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Hematological, biochemical, and histopathological evaluation of the *Morus alba* L. leaf extract from Brunei Darussalam: Acute toxicity study in ICR mice

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Abstract

Background: Studies have reported that the phytochemical content of Mulberry (*Morus alba* Linn.) is influenced by the area where it grows. On the other hand, the study of the bioactivity and toxicity of mulberry leaves from Brunei Darussalam still needs to be completed. In particular, the investigation regarding the safe dose for Mulberry's application from Brunei Darussalam has yet to be studied. Hence, toxicity information must be considered even though the community has used it for generations.

Aim: This study investigated *Morus alba* ethanolic leaf extract (MAE) to observe the acute toxicity in mice.

Methods: In particular, this study utilized 12 female Institute of Cancer Research mice, 8 weeks old, divided into 2 groups: the control group and the MAE group (2,000 mg/kg single dose). Physiology, hematology, biochemistry, and histology were analyzed during the study.

Results: The examination result indicated no mortality and behavioral changes throughout the testing period. However, the mice developed mild anemia and leukopenia, followed by decreased numbers of neutrophils, lymphocytes, and monocytes. In addition, the mice developed a mild hepatocellular injury, indicated by significant ($p < 0.05$) elevations of both alanine aminotransferase (ALT) and aspartate transaminase (AST). The histopathological findings of the liver were also consistent with the increment of ALT and AST, indicating mild hepatocellular necrosis through the eosinophilic cytoplasm and pyknosis ($p > 0.05$).

Conclusion: It was evident that a single oral administration of MAE was not lethal for mice (LD_{50} , which was higher than 2,000 mg/kg). However, the administration of high doses of MAE must be carefully considered.

Keywords: *Morus alba*, Acute toxicity, Hematology, Biochemistry, Histopathology.

Introduction

Mulberry is reported to be the most extensively utilized species, widely cultivated in Asia, the Eurasian continent, Africa, and the United States (Memete *et al.*, 2022). Typically, farmers widely grow Mulberry for its silkworm leaves, fruit for candied jams, bark for paper production, and many other uses in traditional oriental medicine (Chan *et al.*, 2016; Zhang *et al.*, 2018; Giora *et al.*, 2022). The majority of the pharmacological actions were reported on Mulberry leaf extract,

including antidiabetic activity, hypolipidemic activity, reno-protective activity, antioxidant, antimicrobial activity, anticancer activity, and cardioprotective activity (Thaipitakwong *et al.*, 2018). However, before utilizing plant extract as an alternative therapy, the efficacy and safety profile must be carefully considered, especially when administered excessively. In addition, the primary organs, such as the liver and kidneys, might be affected as they play a part in metabolizing and eliminating chemical substances (Hodges and Minich, 2015).

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As well known that the origin of herbal influences the bioactivity, antioxidant, and polysaccharide content of *Morus alba* (MA) Linn (Khan *et al.*, 2019). The soil, altitude, weather, collection period, environment, and species influence the composition of the phytochemicals of plants (Pant *et al.*, 2021), so it can also affect the pharmacodynamics of the herbal (Satoh, 2014). Besides, the extraction method and solvent used play an essential role in the concentration of compounds as well (Sridhar *et al.*, 2021); for instance, microwave-assisted extraction can increase the extraction content's purity and phenolic yield compared to the traditional method (Sridhar *et al.*, 2021) and ethanolic solvent increase the phenolic, flavonoid, and phytochemical content more than aqueous solvent (Sim *et al.*, 2019). In addition, a recent acute toxicity study reported no significant alteration in body weight gain, hematology, and biochemical parameters after oral administration of a single high dose of 15.0 g/kg water extraction of Mulberry leaf extract (Li *et al.*, 2018). However, another study reported some adverse effects in Swiss mice after intraperitoneal administration of a single dose of 2,000 mg/kg of *Morus alba* ethanolic leaf extract (MAE) (de Oliveira *et al.*, 2016). Therefore, the present authors argue that the physiological effects induced by the acute toxicity study of MA leaves in experimental animals, especially rodents, are still diverse due to dosages, treatment routes, extraction method, and the content of ingredients from the leaves of MA, which phytochemical compound varies depending on the location or country. Moreover, studies of the acute toxicity of MA leaves in Southeast Asia, particularly Brunei, have never been conducted. Thus, the acute toxicity study is critical to determine the median lethal dose of 50 (LD₅₀) caused by high-dose administration of MA, leading to the novelty of the present study in determining the toxic effect of *Morus alba* leaf extract on female Institute of Cancer Research (ICR) mice by establishing scientific awareness and safe consumption of *Morus alba* leaf extract. This study used the microwave-assisted extraction of 60% ethanolic extract of MA leaves from Brunei Darussalam to evaluate the acute toxicity effect on ICR mice. Besides, this study investigated body weight gain, feed consumption, relative organ weight, hematology, serum biochemistry, and organ histology.

