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Research Article

Acute toxicity test nanoherbal mahkota dewa fruit (*Phaleria macrocarpa*)

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Abstract

A common plant from Papua, Indonesia, called *Phaleria macrocarpa* (mahkota dewa), has potent therapeutic components. The aim of this study was to evaluate the toxicity level of nanoherbal mahkota dewa and its effect on the changes in hematology, biochemistry, electrolytes, and histopathology of the organ. High Energy Milling (HEM) was used to produce nanoherbal mahkota dewa. LD50 was determined in three stages: dose orientation test (12 mice), preliminary test (20 mice), and LD50 determination (30 mice) for 14 days using the Thomson Weil formula. LC50 was determined using the Brine Shrimp Lethality Test method with concentrations of 1, 10, 100, 1000, and 10,000 ppm. This study revealed that the LD50 value was 1g/kg BW 0,075 and the LC50 value was 2145,0407 ppm. The nanoherbal mahkota dewa affected the histological organs, hematological, biochemical, and electrolyte parameters but did not affect the weight of the organ significantly. From this study, it can be concluded that nanoherbal mahkota dewa belongs to the category of moderate toxicity. In a proper dose, it could be processed as an herbal medicine in the future.

Keywords

Acute toxicity, mahkota dewa, LC50, LD50, nanoherbal

Introduction

Phaleria macrocarpa, from the family of Thymelaceae, commonly known as Mahkota dewa, is a medicinal plant that is indigenous to Indonesia and Malaysia. *Phaleria macrocarpa* (mahkota dewa) is one of the thousand plants in Indonesia that can be used as traditional medicine. *P. macrocarpa* flesh fruits are reported for several pharmacological activities, including anti-tumor, anti-hyperglycemia, anti-inflammation, anti-diarrheal, vasodilator, anti-oxidant, anti-viral, anti-bacterial, and anti-fungal effects. Its stem is used to treat bone cancer; egg shells of seeds are used to treat breast cancer, cervix cancer, preeclampsia, lung diseases, liver and cardiac diseases, while leaves contain constituents that treat impotence, blood diseases, allergies, diabetes mellitus, and tumors (Middleton et al. 2000; Rahmawati et al. 2006; Hending and Ermin 2010; Hendra et al. 2011; Ansari et al. 2013; Simanjuntak et al. 2019a; Rumahorbo et al. 2021a, b).

According to Altaf (2013), *Phaleria macrocarpa* fruit chemical content showed that from extracts of hexane, ethyl acetate, and methanol, the seed shell and the flesh of Mahkota dewa obtained flavonoid compounds, phenols, tannins, saponins, and sterols/terpenes. Isolation is performed to obtain a group of polyphenols with known structures, namely lignans, which are thought to be cytotoxic. In addition, other specific bioactive ingredients such as Phalerin, gallic acid, Icaricide C, magniferin, mahkoside A, dodecanoic acid, palmitic acid, des-acetylflavicordin-A, flavicordin-A, flavicordin-D, flavicordin-A

glucoside, ethyl stearate, and others obtained from *Phaleria macrocarpa* fruit can be used as the reason this plant has strong biopharmaceutical activity.

Herbs have thousands of ingredients and at the same time fight disease (Ekor 2014). Modification of *Phaleria macrocarpa* extract into a new formulation system can overcome problems that often occur in pharmacokinetic mechanisms, such as obstacles to the penetration of bioactive substances from *Phaleria macrocarpa* fruit into cells that are still in the form of crude extract, as described by Thirumurugan et al. (2014). With nano size, it is expected that the penetration kinetics of substances into the membrane can be overcome. Herbs in smaller sizes have the advantages of high loading capacity, better surface, and can be administered in high concentrations (Ajazuddin and Saraf 2010). In addition, based on our literature review, crude extracts of Mahkota dewa fruit that have been tested preclinically in previous studies, obtained from the extraction method that passed through the excessive heating stage, reached more than 600C. Those methods can damage the bioactive compounds contained in them, thereby reducing their work efficiency. With nano technology that does not use heating techniques at all, these problems can be overcome. The use of herbal medicines is also recommended because they have fewer side effects than synthetic drugs (Dewandari et al. 2013).

Although nanoherbal has opportunities in the treatment of various diseases, its safety is still questionable (Hariyadi et al. 2020). Changes in the physicochemical and structural properties of nano-sized materials engineered and decreases in size are responsible for several material interactions that could have toxicological effects (Situmorang et al. 2020). Therefore, a toxicity test was needed on nanoherbal mahkota dewa for an effective dose.

Materials and methods

Materials

Mahkota dewa was obtained from traditional markets in Medan. Mice were obtained from animal cages in the Biology Laboratory, Faculty of Mathematics and Science, Universitas Sumatera Utara, Artemia Viper Eggs (Jeannie Hoo., LTD, China). Flacon, artemia hatchery (local), micropipette (Socorex ISBA S.A), 5-watt lamps (dop), aerator (Niko Nk 1200), 0,9% NaCl from Otsuka, drop pipette, analytical balance (Mettler Toledo AB204), Vortex (Dijkstra), 1 set of surgical instruments (PT. Glorya Medica Abadi), formalin and hematoxylin-eosin stains (PT. Arjuna Utama Kimia), paraffin (PT. Kirana Mitra Abadi), xylene (PT. Anugrah Putra Kencana) and Mayer's albumin (PT. Indo Achitama Chemical Industry).

NanoHerbal mahkota dewa

Nanoherbal mahkota dewa has been made using High Energy Milling (HCl 2M, Tokyo, Japan) at NanoTech Indonesia. The procedure for changing the size of herbal mahkota dewa to nano-size was to put balls as a crushing medium into a larger diameter jar, then small balls and samples. HEM was turned on for 2 hours. The size of this nanoherbal was 246,5 nm ± 55,5. The methods and results of this study are being registered under a simple patent.

Reagents and chemical

DMSO (Sigma Aldrich), water pro-injection (Sigma Aldrich), Hematoxylin and Eosin (Sigma Aldrich), AST kit (Roche), ALT kit (Roche), ALP kit (Roche), Albumin kit (Roche), Total Protein kit (Roche), Bilirubin direct kit (Roche), Urea kit (Roche), Uric acid kit (Roche), and Creatinine kit (Roche).

Lethality test 50 (LC50)

LC50 used the Brine Shrimp Lethality Test (BSLT). Artemia larvae into 5 mL vial bottles containing 1, 10, 100, 1000, and 10,000 ppm nanoherbal mahkota dewa, then calculated using the LC50 Calculator, AAT Bioquest.

