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FULL PAPER

Safety assessment of *Lepisanthes fruticosa* extract in experimental rats

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Abstract

The toxicity study of *Lepisanthes fruticosa* (ceri Terengganu) was carried out using a repeated dose 28-days oral toxicity method on female *Sprague Dawley* (SD) rats. About twenty-four female SD rats with an average body weight of 116.27±11.95 g were divided into four groups. The control group (C) was given a ground normal pellet while treatment groups were given *L. fruticosa* extract incorporated with ground pellet with different dosages (low dose 0.5 g/kg (CEL), medium dose 1.0 g/kg (CEM) and high dose 3.0 g/kg (CEH)). Each rat was given a 25 g pellet per day. Upon arrival, all rats were placed in individual cages and given drinking water via *ad-libitium*. Rats were acclimatized for seven days before treatment started. On day 29, after 12-15 hrs of fasting, rats were euthanized using CO_2 and blood samples were taken from the vena cava for further blood and serum analysis. After 28 days of treatment, the body weight of rats in all treated groups was increased with no significant difference. Administration of *L. fruticosa* extract did not cause any negative effect on blood haematology. Findings from the serum biochemical test showed that the consumption of the extract did not result in liver and kidney failure since no significant changes were observed in enzymatic and blood parameters. Based on these findings, the *L. fruticosa* extract was safe to be taken even at a high dose (3.0 g/kg) as no experiment-related mortality and no toxicity symptom occurred on the experimental animal.

1. Introduction

Malaysia has a large diversity of underutilized fruits that grow wild in the region of Peninsular Malaysia, Sabah and Sarawak. Some of the underutilized fruits are rarely eaten, unknown and unfamiliar. The diversity of our underutilized fruit species has not been fully exploited. Many of them have a broad spectrum of flesh and skin colour. These underutilized fruits may have potential benefits to human health. It is important to include these fruits in health promotion campaigns. Certain of our underutilized plants/fruits and their derivatives have long been used as traditional medicine in Malaysia. Therefore, these fruits have the potential to be used as a functional food ingredient and/or processed as food products for local consumption. There are many kinds of underutilized fruits that are available in Malaysia, such as nam-nam (*Cynometra* sp.), bacang (*Mangifera* sp.), jambu bol (*Psidium* sp.), durian (*Durio* sp.), bidara (*Ziziphus* sp.), mertajam (*Leppisanthes* sp.), longan (*Dimorcarpus* sp.), lenggeng serawak (*Pometia* sp.), belimbing buloh (*Averrhoa* sp.), asam kelubi (Salacca sp.), buah melaka (Phyllanthus sp.), asam gelugor (*Garcinia* sp.), sentol (*Sandoricum* sp.), rambai Sarawak (*Baccaurea* sp.), remia (*Bouea* sp.), pulasan (*Nephelium* sp.) and others. These fruits are usually grown in orchards or fruit gardens around houses, and some grow wild in the rainforest.

Other than that, ceri Terengganu, its scientific name is *Lepisanthes fruticose.* The fruit is a globose berry and the flesh of the fruit taste sweet but a little tart. This fruit has been categorized as underutilized fruit due to its under-exploited potential toward contributing to food security, health, nutritional, medicinal, income generation and environmental service. A previous study has reported on the phytochemical and antioxidant activity of *L. fruticosa* extract (Mirfat *et al.,* 2017). It was found that *L. fruticosa* extract has a high content of phenolic and flavanoid with good antioxidant activity.

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For that, *L. fruticosa* extract might be useful as functional food/ingredient in future research.

In order to develop a new by-product from *L. fruticosa*, it is important for the researcher to determine the safety of the fruits. A risk assessment is a method to determine the adverse effect of any new or uncommonly edible extract/plant including the safe dosage that can be used. The previous in vitro cytotoxicity study carried out by Hadijah *et al.* (2020) reported the safety of dried *L. fruticosa* and its water extract. To date, the safety data of *L. fruticosa* using in vivo method is still lacking. Therefore, the safety/toxicity evaluation study was carried out on *L. fruticosa* extract to determine the toxicity effect of the extract on normal *Sprague-Dawley* rats.