Materials and Methods

Preparation of the leaf extract

First, MA leaves were collected from the Herbal Garden at the University of Brunei Darussalam. Furthermore, the powdered leaves were extracted using the Microwave-assisted extraction method referring to Li *et al.* (2009) with modifications (Li *et al.*, 2009). The *Morus alba* leaves extract was prepared using 100 g of the dried powder in 1,500 ml of 60% ethanol. The solution was heated in a 450 W microwave for

60 seconds and cooled off to 60°C before reheating. The cycle was performed four times. After filtering the mixture through filter paper, the filtrate was evaporated in a rotary evaporator to produce the crude extract. Before being used in subsequent tests, the crude extract was stored at 4°C.

Experimental animal

Experiments were conducted using healthy female ICR mice weighing roughly 25–30 g for an acute 14-day toxicological study obtained from the Faculty of Pharmacy, The MARA University of Technology Malaysia. An acute toxicity study was conducted following the guidelines for testing chemicals, Organization for Economic Cooperation and Development (OECD) test number 423 (OECD, 2002). Experimental animals were divided into two groups of six animals. The *Morus alba* leaf extract (MAE) group was given 2,000 mg/kg 5% dissolved in dimethyl sulfoxide (DMSO), and the control group was given equal treatment of 5% DMSO by oral administration using a standard metal feeding tube. During the first 24 hours, the mice were monitored for their potential deaths, behavioral changes, and signs of toxicity. Daily monitoring was maintained until the 14th day of experimentation.

Body weight gain and feed consumption

The digital weight scale measured the animals weekly for their weight (Professional Digital Scale, China). Daily, the food tray was filled with 100 g of regular feed, and the remaining feed was weighed the following morning at 8 a.m. The amount of group feed consumption was determined by deducting the feed from the remaining residue (Li *et al.*, 2020c).

Hematology and serum biochemical analysis

At the termination of the study, blood was withdrawn through the orbital sinus under anesthesia by using ketamine 100 mg/kg BW (Ket-A-100® Agrovit Peru), xylazine 10 mg/kg BW (Xyla® Interchemie Holland) and Acepromazine 2 mg/kg BW (Castran® Interchemie Holland), injected intraperitoneally and followed by cardiac puncture for euthanasia (Hedenqvist, 2021). An automatic hematology analyzer (Celltac Alpha VET® MEK-6550K, Nihon Kohden, Japan) was utilized to determine the hematological assay. Serums were collected by separating the blood in a microcentrifuge (Eppendorf centrifuge 5424R) at 3,500 rpm for 10 minutes—the measured biochemistry parameters using a biochemistry analyzer (Biolis 24i Premium®, Japan).

Relative organ weight

A complete necropsy was performed after all animals were euthanized. In a further step, the liver, spleen, kidney, uterus, heart, lungs, and brain were all carefully isolated, washed, weighed, and examined for gross abnormalities. The following equation was used to determine the relative organ weight:

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight (g) at the sacrifice}} \times 100$$

Histopathological analysis

The liver and kidney were obtained from each group for histopathological examination and immediately fixed in 10% v/v of neutral buffered formalin. Tissues were processed using standard protocols to perform hematoxylin and eosin (H&E) staining. The histopathological finding was observed under the light microscope (Nikon Eclipse 80i, Japan) and then evaluated using a numerical scoring system with minor modifications (Nurul et al., 2018). The percentage of the severity of hepatocyte or nephron necrosis or degeneration was used in the scoring. The scoring indicated: no percentage of severity was graded as 0; less than 10% of severity was graded as 1; 10%–30% of severity was graded as 1.5; 30%–50% of severity was graded as 2; 50%–70% of severity was graded as 2.5; and more than 70% of severity was graded by 3.