Lethal dose 50 (LD50)

For the LD50 determination we used mice (*Mus musculus*) at around 18–20 g BW. Experimental animals were from the biology animal cages at the Universitas Sumatera Utara. For LD50, 3 stages were used; the dose orientation test (12 Mus musculus) for 24 hours; the preliminary test (20 *Mus musculus*) for 24 hours, and the determination of LD50 (25 *Mus musculus*). 20 mice were used for treatment and 5 mice as controls during 14 days (BPOM RI 2014). In the final stage, the death motility and LD50 values were calculated by the Thomson Weil formula. Histological descriptions of the liver, kidneys, lungs, heart, and brain were observed by the process of Hematoxylin-Eosin staining. The Thomson-Weil formula used is:

$$\text{Log m} = \text{Log D} + \text{d (f+1)} \quad (1)$$

$$\text{LD50 range} = \text{antilog (log m} \pm 2 \times \text{log m)} \quad (2)$$

Description: m is the LD50 value, D is the smallest dose used, d is the log of the multiple of the dose, and f is the factor value from the Thomson Weil table.

Statistics

The Data were calculated by statistical software version 23. The test carried out was a two-way ANOVA ($\alpha = 0,05$) with a 5% significance level.

Hematological analysis

The hematological analysis was conducted at Universitas Sumatera Utara Hospital. The parameters examined

included red blood cells (RBC), white blood cells (WBC), hemoglobin, hematocrit, Mean Corpuscular Volume (MCV), mean cell hemoglobin concentration (MCHC), and mean cell hemoglobin (MCH) using a hematology analyzer (Roche Diagnostic, Switzerland).

Biochemical analysis

The parameters examined in this study were total protein, direct bilirubin, Alanine aminotransaminase (ALT), Aspartate aminotransaminase (AST), Alkaline Phosphatase (ALP), Urea, Creatinine, and Uric Acid using Cobas 6000 (Roche Diagnostic, Switzerland). The measurement of sodium, chloride, and potassium levels was done using Cobas b 221 (Roche Diagnostic, Switzerland) (Fernando et al. 2010; Aasne et al. 2018).

Histopathology

The samples of the liver, lungs, kidney, heart, and brain were collected and then immersed in liquid paraffin at 60 to 70 degrees Celsius for two hours. The paraffin blocks were cut using a microtome with a thickness of 5 to 7 m and connected to slides after being molded and given time to freeze. The organ incision that had been attached to the slide was immediately placed on a heating surface between 56 and 58 °C for about 10 seconds to stretch it and make it adhere to the slide. Adjustments were made to prevent wrinkled or folded organs. Additionally, hematoxylin-eosin staining was done. The preparations were first immersed for 12 minutes in a xylene solution to begin the deparaffination process. After 5 minutes of immersion in 70%, 80%, 90%, and 100% ethanol, the preparations were dehydrated by being washed under running water. After 5 minutes in the hematoxylin solution, the preparations were rinsed under running water, stained with eosin, and then submerged in 70%, 80%, 90%, and 100% ethanol for 10 minutes. In the last step, the preparations were submerged for 12 minutes in xylene and examined with a 100× magnification microscope (Thermo, German).

Data analysis

Data analysis in this study was conducted using SPSS (a statistical program for social sciences) version 23 using the one-way ANOVA (Analysis of Variance) test. If the p-value was less than 0,05, there was a significant difference between groups, and if the p-value was higher than 0,05, there was no difference between groups.

Ethical clearance

This research has been evaluated and received a recommendation for approval from the Animal Research Ethics Committee, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, No. 231/KEPH-FMIPA/2022, July 12, 2022.

Results and discussion

LC50

The lowest larval mortality rate of 3% in LC50 was observed under treatment with 1 ppm nanoherbal mahkota dewa, whereas the highest larval mortality rate of 90% was observed under treatment with 10000 ppm nanoherbal Mahkota dewa (Table 1).

The relationship between the % mortality of *A. franciscana* larvae with nanoherbal in various concentrations was calculated using the LC50 Calculator, AAT Bioquest, and the resulting LC50 value of 2145, 0407 ppm (Fig. 1). Nanoherbal mahkota dewa was moderately toxic (LC50 > 1000 mg/L).

Table 1. LC50 test of nanoherbal Mahkota dewa.

Concentration (ppm)	Log10	% Mortality	Probit
10000	4	90	6,28
1000	3	13	3,87
100	2	13	3,87
10	1	6	3,45
1	0	3	3,12

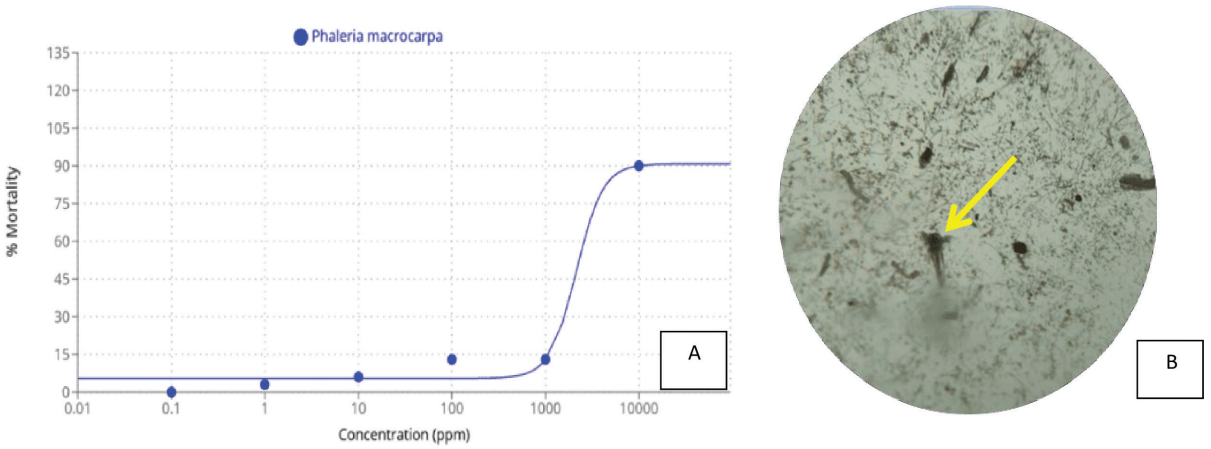

Figure 1. A. Profile of the relationship between % mortality of *A. franciscana* larvae with nano herbal extracts in various concentrations. The graphs were produced using the LC50 Calculator, AAT Bioquest (https7/www.aatbio.com/tools/lc50-caleulator); **B.** The appearance of dead *A. franciscana* larvae from the results of the BSLT test.

LD50

The dose orientation stage (Table 2) used a single dosage test orally by selecting a random dose with 4 dose ranks. The death occurred in a group in observations 24 hours later, carried out to the second stage. The second/preliminary test (Table 2) used 20 mice (5 mice/group). If the death happened at the lowest dose, such as 800 mg/kg BW for 24 hours, then the dose for the third stage (LD50 determination) used a pre-death dose of 400 mg /kg BW. Determination of dose in step 3 (Table 2) used multiples of R dose (BPOM RI, 2014). Based on the calculation of LD50 using the Thomson Weil formula, the LD50 value of the nanoherbal mahkota dewa was 1g/kg BW ± 0,075.

