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Research Article

Oral acute toxicity study of ethanol extract of Mobe leaves (*Artocarpus lacucha* Buch-Ham) in Wistar rats

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Abstract

Many medicinal plants are now being chosen because the treatment is safer and cheaper. *Artocarpus lacucha* Buch-Ham is a plant with many pharmacological activities and is efficacious; its safety has not been studied. Acute oral toxicity evaluation followed Organization for Economic Co-operation and Development guidelines using the fixed dose method. The evaluation began with a preliminary test, then a primary test with three groups: a 2000 mg/Kg BW dose test group, a 5000 mg/Kg BW dose test group, and a control group. The results of visual observations, haematological and clinical examinations, and histological examination of organs (liver, spleen, kidneys, lungs and heart) showed no toxicity in the animals, and they did not die during testing. The findings of this study support the safety of *Artocarpus lacucha* Buch-Ham leaf ethanol extract, which did not produce harmful results in acute toxicity tests.

Keywords

Artocarpus lacucha Buch-Ham, acute toxicity, ethanol extract, plants, Wistar rats

Introduction

The use of natural ingredients for health in Indonesia has developed very rapidly. Various medicinal plants are now chosen as a safer treatment than treatment with chemical drugs (Purwaningsih et al. 2015; Wongon and Limpeanchob 2021). Medicinal plants can synthesize various chemical compounds used to cure various diseases. The World Health Organization (WHO) estimates that 50% of all medications used in medicine are made from natural sources, and around 80% of the world's population relies on medicinal plants. The plant world, rich in secondary metabolites, is the primary source of traditional medicines. Many scientists have tested medical plants' safety and therapeutic uses (Mohan 2018; Wyk and Prinsloo 2018; Ehilé et al. 2021). However, the active ingredients in therapeutic herbs may have adverse or even harmful effects on essential organs, whether or not they are used over a lengthy period (Ekor 2014). Numerous investigations on therapeutic herbs have shown their toxicity and adverse consequences, including clinical signs and symptoms in the nervous, gastrointestinal, and cardiovascular

systems (Kamsu-Foguem and Foguem 2014). Searches for medicinal plants with the potential to be developed into medicines must also adhere to the rules. Understanding drug toxicity mechanisms is essential in drug safety testing to provide a basis for drug risk assessment (Fielden and Kolaja 2008; Husori et al. 2018).

The leaves of *Artocarpus lacucha* Buch-Ham, a member of the *Moraceae* family and a well-known medicinal plant, are native to North Sumatra, Indonesia. Known as Mobe in Indonesia and sometimes nicknamed Monkey Jack. Tropical regions of south and southeast Asia, particularly Nepal, Sri Lanka, India, Myanmar, Indonesia, Vietnam, and Thailand, are home to large populations of this plant. There are many flavonoids and phenolic acids in this plant (Povichit et al. 2010; Gautam and Patel 2014; Hossain et al. 2016; Islam et al. 2019; Sitorus et al. 2022). According to research, this plant has various pharmacological effects that are useful as antimalarial, antiviral (for HSV and HIV), antituberculosis, antiatherosclerotic, antifungal, antiplasmodial, antidiabetic, wound healing, and anticancer due to the presence of active ingredients including artocarpin, oxyresveratrol, phenol, and flavonoids (Saowakon et al. 2009; Jagtap and Bapat 2010; Singhatong et al. 2010; Phoolcharoen et al. 2013; Teanpaisan et al. 2014; Nazliniwaty et al. 2021; Hanafiah et al. 2022; Sitorus et al. 2022). The heartwood has been used as a traditional anthelmintic medicine (Gautam and Patel 2014; Nazliniwaty et al. 2022).

However, the safety of this plant is not known even though it has many benefits. We carried out toxicity effects tests to see whether this plant meets the requirements of a drug regarding its effectiveness and safety (Raynor et al. 2011; Boas et al. 2018; Park et al. 2020; Waruwu et al. 2022). We used the OECD (Organization for Economic Co-operation and Development) guidelines with the fixed dose method, to evaluate toxicity. Female rats were used to evaluate acute oral toxicity because they are more sensitive (OECD 2001).

