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Research Article
Etlingera hemisphaerica Alters One-Dimensional Profile of Serum Proteins Due to Mercury Chloride in Rats (*Rattus norvegicus*)

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Abstract

Background and Objective: Several previous studies have shown that leaf ethanolic extract of *Etlingera hemisphaerica* (LE3H) has the potential to reduce the toxicity and teratogenicity effects of mercury. This study aimed to describe the effects of LE3H on the protein profile of *Rattus norvegicus* serum due to treatment with $HgCl_2$. **Materials and Methods:** Four groups of male rats, K1 was injected intraperitoneally (IP) $HgCl_2$ (5 mg kg^{-1} b.wt.), K2 was injected IP $HgCl_2$ (5 mg kg^{-1} b.wt.) and after 24 hrs it was gavage LE3H (0.27 mg g^{-1} b.wt.) every day for seven days, K3 was injected IP $HgCl_2$ (5 mg kg^{-1} b.wt.), after 24 hrs was gavage LE3H (0.55 mg g^{-1} b.wt.) every day for seven days. The K0 as control, received double-distilled water. On the ninth day, the experimental animals were killed via CD and blood was drawn from the heart to obtain serum. Serum samples were measured for protein content using the Lowry and serum was separated using the One-Dimensional Sodium dodecyl Sulfate-Polyacrylamide gel Electrophoresis (1D SDS-PAGE) technique. **Results:** The electropherograms showed four bands, 264.77, 219.53, 98.57 and 37.29 kDa, whose intensity significantly increased due to $HgCl_2$ treatment and then decreased to close to the control condition with LE3H administration. The results also revealed four bands, 31.95, 28, 06, 26, 29 and 15.09 kDa, whose intensity decreased significantly due to $HgCl_2$ treatment and then increased to close to the control condition by LE3H administration. **Conclusion:** The LE3H change profile of the eight blood serum protein bands due to $HgCl_2$ approximates the control condition in *R. norvegicus*.

Key words: *Etlingera hemisphaerica*, mercury chloride, one-dimensional protein, blood serum, *Rattus norvegicus*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mercury (Hg) is a well-known environmental pollutant that is harmful to both humans and animals[1]. Accidental Hg poisoning can occur in both developing and developed countries. In Bengkulu, Indonesia, Hg poisoning is caused by a lack of information about the hazards of Hg, a lack of efficient governmental supervision and, in particular, gold mining operations. Gold mining in Latin America is also a significant source of Hg poisoning accidents, with adverse health and societal consequences such as neurological impairment, reduced IQ, blood cell destruction and an impaired immune system[2,3]. Meanwhile, 63.8 and 100% of 221 pregnant women in North Carolina, USA, had Hg and lead (Pb) contamination in their blood due to using cosmetics, pesticides and electronic equipment, respectively[4]. Mercuric Chloride ($HgCl_2$) fatal dosages in humans are believed to be between 1 and 4 g. The $HgCl_2$ can induce corrosive damage, gastrointestinal difficulties, circulatory collapse, acute renal failure and death[5]. Furthermore, Hg and its constituents have been related to severe neurotoxicity, particularly damage to the central and peripheral nervous systems[6].

Animal experiments have been conducted to prevent Hg exposure during reproductive age and pregnancy. The Hg detoxification and teratogenicity reduction are equivalent attempts undertaken under distinct conditions, although the same researchers still need to carry them out. Malondialdehyde, reduced glutathione, nitric oxide and total sialic acid levels were examined to see if omega-3 fatty acid (0.5 g kg^{-1} b.wt./day) could protect mice from $HgCl_2$ poisoning (0.4 mg kg^{-1} b.wt./day). According to the findings, omega-3 fatty acids may attenuate $HgCl_2$-induced toxicity in mice by improving antioxidant status and acute-phase response[7]. *Origanum* oil (5 mg kg^{-1}) has antioxidant properties and may protect cells from oxidative damage caused by $HgCl_2$ (4 mg kg^{-1} b.wt.) in *R. norvegicus*[8].

Several ethnic groups in Bengkulu, Indonesia, used Kecombrang or Kunji or Honje forest (*Etlingera hemisphaerica* Blume) as herbal medicine. An ethnomedicinal plant study found that 0.39 mg g^{-1} b.wt., ethanolic leaf extract of *E. hemisphaerica* (LE3H) lowered triglyceride (21.19%) and blood glucose (36.2%) levels in mice with hyperglycemia and hypertriglyceridemia, respectively[9]. The LE3H improved the scoring of spermatogenesis stages in the histopathological picture of *R. norvegicus* testes that was caused by $HgCl_2$[10]. In a prior study, $HgCl_2$ injection boosted leukocytes while lowering erythrocytes, however, $HgCl_2$ administration followed by LE3H therapy conserved the number of blood cells and the control in mice. The formation of a novel 125 kDa protein and overexpression of the 48 kDa protein was caused by $HgCl_2$ treatment. However, this protein profile could be sustained by LE3H treatment in the same way it was in the control condition. The LE3H offers potential protective effects against $HgCl_2$ poisoning in mouse blood. This one-dimensional protein profile of blood serum should be examined further using competent working techniques. As a result, LE3H dietary supplements may benefit persons who have been exposed to $HgCl_2$[11].

This biological modeling study aimed to examine if LE3H may influence the one-dimensional serum protein profile in *R. norvegicus* due to mercury chloride.