Nanoherbal mahkota dewa belongs to the moderate toxic category. It was not difficult to penetrate the body's lipid membrane (Ajazuddin and Saraf 2010; Thirumuruganet al. 2014). Because of its small size, nano-sized Mahkota dewa would bypass several barriers such as gastric acid pH, liver metabolism, and increasing drug circulation into the blood (Ekor 2014; Chayono et al. 2019).

Organs weight

The administration of nanoherbal mahkota dewa did not significantly affect the weight of the liver, lungs, and kidneys ($p > 0,05$) (Table 3). The heaviest liver was in the P3 group (1,68±0.08 g), the heaviest lungs were in P2 (0,59±0.02 g), the heaviest hearts were in P3 and P4 (0,23±0,02 g), the heaviest brain was in P1 (0,47±0,09 g), and the heaviest kidneys were in P2 and P4 (0,39±0,04 g).

The liver was the largest organ in the body, and it was very sensitive when toxins, chemicals, or harmful substances entered the body. The liver acts as a detoxifying organ in the body. When the nanoherbal entered the mice's bodies, the liver responded quickly. The kidneys had a statistical analysis result that was almost similar to the data on the liver because the dissolved compounds that entered the mice's kidneys derived from this nanoherbal mahkota dewa made the kidney organs work harder. The cardiac was an organ that was resistant to stressful conditions. The heart will undergo histological changes when the exposure given was exposure to a relatively severe level of stress.

Table 2. LD50 Dose.

Group	Test Stages								
	Orientation Test			Preliminary Test			LD50 Determination Test		
	mice	Dose (mg/Kg BW)	Mortality	mice	Dose (mg/Kg BW)	Mortality	mice	Dose (mg/Kg BW)	Mortality
Control	–	–	–	–	–	–	5	CMC-Na	0
P1	3	32	0	5	200	0	5	400	0
P2	3	64	0	5	400	0	5	635,2	1
P3	3	108	0	5	800	1	5	1.008,6	2
P4	3	216	1	5	1600	3	5	1601,8	3

Table 3. The weight of organs.

Treatments	Organs				
	Livers	Lungs	Heart	Brain	Kidney
Control	1,48±0,01	0,29±0,02	0,16±0,02	0,43±0,08	0,28±0,02
P1	1,30±0,02	0,59±0,02	0,14±0,07	0,47±0,09	0,35±0,02
P2	1,65±0,03	0,27±0,01	0,20±0,01	0,41±0,05	0,39±0,03
P3	1,68±0,08	0,37±0,07	0,23±0,00	0,46±0,02	0,37±0,03
P4	1,65±0,06	0,30±0,07	0,23±0,02	0,45±0,02	0,39±0,04

Data are presented Mean±SEM.

Hematological analysis

The effect of nanoherbal mahkota dewa on hematological parameters including WBC (white blood cells), RBC (red blood cells), hemoglobin, hematocrit, MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), and MCHC (mean corpuscular hemoglobin concentration) can be seen in Table 4.

From the results that were presented in Table 4, there was a significant difference between the control group and some of the parameters in each group ($p < 0,05$). The value of RBC was a significant difference in P1 and P3, but only the HCT parameter in P4 was a significant difference with control. Meanwhile, in MCV parameters, each group differed significantly from the control. The groups that often had significant differences with the control were P3 and P4. With that dosage, the parameters in hematology can be increased in MCV parameters but decreased in MCH and MCHC parameters.

Hematology parameters such as white blood cells, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin were all evaluated. These measurements are required to determine whether nanoherbal mahkota dewa has an effect on blood parameters. Because hematological parameters are susceptible, if a herb causes toxicity, the hematologic value will change (Olayode et al. 2019). For example, inflammation caused by drug administration causes WBC and RBC parameters to increase; a decrease in hemoglobin level indicates anemia; and a decrease in MCV value indicates thalassemia (Barron et al. 2000; Richard et al. 2016; Orinya et al. 2017). It was discovered in this study that nanoherbal mahkota dewa had an effect on hematology parameters (Table 4). This is in line with the nanoherbal mahkota dewa toxicity evaluation study, which was categorized as moderate liver.

Table 4. Hematological analysis parameters.

Parameters	Units	Groups (Mean±SD)				
		Control	P1	P2	P3	P4
WBC	10^3/uL	7,2± 0,3	8,32±1,07	6,85±0,67	7,26±2,51	8,33±0,58
RBC	10^6/uL	9,3±0,5	12,00±1,29*	7,97±0,94	13,05±1,36*	11,33±0,58
HGB	g/dL	17,4±0,7	15,28±0,69	19,76±0,77	18,32±1,27	17,67±0,58
HCT	%	59,1±5,3	59,95±1,12	57,02±0,63	58,39±0,51	55,00±1,00*
MCV	fL	62,0±3,3	76,37±4,21*	67,14±1,69*	56,68±1,90*	73,33±0,58*
MCH	Pg	27,3±6,3	25,56±1,16	28,02±1,77	22,72±0,87*	22,33±1,53*
MCHC	g/dL	27,4±2,9	26,80±1,84	27,78±0,38	24,90±1,38*	24,63±3,07*

Data are presented Mean±SEM *($p < 0,05$) significant different from normal group.

Serum biochemical parameters

The effect of nanoherbal mahkota dewa on kidney biochemical parameters, including urea, creatinine, and uric acid, showed a significant difference ($p < 0,05$) between the groups.

Based on Table 5, the highest urea value was in the P1 group (22,60±2,48 mg/dL) and it was significantly different from control, while the lowest urea value was in the control group (19,17±1,00 mg/dL). The highest creatinine value was in the P2 group (2,23±0,59 mg/dL), while the lowest creatinine value was in the P3 group (0,780,32 mg/dL). The highest uric acid value was in the P2 group (3,00±0,22 mg/dL), while the lowest uric acid value was in the P1 group (1,31±0,28 mg/dL).

Acute kidney injury will occur if a toxic drug is used that raises the levels of kidney biochemical parameters such as urea, creatinine, and uric acid (Griffin et al. 2019). The results of this study revealed that the levels of urea, creatinine, and uric acid increased with each dose level. It is also consistent with the findings of kidney histology, which revealed changes in kidney morphology (Fig. 6). Several studies have shown that *Phaleria macrocarpa* has nephroprotective properties. *Phaleria macrocarpa* Reduced Nephropathy in Alloxan-Induced Diabetic Rats by Increasing Renal Antioxidant Enzyme Activity (Triastuti et al. 2009).

Liver biochemical parameters

A biomarker examination was needed to ascertain whether the nanoherbal mahkota dewa affected the liver. The biomarkers were total protein, albumin, direct bilirubin, alanine aminotransferase (AST), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were all measured in blood drawn from all groups on day 14. Complete data can be seen in Table 6.

