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Research article

Acute toxicity evaluation of methanol, ethanol and aqueous extracts of *Balanophora latisepala* (V.Tiegh.) Lec.

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Abstract

Balanophora latisepala has been used in traditional medicine in Vietnam for many years with both proven and unproven scientific proofs. This study aimed to evaluate the acute toxicity effects of *B. latisepala* by testing safety parameters of hot water, ethanol and methanol extracts of *B. latisepala* in *Mus musculus*. The acute toxicity was studied according to the World Health Organisations guideline for the evaluation of the safety and efficacy of herbal medicines. During study, a single dose of 1000, 2000 and 5000 mg/kg of each extract was orally administered to Swiss mice. To determine the median lethal dose, experimental mice were observed in behavior and mortality for 72 hours. Data of organ weight, histopathology, biochemical and hematology were also collected. The results showed that hot water, ethanol and methanol extracts at a dose of 5000 mg/kg did not induce mortality in experimental mice; therefore, LD_{50} is not determined. Insignificant changes were found in relative organ weight at dose 5000 mg/kg for all of the extracts. Similarly, no significant differences were observed in biochemical indices and organ histology. However, changes in hematological indices in both male and female mice were noticed. In male mice, it is likely that all *B. latisepala* extracts induced anemia. Moreover, clotting or bleeding abnormalities were also observed in female mice. Methanol extracts had the highest effect to hematology indices ($p<0.05$). Therefore, *B. latisepala* in different doses was shown its safety under acute toxicity studies with promising applications in drug therapy.

Keywords: Acute toxicity, *Balanophora latisepala* (*B. latisepala*), Ethanol extract, Hot water extract, LD_{50}, Methanol extract

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INTRODUCTION

Medicinal plants are widely used for the treatment of a variety of diseases traditionally and have played significant roles in the pharmacological research studies and development of modern drugs (Saad et al., 2006; Wu et al., 2013; El Moussaoui et al., 2020). Medicinal plants contain many bioactive compounds elaborated under secondary metabolism pathways (Gurib-Fakim, 2006) providing effectiveness in treating and preventing diseases with fewer or no side effects (EI Moussaoui et al., 2020). However, there is non-standardized dose in the use of traditional medicine and most of the plants have not been evaluated for toxicity (Nabukenya et al., 2014). Studies show that using medicinal plants without evaluating their efficacy and safety profile could result in adverse effects on different organs (Abid and Mahmood, 2019). Therefore, in order to ensure the safety of any medicinal plant, studies on its safety and reasonable dose should be performed prior to usage (Da Silva Moreira et al., 2019; Nfozon et al., 2019).

Balanophora J.R. Forster & G. Forster is a genus belonging to the family *Balanophora*ceae which includes about 120 species all over the world (Hemsl, 2017). They are distributed in temperate and tropical Asia, the Pacific, tropical Australia, the Comoros, Madagascar, tropical Africa, China, India, Indonesia, Myanmar and Vietnam (Nguyen et al., 2017). In Viet Nam, there have been 9 species of the genus *Balanophora* (*B. harlandii, B. abbreviata, B. elongata, B. fungosa var. fungosa, B. fungosa subsp. indica, B. latisepala, B. laxiflora, B. subcupularis and B. tobiracola*) have been classified. The classification was mainly based on the comparison of morphological characteristics described in the available scientific literature (Bui et al., 2018). Plants of *Balanophora* have been used as medicine for treating many diseases such as diabetes, gonorrhea, abdominal pain, and cough. This plant is also used to stop bleeding, detoxify, reduce fever, clear heat, regulate menstruation, or prevent hair loss (Chen et al., 2012; Hemsl, 2017; Fang et al., 2018; Huu Tai et al., 2020). It is shown that the species of this genus also contain anti-oxidant, HIV inhibitory, anti-inflammatory, hepatoprotective, anticancer, and antibacterial activities (Ho et al., 2010; Ho et al., 2012; Wang et al., 2012; Nguyen et al., 2017; Wei et al., 2017).

Although plants of the *Balanophora* genus, including *B. latisepala* are widely used in Vietnamese traditional medicine, their safety has not been scientifically evaluated. This paper is to elaborate on potential risks, including side effects, overdosing, and poisoning when using *B. latisepala* as traditional medicine.