MATERIALS AND METHODS

Study area: This ethnomedicinal plant study was conducted during 2019-2021 at Bengkulu University, Indonesia, SBIH Ruyani, Bengkulu, Indonesia and Kendrick Laboratories Inc., (1202 Ann St., Madison WI 53713) USA.

Extract preparation: Plant identification was confirmed with the help of the Indonesian Institute of Sciences, Research Center for Plant Conservation and Botanic Garden, Bogor, Indonesia (Number B-1750/IPH.3./KS/V/2019). Research plant materials were obtained from Curup City, Rejang Lebong District, Bengkulu Province, Indonesia. The leaves were washed before being cut into small bits. Wind-drying 3000 g of fresh *E. hemisphaerica* leaves yielded 800 g of dried leaves. The powder was macerated in 2 L of 96% ethanol for seven days before condensing[12] with a rotary evaporator (RE-52A Rotary Evaporator, Bengkulu University, Indonesia) to yield a concentrated extract weighing 3 g. Once the ethanol in the concentrated extract has evaporated, 2 g of crude extract could be utilized as a test sample for this investigation[11,13,14].

Dosage determination and conversion: Previous research found that 0.39 mg g^{-1} b.wt., of forest honje (*E. hemisphaerica*) leaf extract was beneficial in mice[11,13,14]. Because rats were utilized as test animals in this investigation, the dose was converted from 20 g mice to 200 g *R. norvegicus*, namely 7[15] Dose 1: 0.39 mg g^{-1} b.wt., mice = 7.8 mg/20 g b.wt., mice for *E. hemisphaerica* leaf extract. The rat conversion dose is 7.8 mg×7 = 54.6 mg/200 g b.wt., rats, similar to 0.27 mg g^{-1} b.wt., rats. Dose 2: 0.78 mg g^{-1} b.wt., mice = 15.6 mg/20 g b.wt., mice. The rat conversion dose is 15.6 mg×7 = 109.2 mg/200 g b.wt., rats, similar to 0.55 mg g^{-1} b.wt., rats. Finally, LE3H working dosages for rats were set at 0.27 and 0.55 mg g^{-1} b.wt., respectively.

Table 1: One-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (1D SDS-PAGE) separated *R. norvegicus* serum protein samples K0, K1, K2 and K3

Lane No.	Serum sample protein	Bicinchoninic acid (BCA) assay result (mg mL^{-1})	n	Separated protein levels (µg)
1	Molecular weight standards	-		
2	Sample buffer blank	-		
3	K0: Control, double-distilled (DD) water, after 24 hrs given DD water for 7 days	32.40	1	50
4	K0: Control, double-distilled (DD) water, after 24 hrs given DD water for 7 days	32.40	1	50
5	K0: Control, double-distilled (DD) water, after 24 hrs given DD water for 7 days	32.40	1	50
6	K1: 5 $HgCl_2$ (5 mg kg^{-1} b.wt.) IP, after 24 hrs given DD water for 7 days	24.1	1	50
7	K1: 5 $HgCl_2$ (5 mg kg^{-1} b.wt.) IP, after 24 hrs given DD water for 7 days	24.1	1	50
8	K1: 5 $HgCl_2$ (5 mg kg^{-1} b.wt.) IP, after 24 hrs given DD water for 7 days	24.1	1	50
9	K2: $HgCl_2$ (5 mg kg^{-1} b.wt.) IP, after 24 hrs given LE3H (0.27 mg g^{-1} b.wt.) for 7 days	26.43	1	50
10	K2: $HgCl_2$ (5 mg kg^{-1} b.wt.) IP, after 24 hrs given LE3H (0.27 mg g^{-1} b.wt.) for 7 days	26.43	1	50
11	K2: $HgCl_2$ (5 mg kg^{-1} b.wt.) IP, after 24 hrs given LE3H (0.27 mg g^{-1} b.wt.) for 7 days	26.43	1	50
12	K3: $HgCl_2$ (5 mg kg^{-1} b.wt.) IP, after 24 hrs given LE3H (0.55 mg g^{-1} b.wt.) for 7 days	21.18	1	50
13	K3: $HgCl_2$ (5 mg kg^{-1} b.wt.) IP, after 24 hrs given LE3H (0.55 mg g^{-1} b.wt.) for 7 days	21.18	1	50
14	K3: $HgCl_2$ (5 mg kg^{-1} b.wt.) IP, after 24 hrs given LE3H (0.55 mg g^{-1} b.wt.) for 7 days	21.18	1	50
15	Sample Buffer blank	-		

n: Number of lD SDS-PAGE repetitions

Experimental animal: As an experimental animal, 16 male Sprague Dawley rats (*Rattus norvegicus*) from the Animal Test Center, School of Life Sciences and Technology (SITH), Bandung Institute of Technology (ITB) were employed. There were four groups of male *R. norvegicus* (K0, K1, K2 and K3), each with four *R. norvegicus*[16]. The K2 was injected with IP $HgCl_2$ (5 mg kg^{-1} b.wt.) and the rats were subsequently given gavage LE3H (0.27 mg g^{-1} b.wt.) every day for seven days. The K3 was injected with IP $HgCl_2$ (5 mg kg^{-1} b.wt.) and the rats were subsequently given gavage LE3H (0.55 mg g^{-1} b.wt.) every day for seven days. In the same way, K0, the control, received double-distilled water. The change in body weight of *R. norvegicus* was measured by weighing it before and after treatment on day 1 (D1).