Liver biochemistry was assessed from the parameters such as total protein, albumin, direct bilirubin, AST, ALT, and ALP (Table 8). The highest total protein value was in the P4 group (7,12±0,69 g/dL), while the lowest protein value was in the P1 group (4,91±0,44 g/dL). The highest albumin value was in the P1 group (5,62±0,52 g%), while the lowest albumin value was in the P2 group (2,96±0,42 g%). The highest direct bilirubin value was in the P2 group (0,38±0,52 mg/dL), while the lowest direct bilirubin values were both in the control and P1 groups (0,01±0,01 mg/dL); there was a statistically significant difference between the control and P3 groups ($p < 0,05$). The highest AST value was in the P4 group (117,00±3,06 U/L), while the lowest bilirubin AST value was in the P2 group (70,00±3,06 U/L). The highest ALT value was in the P4 group (58,00±5,13 U/L), while the lowest ALT value was in

Table 5. Kidney biochemical parameters.

Parameters	Units	Groups (Mean±SD)				
		Control	P1	P2	P3	P4
Urea	mg/dL	19,17±1,00	22,60±2,48*	21,98±1,57	21,28±1,11	21,69±1,62
Creatinine	mg/dL	0,93±0,73	1,09±0,10	2,23±0,59*	0,78±0,32	0,98±0,13
Uric Acid	mg/dL	1,48±0,54	1,31±0,28	3,00±0,22*	2,01±0,90	1,61±0,45

Data are presented Mean±SEM *($p < 0,05$) significant different from normal group.

Table 6. Liver biochemical parameters.

Parameters	Units	Groups (Mean±SD)				
		Control	P1	P2	P3	P4
Total protein	g/dL	5,3±1,0	4,9±0,4	6,6±0,7	5,9±0,3	7,1±0,6*
Albumin	g %	3,0±0,2	5,6±0,5*	2,9±0,4	3,6±0,5	2,9±0,4
Billirubin direct	mg/dL	0,01±0,01	0,0±0,0	0,09±0,4	0,3±0,5*	0,01±0,0
AST	U/L	111,6±5,0	105,0±9,5	70,0±3,0*	92,6±11,3*	117,0±3,0
ALT	U/L	51,3±3,5	51,3±4,9	21,0±2,5*	37,3±6,6*	58,0±5,1
ALP	U/L	154,0±7,2	204,0±7,9*	120,0±3,2*	210,3±14,1*	218,0±13,6*

Data are presented Mean±SEM *($p < 0,05$) significant different from normal group.

the P2 group (21,00±2,52 U/L). There was a statistically significant decrease in ALT and AST levels in the P2 and P3 groups compared to the control group, respectively ($p < 0,05$). The highest ALP value was in the P4 group (218,00±13,65 U/L), while the lowest was in the P2 group (120,00±3,21 U/L). The ALP value in all treatment groups had a statistically significant increase compared to the control group. In addition, the ALP value in group P2 even decreased ($p < 0,05$).

Some biochemical parameters, such as AST, ALT, Total Protein, Albumin, Bilirubin Direct, and ALP, can be used to assess liver toxicity (Abou, 2016). These parameters typically rise when there is liver damage. It is because the liver is a vital organ in the metabolsm of compounds. When injuries occur, AST and ALT levels rise. Additionally, ALT is more sensitive than AST to liver damage (Jeschke 2009). There was an increase in the parameters in this study (Table 6). This is also strongly related to the histological findings of the liver, which revealed damage (Fig. 2). Previous research has shown that Mahkota dewa has a hepatoprotective effect by significantly protecting against Carbon Tetrachloride-Induced Liver Fibrosis in Rats: Role of TNF-and TGF-1 (Sundari et al. 2018).

Electrolyte parameters

On day 14, all groups in this study had their Na+, K+, and Cl-levels measured (Table 7). The highest sodium value was in the P4 group (117,71±15,58 mmol), while the lowest value was in the P1 group (104,35±7,50 mmol). The highest potassium value was in the P4 group (6,81±0,53 mmol), while the lowest potassium value was in the P1 group (3,08±0,17 mmol). There was a statistically significant difference ($p < 0,05$) between the P4 and MC groups. The highest chloride value was in the P3 group (94,47±5,95 mmol), while the lowest chloride value was in the control group (68,96±3,72 mmol). All treatment groups in chloride parameters were significantly different from the control group.

Histology of the liver after giving nanoherbal mahkota dewa

There was a significant difference ($p < 0,05$) in normal hepatocyte cells, parenchymatic degeneration, hydropic degeneration, and necrosis (Table 8). Normal hepatocyte cell values in the control group and P2 group have almost the same average. The highest parenchymal degeneration was

Table 7. Electrolyte parameters.

Parameters	Units	Groups (Mean±SD)				
		Control	P1	P2	P3	P4
Sodium	mmol	108,6±7,4	104,3±7,0	113,1±11,3	110,7±16,8	117,7±15,5*
Potassium	mmol	2,6±0,4	3,0±0,1	6,0±0,8*	5,2±0,5*	6,8±0,5*
Chloride	mmol	68,9±3,7	86,0±6,3*	81,1±1,8*	94,4±5,9*	84,55±5,5*

Data are presented Mean±SEM *($p < 0,05$) significant different from normal group.