MATERIALS AND METHODS

Preparation of plant extract

Plant collection and identification

Balanophora is a flowering plant that parasitizes roots of trees. Flowering *B. latisepala* was collected from Cam Mountain, An Giang province in November 2021. The sample was identified by Dr. Luu Hong Truong (Southern Institute of Ecology - Vietnam Academy of Science and Technology) and the specimens (No.2021.*Balanophora-latisepala*) have been kept at Plant Lab, Biology Department, School of Education, Can Tho University.

Figure 1 Balanophora latisepala

Preparation of the extract

Whole plants of *B. latisepala* were dried in an air-circulating oven at 60°C to a constant weight and then grounded using a laboratory blender.

Hot water extract: The powdered sample (10 g) was macerated in 100 mL of hot distilled water for 1h at 100°C. The hot water extract was filtered using filter paper. The extracts were stored in airtight containers at 4°C prior to further experiments (Taziebou et al., 2007; Nfozon et al., 2019).

Methanol and ethanol extracts: The plant powder (10 g) was put in 100 mL of methanol or ethanol for 3h. The methanol and ethanol extracts were filtered using filter paper. The entire extracts were concentrated to dryness using a Rotary Evaporator (SCI100, Scilogex Pro, USA) by removing ethanol or methanol at 55°C under a reduced pressure (Alelign et al., 2020).

The extraction yield was determined by the formula:

$$\frac{Powder\ weight - Extract\ weight}{Powder\ weight}\ x\ 100$$

Experimental animals

Forty *Mus musculus* mice (8-10 weeks old) weighing 30-32 g were purchased from Pasteur Institute (Ho Chi Minh City, Vietnam). Animals were kept at standard laboratory conditions of temperature and 12h:12h light/dark cycle. Food and water were freely accessed. Toxicity assays were conducted in accordance with the standard guidelines of the Organization for Economic Cooperation and Development (OECD) for use of animals in scientific research (Alelign et al., 2020). Ethical approval was granted by the Animal Welfare committee with Assessment No. AWA2021-08/KSP.

Acute toxicity study

The median lethal dose (LD50) is used to determine Acute toxic dose that kills 50% of experimental mice (Gadanya et al., 2011). The acute toxicity study was conducted according to the World Health Organizations (WHO) guideline for the evaluation of the safety and efficiency of herbal medicines (WHO, 2000) and OECD guidance (OECD, 2001). In acute toxicity study, mice were housed together in four groups (control, 1000 mg/kg, 2000 mg/kg and 5000 mg/kg), each group composed of ten animals, five male and five female (Sung et al., 2017). Mice were kept for 24 hours fasting to acclimatize to cage conditions before experimenting with free access to water. Mice were given treated concentrations using a syringe with a curved tip. The dosing volume was 10 mL/kg of body weight (Alelign et al., 2020). Signs of toxicity (behavior and physical condition) and mortality were observed up to 72 hours (Nfozon et al., 2019). The tested mice were observed for clinical signs of mortality to determine LD_{50} and behavioral changes (food intake, water intake, respiration, convulsion, increased motor activity, salivation, tremor, body temperature, coma, constipations, changes in eye colors, and skin colors) (Nfozon et al., 2019; Taziebou et al., 2007; Sung et al., 2017). After 72 hours experiment, mice were anaesthetized using diethyl ether after fasting for last night. Blood samples were taken for biochemical and hematological analysis. Finally, body organs were collected for detailed gross anatomy, organ weight and histopathological investigation (Iserhienrhien and Okolie, 2020).

Mice were dissected to obtain heart, liver, lungs, kidneys, spleen, pancreas, testes and ovaries. Organ's weights were determined to detect possible toxic effects of *B. latisepala* at the histological level. Relative organs weights were determined according to the formula: Relative Organ Weight (%) = [Absolute weight of organ (g)/weight of mouse on sacrifice day (g)] x100 (Sung et al., 2017; Abid and Mahmood, 2019; Iserhienrhien and Okolie, 2020).

Haematological analysis

Blood samples were analysed by CELL-DYN Ruby Automated Hematology Analyzer (Abbott, USA) included red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb), hematocrit (HCT), mean cell hemoglobin (MCH), mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), corpuscular volume (CV), lymphocytes (LYM), neutrophils (NEU), monocytes (MONO), thrombocyte count, and basophils (BASO) (Iserhienrhien and Okolie, 2020; Taziebou et al., 2007).