Materials and methods

Preparation of plant extracts

The dry powder of *Artocarpus lacucha* Buch-Ham leaves was extracted using a maceration process and 96% ethanol (Merck). The filtrate was collected and a viscous extract was obtained by evaporation under low pressure and then aerated to dry (Harahap et al. 2018; Dalimunthe et al. 2022).

Preparation of *Artocarpus lacucha* Buch-Ham ethanol extract suspension

The ethanol extract suspension of *Artocarpus lacucha* Buch-Ham was carried out in the following way: 6000 mg of the extract was put into a mortar, and the developed Na-CMC suspension was added (Na-CMC 0.125 g was put into 10 ml of hot distilled water), then homogenized, then add distilled water up to 30 ml. Extract suspension preparation was carried out every day during testing.

Animals

The acute toxicity test in this study used 20 female Wistar rats (*Rattus norvegicus* L.) weighing 150–180 grams—five for the preliminary test and 15 for the primary test. Rats were kept in temperature-controlled spaces with access to food and water. The animal must be housed in the finest conditions possible, in a clean, well-ventilated cage, two weeks before testing. Each set of animals was separated and treated individually for each experiment, with one tail per cage to make observation easier. All animal operations and treatments were performed at room temperature (between 20 and 22 °C), and additional precautions were made to prevent environmental disturbances that may alter the animals' reactions. Prior to treatment, rats were fasted, and their weight was measured.

Ethical clearance

This study's experimental techniques were all carried out by OECD recommendations. This research adhered to the recommendations and received clearance from the Faculty of Mathematics and Science at the Universitas Sumatera Utara's Animal Research Ethics Committee (AREC) under the designation 0226/KEPH-FMIPA/2023.

Acute toxicity evaluation

Evaluation of acute toxicity using the fixed dose method was based on OECD guidelines. The evaluation begins with a preliminary test to determine the dose for the main trial. This test used one female rat in each dose group. The initial dose in the preliminary test was chosen from the fixed-dose levels 5, 50, 300, and 2000 mg/kg BW, which are the doses expected to cause toxic effects. Observations are carried out for 24 hours; if there were no toxic symptoms or death, the evaluation could be continued with the primary test (OECD 2001; Waruwu et al. 2022).

The test was continued with the primary test based on the initial test dose, using female rats consisting of five rats in each group. The division of the test animal groups is as follows:

C: Control, given CMC Sodium 0.5%
TI: Treatment, given test preparations at a dose of 2000 mg/Kg BW
TII: Treatment, given test preparations at a 5000 mg/Kg BW dose.

The test preparation was administered orally using an oral probe, and symptoms of toxicity were observed in each group and compared with controls. Toxic symptoms recorded include hair and skin, eyes, saliva, breathing, urine (colour), stool consistency, somatomotor activity

and behavioural patterns, sleep, mucous membranes, convulsions and tremors, itching, coma, and death (Vinay et al. 2021).

Monitoring for signs of acute toxicity

Clinical symptoms were monitored during the first 24 hours after therapy until the next 14 days. If an animal dies within this period, it is recorded, and an autopsy and observation are immediately carried out (Dalimunthe et al. 2022). Body weight changes were analyzed once a week. Animals alive at the end of the experiment were measured, killed, and autopsied.

Observation of haematology and biochemical analysis

The animal's neck was dislocated, then blood was taken slowly through the heart (intracardiac) using a sterile syringe as much as 1–3 ml, and then a haematological examination was carried out. Separately, 1 ml of blood was centrifuged for 10 minutes at 3000 rpm until serum was produced and examined for clinical and biochemical levels.

Observation of organs and histological examination

Animal organs, including the liver, spleen, kidney, lung, and heart, were cleaned to check their colour, consistency, and surface. In order to calculate the relative weight, it is dried and weighed as follows:

Relative weight = Organ weight / Animal weight

Immediately after being separated, the organs were immersed in a 10% formalin buffer solution, where histopathological preparations using hematoxylin-eosin staining were produced and inspected under a microscope (Anto et al. 2022; Satria et al. 2022).

Result

Observation of toxic symptoms in the preliminary test

Based on our observations, the animals showed normal activity. They did not show symptoms of toxicity or death in either the control group or the highest dose test group (2000 mg/Kg BW) after the preliminary test so that evaluation of acute toxicity could be continued in the primary test.