Statistical analysis

All experimental data, except histological scoring results, were analyzed using a student’s *t*-test. In addition, liver and kidney lesion scores were analyzed using the nonparametric Mann–Whitney. Data were displayed as mean ± SEM (standard error of the mean). A *p*-value of 0.05 or less was considered statistically significant. SPSS statistical version 26 was utilized to analyze the computed data for statistical analysis.

Ethical approval

Ethical clearance for the animal study was approved by a committee member of Animal Care Universiti Putra Malaysia with certificate no. UPM/IACUC/AUP-R024/2022.

Results

Body weight, feed intake, and relative organ weight

Both groups increased in body weight in week 2 compared to week 1. However, no significant differences

existed between the MAE and the control groups across the 14-day experimental period (Table 1). The feed intake was also similar throughout the study period (Table 1). The same results were shown in relative organ weight in treated and control groups (Table 2).

Effects of *Morus alba* leaf extract on hematological variables

According to the results of the student’s *t*-test, there were significant (*p* < 0.05) differences in the hematological parameters between the MAE group and the control group (Table 3). The MAE group treated with a single high dose of 2,000 mg/kg of MA leaf extract indicated mild anemia with a significantly decreasing number of red blood cells (RBCs), hemoglobin (Hb), and packed cell volume (PCV). In addition, leukopenia in the MAE group was indicated by a decline in white blood cells (WBCs) followed by a lower number of neutrophils, lymphocytes, and monocytes.

Effects of MA leaf extract on biochemical variables

Table 4 illustrates that several biochemical parameters significantly differed between the MAE and the control groups. For instance, mice treated with 2,000 mg/kg of MA leaf extract indicate significantly higher alanine aminotransferase (ALT) and aspartate transaminase (AST) levels (*p* < 0.05) than those in control mice, marking that mice develop a mild hepatocellular injury. Meanwhile, creatinine kinase (CK) also increases significantly (*p* < 0.05) in the MAE group compared to the control group. On the other hand, kidney injury parameters, including serum urea and creatinine of the mice, are within normal limits.

Effects of single high-dose oral administration of MA leaf extract on liver and kidney histological scoring

The histological finding of the livers in the MAE group indicated mild hepatocellular injury, depicted by eosinophilic cytoplasm, inflammatory cell infiltration,

Table 1. Effect of single high dose oral administration of *Morus alba* leaf extract on weekly body weight and daily feed intake.

Groups	Body weight (g)		Feed intake (g/day/mouse)	
	Week 1	Week 2	Week 1	Week 2
Control	0.76 ± 0.38	0.85 ± 0.48	4.93 ± 0.28	4.58 ± 0.18
MAE (2,000 mg/kg)	0.77 ± 0.19	1.31 ± 0.21	4.92 ± 0.11	4.91 ± 0.18

The data in the table are demonstrated as the mean ± SEM. No significant differences were observed.

Table 2. Effect of single high dose oral administration of *Morus alba* leaf extract on relative organ weight.

Groups	Liver	Spleen	Left kidney	Right kidney	Uterus	Heart	Lung	Brain
Control	3.97 ± 0.18	0.53 ± 0.03	0.57 ± 0.02	0.60 ± 0.01	1.61 ± 0.12	0.49 ± 0.02	0.72 ± 0.02	1.43 ± 0.05
MAE (2,000 mg/kg)	3.97 ± 0.13	0.49 ± 0.03	0.58 ± 0.02	0.66 ± 0.02	1.44 ± 0.12	0.40 ± 0.02	0.73 ± 0.02	1.47 ± 0.02

The data in the table are demonstrated as the mean ± SEM. No significant differences were observed.

Table 3. Effect of single high dose oral administration of *Morus alba* leaf extract on hematological parameters.