Biochemical analysis

For biochemical analysis, mouse serum was aspirated with a micro-pipette into sample bottles for the various biochemical analyzer (the Cobas clinical chemistry automatic analyzer - La Roche Ltd., Japan). Biochemical studies were carried out for liver function (in terms of aspartate transaminase (AST) and alanine transaminase (ALT)) and kidney function (Uric acid) (Abid and Mahmood, 2019; Nfozon et al., 2019; Ajij et al., 2020).

Histopathological examination

Organs including heart, liver, lungs, kidneys, spleen, pancreas, testes, ovaries of mice were weighed and fixed with Bouin's solution. Following fixation, tissues were cleansed in graded series of alcohol, washed in xylene and inserted into paraffin. Sections of 5 µm thickness were prepared using a Accu-cut SRM microtome, processed in alcohol- xylene series, stained with hematoxylin and eosin Y (H&E) and observed under the light microscope (Abid and Mahmood, 2019; Alelign et al., 2020; Iserhienrhien and Okolie, 2020).

Statistical analysis

Data were analyzed using ANOVA with the aid of IBM SPSS 22 for Windows. Data obtained were expressed as mean ± SD. Multiple comparisons of the means were done using the Duncan Multiple Range Test at 5% probability.

RESULTS

Extraction efficiency

Methanol and ethanol extraction efficiency (%) of *B. latisepala* species were relatively high (Table 1) while the aqueous extract was the lowest extraction efficiency (17.17±0.38%, $p<0.05$). The crude extract yield of methanol and ethanol was higher than that of hot water of over 42%.

Table 1 Extraction efficiency of *B. latisepala* extracts.

Extract	N	Power weight (g)	Efficiency (%)
Hot water	3	10	17.17±0.38a
Ethanol	3	10	42.17±0.51b
Methanol	3	10	42.07±1.10b

Data are expressed as mean ± SD and analyzed by one-way ANOVA, followed by Dunnett's test ($p < 0.05$) as compared to respective parameter values of control groups. Values column having the same letter are not significantly different

Behavior observation and LD50 determination

Mice were tested for acute toxicity after oral administration of extracts at single doses of 1000, 2000, and 5000 mg/kg. No death and clinical changing (including digestion, food intake, urination, rate of respiration, change in skin, drowsiness, sedation, eyes color, diarrhea, general physique, coma, death, grooming, convulsion, tremors, and sleep) were observed in any group throughout the experiment. Therefore, the value LD_{50} is not determined (the median lethal dose $LD_{50} > 5000$ mg/kg).

Relative organ weight

No significant differences occurred in relative organ weights of treated and control groups, and no changes were noticed between male and female mice (Table 2). However, in both sexes, relative organ weight of pancreas of treatment groups decreased significantly compared to the control group ($p<0.05$).

Table 2 Effect of extract of *B. latisepala* on relative organ weight in mice in acute toxicity study.

Organ	Relative weight of organs (%)			
	Control	Extract (dose 5000 mg/KgBW)		
		Hot Water	Methanol	Ethanol
Male				
Heart	0.48±0.08^{a}	0.50±0.07^{a}	0.41±0.05^{a}	0.46±0.05^{a}
Liver	4.16±0.45^{a}	4.48±0.52^{a}	4.37±0.21^{a}	4.53±0.32^{a}
Lungs	0.77±0.09^{a}	0.82±0.27^{a}	0.53±0.09^{a}	0.66±0.17^{a}
Kidney	1.18±0.22^{a}	1.24±0.07^{a}	1.17±0.16^{a}	1.25±0.15^{a}
Spleen	0.45±0.08^{a}	0.43±0.09^{a}	0.40±0.11^{a}	0.58±0.25^{a}
Pancreas	0.97±0.33^{b}	0.59±0.23^{ab}	0.50±0.11^{a}	0.40±0.19^{a}
Female				
Heart	0.49±0.05^{a}	0.40±0.03^{a}	0.40±0.02^{a}	0.44±0.12^{a}
Liver	4.53±0.09^{b}	3.82±0.14^{a}	4.43±0.18^{b}	4.78±0.48^{b}
Lungs	0.80±0.12^{a}	0.53±0.15^{a}	0.68±0.14^{a}	0.84±0.30^{a}
Kidney	1.01±0.10^{a}	0.88±0.03^{a}	0.92±0.08^{a}	1.01±0.17^{a}
Spleen	0.46±0.16^{a}	0.38±0.10^{a}	0.56±0.06^{a}	0.73±0.29^{a}
Pancreas	1.07±0.11^{b}	0.61±0.26^{a}	0.51±0.08^{a}	0.53±0.28^{a}

