ORIGINAL ARTICLE

Cytotoxicity and Acute Toxicity Assessment of *Basella Alba* Leaf Extract

Shamala Salvamani[1], Asveene Chandran[2], Gunasekaran Baskaran[2], Vaidehi Ulaganathan[3], Mohd Yunus Shukor[4]

[1] Division of Applied Biomedical Science and Biotechnology, School of Health Sciences, International Medical University, Kuala Lumpur, Malaysia
[2] Department of Biotechnology, Faculty of Applied Sciences, UCSI University, Kuala Lumpur, Malaysia
[3] Department of Food Science with Nutrition, Faculty of Applied Sciences, UCSI University, Kuala Lumpur, Malaysia
[4] Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43600 Serdang, Selangor, Malaysia

ABSTRACT

Introduction: Basella alba is widely used in ayurveda and traditional medicine to treat various types of diseases. The toxicity evaluation of *B. alba* has not been studied up to date. The toxicity assessment is important since medicinal plant extract can cause adverse effects on humans. Hence, in this study the toxicity of *B. alba* leaf extract was investigated. **Materials and Method:** The toxicity of *B. alba* extract was evaluated by cytotoxicity test on Vero and WRL-68 cell lines via Sulforhodamine B (SRB) assay , and acute toxicity on mice at a single dosage of 2000 mg/kg body weight by oral gavage. The chosen concentrations were based on preliminary screening and previous literature, ensuring that they are within a biologically relevant range. **Results:** SRB assay revealed a non cytotoxic effect of *B. alba* with IC50 value of more than 625 µg/ml. Throughout 14 days treatment of *B. alba* extract, no changes noted in behavior, outer appearance, clinical sign, body and relative organ weight in control and treatment groups. The biochemical liver function test showed no significant elevation of TBIL, ALT, ALP and AST in *B. alba* extract treated mice compared to the control group. Histopathological examination revealed normal structure and coordination of cells, and no harmful effects noted in liver and kidney. **Conclusion:** The cytotoxicity and acute toxicity studies confirm that *B. alba* extract is non-toxic at the tested dosage. However, limitations exist, including a small sample size and the absence of subchronic or chronic toxicity assessments. Future studies should explore extended exposure periods and higher dosages.
Malaysian Journal of Medicine and Health Sciences (2026) 22(1): 1-8.doi:10.47836/mjmhs.v22.i1.1512

Keywords: Basella alba, Cytotoxicity, Vero cell, WRL-68 cell, Acute toxicity, Histopathology

Corresponding Author:
Baskaran Gunasekaran, Assoc. Prof
Email: baskaran@ucsiuniversity.edu.my
Tel: +603 - 9101 8880

INTRODUCTION

Medicinal plants are extensively used in promoting health and treating various illnesses throughout the world [1]. Investigation on medicinal plants revealed its nutritional values, medicinal properties and pharmacological activities like antioxidant, anti-inflammatory, anti-diabetic, anti-thrombotic, anti-cancer, anti-atherogenic, cardioprotective effects and many more [2-4]. The World Health Organization (WHO) reported that about 80% of the human population relies on alternative medicine for the initial treatment of diseases due to the adverse effects of synthetic drugs [5]. Medicinal plants are rich in bioactive compounds which act as potent effectors of biological processes and capable of suppressing the risk of diseases via complementary and overlapping mechanisms [6].

Despite the therapeutic advantages, scientific investigation revealed that some constitutes of the medicinal plants possess cytotoxic, genotoxic, mutagenic and carcinogenic effects [7,8]. Nevertheless, the toxicity assessment of many medicinal plants was not investigated. Therefore, the toxicity evaluation of medicinal plants is crucial to ensure the safety before use in humans and animals.

Basella alba L (*B. alba*) is a wild leafly medicinal plant belonging to the family of Basellaceae. *B. alba* most probably originated from India or Indonesia and it is abundant in Malaysia, Philippines and tropical Africa [9].

B. alba which is known as Indian spinach or Remayung locally has been used from ancient times in Asian countries due to its medicinal benefits [10]. The leaves and stems of *B. alba* are edible and traditionally used to treat digestive disorder, hemorrhage, ulcer, anemia, hypertension, malaria and to reduce inflammation [11,12]. *B. alba* is used in ayurvedic treatment against diseases like leukemia, melanoma and oral cancer [13]. *B. alba* has been proven to be rich in nutrients, vitamins, minerals and possesses beneficial therapeutic effects [14]. Several studies have examined the toxicity of medicinal plants from the Basellaceae family and other commonly used medicinal plants [11]. For instance, studies on Anredera cordifolia and Talinum triangulare, both from the same family, revealed minimal toxic effects. Additionally, commonly used medicinal plants such as Moringa oleifera and Ocimum sanctum have demonstrated low toxicity profiles in acute and subchronic toxicity studies [14]. These findings further support the need for comparative toxicological evaluations to ensure the safety of medicinal plants in therapeutic applications.