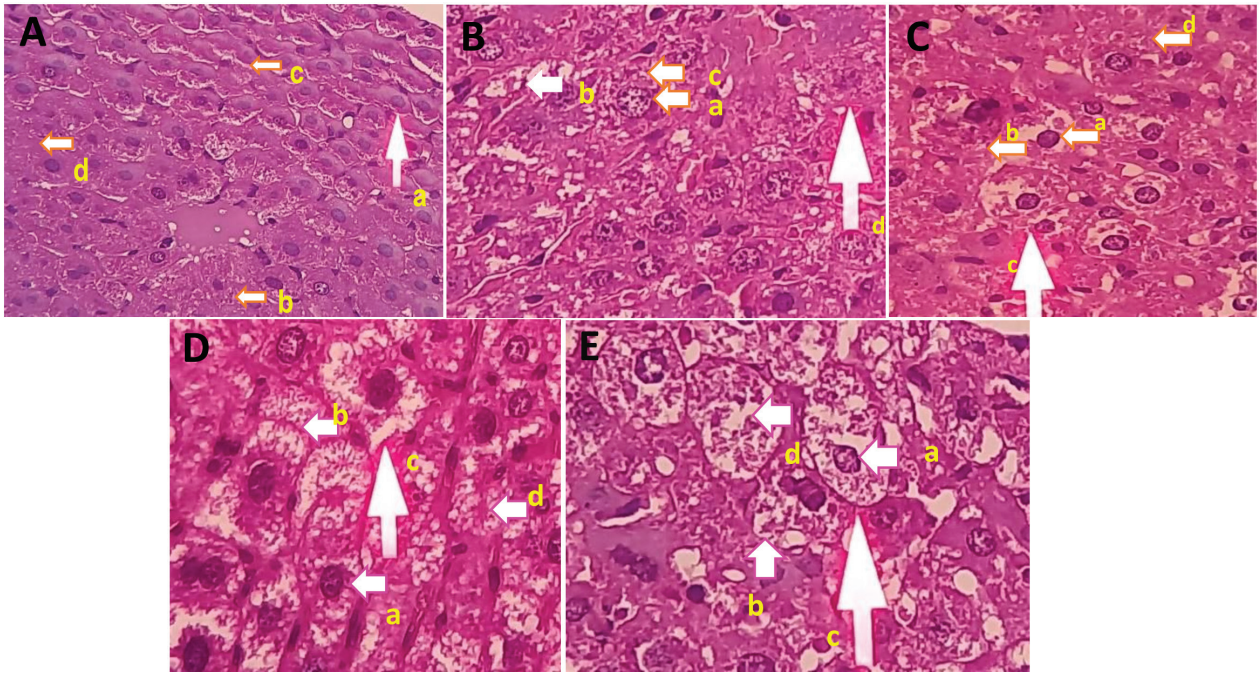

Figure 2. Histology of liver damages. **A.** Control; **B.** Pl(400 mg/KgBW); **C.** P2 (635.2 mg/KgBW); **D.** P3 (1.008.6 mg/KgBW); **E.** P4(1601.8 mg/KgBW); **a.** Normal hepatocytes (400X); **b.** Parenchymal Degeneration; **c.** Hydropic Degeneration, d. Necrosis cell (40×).

Table 8. Average of damages degree in liver.

Treatments	Normal Hepatocytes (400X)	Parenchymal Degeneration (%)	Hydropic Degeneration (%)	Necrosis (%)
Control	233.67±2.44	36.67±17.32	25±15.21	8.1±3.82
P1	216.44±1.07	34.44±20.68	22.2±21.52	9.4±5.27
P2	230.67±3.80	24.44±16.09*	8.3±4.33*	10.7±8.97
P3	75.11±3.62*	50.00±8.66*	36.6±5*	11.1±2.20*
P4	172.78±2.29*	32.22±23.19	20±18.54	10.6±5.27

Data are presented Mean±SEM *($p < 0,05$) significant different from normal group.

found in the control group and followed by the P3 (dose 1.008,6 mg/Kg BW), the same in hydropic degeneration cases also found in the P3. Giving nanoherbal mahkota dewa to mice at different doses causes damage to hepatocyte cells. The average number of normal hepatocytes in control and P2 was almost the same. The lowest value on the normal hepatocyte liver was in P3, and the highest parenchymal degeneration, hydropic degeneration, and necrosis levels were also found in P3. It was indicated that the level of damage happened in the 1.008,6 mg/Kg BW range of dose.

Based on the observations of the liver (Fig. 2), the control group is the group that contains the most normal hepatocyte cells and blood vessels were still visible. In the P1 group, liver damage with parenchymous degeneration was seen more than hydropic degeneration and necrosis. P2 also had significant, but not as severe, parenchymal degeneration as P1. P3 had the most hydropic degeneration compared to the other doses, and the shape of the hepatocyte cells in the liver is becoming more irregular, as is the density between cells. This dose showed the onset of an increase in severe damage to liver cells. The necrosis was clear and numerous. P5 was the highest dose in this study, and it appears that the damage was getting worse. The relationship between cells had a short distance and was very toxic.

Many cell nuclei emerge from hepatocyte cells in the liver. The dead cells of the nuclei appear smaller, chromatin, and reticular fibers multiply. Increased hepatocyte damage by chemical compounds in mahkota dewa fruit A study also proved that hepatic protectors are widely known to protect the liver from damage (Kaplowits 2002). However, herbal liver protection was preferred by the public. Terpenoid compounds can be used as insecticides and are highly toxic to animals (Anderson et al. 1993). The damage would cause an immune response and directly affect cell biochemistry. The occurrence of liver cell necrosis could be identified by changes in the cytoplasm and nuclear nucleus (Plopper and Dungwort 1987).

Histology of the lungs after giving nanoherbal mahkota dewa

Based on Table 9, the degree of alveolar inflammation, lung parenchyma damage, and alveolar lumen narrowing in each group did not show any difference. Between control and P1, P2, and P3, all experienced inflammation of the alveoli, parenchymal damage, and narrowing of the alveoli in the 3rd degree, which was more than 60%. While in the P4 group, the group with the treatment at a dose of 1601.8 mg/Kg BW had a difference of 0,67% lower than the other groups in the three parameters (Fig. 3). Based on Fig. 3, the symptoms of inflammation were characterized by cellular infiltrates, congestion, and/or edema but no exfoliation or cell debris. If the foreign body persists, the inflammatory response will gradually evolve into chronic inflammation dominated by macrophages with varying numbers of other cell types. Fibrosis may also be present.

Inflammation degree: 0 = normal, 1 = light, 2 = medium, 3 = weight. Parenchymal Damage: 0 = normal, 1 = 0–30% damage, 2 = 31–60% damaged, 3 = > 61% damaged. Alveolar shape: 0 = normal, 1 = 0–30% have narrowing, 2 = 31–60% have narrowing, 3 = > 61% have narrowing.

The alveolar pattern of inflammation was related in part to the fact that the terminal bronchioles, alveolar ducts, and adjacent alveoli were the sites of maximum deposition of inhaled small particles (Schnellmann and Goldstein 2001). In this case, the control group had the same damage in the microanatomy lung as P1, P2, and a little different with P4. The relationship between alveoli in the pulmonary organ control group showed that the extracellular matrix consists of damaged collagen and elastin fibers. The alveolar lumen looked different and damage to the structure of the lung microanatomy was caused by damage to epithelial and endothelial cells in the alveoli.

Physiologically, free radicals or incoming toxic substances are detoxified by macrophages, neutrophils, and eosinophils. However, an excessive increase in the airway would trigger the movement of macrophages, neutrophils, and eosinophils, which could cause an inflammatory reaction that could lead to more cell damage and death (Robbinson and Kumar 1995); Gutowski and Kowalczyk 2013). The pathologically altered lungs cause airway narrowing and obstruction (Silva and Bercik 2012). However, the dose-induced variation did not cause significant differences in the lung anatomic features of the five experimental groups. Lung damage may be caused by other external factors.

Table 9. Average of damages degree in alveolus lungs.

Treatments	Alveolar inflammation	Lung Parenchyma Damage (%)	Alveolar lumen narrowing
Control	3,00±0.00	3,00±0.00	3,00±0.00
P1	3,00±0.00	3,00±0.00	3,00±0.00
P2	3,00±0.00	3,00±0.00	3,00±0.00
P3	3,00±0.00	3,00±0.00	3,00±0.00
P4	2,33±1.15	2,33±1.15	2,33±1.15

Data are presented Mean±SEM.