Data are expressed as mean ± SD and analyzed by one-way ANOVA, followed by Dunnett's test ($p < 0.05$) as compared to respective parameter values of control groups. Values rows having the same letter are not significantly different.

Haematological parameters

Blood parameters are relevant indicators for potential health hazards and have a higher predictive value for toxicity (Arsad et al., 2013; Ghadirkhomi et al., 2016). Therefore, it can be used to provide information about the toxicity mechanism/safety of a therapeutic agent (Tousson et al., 2011).

In regards to the observed hematological values, most of the values including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), Procalcitonin (PCT), platelet distribution width (PDW), white blood cell (WBC), neutrophil (NEU), monocyte (MON), eosinophil (EOS) in both male and female mice shown in the treated groups (hot water, ethanol and methanol extracts) were similar with the control group (Table 3). However, some values were significantly different from those of the control group and both sexes, such as those pertaining to red blood cell (RBC), Hemoglobin (HBG), hematocrit (HCT), mean platelet volume (MPV), and Lymphocyte (LYM).

Table 3 Effect of acute toxicity administration of B. latisepala extract haematological parameters in mice.

Parameter	Unit	Control	B.L extract (dose 5000 mg/Kg)		
			Hot Water	Methanol	Ethanol
Male					
RBC	10^6/mL	9.72±0.34^c	9.34±0.94^{bc}	8.06±0.36^a	8.21±0.58^{ab}
HBG	g/dL	15.40±0.89^c	13.60±0.26^b	12.27±0.76^a	12.77±0.50^{ab}
HCT	%	55.97±3.54^b	50.73±0.81^a	45.83±3.26^a	46.63±2.63^a
MCV	µm^3	57.57±2.06^a	56.23±2.71^a	56.90±4.48^a	56.83±1.65^a
MCH	pg	15.80±0.53^a	15.40±0.35^a	15.23±1.04^a	15.60±0.53^a
MCHC	g/dL	27.47±0.15^a	27.13±0.51^a	26.73±0.32^a	27.40±0.92^a
PLT	10^3/mL	782.3±64.8^a	734.3±50.1^a	703.0±132.9^a	583.7±231.0^a
PCT	%	0.49±0.04^a	0.53±0.13^a	0.49±0.10^a	0.41±0.14^a
MPV	µm^3	6.20±0.10^a	6.37±0.06^a	7.00±0.00^b	7.03±0.45^b
PDW	µm^3	5.93±0.42^a	6.63±0.23^a	7.90±0.44^b	7.87±0.90^b
WBC	10^3/mL	5.39±1.89^a	4.05±1.80^a	5.89±3.63^a	4.72±1.08^a
NEU	10^3/mL	0.42±0.35^a	0.29±0.25^a	0.27±0.20^a	0.32±0.28^a
LYM	10^3/mL	4.69±1.68^a	3.14±1.00^a	5.46±3.35^a	4.06±0.85^a
MON	10^3/mL	0.08±0.07^a	0.03±0.01^a	0.04±0.03^a	0.19±0.27^a
EOS	10^3/mL	0.00±.006^a	0.01±0.01^a	0.03±0.04^a	0.00±0.00^a
Female					
RBC	10^6/mL	9.08±1.13^a	8.81±0.51^a	8.11±0.58^a	7.62±1.32^a
HBG	g/dL	14.07±0.97^a	14.23±0.76^a	12.30±0.70^a	11.90±1.97^a
HCT	%	50.70±4.33^a	50.07±2.42^a	44.17±3.07^a	42.80±7.65^a
MCV	µm^3	56.03±2.36^a	56.83±0.91^a	54.57±3.88^a	56.10±2.14^a
MCH	pg	15.53±1.03^a	16.13±0.06^a	15.27±1.10^a	15.63±1.08^a
MCHC	g/dL	27.73±1.54^a	28.40±0.36^a	27.97±0.40^a	27.80±1.00^a
PLT	10^3/mL	706.67±119.07^a	812.33±164.81^a	633.33±242.28^a	487.67±385.09^a
PCT	%	0.45±0.07^a	0.52±0.11^a	0.46±0.12^a	0.32±0.24^a
MPV	µm^3	6.40±0.10^a	6.33±0.15^a	7.50±0.70^b	6.80±0.52^{ab}
PDW	µm^3	6.27±0.15^a	6.30±0.44^a	11.17±4.02^b	7.47±1.50^{ab}
WBC	10^3/mL	3.02±1.12^a	3.93±1.40^a	5.06±1.42^a	3.83±0.76^a
NEU	10^3/mL	0.33±0.07^a	0.34±0.15^a	0.36±0.11^a	0.18±0.06^a
LYM	10^3/mL	2.55±1.03^a	3.43±1.21^{ab}	5.00±1.51^b	3.36±0.81^{ab}
MON	10^3/mL	0.06±0.06^a	0.04±0.03^a	0.15±0.15^a	0.16±0.22^a
EOS	10^3/mL	0.00±0.01^a	0.00±0.00^a	0.00±0.01^a	0.02±0.03^a