2. Materials and methods

2.1 Sample preparation

Lepisanthes fruticosa were sampled at the ripe stage (index 8) from MyGeneBank™ research plot at MARDI, Malaysia. The fruits were separated from seeds and the edible portion was then cut into small pieces and dried at 40°C for 3 days. Dried samples were ground and sieved with 1mm mesh. The *L. fruticosa* powder was then extracted using deionised water (1:10 w/v). The mixture was shaken using an orbital shaker at room temperature for 12 hrs and filtered through a thin cloth. The separated solid was re-extracted two times and the filtrate was combined and concentrated using a rotary evaporator at 40°C. The concentrated crude extracts were added with maltodextrin at final content of not more than 40% in the solid crude extract. The mixture was lyophilized to powder and stored at -20°C.

2.2 Animal husbandry

All procedures concerning the use of animals were approved (20170717/R/MAEC00020) by the Animal Ethics Committee MARDI (AEC-MARDI). The animals were housed in a controlled environment, with a temperature of 24±2°C and relative humidity of 30-70%. The rooms were illuminated with 12 hrs artificial fluorescent light and 12 hrs darkness per day. The animals were provided with a standard pelleted laboratory animal diet and distilled water *ad libitum*. The animals were allowed to acclimatize for seven days before the treatment started.

2.3 Sub-acute study

The toxicity study of *L. fruticosa* extract was carried out using a repeated dose 28-days oral toxicity method (OECD 407) on female *Sprage-Dawley* (SD) rats. About 24 female SD rats with an average initial body weight of 116.27±11.95 g were divided into four groups. The control group (C) was given a ground normal pellet while the treatment group were given CE powder incorporated with ground pellet with different dosage (low dose 0.5 g/kg, medium-dose 1.0 g/kg and high dose 3.0 g/kg). Each rat was given a 25 g pellet per day. Upon arrival, all rats were placed in individual cages and given drinking water via *ad-libitium*. Rats were acclimatized for seven days before treatment started. On day 29, after 12-15 hrs fasted, rats were euthanized using carbon dioxide gas and blood sample were taken from the vena cava for further analysis.

2.4 Blood analysis

The rats fasted for 12-15 hrs and blood samples were withdrawn from the posterior vena cava. Samples of blood for haematological and biochemistry analyses were withdrawn right after being euthanized by carbon dioxide gas.

For the evaluation of biochemical parameters, one aliquot of blood per animal was placed in a 5 mL Z-serum tube (Bacton Dickinson, BD Vaccutainer) and centrifuged at 4500 rpm for 10 mins and serum was collected for further enzymatic and clinical analysis namely alanine transferase (ALT), aspartate aminotransferase (AST), total protein (TP) and albumin (ALB). All parameters were measured using a Blood Clinical Analyzer (DIRUI CS-300, China). The reagents for the tests were obtained from Randox (Randox Laboratories Ltd, Antrim, United Kingdom). While blood kept in the K_3EDTA container was used to analyse the full blood profile namely red blood cell (RBC), hematocrit (HCT), platelet (PLT), white blood cell (WBC) and haemoglobin (HGB) using Exigo Blood Hematology Analyzer, Sweden.

2.5 Statistical analysis

The completely randomized design (CRD) was used in the experiment and all data were analysed using ANOVA/Duncan (SAS V9.3). All values are expressed as group mean ± standard error of the mean (SEM). The data is considered statistically significant at $p < 0.05$.

3. Results

After 28 days, the bodyweight of rats in all groups showed an increment with no significant difference between *L. fruticosa* extract (Figure 1). This is a positive indicator in toxicology studies and has proven a good health status and no toxicity effect. The decreased body weight was one of the toxicity symptoms. There was no experiment-related mortality of animals at any dose level tested within a 28-day repeated feeding study of *L. fruticosa*. In addition, there were no abnormalities with

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respect to hair coat, eye colour, rashes and skin irritation. Besides body weight, organ weight is also one of the most sensitive toxicity indicators. Five organs were weighed namely the heart, kidney, spleen, lung and liver. Table 1 shows relative organ weight in rats treated with different *L. fruticosa* dosage. The results showed that there were no significant differences in all organ weights between the treated and control group.