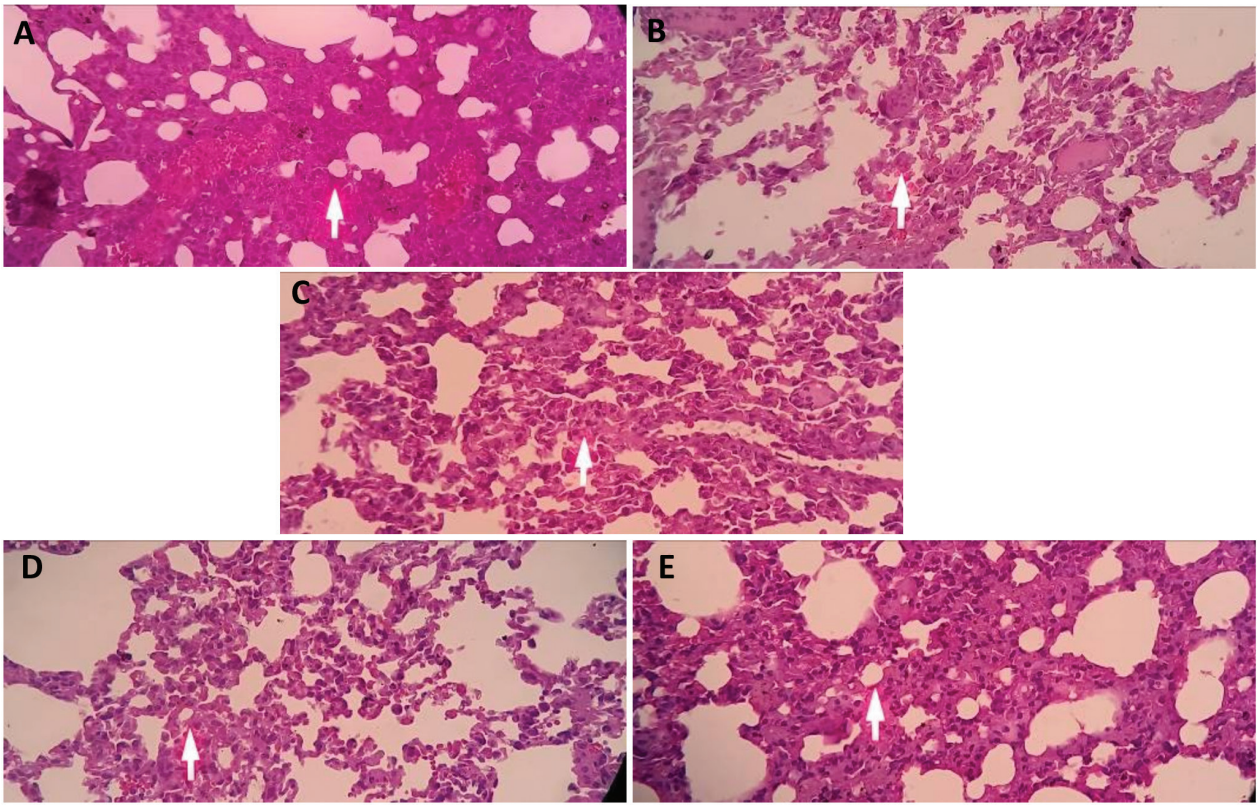

Figure 3. Histology of lungs. The arrow showed parenchymal damage/inflammation. **A.** Control; **B.** PI (400 mg/KgBW); **C.** P2 (635.2 mg/KgBW); **D.** P3 (1.008.6 mg/KgBW); **E.** P4(1601.8 mg/KgB W).

Histology of the cardiac after giving nanoherbal mahkota dewa

Cardiac damage affects cardiac muscle cells after giving nanoherbal mahkota dewa (Table 10). They had damage, with varying degrees of damage at each dose level. There was a significant difference ($p < 0,05$) in vacuolar degeneration, parenchymal degeneration, and necrosis, but normal cardiocytes could not be measured because the boundary between the cardiocyte cells was not clear, so a normal cardiocyte count could not be performed. The highest parenchymal degeneration and necrosis degrees were in the P4 dose, while vacuolar degeneration was in the P2. Histology of cardiac cells (Fig. 4) showed that at each dose level, parenchymal degeneration, hydropic degeneration, and necrosis in cardiac muscle cells became more irregular and the density between cells decreased.

Parenchymal degeneration was reversible, and cells could return to their original state. However, in severe or persistent stress and injury, irreversibility occurs. Cardiac muscle is a target for autoimmune inflammation. Old age, thymoma, and anti-CV1 antibodies appear to be risk factors that may lead to recognizing cardiac involvement (Davidson and Diamond 2001; Ding et al. 2007). The activity of this herb with the appropriate dose could be used as an antioxidant by inhibiting the formation of radical oxygen species (ROS) that could induce cancer and various types of inflammation (Goodlett and Horn 2001).

Parenchymal and hydrophic degeneration: 0 = normal, 1 = 0–30% degenerate, 2 = 31–60% degenerate, 3 = > 61% degenerate. Necrosis: 0 = normal, 1 = 0–30% have Necrosis, 2 = 31–60%, Necrosis3 = > 61% have necrosis.

Histology of brain after giving nanoherbal mahkota dewa

A Purkinje cell is a class of GABAergic inhibitory neurons located in the cerebellum. However, in this preparation,

Table 10. Average of damages degree in cardiac.

Treatments	Normal Cardiocytes	Parenchymal Degeneration (%)	Vacuolar Degeneration (%)	Necrosis (%)
Control	The boundary between the cardiocyte cells is not clear, so a normal cardiocyte count cannot be performed	0,78±0,44	0,89±0,33	0,67±0,50
P1		0,78±0,44	0,89±0,33	0,00±0,00*
P2		1,00±0,00*	1,22±0,44*	0,67±0,50
P3		0,89±0,33	0,89±0,33	0,67±0,50
P4		1,44±0,53*	1,11±0,33*	1,22±0,44*

Data are presented Mean±SEM.