Data are expressed as mean ± SD and analyzed by one-way ANOVA, followed by Dunnett's test ($p < 0.05$) as compared to respective parameter values of control groups. Values rows having the same letter are not significantly different.

Biochemical parameters of liver and kidney

The kidney and liver are more predisposed to toxic effects of xenobiotics during their metabolism and excretion (Dybing et al., 2002; George, 2011; Saad et al., 2006b). The results of the experiment suggested that kidney and liver functions were not altered in both treated male and female mice (Table 4). There was no statistically significant difference in acid uric, AST, and ALT levels between control and treated mice.

Table 4 Effect of acute toxicity administration of B. latisepala extract biochemical parameters in mice.

Parameter	Unit	Control	B.L extract (dose 5000 mg/Kg BW)		
			Hot Water	Methanol	Ethanol
Male					
AST	U/L	54.33±5.86[a]	50.33±10.12[a]	42.67±1.15[a]	49.67±9.02[a]
ALT	U/L	23.00±6.00[a]	22.33±4.73[a]	23.33±2.52[a]	22.00±2.65[a]
Uric acid	µmol/L	88.00±26.23[a]	86.00±12.12[a]	158.67±27.68[a]	159.00±75.32[a]
Female					
AST	U/L	50.33±4.16[a]	64.33±4.93[a]	60.67±21.39[a]	70.33±15.50[a]
ALT	U/L	29.00±8.66[a]	20.00±1.73[a]	20.67±4.51[a]	24.33±1.15[a]
Uric acid	µmol/L	92.00±38.69[a]	95.33±20.40[a]	155.33±34.00[a]	164.67±48.75[a]

Data are expressed as mean ± SD and analyzed by one-way ANOVA, followed by Dunnett's test ($p < 0.05$) as compared to respective parameter values of control groups. Values rows having the same letter are not significantly different.

Histopathological examination

Gross anatomical examination of the vital organs (heart, liver, lungs, kidneys, spleen, pancreas, testes and ovaries) in acute toxicity study did not reveal any gross pathological lesion in both male and female mice treated by hot water, ethanol and methanol extracts (Figure 2).