On D9, test animals were sedated with sodium pentobarbital[17], then killed by cervical dislocation D9 and blood was taken from the heart to obtain serum. The material might be rendered non-infectious by diluting it in an SDS sample buffer (5.0% SDS, 10% glycerol and 60 mM Tris, pH 6.8)-1 part serum to 2 parts SDS sample buffer and 5 min in boiling water bath. Blood serum samples were stored at -40°C until needed in the following step.

Protein content analysis and electrophoresis: Serum samples were frozen on ice and centrifuged for 15 min at 2,000×g at 4°C. Supernatants were transferred to Eppendorf tubes, then separated by centrifugation at 14,000×g for 15 min at 4°C and supernatants were put in prelabeled Eppendorf tubes. The protein content of the samples was determined using the bicinchoninic acid (BCA) test[18]. The samples were dissolved until the protein concentration was 5 mg mL^{-1} in a sample buffer that had 5.0% sodium dodecyl sulfate (SDS), 10% glycerol, 50 mM dithiothreitol and 63 mM Tris, pH 6.8. Within 10 min of adding the buffer, the samples were heated in a digital dry bath at 95°C.

One-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (1D SDS-PAGE, Table 1) was performed with a 10% acrylamide slab gel (125 mm length×150 mm width×0.75 mm thickness) overlaid with a 25 mm stacking gel using the Laemmli method[19] as described second dimension by Burgess-Cassler. After 3.5 hrs of electrophoresis at 15 mAmp/gel, the bromophenol blue front had moved to the end of the slab gel. The gel was coloured with Coomassie blue before being suspended in 10% acetic acid and dried between cellophane sheets[20].

Myosin (220,000), phosphorylase a (94,000), catalase (60,000), actin (43,000), carbonic anhydrase (29,000) and lysozyme (14,000) were put to a well as molecular weight standards. Using a calibrated GE Healthcare ImageScanner III ImageScanner III (LabScan 6.0 User Manual 28-9173-83 Edition AB, the stained gel was digitized over the necessary optical density range. Phoretix 1D software (version 11.2) and a Windows 10 compatible computer were used to calculate stain density in each protein bands as a percentage of total stain per lane.

Statistical analyses: The data from this investigation was generalized using multiple comparisons (Analysis variance, the one-way classification) and the least significant difference (LSD, Waller-Duncan's Test) was computed at a significance level of 95%[21].

Ethical statement: The five freedoms (F) are defined as (a) Freedom from hunger and thirst, (b) Freedom from discomfort, (c) Freedom from pain, injury and disease,

(d) Freedom from fear and chronic stress and (e) Freedom to exhibit normative behavioral patterns[22,23]. This investigation emphasizes the ethics of animal use and human treatment aspects. The National Institutes of Health's Guide for the Care and Use of Laboratory Animals was followed in this investigation. The protocol was authorized (No.21/UN.30.14.9/LT/2018, March 31, 2018) by the Bengkulu University Committee on the Ethics of Animal Experiments.

RESULTS

The treatment of $HgCl_2$ (5 mg kg^{-1} b.wt., K1) significantly reduced *R. norvegicus* body weight compared to the control (K0). Meanwhile, following 24 hrs of treatment with $HgCl_2$ (5 mg kg^{-1} b.wt.), followed by LE3H (0.27 mg g^{-1} b.wt., K2 and 0.55 mg g^{-1} b.wt., K3) every day for seven days, body weight recovered closer to K0 conditions (Table 2).

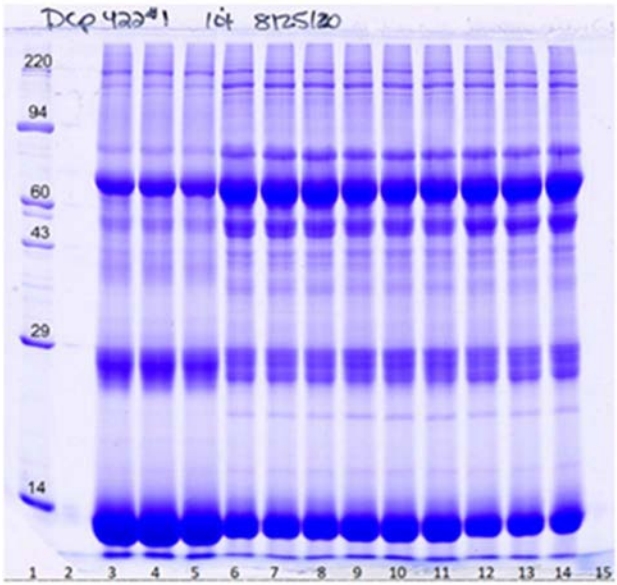

Fig. 1: Original electropherogram of One-Dimensional Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (1D SDS-PAGE)
Separated *R. norvegicus* blood serum samples K0, K1, K2 and K3 and Lane 1, 2, 3,……15

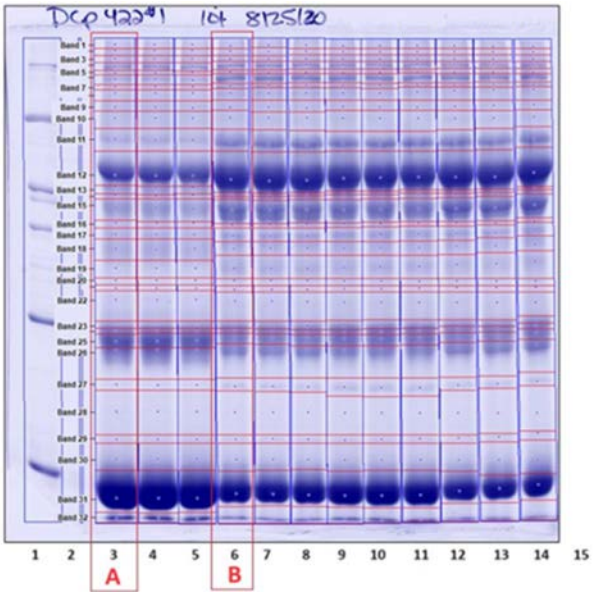

Fig. 2: Electropherogram with a densitometer image of One-Dimensional Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (ID SDS-PAGE)
Separated *R. norvegicus* blood serum samples K0, K1, K2 and K3. Lane 1, 2, 3,……15 (Table 1). Band 1, 2, 3……32 (Table 3). A and B are examples of two areas (Fig. 3)