The result of the haematology examination is shown in Table 2. As for rats treated with *L. fruticosa* extract, all haematology parameters were not significantly different as compared to control groups. The enzymatic analysis (ALT, AST, ALP), TP and ALB for liver function test in Table 3 is considered as no liver damage. The levels for these parameters did not show any significant differences between the control and all treatment groups. Rats treated with a medium dose of *L. fruticosa* (CEM) showed a decreased value in ALB, but according to Petterino and Argentino-Sterino (2006), the decreased value of ALB (37.46±1.87g/ L in medium dose were still within the normal value range (ALB 32-47g/L).

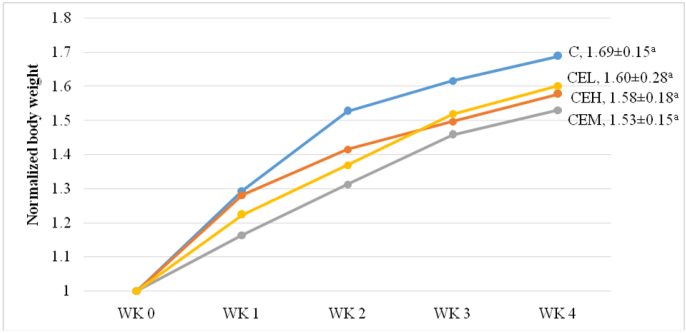

Figure 1. Normalized body weight of rats treated with *L. fruticosa* u extract (n = 6). Values with different superscript are significantly different (p<0.05). C: control dose, CEL: *L. fruticosa* extract low dose, CEM: *L. fruticosa* extract medium dose, and CEH: *L. fruticosa* extract high dose.

Table 1. Percentage of organ relative weight of rats treated with *L. fruticosa* extract (n = 6)

Analysis	C	CEL	CEM	CEH
Heart	4.11±0.10^{a}	3.82±0.20^{a}	4.02±0.15^{a}	4.02±0.25^{a}
Liver	3.07±0.45^{a}	3.15±0.46^{a}	3.13±0.42^{a}	3.17±0.40^{a}
Lung	0.78±0.18^{a}	0.74±0.07^{a}	0.81±0.06^{a}	0.71±0.09^{a}
Spleen	0.22±0.01^{a}	0.24±0.01^{a}	0.21±0.04^{a}	0.20±0.02^{a}
Kidney	0.82±0.05^{a}	0.84±0.04^{a}	0.84±0.06^{a}	0.84±0.04^{a}

Values with different superscript within the same row are significantly different (p<0.05). C: control dose, CEL: *L. fruticosa* extract low dose, CEM: *L. fruticosa* extract medium dose, and CEH: *L. fruticosa* extract high dose.

Table 2. Blood haematology of rats treated with *L. fruticosa* extract (n = 6)

Analysis	C	CEL	CEM	CEH
RBC (10^{12}/L)	9.55±0.66^{a}	9.30±0.69^{a}	10.10±0.64^{a}	10.03±1.14^{a}
HCT (%)	50.62±2.61^{a}	50.27±4.21^{a}	54.28±2.37^{a}	53.63±4.29^{a}
PLT (10^{9}/L)	1017.75±202.93^{a}	982.40±161.45^{a}	1060.00±285.66^{a}	1027.20±247.34^{a}
WBC (10^{9}/L)	8.08±1.33^{a}	9.05±1.61^{a}	9.55±2.14^{a}	9.07±1.73^{a}
HGB (g/L)	17.88±1.12^{a}	17.81±1.44^{a}	18.81±0.66^{a}	18.68±1.55^{a}

Values with different superscript within the same row are significantly different (p<0.05). C: control dose, CEL: *L. fruticosa* extract low dose, CEM: *L. fruticosa* extract medium dose, CEH: *L. fruticosa* extract high dose, RBC: red blood cell, HCT: hematocrit, PLT: platelet, WBC: white blood cell, HGB: hemoglobin

Table 3. Liver function analysis of rats treated with *L. fruticosa* extract (n = 6)