Observation of toxic symptoms in the primary test

Table 1 shows that the animals had normal activities and did not show toxic symptoms in the control and treatment groups after administering the ethanol extract of *Artocarpus lacucha* Buch-Ham leaves. No animals died when the test preparation was within the 14-day observation period.

Table 1. Observation results of toxic symptoms in the primary test.

Parameters	Groups														
	C	TI	TII	C	TI	TII	C	TI	TII	C	TI	TII	C	TI	TII
	1 hour			24 hour			48 hour			7th day			14th day		
Fur and skin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Eyes	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Salivation	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Respiration	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Urination (colour)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Feces consistency	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Somatomotor activity and behavior pattern	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sleep	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Mucous membrane	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Convulsions and tremors	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Itching	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Coma	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Mortality	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

C: Normal control group treated with CMC Sodium 0.5%, TI: Treatment group treated with 2000 mg, TII: Treatment group treated with 5000 mg, – : No signs of toxicity were found.

Body weight monitoring

Each rat's body weight was measured before and after being administered the ethanol from *Artocarpus lacucha* Buch-Ham leaf extract. Table 2 demonstrates no difference in weight growth between the groups given the ethanol extract of *Artocarpus lacucha* Buch-Ham leaves, and the control group ($p > 0.05$), which indicates that the ethanol extract of *Artocarpus lacucha* Buch-Ham leaves given to rats had no impact on their weight development.

Table 2. Rat's body weight was affected by *Artocarpus lacucha* Buch-Ham leaves in research on acute oral toxicity.

Groups	Units	Body weight (Mean ± SD)		
		1st day	7th day	14th day
C	g	160 ± 0.70	164 ± 1.22	167 ± 1.58
TI	g	161.2 ± 1.30	163 ± 1.58	166.8 ± 1.64
TII	g	160.78 ± 0.91	163.4 ± 1.34	167.9 ± 1.02

Values are expressed as Mean ± SEM. C: Normal control group treated with CMC Sodium 0.5%, TI: Treatment group treated with 2000 mg, TII: Treatment group treated with 5000 mg.

Haematological observations

The haematological parameters in rats shown in Table 3 show significant differences in MCHC and Neutrophil levels between the 2000 mg/Kg BW group and the control ($p < 0.05$). There were no significant differences between the control and test groups in other haematological measurements ($p > 0.05$).

Observation of biochemical analysis

As seen in Table 4, the biochemical analysis showed no significant difference ($p > 0.05$).

Table 3. Results of haematological observations.

Parameters	Unit	Groups (Mean ± SD)			Reference Value (CLS)
		C	TI	TII	
Hemoglobin (Hb)	g/dL	15.24 ± 0.58	14.95 ± 1.01	14.32 ± 2.04	13.7–16.8
Hematocrit (HCT)	%	45.02 ± 3.03	43.16 ± 3.31	44.52 ± 4.26	37.9–49.9
White blood cells (WBC)	10^3/µL	3.52 ± 0.97	3.99 ± 1.70	4.7 ± 1.57	1.13–7.49
Red blood cells (RBC)	10^6/µL	8.33 ± 0.93	7.51 ± 0.27	8.36 ± 0.66	7.07–09.03
Platelet	10^3/µL	781.4 ± 115.4	787.4 ± 67.54	871.2 ± 63.94	680–1200
Mean corpuscular volume (MCV)	fL	54.14 ± 3.29	52.78 ± 2.49	55.86 ± 1.42	49.9–58.3
Mean corpuscular hemoglobin (MCH)	pg	19.09 ± 0.87	19.8 **± 0.56**	18.98 ± 1.22	17.8–20.9
Mean corpuscular hemoglobin concentration (MCHC)	g/dL	33.9 ± 1.5	37.02 ± 1.24	34.9 ± 2.27	33.2–37.9
Eosinophils	%	1.22 ± 0.49	1.99 ± 0.91	2.78 ± 1.29	0.5–4.5
Monocytes	%	2.16 ± 1.03	2.58 ± 1.32	2.66 ± 1.24	0.8–3.9
Basophils	%	0.36 ± 0.34	0.56 ± 0.32	0.52 ± 0.22	0–0.8
Limphocytes	%	67.02 ± 2.96	60.32 ± 6.16	64.38 ± 2.27	62.2–90
Neutrophils	%	18.3 ± 5.31	26.18 ± 3.93	14.9 ± 1.81	7.1–33.2

Values are expressed as Mean ± SEM. C: Normal control group treated with CMC Sodium 0.5%, TI: Treatment group treated with 2000 mg, TII: Treatment group treated with 5000 mg.

