL; Q4: 3.50 ± 0.50 mL) consistent with the given extract concentrations (Table 6; Figure 3). Meanwhile Hg toxification induction by gavage of 5 mg/kg bw HgCl₂ and detoxification efforts using three times administration for each dosage of 0.13, 0.26, and 0.39 mg/g bw LE3H in d-16 did not influence statistically to volume (Table 7; Fig.5) of liver. According to these facts it could be stated that LE3H not able to recovery the volume of liver which was induced by HgCl₂. Liver histological incisions are important data on the study of hepatotoxicity (Ghate *et al.*, 2015). Thin liver sections of *M. musculus* were stained by hematoxylin and eosin with the standard working techniques. Oral administration of 5 mg/kg bw HgCl₂ resulted in swelling of the tissue and a decrease in the extracellular space (Q1) compared with the control (Q0), and then the symptoms were reduced by the triple dosage of 0.39 mg/g bw (Q4) LE3H to reach nearly the control condition (Q0; Figure 6). Further quantitative analysis of the changes in the tissues can be performed by ant antibody against F-80 works well for showing the Kupffer cells (Robertson, 2015; the University of California; personal communication). It should be stated here that LE3H has potential to recovery histopathological effects of HgCl₂ on mice liver.

The result of qualitative phytochemical test showed that LE3H contains tannins (++) , saponins (+), alkaloids (+), and flavonoids (+; Ruyani *et al.*, 2016). Flavonoids have also been proposed to inhibit the pro-inflammatory activity of enzymes involved in free radical production, such as cyclooxygenase, lipoxygenase or inducible nitric oxide synthase (Izzi *et al.*, 2012; Gomes *et al.*, 2012) and to modify intracellular signaling pathways in immune cells (Gomes *et al.*, 2012). It is well known that class of flavonoids such as proanthocyanidins (PCs) and procyanidins. An investigation revealed that pretreatment with PCs can inhibit reactive oxygen species (ROS) production, protected antioxidant enzymes, and reversed hepatotoxicity. PCs had hepatoprotective effects on HgCl₂-induced toxicity by antagonizing oxidative stress in rat liver (Deng *et al.*, 2012). Previous research indicated that ROS generation was decreased by 27.63% and apoptosis was also decreased in procyanidins groups respectively, and then pathological changes were much better as well (Yang *et al.*, 2011). Meanwhile methanolic extract of *D. burmannii* (DBME), which is contained *tannins*, could significantly normalize serum enzyme levels and restored liver antioxidant enzymes levels in mice. The findings suggested that the constituents present in DBME contributed to its iron chelation activity. DBME lowered also the raised levels of liver damage parameters, also reflected from the morphological analysis of the liver sections (Ghate *et al.*, 2015).

LE3H was predicted to have detoxification mechanisms, namely; (a) HgCl₂ chelation and (b) antioxidant activities. Hence both these activities, it has the potential to recovery HgCl₂ toxicity effects in *M. musculus* liver. This detoxification mechanism is still need to be studied in more detail at molecular level; it may be performed through immunohistochemistry and toxicoproteomics techniques.

Conclusions

The leaf ethanolic extract *E. hemisphaerica* Blume (LE3H; 0.39 mg/g bw) has hepatoprotective effects to recovery HgCl₂ (5 mg/kg bw) toxicity on *M. musculus*. Therefore, daily medication of LE3H may be useful for detoxication in persons whom are exposed to HgCl₂.

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Authors' Contributions

AR conceived of the study, and participated in its design and coordination and helped to draft the manuscript. DM participated in the preparing of experimental animals. RZEP carried out in the histopathological observation. TY participated on the study of Hg detoxification on liver. AS carried out extract preparation and phytochemical test. All authors read and approved the final manuscript.

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