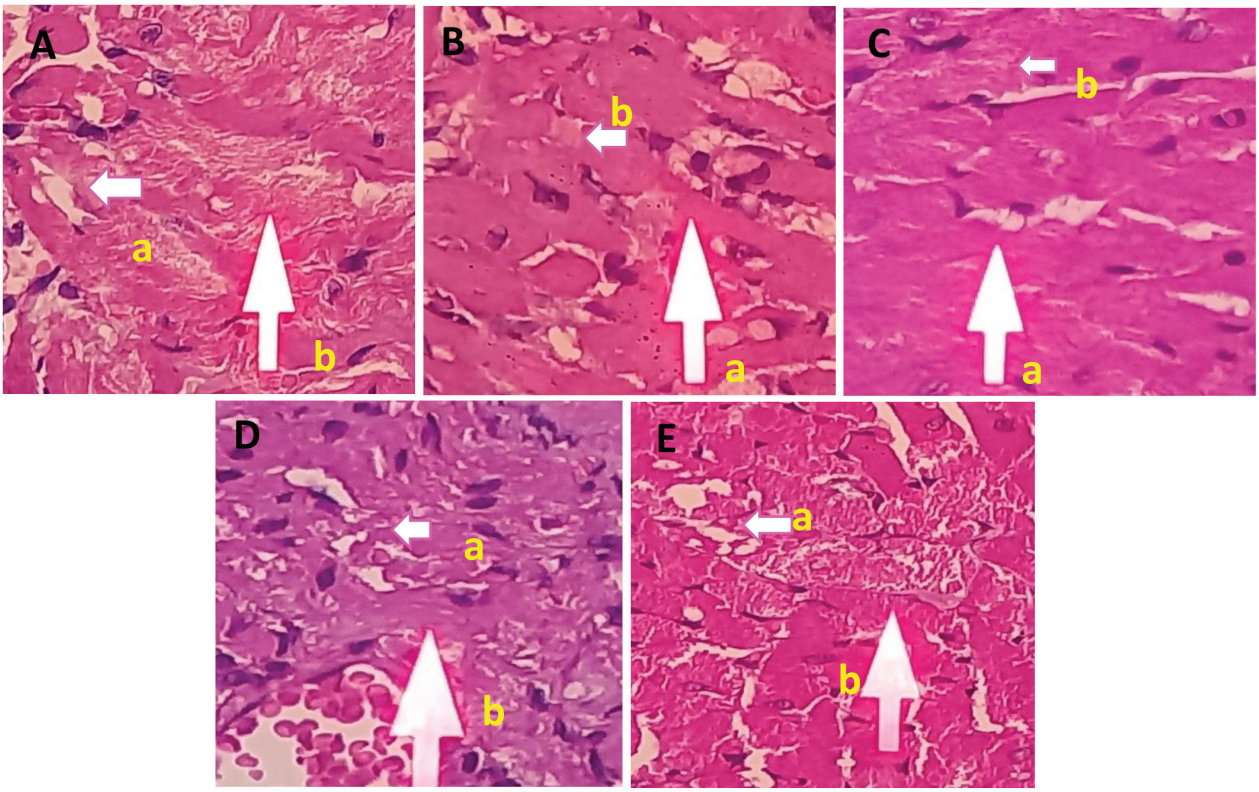

Figure 4. Histology of cardiac Damages. **A.** Control; **B.** PI (400 mg/KgBW); **C.** P2 (635.2 mg/KgBW); **D.** P3 (1008,6 mg/KgBW); **E.** P4(1601,8 mg/KgBW); **a.** Parenchymal damagc/Vacuolar degeneration and **b.** necrosis.

no slices were found, indicating the presence of a cerebellum due to cerebellar tissue that could have been damaged during surgical procedures or other extrinsic factors. There was a significant difference ($p < 0,05$) in the number of Purkinje cells. However, there was no significant difference ($p > 0,05$) in the number of necrotic cell parameters. The nanoherbal of mahkota dewa affects the number of Purkinje cells in the brains of mice. The number of Purkinje cells in the control group had the highest value compared to all other treatment groups. The higher the dose used, the lower the number of Purkinje cells found.

Based on statistical tests, there was a significant difference ($p < 0,05$) in the necrosis cell of the mouse brain after giving nanoherbal mahkota dewa at each dose level (Table 11). Based on the histological observations of brain cells (Fig. 5), the control group had many *Purkinje* cells. Purkinje cells in P2 and P3 reduce cell-to-cell connections so far and increase necrosis. P4 had extensive cell necrosis and a long-distance relationship between cells. This dose could interfere with the central nervous system in mice.

Table 11. The number of Purkinje cells and necrosis cells in the brain.

Treatments	Purkinje cell	Necrosis
Control	30,78±0,54	2,17±0,11
P1	26,56±0,89*	3,48±0,08
P2	27,11±0,73	10,32±0,23*
P3	22,22±0,52*	8,88±0,17*
P4	21,00±1,01*	12,22±0,87*

The cerebellum and hippocampus were the parts of the brain that were the most susceptible to damage from oxidative stress. Some researchers claim that oxidative stress could induce the formation of free radicals that cause cell damage and death (Maier and West 2001; Martinc et al. 2014). The higher the mahkota dewa dose, the lower the number of Purkinje cells. The herbs of nanoherbal mahkota dewa research also play a role in preeclampsia (Simanjuntak et al. 2019b). This study concludes that solid dispersion acute toxicity testing is important to evaluate because it guarantees the safety and effectiveness of the formulation.

Histology of kidney after giving nanoherbal mahkota dewa

In this study, the histopathological features of the kidneys were assessed using experiments as described below. Based on Table 13, there was no severe damage found in the kidneys of mice after exposure to the nanoherbal mahkota dewa in graded doses. Referring to the histology assessment instrument of kidney tissue in Table 12, it was found that there was level 1 damage to the tubular and endothelium of group P1. It was characterized by the loss of the brush border that was less than 25% in the tubular cells, while the integrity of the basement membrane was intact.