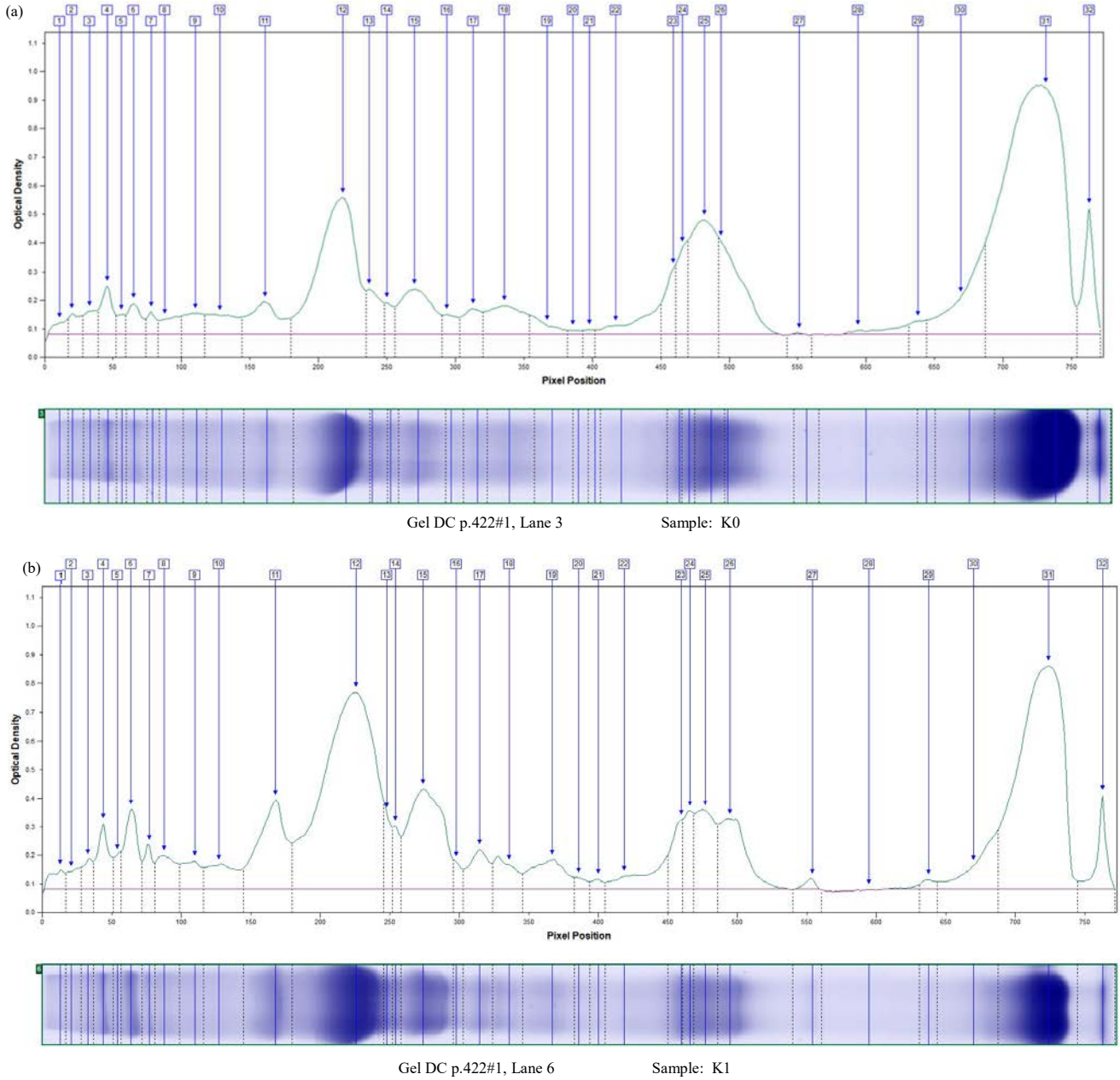

Fig. 3: Two examples of protein band thickness determination with the densitometer

R. norvegicus blood serum samples for K0 (Lane 3, A) and K1 (Lane 6: B) from the 1D SDS-PAGE protein electropherogram see Fig. 2

Table 2: Difference in body weight of *R. norvegicus* was determined by weighing it before day 1 (DI) and day 9 (D9) after treatment

Experimental animal group	N	Body weight on day 1 (D1) (X±SD) g	Body weight on day 9 (D9) (X±SD) g	Weight difference (D9-D1) (X±SD) g
K0: Control, double-distilled (DD) water, after 24 hrs given DD water for 7 days	4	286.50±1.29	300.00±0.82^{a}	13.50±1.00^{p}
K1: 5 $HgCl_2$ (5 mg kg^{-1} b.wt.) IP, after 24 hrs given DD water for 7 days	4	286.00±4.97	257.00 ± 17.93^{b}	-29.00±17.98^{q}
K2: $HgCl_2$ (5 mg kg^{-1} b.wt.) IP, after 24 hrs given LE3H (0.27 mg g^{-1} b.wt.) for 7 days	4	287.75±1.71	279.00±5.715^{b}	-8.75±5.12^{q}
K3: $HgCl_2$ (5 mg kg^{-1} b.wt.) IP, after 24 hrs given LE3H (0.55 mg g^{-1} b.wt.) for 7 days	4	286.25±4.35	275.50±11.12^{b}	-10.75±6.80^{q}