Hematological parameters	Control	MAE (2,000 mg/kg)
RBC ($\times 10^{12}/l$)	8.22 \pm 0.17	6.91 \pm 0.43*
Hb (g/l)	135 \pm 2.84	115 \pm 0.640*
PCV (%)	39.05 \pm 0.88	33.23 \pm 1.82*
MCV (fl)	47.48 \pm 0.35	48.23 \pm 0.71
MCH (pg)	16.42 \pm 0.12	16.75 \pm 0.27
MCHC (g/l)	345.67 \pm 0.88	347 \pm 0.2.41
Platelets ($\times 10^3/\mu l$)	620.50 \pm 42.14	521.67 \pm 124.13
WBC ($\times 10^9/l$)	6.33 \pm 0.42	2.33 \pm 0.36*
Neutrophils ($\times 10^9/l$)	0.85 \pm 0.11	0.42 \pm 0.11*
Lymphocytes ($\times 10^9/l$)	5.08 \pm 0.38	1.76 \pm 0.29*
Monocytes ($\times 10^9/l$)	0.34 \pm 0.07	0.10 \pm 0.02*
Eosinophils ($\times 10^9/l$)	0.06 \pm 0.02	0.06 \pm 0.02
Basophils ($\times 10^9/l$)	0.00 \pm 0.00	0.00 \pm 0.00

*Significant difference at $p < 0.05$ versus the control group. All values are presented as mean \pm SEM.

Table 4. Effect of single high-dose oral administration of *Morus alba* leaf extract on serum biochemical parameters.

Groups	Creatinine ($\mu\text{mol/l}$)	Urea (mmol/l)	ALT (U/l)	AST (U/l)	CK (U/l)	Total protein (g/l)	Albumin (g/l)	Globulins (g/l)
Control	23.83 \pm 1.83	8.44 \pm 0.41	44.47 \pm 5.92	141.37 \pm 19.26	728.37 \pm 232.17	59.77 \pm 1.14	31.48 \pm 0.47	28.28 \pm 1.09
MAE (2,000 mg/kg)	22.53 \pm 0.61	9.07 \pm 0.53	73.58 \pm 6.98*	396.20 \pm 101.71*	1,684.68 \pm 283.56*	60.33 \pm 1.18	31.58 \pm 0.74	28.75 \pm 1.29

*Significant difference at $p < 0.05$ versus the control group. The data in the table are demonstrated as the mean \pm SEM.

and pyknosis (Fig. 1). *Morus alba* extract activated Kupffer cells. It was scored, albeit it was statistically insignificant ($p > 0.05$) (Table 5). Moreover, the MAE group experienced mild morphological changes in kidney tissues, such as tubular injury, loss of brush border, and hydropic degeneration (Fig. 2). However, the scoring value of the lesion in the kidney tissue was within the normal limit ($p > 0.05$) (Table 6).

Discussion

Acute toxicity studies are implemented to identify the dose that will result in a fatality or significant toxicological effects when administered once or throughout several administrations in a short period (Saganuwan, 2017). In this study, neither the control nor the extract-treated mice at a dose of 2,000 mg/kg MA leaf extract exhibited any mortality, behavioral changes, or abnormal appearance throughout the experiment. As a result, this study reported no acute toxicity in mice administered by the leaf extract of MA, and the approximate median LD₅₀ MA was found to be greater than 2,000 mg/kg, which was in

accordance with previous studies, such as Hwang *et al.* (2016) (LD₅₀ > 2,000 mg/kg) (Hwang *et al.*, 2016) and Figueredo *et al.* (2018) (LD₅₀ > 2,000 mg/kg) (Figueredo *et al.*, 2018). Moreover, there are no remarkable differences in body weight, feed intake, and relative weight organs. Based on this result, the study concluded that a single high-dose administration of *Morus alba* leaf extract had no adverse effects on the nutritional advantages, including weight gain and appetite stability, and it did not affect organ growth. In toxicity studies, the parameters of the hematological evaluation system are considered the most sensitive targets for poisons or their metabolites related to cellular components. Therefore, hematological changes indicate a higher predictive value for human and animal toxicity studies (Arika *et al.*, 2016). The present study reported lower RBC, Hb, and PCV levels in the MAE group treated with 2,000 mg/kg of *Morus alba* leaf extract than those in the control group, implying that the MAE group suffered from mild anemia. Furthermore, a lower total of WBC, specifically on neutrophils, lymphocytes, and monocytes, was also observed in the MAE group