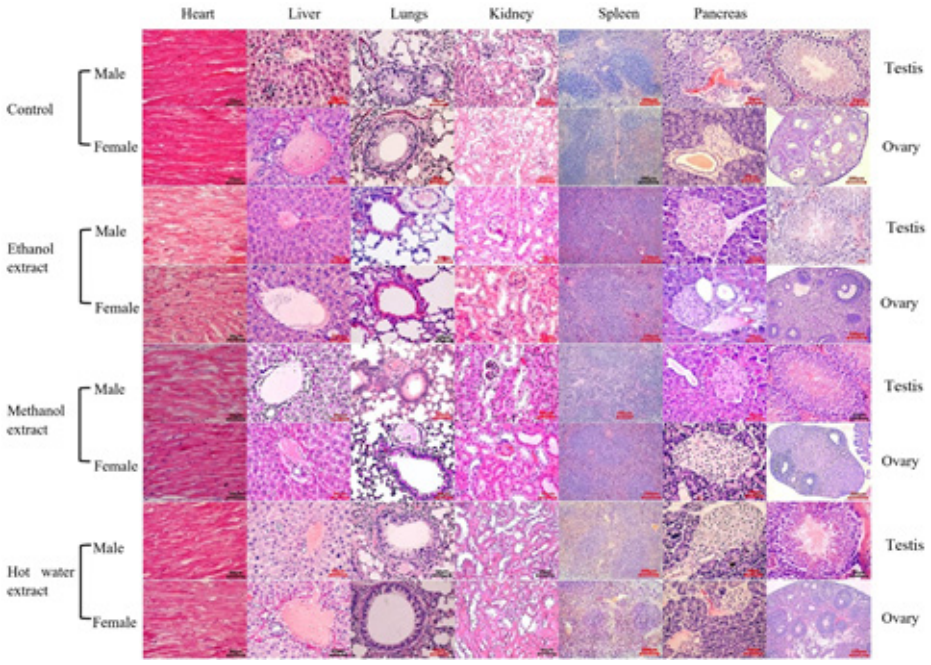

Figure 1 Effect of extracts at 5000 mg/kg on the histological structures of different organs of mice (H&E staining).

Histologic examination of heart showed that there was a normal myofibrillar structure with striations, branched appearance in both *B. latisepala* treated mice and the relative control. There was no sign of cardiac necrosis, haemorrhage, infiltration, or other damage to be observed.

Throught themicroscopic examination, liver sections of normal control tissues showed healthy hepatic cells without fatty lobulation, well-preserved cytoplasm, prominent nucleus and intact central vein. There was no sign of injury, necrosis, congestion, fatty acid accumulation, or hemorrhagic areas around the central vein or sinusoids of the liver in mice treated by hot water, ethanol, and methanol extract of *B. latisepala*.

In both male and female mice, multiple sections of lungs showed normal cellular structure in the treatment group and in the control group. There was no bronchi or alveoli collapse and no inflammatory cell infiltration surrounding the bronchi.

The current study, the kidney showed normal morphological architecture among the different dose levels and controls. There was also no interstitial and intraglomerular congestion or tubular atrophies in all samples. The nephron cells showed normal structure and there was no degeneration, bleeding, or necrosis infiltration to be observed.

In other organs, the cross-section of the spleen showed normal tissue structure. There was no hemorrhage nor pathological change in the spleen sinus. In both male and female mice, pancreas tissues showed normal structure. There were negligible abnormalities to be observed in the both pancreatic acini and islets. Testicles in male and ovaries in female mice showed normal pathology for both the control group and the extract-treated group. There was no sign of necrosis, edema, inflammation or congestion in the seminiferous tubules. Similarly, the ovaries were free from necrosis, edema, inflammation, and congestion. The follicle tissue also showed normal developmental structure.

DISCUSSION

The study of Nguyen et al., (2021) showed that the gum was found much in this methanol and ethanol extracts of this species. In high water temperature conditions, the adhesion of gum increases as this component adhesives physical components in medicinal materials, hence, the extraction efficiency in water was significantly reduced, significantly lower than other solvents.

When performing acute toxicity tests in mice, LD_{50} value was not determined because none of the mice died at the highest dose. According to the Globally Harmonized System (GHS) of Classification and Labelling of Chemicals, the substances with oral LD_{50} values of > 5000 mg/kg are considered relatively safe following acute exposure (Abid and Mahmood, 2019b). Moreover, the physical observation of the treated mice indicated by extract dose 5000 mg/kg, none of them showed observable signs of toxicity. These results, therefore, suggest that the ethanol, methanol and hot water extracts of *B. latisepala* are safe in laboratory mice with an LD_{50} > 5000 mg/kg.