Different superscript letters in one column show statistically significant differences between K0, K1, K2 and K3 at a significance level of 95%[21], N: Number of rat repetitions, X: Mean value, SD: Standard deviation and IP: Intraperitoneal

The 1D SDS-PAGE separation findings of four serum protein samples (K0, K1, K2 and K3) are exhibited on a one-dimensional protein profile (Fig. 1). The one-dimensional protein profile provided protein band numbering for each significant band in the samples. Four instances of protein band thickness determination with a densitometer of *R. norvegicus* blood serum samples for K0 (Lane 3), K1 (Lane 6), K2 (Lane 9) and K3 (12) from the 1D SDS-PAGE protein electropherogram (Fig. 2). Two examples of protein band thickness determination using the densitometer of *R. norvegicus* blood serum samples for K0 (Lane 3) and K1 (Lane 6) from the 1D SDS-PAGE protein electropherogram (Fig. 3).

Table 3 listed the average integrated volumes for each band in the samples. The total band volume for K0 is 7827.0, followed by 7202.2 for K1, 7547.1 for K2 and 6867.2 for K3. The entire band volume is divided to determine the percentage of each band out of the 32 formed bands. It displays the average percentage of total protein compared to total stain density per sample (Table 4).

The protein electropherogram from 1D SDS-PAGE displays 32 protein bands. Treatment with $HgCl_2$ (5 mg kg^{-1} b.wt., K0 = K1, 3.125%) did not affect one band (band 2). Seventeen protein bands (bands 1, 4, 5, 6, 7, 9, 10, 11, 12, 14, 15, 17, 19, 20, 21, 23 and 27) increased in intensity after treatment with $HgCl_2$ (5 mg kg^{-1} b.wt., K0>K1, 43.750%),

Table 3: Average integrated band volume for each of the major bands was shown in Fig. 2 for gel DC p.422#1

				Group of experimental animals							
				K0		K1		K2		K3	
Band	Molecular weight (kDa)	N	n	Average band volume	±SD	Average band volume	±SD	Average band volume	±SD	Average band volume	±SD
1	264,770	4	3	33.2	1.3	46.7	2.8	48.2	0.6	43.0	0.9
2	252,010	4	3	44.1	4.0	40.2	3.5	37.6	1.3	34.1	0.7
3	235,170	4	3	56.8	2.9	48.5	2.4	53.5	0.8	47.0	1.2
4	219,530	4	3	107.1	2.3	119.0	4.8	108.1	1.0	96.6	1.1
5	203,030	4	3	30.9	3.7	42.5	3.0	41.9	3.6	49.9	1.8
6	190,280	4	3	84.0	3.5	169.1	6.2	154.8	1.5	141.2	2.6
7	172,950	4	3	38.8	1.3	60.3	3.6	56.4	3.4	53.8	2.2
8	157,800	4	3	67.0	3.1	108.9	0.9	102.9	6.0	103.1	6.7
9	124,820	4	3	82.8	3.8	89.4	1.0	76.6	4.2	74.1	0.9
10	98,570	4	3	120.2	5.2	119.3	9.1	123.7	7.0	104.1	1.5
11	83,260	4	3	190.9	9.6	395.1	2.4	349.8	5.6	371.4	4.2
12	66,660	4	3	1012.3	39.4	1696.3	10.3	1547.0	4.5	1650.8	50.4
13	60,300	4	3	122.6	9.9	83.0	7.2	69.9	5.2	72.4	2.5
14	58,120	4	3	56.4	1.3	81.6	7.4	86.0	7.7	76.5	7.7
15	52,210	4	3	298.1	16.9	586.2	5.6	534.8	2.9	642.9	26.2
16	45,720	4	3	51.9	5.0	37.8	3.0	43.5	0.5	39.5	3.1
17	42,270	4	3	90.0	4.9	125.7	1.7	152.4	1.2	132.2	6.2
18	40,190	4	3	204.3	11.8	109.8	0.8	121.4	2.2	109.5	4.4
19	37,290	4	3	72.8	5.5	163.8	1.0	170.9	1.8	152.2	8.8
20	35,130	4	3	10.9	1.1	22.8	0.2	28.0	0.5	20.2	1.5
21	33,990	4	3	10.0	0.7	19.2	0.1	25.2	0.3	18.7	2.0
22	31,950	4	3	146.2	3.3	125.2	6.3	143.4	5.2	131.0	7.1
23	28,470	4	3	127.2	2.2	126.8	6.9	163.4	7.5	114.7	4.7
24	28,060	4	3	157.3	16.1	120.9	0.8	155.0	9.3	141.3	9.1
25	27,310	4	3	564.9	23.9	261.1	7.1	317.9	1.1	236.5	12.1
26	26,290	4	3	459.1	39.8	322.7	7.2	367.3	3.0	348.6	10.6
27	22,520	4	3	3.2	1.1	18.5	0.9	45.9	0.5	23.4	2.4
28	19,830	4	3	53.5	5.9	5.8	0.4	24.6	2.1	8.1	5.2
29	17,220	4	3	40.3	2.0	20.9	0.1	30.9	0.4	21.8	2.7
30	15,090	4	3	382.5	6.7	193.6	15.2	226.8	5.5	314.7	105.0
31	11,660	4	3	2859.1	155.5	1696.1	33.5	1945.0	32.1	1364.6	151.3
32	9,150	4	3	248.6	9.8	145.4	5.6	194.3	10.4	129.3	12.7
Total				7827.0		7202.2		7547.1		6867.2	