Analysis	C	CEL	CEM	CEH
ALT (U/L)	37.80±7.08^{a}	38.80±4.55^{a}	38.83±3.37^{a}	33.20±4.92^{a}
ALP (U/L)	181.00±34.42^{a}	166.50±30.23^{a}	178.50±17.40^{a}	173.20±33.45^{a}
AST (U/L)	98.00±11.98^{a}	109.00±13.22^{a}	96.00±7.56^{a}	93.83±13.73^{a}
TP (g/L)	79.64±4.50^{ab}	80.64±2.50^{a}	75.45±3.22^{b}	81.13±3.60^{a}
ALB (g/L)	41.29±2.46^{a}	40.63±1.71^{a}	37.46±1.87^{b}	41.77±2.75^{a}

Values with different superscript within the same row are significantly different (p<0.05). C: control dose, CEL: *L. fruticosa* extract low dose, CEM: *L. fruticosa* extract medium dose, CEH: *L. fruticosa* extract high dose, ALT: alanine transferase, ALP: alanine phosphatase, AST: aminoaspartate transferase, TP: total protein, ALB: albumin

Table 4. Kidney function analysis of rats treated with *L. fruticosa* extract (n = 6)

Analysis	C	CEL	CEM	CEH
Urea (mmol/L)	6.72±0.71^{a}	7.00±1.12^{a}	6.93±0.93^{a}	7.06±0.50^{a}
Creatinine (µmol/L)	77.00±5.14^{a}	76.60±3.43^{b}	74.83±5.53^{a}	80.67±7.14^{a}

Values with different superscript within the same row are significantly different (p<0.05). C: control dose, CEL: *L. fruticosa* extract low dose, CEM: *L. fruticosa* extract medium dose, CEH: *L. fruticosa* extract high dose.

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To determine the kidney function, two parameters were analyzed namely urea and creatinine. As presented in Table 4, rats treated with *L. fruticosa* extracts showed no significant difference in both urea and creatinine values as compared to the control group.

4. Discussion

The toxicology evaluation study was carried out to ensure that *L. fruticosa* extract is safe and does not affect normal body function. According to Perk *et al.* (2013), safety and toxicity study involving plants and their extracts has become the main focus among researchers. A safety study is an important tool in establishing the safety and effectiveness of new plants and their byproducts. The toxicity determination through a 28-days repeated dosage test was a basic assessment in various safety/toxicity studies (Yamakoshi *et al.,* 2002). An early indicator in determining whether the substance is toxic or safe is through physical observation at the beginning of the study period such as hair loss, dry hair, redness in the skin, bleeding, diarrhoea, behavioural changes like weakened or violent and/or death (Delaney *et al.,* 2008).

There was neither mortality nor emergence of any toxic symptoms seen in rats given *L. fruticosa* extract even in high dose (CEH) throughout the study period. Bodyweight data in this study show a positive weight gain and there was no reduction in weight recorded. Bodyweight is early detection if there is any toxic effect in the sample. According to Teo *et al.* (2002), weight changes are used as the main indicator of negative effects in the study materi*al.* Any changes to body weight are usually due to some physiological changes such as drastic changes in liver function and hormones. It is also due to the malabsorption of protein and amino acids (Perk *et al.,* 2013). Repeated exposure to toxic material will cause weight loss. Weight loss indicates a negative reaction between rats and studied samples (El Hilaly *et al.*, 2004). After 28 days of repeated dosage of *L. fruticosa*, the bodyweight of treated rats was not significantly different from the control group. This is a positive sign for body weight and proves that *L. fruticosa* did not affect any physiological activity in the body.