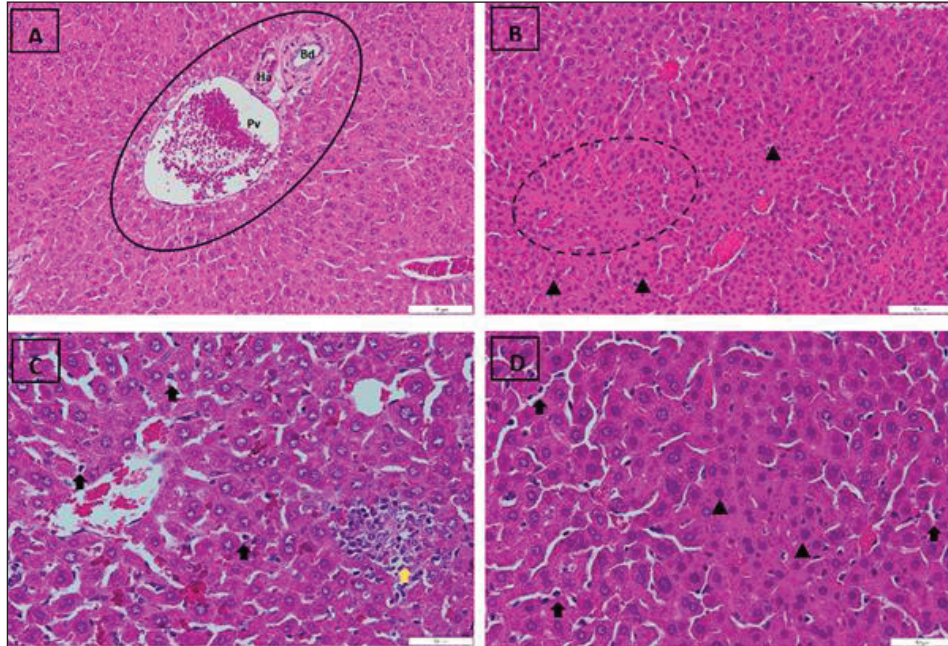


Fig. 1. Microphotograph histology of mouse liver on the acute toxicity of MAE on H&E staining. Key (A) Portal triad (encircled) structure of normal liver mouse in the control group (scale bar =100 μ m); (B) mouse liver treated with 2,000 mg/kg of MAE indicating eosinophilic cytoplasm (dotted encircle) and pyknosis (arrowhead) (scale bar = 100 μ m); (C) Mouse liver sinusoids with increased activation of Kupffer's cells (arrow) and inflammatory cell infiltration (yellow arrow) after 2,000 mg/kg of MAE were pictured in higher magnification (scale bar = 50 μ m). (D) Mouse liver at higher magnification showing pyknosis (arrowhead) and activated Kupffer cells (arrow) (scale bar = 50 μ m). Key: Pv = hepatic portal vein, Ha: Hepatic artery, Bd = Bile duct.

Table 5. Liver lesion scores of ICR mice on the oral treatment of 2,000 mg/kg of *Morus alba* leaf extract.

Lesion	Eosinophilic cytoplasm	Pycnotic	Karyolysis	Enlarged sinusoid	Activated Kupffer cell	Inflammation
Control	0.67 \pm 0.21	0.58 \pm 0.20	0.17 \pm 0.17	0.42 \pm 0.27	0.33 \pm 0.21	0.17 \pm 0.17
MAE (2,000 mg/kg)	1.33 \pm 0.00	1.25 \pm 0.31	0.50 \pm 0.22	0.50 \pm 0.34	0.67 \pm 0.33	0.50 \pm 0.22

The data in the table are demonstrated as the mean \pm SEM; there were insignificant ($p > 0.05$) differences between the groups.