N: Number of rat repetitions, n: Number of ID SDS-PAGE repetitions, Values are band volume in individual protein bands as an average band volume of total stain per lane±Standard Deviations, Each value is the average of three lanes and Each molecular weight is an average of twelve lanes

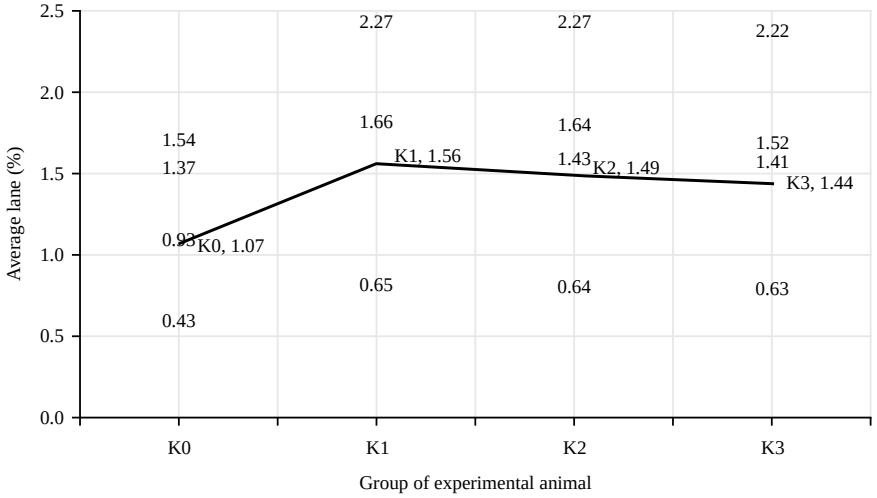

Fig. 4: Average expression of the four increased protein bands (K1) was greater than the control (K0)

Meanwhile, treatment with $HgCl_2$ (5 mg kg^{-1} b.wt.) was followed by LE3H (0.27 mg g^{-1} b.wt.) (K2) and LE3H (0.55 mg g^{-1} b.wt.) for 7 days and the expression of protein bands tended to decrease towards K0 condition

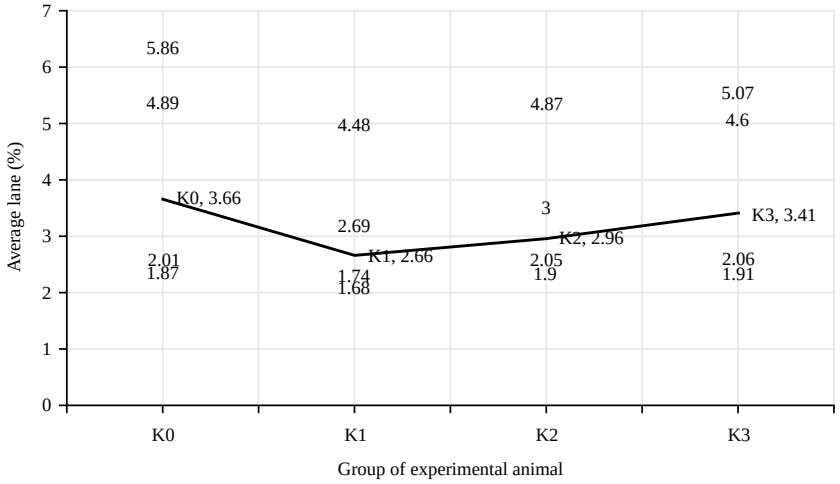

Fig. 5: Average expression of the four decreased protein bands (K1) was smaller than the control (K0)

Meanwhile, treatment with $HgCl_2$ (5 mg kg^{-1} b.wt.) was followed by LE3H (0.27 mg g^{-1} b.wt.), (K2) and LE3H (0.55 mg g^{-1} b.wt.) and (K3) for 7 days the expression of protein bands tended to increase closer to the K0 condition

whereas fourteen protein bands (bands 3, 8, 13, 16, 18, 22, 24, 25, 26, 28, 29, 30, 31 and 32) decreased. Furthermore, the electropherogram revealed four bands with intensities of 264.77, 219.53, 98.57 and 37.29 kDa that significantly increased with $HgCl_2$ treatment and then dropped to near control with LE3H delivery (Fig. 4). The results also revealed four bands with intensities of 31.95, 28, 06, 26, 29 and 15.09 kDa that reduced dramatically after $HgCl_2$ treatment and then increased to close to the control condition after LE3H delivery (Fig. 5). The $HgCl_2$-induced LE3H alteration profile of the eight blood serum protein bands is similar to the control condition in *R. norvegicus*.

DISCUSSION

In a prior ethanolic leaf extract of *E. hemisphaerica* (LE3H) toxicity trial in mice, administration of LE3H at a dose of 0.39 mg g^{-1} b.wt., resulted in no significant difference in the number of leukocytes compared to the control[11]. Based on these findings, this study did not establish a separate LE3H group without the $HgCl_2$ therapy.