Thus, the present study is designed to investigate the toxicity of *B. alba* leaf extract using cytoxicity test against normal kidney and liver cell lines, and acute toxicity test in mice under the Organization for Economic Cooperation and Development (OECD 420) guidelines. This is the first report on the toxicity assessment of *B. alba* leaf extract and we hypothesize that *B. alba* leaf extract is non-toxic based on its traditional use and compositions.

MATERIALS AND METHOD

Plant sample

B. alba leaves were procured from a local market in Seri Kembangan, Malaysia, and authenticated (voucher no. SK 2087/12). The leaves were air-dried, ground, and extracted using methanol under controlled conditions. The extract was concentrated using a rotary evaporator at 40°C.

Extraction procedure

B. alba leaves were washed thoroughly and air dried overnight at room temperature. The leaves were grounded using a blender (Panasonic MX 8967) and soaked in methanol (250 ml) under shaking conditions at 25 C for 48 h. After filtration, removal of solvent from the extract was done using a separatory funnel. The extract of *B. alba* was then concentrated using a rotary evaporator (Heidolph) under reduced pressure at 40 C.

Cytotoxicity study

Cell lines

A normal kidney cell line (vero cells; Sigma, Missouri, USA) and a hepatic human cell line (WRL-68 cells; Sigma, Missouri, USA) was maintained in Dulbecco's Modified Eagle Medium (DMEM; Life Technologies, Carlsbad, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Life Technologies, Carlsbad, USA), glutamine (2 mM), penicin (100 U/ml) and streptomycin (100 µg/ml) at 37 C in a 5% CO2 [15].

Sulforhodamine B (SRB) assay

Cytotoxicity effect of *B. alba* leaves extract was estimated through the sulforhodamine B (SRB) assay as described by Skehan et al. [16] and Patel et al. [17] Vero cells and WRL-68 cells were diluted to 1 Ч 105 cells/ml using DMEM medium containing 10% new born sheep serum. The diluted cell suspension (100 µl; approximately 10,000 cells) was added into the wells of the microtiter plate. The plates were incubated for 24 h at 37 °C in 5% CO2 incubator to allow cells attachment to the wells. After incubation, when a partial monolayer was observed, the supernatant was flicked, washed and different concentrations of *B. alba* extract ranged from 0.0001- 625 µg/ml (100 µl) were added to the wells in the plates. The concentrations used in this study were selected based on a combination of preliminary screenings, existing literature, and standard toxicity testing guidelines. The chosen cytotoxicity assay concentrations (ranging from 0.0001 to 625 µg/ml) were designed to encompass a broad range, allowing assessment of both subtoxic and potentially toxic effects [16, 17]. Paclitaxel (cytotoxic drug) was used as the positive control at concentration 0.0001 to 625 µg/ml (100 µl). The plates were further incubated for 72 h and microscopic examination was then performed. Upon incubation, trichloroacetic acid (50% ; 25 µl) was added gently into the wells so that it forms a thin layer over the *B. alba* extract to form a final concentration of 10%. The plates were incubated at 4 °C for 1 h. The plates were flicked, washed with tap water five times to remove traces of medium and sample, and air-dried. The air-dried plates were then stained with SRB (100 µl; Sigma, Missouri, USA) and kept for 30 min at room temperature. The unbound dye was removed by washing a few times with 1% acetic acid. The plates were air dried and Tris base (10 mM; 100 µl) was added into the wells to solubilize the dye. The plates were then shaken vigorously for 5 min. The A540 was measured with a microtiter plate reader (Biotek, Winooski, USA). The percentage growth inhibition was calculated using the following formula:

% cell viability= (At-Ab)/ (Ac-Ab) x100
Where,
At= Absorbance value of test compound
Ab= Absorbance value of blank
Ac=Absorbance value of control
IC50 values were determined from the corresponding dose response curves and were expressed as inhibition 50% of cell viability ± SEM.

Acute toxicity study

Experimental animals

10 healthy female ICR mice (8 weeks; weight 28-35 g) were purchased from local suppliers. The animals were housed in polypropylene cages equipped with grill tops (stainless steel) and provided sterile paddy husks as bedding material at the animal house of Veterinary Faculty, Universiti Putra Malaysia (UPM). The animal ethics approval was obtained from Animal Ethics Committee, UPM (reference no: UPM/ IACUC/ AUP-R011/2013) and the experiments were conducted in accordance with the guidelines of Institutional Animal Care and Use Committee (IACUC), UPM. The acute toxicity study was carried out for 14 days.

Treatment

The female mice were fasted overnight before the treatment and food was given 1h after the treatment. It is expected that *B. alba* would be relatively safe because they are edible medicinal plant. Thus, a single high dose, as recommended by OECD 420 [18] guidelines of 2000 mg/kg body weight of *B. alba* extract was administered by gavage to 5 female mice and water was given to another 5 female mice as a control group. After a single administration, observations were made for every hour up to 6 hours and every day up to 14 days. The mice were weighed daily and observed for any toxicity signs and mortality for 14 days [19,20]. The visual observations included changes in the eyes and mucous membranes, skin and fur, somatomotor activity, behavioral pattern, tremors or convulsions, salivation, diarrhea, lethargy and coma. After 14 days of observation periods, the mice were sacrificed by cervical dislocation. Heart, lung, liver, kidneys, spleen and intestine were removed and weighed.