Table 4. Observation results of biochemical parameters.

Parameters		Units	Groups (Mean ± SD)			Reference Value (CLS)
			C	TI	TII	
Liver function	Total protein	g/dL	6.41 ± 0.72	5.66 ± 0.08	6.29 ± 0.53	5.5–7.7
	Billirubin direct	mg/dL	0.04 ± 0.02	0.04 ± 0.01	0.05 ± 0.01	0.03–0.06
	SGOT/AST	U/L	102.2 ± 24.19	104.8 ± 23.34	108 ± 8.51	65–203
	SGPT/ALT	U/L	40.4 ± 4.72	43 ± 2.24	38.4 ± 8.17	16–48
	Alkaline phospatase	U/L	127.6 ± 8.5	133.4 ± 2.97	139.4 ± 19.42	65–203
Kidney function	Urea	mg/dL	21.72 ± 1.54	18.6 ± 1.21	19.28 ± 2.96	13.2–27.1
	Creatinine	mg/dL	0.35 ± 0.08	0.33 ± 0.03	0.42 ± 0.08	0.2–0.6

Values are expressed as Mean ± SEM. C: Normal control group treated with CMC Sodium 0.5%, TI: Treatment group treated with 2000 mg, TII: Treatment group treated with 5000 mg.

Observation results of organs

Organ observations based on Table 5 that have been carried out include observing the colour, surface and consistency of the liver, kidneys, heart, spleen and lungs, showing no differences between the test and control groups.

Table 5. Observation of colour, surface, and consistency of organs.

Organs	Group	Colour	Surface	Consistency
Liver	C	Deep red	Slippery	Chewy
	TI			
	TII			
Heart	C	Red brown	Slippery	Chewy
	TI			
	TII			
Spleen	C	Red brown	Taper	Chewy
	TI			
	TII			
Lungs	C	Red brown	Taper	Chewy
	TI			
	TII			
Kidney	C	Red brown	Slippery	Chewy
	TI			
	TII			

C: Normal control group treated with CMC Sodium 0.5%, TI: Treatment group treated with 2000 mg, TII: Treatment group treated with 5000 mg.

Based on Table 6, the relative organ weight ratio parameters between the control and treatment groups were not significantly different ($p > 0.05$).

Table 6. Observations on relative organ weights.

Organs	Relative organ weight (%)		
	C	TI	TII
Liver	3.75 ± 0.34	4.83 ± 1.89	4.25 ± 0.52
Heart	0.71 ± 0.22	0.86 ± 0.04	0.81 ± 0.09
Spleen	0.81 ± 0.19	0.99 ± 0.08	0.984 ± 0.08
Lungs	1.85 ± 0.41	1.99 ± 0.08	1.89 ± 0.04
Right Kidney	0.77 ± 0.2	0.79 ± 0.05	0.71 ± 0.02
Left Kidney	0.82 ± 0.33	0.83 ± 0.04	0.69 ± 0.04

Values are expressed as Mean ± SEM. C: Normal control group treated with CMC Sodium 0.5%, TI: Treatment group treated with 2000 mg, TII: Treatment group treated with 5000 mg.

Fig. 1 displays the findings of a microscopic study of the organ. The control and test groups had normal tissue conditions based on microscopic examination of liver tissue. There is no hydropic necrosis or degeneration, and instead, the hepatocytes are distributed radially within the liver lobules. Kidney tissue from the control and test groups both had normal tissue conditions. Cardiac histology also revealed that cardiac muscle cells in the control and test groups were not harmed and had normal myocyte and myofibril structures. The organization of the white and red pulp parenchyma appeared normal when the spleen tissues of the control and test groups were examined. On viewing the lungs, there was no inflammatory cell infiltration, oedema, or congestion, confirming that the lung tissue is still normal after treatment with the leaf extract.