Other than body weight, organ weight is also one of the important parameters in the indication of toxic effects. Organ weight measurement is another important guide to assess general toxicity. Changes in organ weight are an indicator of toxicity since organ weight will be affected by the suppression of body weight (Heywood, 1983). The purpose of determining organ relative weight is because the bodyweight of animals normally will vary within the group, so each organ weight should be related to the bodyweight of the animal from which it was removed (Heywood, 1983). In the toxicity assessment involving *L. fruticosa* extract, organ relative weight for each dosage did not differ much and produced any significant difference as compared to the control group. When any significant difference occurred in organ relative weight between the treatment and control group, further inspection needs to be done to confirm the toxicity effect of the substance or any organ failure. Any reduction of body and internal organ weight that occurred during the experimental period were strongly related to toxic substance exposure. According to Farah Amna *et al.* (2013), two main organs that are considered useful in toxicity studies are the liver and kidney. These organs play an important role as toxic indicators and are very sensitive in predicting toxicity. Both organs will affect the serum enzymatic parameters that lead to histopathological changes. It is also known that the liver is a primary detoxification organ. Any substance ingested will go through the liver for metabolism reaction (De Souza *et al.,* 2015). The blood clinical analysis showing level of enzymatic and other parameters are important tool in determining the organ status, which correlates with the safety of the extract tested.

Analysis involving blood was performed to prove the safety of the sample. The analysis involved blood haematology and blood clinical analysis. According to Adeneye *et al.* (2006), the haematology system is one of the most sensitive targets for toxic compounds. It is also a major index of physiological and pathological status in humans and animals. Blood haematology assessment is closely related to the assessment of toxicity risks in drastic changes in blood parameters such as red blood cells, white blood cells, platelets and so on (Olson *et al.,* 2000). Blood is a major transport in the body and is also involved in the body's metabolic process. Any changes in blood parameters were related to the body's response to any stress or deprivation. In this study, haematology parameters measured in Table 2 did not differ significantly between the treated and control groups. These results can imply that *L. fruticosa* extract did not have a toxic effect on haematopoiesis and blood cell counts.

In blood clinical analysis, the parameter analysed was divided into two which were the liver function and the kidney function test. Table 3 lists the enzymes for liver function namely ALT, AST and ALP and also other parameters for the liver that as TP and ALB. It is shown that *L. fruticosa* extract given to the rats even in high doses did not affect the normal value on each parameter. The malfunction of the liver was associated with an increasing level of enzyme ALT, AST and ALP. The AST enzyme will be released in blood circulation when

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it is damaged or injured in an organ such as the liver and heart (Patel *et al.*, 2008). The hepatic damage or changes in the hepatocyte membrane is associated with a high level of ALT and AST in blood circulation (Perk *et al.,* 2013). In addition, another sign of liver damage/injury is the decreased protein level. The level of TP and ALB in the blood is used to evaluate the synthesis function in the liver. Decreased level of TP is due to protein loss, it might be due to either malabsorption, poor nutrition or kidney or liver injury. Since the level of hepatic biomarkers and liver relative weight did not differ from the control group, it can be concluded that *L. fruticosa* extract did not induce hepatotoxicity.

Other than the liver, the kidney is another vital organ in toxicity studies. Urea and creatinine are commonly used as a measurement for kidney function. Table 4 shows the level of urea and creatinine in rats supplemented with *L. fruticosa* extract. Urea is the end product of protein and amino acid catabolism, produced by the liver and distributed throughout the intracellular and extracellular fluid, and it was then filtered out of the blood by glomeruli and partially reabsorbed with water. The concentration of urea in the serum is the most frequently clinical indices for estimating renal function. It is useful in the differential diagnosis of acute renal failure and pre-renal conditions where the blood urea nitrogen–creatinine ratio is increased (Gowda *et al.,* 2010). While creatinine is a breakdown product of creatine phosphate in muscle and is usually produced at a fairly constant rate by the body depending on muscle mass. The National Kidney Disease Education Program recommends calculating the glomerular filtration rate from serum creatinine concentration (Miller *et al.,* 2005). The creatinine clearance test is used to monitor the progression of renal disease. The diagnosis of renal failure is usually suspected when serum creatinine is greater than the upper limit of the "normal" interval (Saka *et al.,* 2017).

4. Conclusion

The administration *L. fruticosa* extract even in high doses did not affect the body system and no toxicity symptoms occurred physically and clinically. It may not lead to haematological, hepatic and/or renal toxicity. Based on this finding, it is suggested that *L. fruticosa* is safe to be taken as no kidney and liver failure was detected in the study.

Conflict of interest

There is no conflict of interest involved in this study.

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