Table 4: Average percent of total protein relative to stain density for each of the major bands was shown in Fig. 2 for gel DC p.422#1

				Group of experimental animals							
				K0		K1		K2		K3	
Band	Molecular weight (kDa)	N	n	Average Lane (%)	±SD	Average Lane (%)	±SD	Average Lane (%)	±SD	Average Lane (%)	±SD
1[a]	264,770	4	3	0.43	0.01	0.65	0.04	0.64	0.01	0.63	0.01
2	252,010	4	3	0.56	0.04	0.56	0.05	0.50	0.02	0.50	0.02
3	235,170	4	3	0.73	0.01	0.67	0.03	0.71	0.01	0.68	0.03
4[a]	219,530	4	3	1.37	0.03	1.65	0.07	1.43	0.01	1.41	0.04
5	203,030	4	3	0.39	0.03	0.59	0.04	0.56	0.05	0.73	0.04
6	190,280	4	3	1.07	0.01	2.35	0.09	2.05	0.02	2.06	0.02
7	172,950	4	3	0.50	0.01	0.84	0.05	0.75	0.05	0.78	0.04
8	157,800	4	3	0.86	0.04	1.51	0.00	1.36	0.08	1.50	0.12
9	124,820	4	3	1.06	0.02	1.24	0.02	1.02	0.06	1.08	0.02
10[a]	98,570	4	3	1.54	0.01	1.66	0.12	1.64	0.09	1.52	0.01
11	83,260	4	3	2.44	0.04	5.49	0.02	4.63	0.07	5.41	0.03
12	66,660	4	3	12.94	0.08	23.55	0.10	20.50	0.09	24.04	0.44
13	60,300	4	3	1.57	0.06	1.15	0.09	0.93	0.07	1.06	0.02
14	58,120	4	3	0.72	0.05	1.13	0.10	1.14	0.10	1.11	0.10
15	52,210	4	3	3.81	0.14	8.14	0.04	7.09	0.03	9.36	0.28
16	45,720	4	3	0.66	0.05	0.52	0.04	0.58	0.01	0.57	0.05
17	42,270	4	3	1.15	0.02	1.74	0.03	2.02	0.02	1.93	0.10
18	40,190	4	3	2.61	0.06	1.52	0.01	1.61	0.03	1.60	0.09
19[a]	37,290	4	3	0.93	0.05	2.27	0.00	2.27	0.02	2.22	0.16
20	35,130	4	3	0.14	0.01	0.32	0.00	0.37	0.01	0.29	0.02
21	33,990	4	3	0.13	0.01	0.27	0.00	0.33	0.00	0.27	0.03
22[b]	31,950	4	3	1.87	0.10	1.74	0.09	1.90	0.07	1.91	0.07
23	28,470	4	3	1.63	0.07	1.76	0.10	2.16	0.10	1.67	0.09
24[b]	28,060	4	3	2.01	0.20	1.68	0.02	2.05	0.12	2.06	0.11
25	27,310	4	3	7.22	0.11	3.63	0.09	4.21	0.01	3.45	0.22
26[b]	26,290	4	3	5.86	0.28	4.48	0.10	4.87	0.03	5.07	0.08
27	22,520	4	3	0.04	0.01	0.26	0.01	0.61	0.01	0.34	0.04
28	19,830	4	3	0.69	0.10	0.08	0.01	0.33	0.03	0.12	0.08
29	17,220	4	3	0.52	0.04	0.29	0.00	0.41	0.01	0.32	0.04
30[b]	15,090	4	3	4.89	0.22	2.69	0.21	3.00	0.07	4.60	1.60
31	11,660	4	3	36.52	0.57	23.55	0.44	25.77	0.45	19.85	1.90
32	9,150	4	3	3.18	0.02	2.02	0.08	2.58	0.14	1.88	0.17
Total				100.0%		100.0%		100.0%		100.0%	

N: Number of rat repetitions, n: Number of ID SDS-PAGE repetitions, [a]Four increased expressions of protein bands (K1) were greater than the control (K0) due to treatment with $HgCl_2$ (5 mg kg^{-1} b.wt., Fig. 4), [b]Four decreased protein bands (K1) were smaller than the control (K0) due to treatment with HgCl2 (5 mg kg^{-1} b.wt., Fig. 5), values are stain density in individual protein bands as a percentage of total stain per lane±Standard Deviation. Each value is the average of three lanes, each molecular weight is an average of twelve lanes

Animals were subjected to inorganic mercury (mercury chloride, $HgCl_2$), which produced reactive oxygen species (ROS) and oxidative stress, resulting in cell death. Reduced ROS removal by scavenging mechanisms may result in oxidative damage, which is detrimental to male animal systems[24]. The current investigation found that $HgCl_2$ treatment considerably lowered rat body weight compared to the control group. Furthermore, after $HgCl_2$ treatment, followed by LE3H every day for seven days, body weight tended to rebound closer to control levels (Table 2). This conclusion was consistent with a previous study that discovered *C. album* Linn. and vitamin C to help protect male rats' reproductive organs from mercury-induced oxidative stress[25]. The oral dose of $HgCl_2$ was also observed to significantly lower body and kidney weights. Pretreatment with quercetin (QC) decreased the deleterious effects of $HgCl_2$ by returning body and kidney weights to control values[26]. As a result, LE3H and *C. album* Linn. have the same therapeutic effect, whereas QC protects male *R. norvegicus* against oxidative stress caused by Hg.