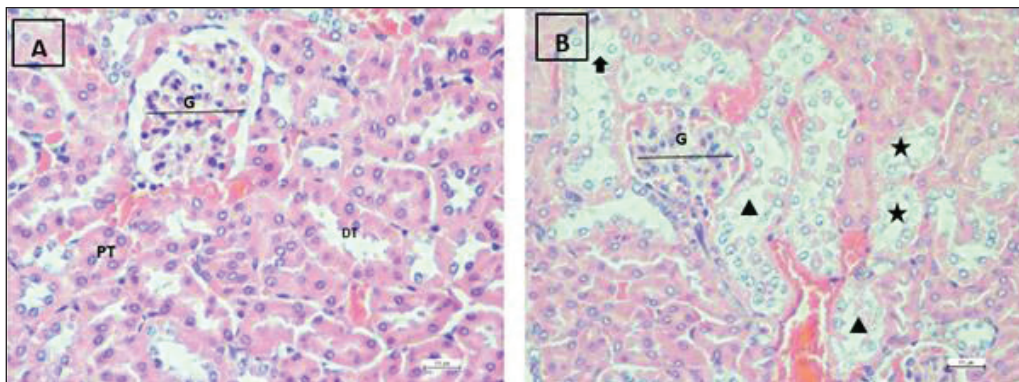


Fig. 2. Microphotograph histology of mouse kidney on the acute toxicity of MA leaf extract on H&E staining. Key (A) glomerulus and interstitial tubule of normal mouse kidney in the control group (scale bar =10 μ m); (B) microphotograph of mouse kidney treated with 2,000 mg/kg MAE indicating tubular injury (arrowhead), loss of brush border (star), and hydropic degeneration (arrow) (scale bar =10 μ m): G = glomerulus, PT = proximal tubules, DT = distal tubules.

Table 6. Kidney lesion scores of ICR mice on the oral treatment of 2,000 mg/kg of *Morus alba* leaf extract on acute toxicity study.

Lesion	Hydropic degeneration	Eosinophilic cytoplasm	Pycnotic	Karyolysis	Tubular injury	Protein Cast
Control	0.00	0.17 ± 0.17	0.00	0.00	0.00	0.00
MAE (2,000 mg/kg)	0.33 ± 0.21	0.33 ± 0.21	0.33 ± 0.21	0.00	0.25 ± 0.25	0.17 ± 0.17

The data in the table are demonstrated as the mean ± SEM; there were insignificant ($p > 0.05$) differences between the groups.

compared to the control group. Based on prior studies, the leukocyte was included in the innate immune system, providing the first line of defense against various common microorganisms (Marshall *et al.*, 2018), corresponding with inflammatory responses and tissue damage (Chen *et al.*, 2018). In the present study, the hematological data suggested that oral administration of 2,000 mg/kg of MA leaf extract might interfere with hemopoietic in mice in the short term, causing bone marrow dysfunction, as portrayed by the evidence and mild normocytic normochromic non-regenerative anemia, consistent with anemia related to bone marrow disorders. As such, the suggestion of this study was in line with previous studies reporting the suppression of bone marrow portrayed by reducing the white cell count; neutropenia, lymphopenia, and monocytopenia causing life-threatening adverse events (Pujari and Bandawane, 2022; Donadieu and Bellanné-Chantelot (2022).

The evaluation of kidney and liver function becomes one of the toxicity assessments of plant extracts, as both are responsible for an array of functions to maintain the survival of an organism (Al-Afifi *et al.*, 2018). The MAE group indicated mild hepatocellular necrosis by significantly elevating ALT and AST. The histopathological findings of the liver were also consistent with the increasing ALT and AST, indicating mild hepatocellular necrosis, such as the eosinophilic cytoplasm and pyknosis, which was statistically insignificant ($p > 0.05$). A single MAE insult was on day 1 of the experiment, and liver tissues were sampled only on day 14 post-insult. The regeneration of hepatocytes during 14 days reduced the severity of the hepatocellular necrosis from severe/moderate to mild. Moreover, a significantly increasing CK muscle enzyme in the MAE group compared with the control animals indicates a muscle injury or disease in the mice. Another study conducted with another plant, such as the herbal moonwort, reported that it caused muscle injury, characterized by increased creatine kinase and rhabdomyolysis in an acute toxicity case study (Li *et al.*, 2020a; Olorunnisola *et al.*, 2012).