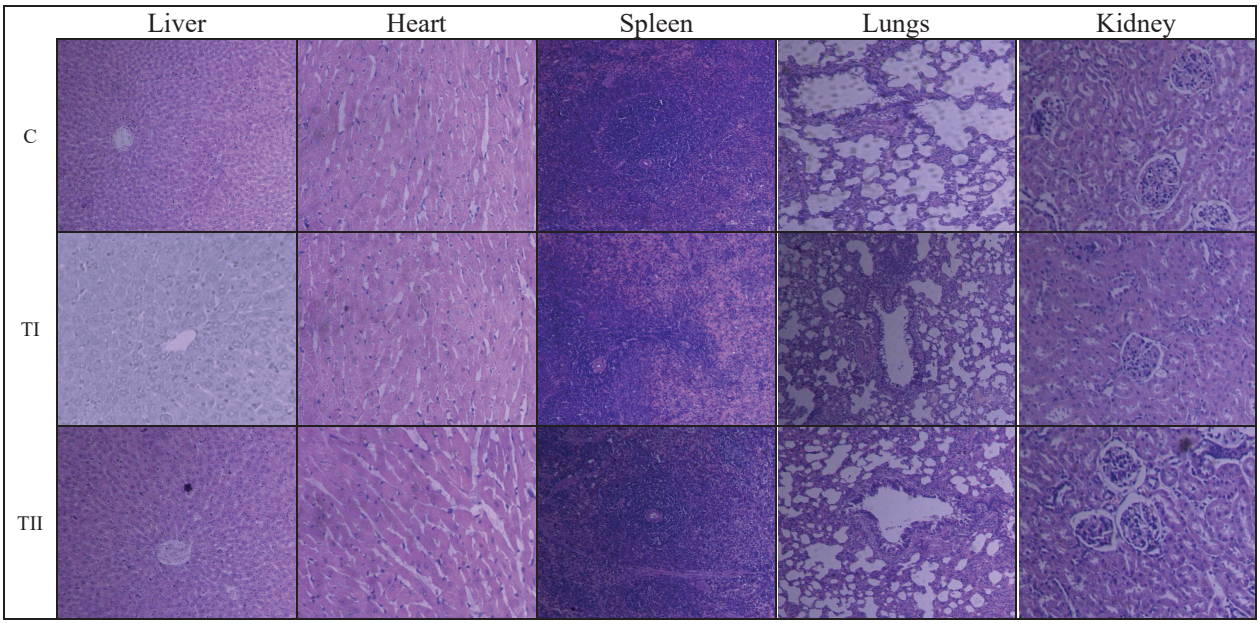

Figure 1. Organ histopathology (Staining: Hematoxylin and eosin). (C: Normal control group treated with CMC Sodium 0.5%, TI: Treatment group treated with 2000 mg, TII: Treatment group treated with 5000 mg).

Discussion

Artocarpus lacucha Buch-Ham contains phenolic compounds, including flavonoids, phenolic acids, or phenolic derivatives. In addition, according to research, it has many beneficial pharmacological activities (Gautam and Patel 2014; Sitorus et al. 2022). Testing of toxic effects in acute toxicity carried out in this study proves its safety. The principle of acute toxicity testing is to look for toxic effects that appear quickly after administering the test preparation orally in a single dose or several doses over 24 hours (OECD 2001; BPOM 2022). The substance being evaluated was administered once, twice, or more within 24 hours to perform an acute toxicity test. This preclinical test aims to measure a substance's toxicity level within a certain period of time after administering a single dose. A quantitative benchmark often used to express the lethal dose range in acute toxicity tests is LD_{50} (Dibua et al. 2022).

After administration of *Artocarpus lacucha* Buch-Ham ethanol extract leaves at 2000 and 5000 mg/Kg BW doses, no animals died while administering the test preparation during the 14-day observation period. According to OECD (2001), if no deaths occur at a 5000 mg/Kg BW dose, the LD_{50} value is more than > 5000 mg/Kg BW and is included in the practically non-toxic criteria. Death is a state of dying or the inability to survive (OECD 2001; Satria et al. 2022). Animals also had normal activity, and no toxic symptoms were seen in any dose of the treatment group. Dosage determines the toxic properties of a compound. Increasing the dose will usually cause more organ systems to be affected and will have very different working effects (Kruk et al. 2022).