The relative organ weight index had been used as another basic indicator to assess the deleterious effects of the plant metabolites. The effect of toxic substances on the internal organs could be identified by assessing the relative organ weight as the index that gives a preliminary insight to the swelling or damage caused by any harmful agents (Yam et al., 2013). After 72h of experiment, relative organs weights of mice were determined, however, there was no significant difference in organs mass between treatments. It proves that the extracts of *B. latisepala* did not cause swelling, inflammation, or damage to the organs of the laboratory mice. The pancreas was the only organ that experienced a loss of mass in the examined organs when the extract was administered to rats. This may contribute to the determination of the target of natural compounds, which is the pancreas of the experimental organism.

The hematological parameters of tested mice were also determined for the purpose of evaluating the overall effect of various extracts on the cellular composition of the blood, thereby determining the factors that ensure the homeostasis. The RBC, HGB, HCT values in female mice treated with *B. latisepala* extracts were not different from those in the control group, whereas male mice treated with hot water extracts, ethanol and methanol extracts had these values reduced in comparison with the control group. Specifically, the number of red blood cells (RBC) of mice treated with hot water extract, ethanol and methanol decreased by 9.34±0.94, 8.21±0.58, 8.06±0.36 mil/mL respectively compared with the control group 9.72±0.34 mil/mL ($p<0.05$). The Hb content of the control group was 15.40±0.89 g/dL, the treatment groups decreased in order of hot water (13.60±0.26 g/dL), ethanol (12.77±0.50 g/dL), and methanol (12.27±0.76 g/dL) ($p<0.05$). Similarly, mice treated with the extracts had a different decrease in HCT content compared with the control group. Reductions in these indices showed that the extract interfered with the normal production of Hb and its concentrations within RBCs. Thus, *B. latisepala* extract may possess the potential to induce anemia in male mice (Olorunnisola et al., 2012; Ferreira et al., 2014). Among the three experimental extracts, the methanol extract reduced more RBC, HGB, and HCT than the hot water and ethanol extracts. MPV of mice treated with methanol extract in both sexes increased compared with controls ($p<0.05$). PDW in female mice treated by methanol extract (11.17±4.02 µm3) also increased more than the control group (6.27±0.15 µm3). An increase in both platelet volume (MPV µm3) and platelet distribution width (PDW) due to platelet activation, resulting from platelet swelling and pseudopodia formation (Vagdatli et al., 2010). Therefore, it is observed that in female mice, methanol extract results in clotting and bleeding abnormalities.

The kidney and liver are useful in predicting toxicity effects of phyto-therapeutic products or drugs (Bello et al., 2016). Liver function parameters such as AST and ALT were determined, and the level of uric acid was also measured to assess the kidney function. High levels of AST and ALT enzymes were determined when the liver was diseased or hepatotoxicity (Nfozon et al., 2019). High level of uric acid in serum can be induced by acute kidney injury and reduced glomerular filtration rate (Giordano et al., 2015; Hahn et al., 2017). No differences were identified between the uric acid, AST

and ALT levels of control mice and mice that received the extracts. This indicates that hot water, ethanol and methanol extracts of *B. latisepala* did not cause any deleterious effects on mouse liver and kidney functions.

To more comprehensively evaluate the effects of the extract on the organs of the body, histological studies of the organs were performed. The results of histological analysis showed that the extracts did not have any obvious effect on the structure of the organs. The histology of heart, liver, kidney, pancreas, spleen, lung and gonads were normal and did not differ from histology of control mice.

CONCLUSIONS

B. latisepala extracts almost did not cause any significant toxicity resulting in death, nor produced any hematological, serum chemical alteration and histopathological derangements in experimental mice. The results from this acute oral toxicity study suggested that hot water, ethanol and methanol extracts of dried *B. latisepala* were relatively nontoxic and there was no adverse effect level of *B. latisepala* to be determined as 5000 mg/Kg body weight/day. However, further toxicity assessments such as subacute, chronic, or genotoxic studies using repeated doses of *B. latisepala* should be conducted to confirm its safety for a long-term use.

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AUTHOR CONTRIBUTIONS

Nguyen Trong Hong Phuc; conceptualization and design the experiment, supervision, editing and finalization

Pham Dong Hai; conceptualization and design the experiment, investigation

Phan Thanh Dat; design the experiment, investigation

Nguyen Thi Yen Lan; investigation, statistical analysis

Phung Thi Hang; analysis of plant samples

Dang Minh Quan; Investigation, methodology, formal analysis, manuscript preparation

CONFLICT OF INTEREST

We have no conflict of interest.

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