Kidney lesion score indicates that kidney injury parameters were within normal limits on kidney histology in the MAE group, consistent with average biochemical values, including serum urea and creatinine. In addition, the kidney tissue observation confirmed that tubular injury, loss of brush border, and hydropic degeneration occurred in the cortex

area. However, the result was statistically insignificant on lesion-scoring changes triggered by 2,000 mg/kg MA leaf extract. This result was consistent with a previous study in which MA 2,000 mg/kg can alter renal histology by shrinking the subcapsular space and swelling the tubules (de Oliveira *et al.*, 2016). However, one study provided different results, showing that acute toxicity of *Morus alba* leaf extract at 15,000 mg/kg did not affect renal histological changes (Li *et al.*, 2018). The difference in results from previous studies could be due to the fact that they used the aqueous extraction method, while this study used the ethanol extraction method. Differences in extraction solvents can affect the yield composition of the plant (Lefebvre *et al.*, 2021). Animal testing is commonly used to predict human toxicity in pharmaceutical research (Van Norman, 2019). For human intake application, since the MAE caused mild anemia and leukopenia, it should be noted that the administration of MA leaves must be carefully considered, especially among pregnant women and in old age conditions. In addition, white mulberry root extract has been reported to have an abortive effect in mice at 50–90 mg/kg (Yoshida *et al.*, 2016). Other herbs, such as parsley (*P. crispum*), cinnamon (*C. verum*), and saffron (*C. sativus*), should be avoided during pregnancy as they are reported to have abortifacient effects (Aljoher *et al.*, 2018; Eid *et al.*, 2020). Another study reported that pregnant women taking herbal medicines in the first trimester are at risk of congenital malformations of the infant's nervous system, musculoskeletal system, eyes, and connective tissue (Chuang *et al.*, 2006). The occurrence of anaemia can also be caused by excessive consumption of coffee (*C. arabica*) and green tea (*C. sinensis*), as the caffeine contents can inhibit the absorption of calcium and iron (Eid *et al.*, 2020).

MA methanol extract includes active chemical components such as coumarins and moracin (Dugo *et al.*, 2009; Li *et al.*, 2020b). Coumarin belongs to the class of benzopyrone compounds. Coumarin has the potential as an anticancer drug used in treating kidney, prostate, and leukemia, reducing the side effects of radiotherapy (Akkol *et al.*, 2020). Despite the phytotherapy benefits contained in coumarin compounds, previous studies have reported that coumarin could cause moderate liver toxicity characterized by degeneration, stimulation of apoptosis, and necrosis in hepatocytes (Lake and Grasso, 1996). Coumarin was reported to

contain a median LD₅₀ of 196–780 mg/kg BW in mice, characterized as hepatotoxic (Lake, 1999). Furthermore, the study reported that the coumarin phytochemical in MA might have played a role in the hepatotoxic and hematologic alterations observed in the mice acute toxicity research (de Oliveira *et al.*, 2016). However, the diversity and concentration of a compound in the extract are influenced by the geography, environment, extraction method, and part of the plants (Khan *et al.*, 2019; Chen *et al.*, 2022) Moraceae. Therefore, the present authors encourage future studies to investigate the main compounds and quantification of *Morus alba* extract.

Conclusion

In conclusion, a single oral administration of 2,000 mg/kg *Morus alba* leaf extract was reported to be not lethal to the mice. Nonetheless, it disrupts the hematopoietic system, marked by evidence of mild normocytic normochromic non-regenerative anemia and compromising normal liver functioning. Due to the limitations of this study, additional research is encouraged to determine the specific compound of *Morus alba* leaf extract that causes such changes.

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Authors contribution

Conceptualization, AF, HH, and AA., Methodology, AF, HH, NK., Investigation and data curation, AF, HH., Funding acquisition, AA, NK., Writing–review and editing AF, HH, AA. Project administration, AF, NK, AA., Supervision, and validation, HH, AA, MHMN.

Conflict of interest

The authors declare that there is no conflict of interest.

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Data availability

The article includes all data supporting the study's findings. In case additional data is needed, the author can provide it upon reasonable request.

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