Body weight is a sensitive indicator of toxic symptoms and said to be toxic if there was a change in body weight of up to 10% (Bhardwaj and Gupta 2012; Hidayat et al. 2022; Oriakhi and Ikponmwosa-Eweka 2023). Based on statistical tests, there was no real change in body weight growth. The rat's body weight did not fluctuate more than 10%, either up or down, indicating that administration of *Artocarpus lacucha* Buch-Ham leaf ethanol extract at doses of 2000 and 5000 mg/Kg BW did not affect the rat's body weight growth (OECD 2001).

Furthermore, there were no significant differences in blood biochemical parameters. A good blood profile and its components within the normal range will indicate that the body is in good physiological health (Astuti et al. 2022). Damage to liver and kidney cells will affect blood biochemical values. The SGPT and SGOT enzyme values measure damage to liver cells or liver tissue. Damage that has occurred to liver cells causes increased levels of liver enzymes in the bloodstream, which are used to assess liver activity; this occurs due to disruption of the structure and function of the liver cell membrane. In cases where liver inflammation has resulted in damage, there is an increase in ALT activity earlier and more rapidly compared to AST levels (Kim and Wu 2020; Fu et al. 2023).

Meanwhile, changes in creatinine and urea are markers of kidney cell damage (Albrakati 2021; Luft 2021). Significant differences in MCHC and neutrophil levels did not affect the results of the haematological examination. Based on Charles River Laboratories, blood chemistry measurements used in acute toxicity tests are still within the normal range (CRL 1998; Harahap et al. 2018).

Macropathological examinations of the liver, kidneys, heart, spleen, and lungs showed no significant colour differences compared to the control group. There was also no significant difference in the relative organ weight ratio compared to the control group. Changes in an organ's colour

and weight can indicate toxic consequences. The purpose of the organ observations that have been carried out is to collect data about the toxicity of a test chemical for the particular organ being studied and its impact on that organ (Farag et al. 2015; Elizalde-Velázquez and Gómez-Oliván 2021).

The histopathological picture was still typical in both the control and test groups. In the liver, hepatocytes were arranged radially in the hepatic lobules, and no hydropic degeneration or central venous necrosis was seen. The gaps between these plates contain capillary sinusoids called hepatic sinusoids. Sinusoids are blood vessels that expand irregularly and consist of only one continuous layer of endothelium (Junqueira et al. 2016). The kidney histology observed in the control group and those given an ethanol extract of *Artocarpus lacucha* Buch-Ham leaves was still normal. Bowman's capsule, proximal tubule, distal tubule, and glomerulus appeared healthy, which shows that the ethanol extract of *Artocarpus lacucha* Buch-Ham is not harmful to the kidneys (Razmpoosh et al. 2020; Albrakati 2021). Histological observations of the heart, including myocytes and myofibrils, showed no damage to cardiac muscle cells. Cardiac muscle cells show the typical shape of myocytes and myofibrils. Lung tissue was examined and shown to be normal; there was no inflammatory cell infiltration, oedema, or congestion. Observations on the spleen also showed that the white and red pulp parenchyma appeared normal (Beegam et al. 2020; Belbellaa et al. 2020; Karami et al. 2022); which indicates that administration of *Artocarpus lacucha* Buch-Ham leaf ethanol extract at a dose of 2000 and 5000 mg/Kg, BB does not cause any harmful effects on body organs.

Conclusion

The results of this study prove the safety of the ethanol extract of *Artocarpus lacucha* Buch-Ham leaves in rats. No animals died after administering extract doses of 2000 and 5000 mg/Kg BW. The LD_{50} is > 5000 mg/Kg BW, included in the practically non-toxic criteria. The changes in body weight that have been observed are less than 10%, and they also have a good blood profile and are within normal limits. Macroscopic and histopathological observations of the organs were still good and showed no differences with the control group. For the future, more research is suggested to estimate the consequences of the leaf extract applications in the pharma industry.

Conflict of interest

The authors have no conflicts of interest regarding this investigation.

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