The serum contains diverse proteins, some unique to blood and others created in the blood by tissues and organs. Tissue leakage factors give information about the tissue from which they originate and hold great promise as disease biomarkers or illness-related protein alterations[27]. Because proteins are active gene products, their presence properly represents the state of the separated cells or tissues. As a result, in eukaryotic organisms, gene transcription (mRNA) outcomes are not directly translated into proteins, instead, changes in the appearance of proteins, rather than mRNA, are

more efficient in learning the sick condition or selecting toxicity. Then there is the toxicological application of protein profiling[28]. Meanwhile, the protein composition of blood cells could be connected to the $HgCl_2$ poisoning mechanism. The $HgCl_2$ administration caused the development of a novel 125 kDa protein and the overexpression of the 48 kDa protein, which could be protected by LE3H therapy in the same way as the control condition[11].

The $HgCl_2$ has a high affinity for biomolecules containing the SH group, such as sulfhydryl proteins and Glutathione (GSH), which may contribute to its toxicity[29]. As the $HgCl_2$-GSH complex, $HgCl_2$ can infiltrate the blood or lymphatic system and persist in tissues. $HgCl_2$ can induce oxidative stress[30] due to its pro-oxidant nature. It occurs when reactive oxygen species (ROS) such as superoxide anions (NO_2), hydroxyl radicals (NOH) and Hydrogen Peroxide (H_2O_2) overcome the body's protective mechanisms, causing DNA macromolecules, lipids and proteins to deteriorate[31]. The $HgCl_2$ treatment resulted in eight protein bands that differed substantially from the control (Table 4). In addition, the eight protein bands appeared to recover closer to the control condition (Fig. 4 and 5) after receiving LE3H protection. The extract LE3H contains tannins (++), saponins (+), alkaloids (+) and flavonoids (+). It is known that 0.39 mg g^{-1} b.wt., LE3H has the same effect as 0.20 mg g^{-1} b.wt., Immunos TM (ingredients: selenium 15 mcg, ascorbic acid 50 mg, echinacea 500 mg, zinc picolinate 10 mg), a nutritional supplement to stimulate the body's immune system during acute and chronic infections that have been circulating on the market. The effect of LE3H gavage treatment is comparable to that of Immunos TM in terms of (a) $HgCl_2$ chelation and (b) antioxidant activity. These actions can prevent $HgCl_2$ poisoning in the organism[11].

Serum creatinine (sCr), blood urea nitrogen (BUN) and Aspartate Aminotransferase (AST) levels were substantially higher in the $HgCl_2$-treated group than in the control group. Neutrophil Gelatinase-Associated Lipocalin (NGAL), clusterin, kidney injury molecule-1 (KIM-1), Monocyte Chemoattractant Protein-1 (MCP-1), Vascular Endothelial Growth Factor (VEGF) and tissue inhibitor of metalloproteinases 1 (TIMP-1) were significantly reduced in urine in response to $HgCl_2$ administration by QC pretreatment compared to the $HgCl_2$-treated group[26]. The QC is a natural flavonoid with medicinal and anti-mercury properties[32,33]. Based on these facts, it is known that (a) Treatment with $HgCl_2$ causes the expression of specific proteins to increase or decrease compared to controls, (b) LE3H and QC have similar properties, namely potential protective effects against mercury-induced and (c) QC is a natural flavonoid[26] and flavonoids are one of the secondary metabolites found in the crude extract of LE3H[11].

The preceding three concerns (a, b and c) are considered when interpreting the findings of this study. According to the results of this study, the LE3H alteration profile of the eight blood serum protein bands induced by $HgCl_2$ in rats closely resembles the control profile (Fig. 4 and 5). According to the results of 1D SDS-PAGE, each blood serum protein band may be composed of numerous distinct protein regions with varying isoelectric points (pI)[34-36]. Using a Two-Dimensional Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (2D SDS-PAGE) technique, the eight blood serum protein bands were separated further, allowing each protein location to be defined and subsequently identified[28,37-39]. Using proteome analysis (toxicoproteomics), it will be easier to fathom the molecular mechanism of the LE3H alteration profile of the eight blood serum protein bands due to $HgCl_2$ compared to the control condition in *R. norvegicus*[40,41]. If the crude extract contained QC[42,43] evidence of the natural component LE3H's ability to protect against mercury-induced toxicity would be more apparent. Recent research indicates that the QC level in LE3H was 7.47±0.2 μg mL^{-1} [44] QC is a bioflavonoid commonly found in edible fruits and vegetables, including onions, grapes, apples and tea[45] and LE3H[44]. Assume that QC has been identified as being present in LE3H. Subsequently, it is hypothesized that the fruit ethanolic extract of *E. hemisphaerica* (FE3H), which comprises flavonoids in greater quantities, possesses QC levels proportional to their abundance[46]. Due to its exceptional antioxidant and metal ion chelating characteristics, QC has demonstrated efficacy in combating allergies, inflammation, atherosclerosis and cancer[47].

CONCLUSION

This study is limited by the fact that each blood serum protein band resulting from a one-dimensional serum protein profile may consist of several individual protein patches with a differing pI. The eight blood serum protein bands should be further separated using two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (2D SDS-PAGE) so that each protein location can be characterized and identified.

SIGNIFICANCE STATEMENT

There is yet no clear answer for the worldwide issue of mercury's toxicity and teratogenicity and we are continuously searching for one. Ethanolic extract of *Etlingera hemisphaerica* leaves (LE3H) has been demonstrated in a number of earlier research to have the capacity to lessen mercury's teratogenicity and toxicity. With its few negative effects, ease of use and low cost, this natural substance is intended to be an alternate treatment.

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