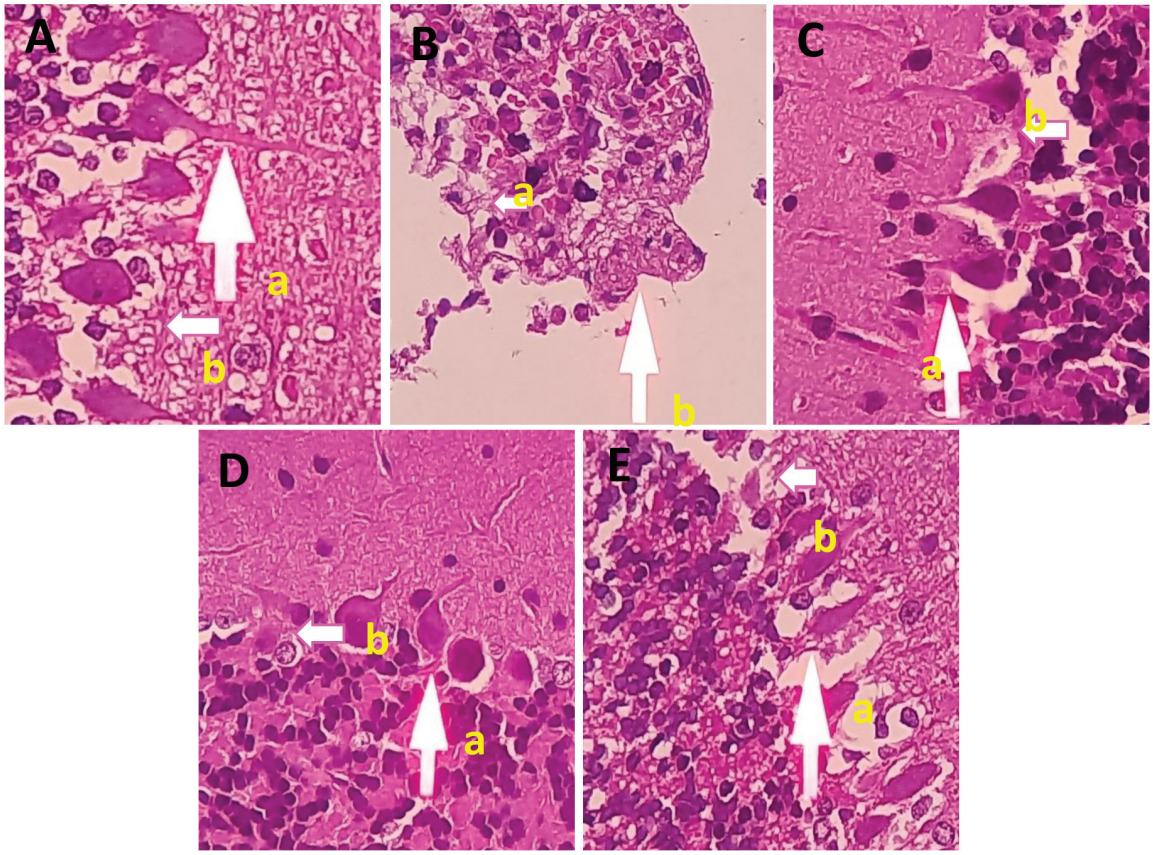

Figure 5. Histology of necrosis cells in brains. **A.** Control; **B.** PI (400 mg/kg BW), **C.** P2 (635.2 mg/kg BW), **D.** P3 (1.008.6 mg/kg BW), **E.** P4(1601.8 mg/kg BW). **a.** Purkinje cells andb. Necrosis.

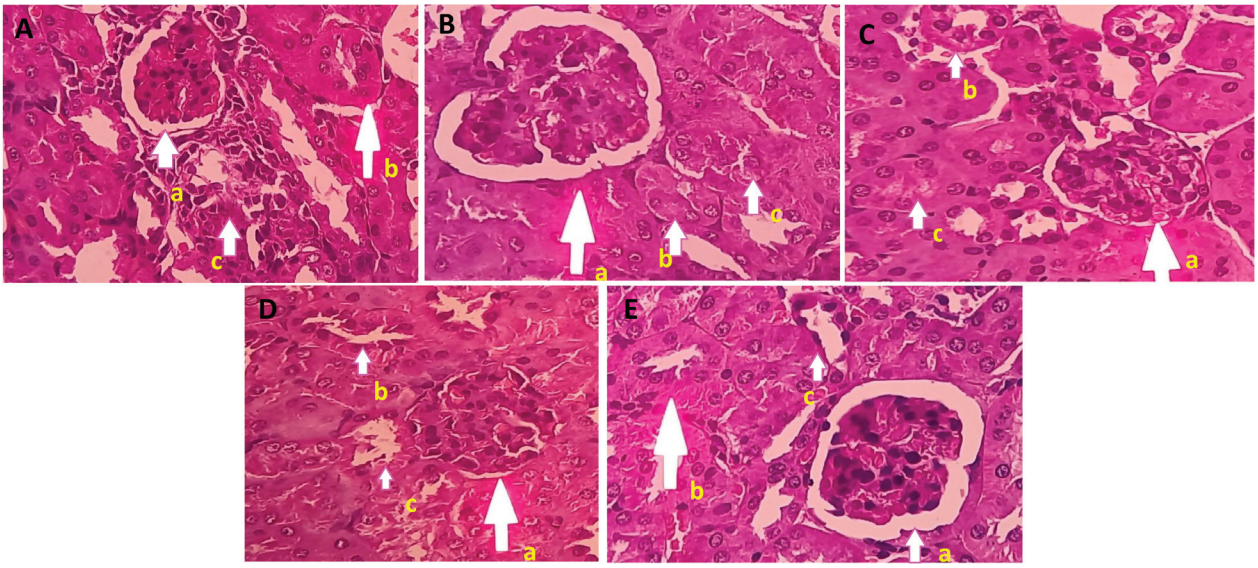

Figure 6. Histology of kidney. **A.** Control; **B.** PI (400 mg/kg BW); **C.** P2 (635.2 mg/kg BW); **D.** P3 (1.008.6 mg/kg BW); **E.** P4(1601.8 mg/kg BW); **A.** Tubular; **b.** Endothelium + Glomerulus; **c.** Tubulo-intcrstitiaL; **a.** Tubules; **b.** Endothelium + Glomerulus; **c.** Tubulointcrstitial

The endothelium is characterized by swelling of the endothelium (Fig. 6). Other minor damage could be seen in Table 13, which was interpreted in Fig. 6. Kidneys are organs that have an important role in maintaining stability in the body, such as the balance of body fluids, electrolytes, and acid bases in the body through blood filtration (Hoening and Zeidel 2014). The forms of toxicity commonly found in the kidneys include cell necrosis, which is cell death. Cells in the kidney that undergo necrosis could be caused by several factors, including strong toxins (e.g., phosphorus, poisonous mushrooms such as arsenic, and others), disturbances in metabolic activity, and viral infection that causes the fulminant form, commonly known as a malignant virus (Beaumier et al. 2016).

Table 12. Kidney histology assessment instrument.

Types of tissue	Description	Score
Tubular	No damage	0
	The loss of the brush border was less than 25% in the tubular cells. The integrity of the basement membrane is intact.	1
	Over 25% of tubular cells have lost their brush border. Basement membrane thickening	2
	Inflammation cast formation, necrosis of up to 60% of tubular cells.	3
	(Additionally, more than 60% of tubular cells are necrotic.)	4
Endothelium	No damage	0
	Swelling of the endothelium	1
	Endothelial disorders	2
	Endothelial loss	3
Glomerulus	No damage	0
	Bowman's Capsule Thickening	1
	Retraction of the juxtaglomerular apparatus	2
	Fibrosis of the glomerulus	3
Tubulointerstitial	No damage	0
	Inflammation, hemorrhage of less than 25% of tissue	1
	Necrosis in less than 25% of tissue	2
	Necrosis up to 60%	3
	Necrosis more than 60%	4

Considering that the nanoherbal mahkota dewa has a moderate toxicity value, the tested dose exposure did not cause significant tissue damage to the kidneys. In addition, exposure that was only carried out for 2 weeks gave only mild inflammation (Level 1) and hemorrhage of less than 25% of the tissue in the entire dose treatment group (Table 11, Fig. 6).

Table 13. Kidney histology assessment score.

Treatments	Tubular	Glomerulus	Endothelium	Tubulointerstitial
Control	0	0	0	0
P1	1	0	1	1
P2	0	0	0	1
P3	0	0	0	1
P4	0	1	0	1

Conclusion

In short, this study provides information that the LC50 and LD50 values of nanoherbal *Phaleria macrocarpha* fruit are 2145,0407 ppm and 1g/kg BW±0,075. Nanoherbal *Phaleria macrocarpha* fruit affects the histological changes in the organs, biochemical parameters of the kidney, liver, and electrolytes. In the future, a chronic toxicity study is recommended to confirm the safe use of *Phaleria macrocarpa*.

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