Histopathology of liver and kidney

The liver and kidney tissues were fixed in 10% buffered formalin. The tissues were dehydrated in alcohol, cleared in xylene and embedded in paraffin wax. For histological analysis, paraffin-embedded tissue sections were cut at 5 µm thickness, mounted on slides and stained with hematoxylin and eosin.

Serum biochemical analysis

After 14 days of treatment with *B. alba* extract, the liver function was evaluated with TBIL, ALT, ALP and AST levels in mice serum.

Statistical analysis

Statistical Package for Social Sciences (SPSS) software was used for statistical analysis. Data expressed as the Mean ± S.E.M; t-test was performed for statistics and p values less than 5% were considered significant ($p < 0.05$).

RESULTS

Cytotoxicity Study of *B. alba*

The cytotoxicity activity of *B. alba* extract was investigated against kidney cell line (vero cell) and hepatic human cell line (WRL-68 cell) in SRB assay. The effect of various concentrations of *B. alba* on vero and WRL-68 cells viability for 72 h were shown in Figure 1 and Figure 2, respectively. The extract shows no significant inhibition towards cell viability in both cell lines up to concentration of 625 µg/ml. Meanwhile, paclitaxel (positive control) is an established drug that is proven to be cytotoxic, inhibits about 50% of vero cell viability at the concentration as low as 0.01 µg/ml and almost 100% inhibition was recorded at 1 µg/ml. Furthermore, in the WRL-68 cell line, paclitaxel caused approximately 60% inhibition in cell viability at the concentration of 0.01 µg/ml, followed by 100% inhibition at 1 µg/ml. The IC50 values of *B. alba* extract and paclitaxel in vero and WRL-68 cells are presented in Table 1. *B. alba* extract showed no cytotoxic effect on both cell lines with IC50 values more than 625 µg/ml in 72 h of treatment compared to paclitaxel with IC50 values of 0.045 µg/ml on vero cell and 0.014 µg/ml on WRL-68 cell.

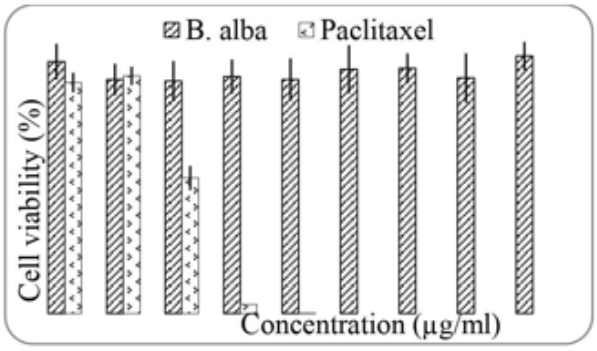


Figure 1: Viability of vero cells receiving various concentration of *B. alba* and paclitaxel

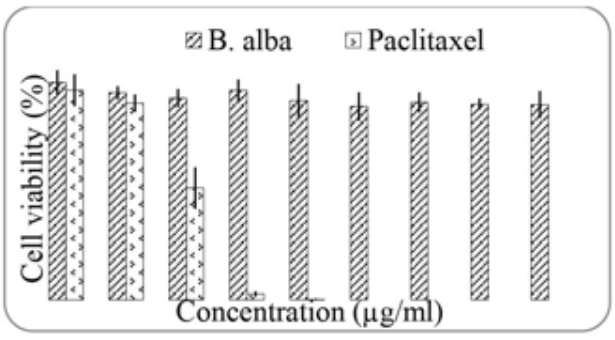


Figure 2: Viability of WRL-68 cells receiving various concentration of *B. alba* and paclitaxel

Table I Median inhibitory concentration (IC_{50}) of *B. alba* and paclitaxel after 72 h

Samples	IC_{50} (µg/mL)	
	Vero	WRL-68
B. alba	> 625	> 625
Paclitaxel [a]	0.045 ± 0.00	0.014 ± 0.00

IC_{50} values were determined from the corresponding dose response curves were expressed as inhibition 50% of cell viability ±_ S.E.M

Acute Toxicity Study of *B. Alba*

Toxicity screening in medicinal plants is essential to assure the safety for the usage in clinical application. Acute toxicity study and histopathology are the commonly used toxicological analysis to evaluate the medicinal plants [18].

Acute Toxicity Response and Behavioural Analysis

Throughout the 14 days observation period, there were no mortality, changes in behavior (hypoactivity and hyperactivity) or changes on outer appearance (eye, fur, skin, mucus membranes). The *B. alba*-treated mice also did not show any observable signs of toxicity (diarrhea, vomiting, constipation, tremors, convulsions, salivation, lethargy and coma). Overall, the condition of treated mice was similar to that of the control mice (Table 2).

Table II: Acute toxic response of ICR female mice of control and treatment group

Dose (mg / kg)	Symptoms	Time vs number of mice (n)						Toxicity signs
		30 min	4 hours	24 hours	48 hours	72 hours	14 days	
Control	Normal	5	5	5	5	5	5	Nil
	Not active	0	0	0	0	0	0	
	Died	0	0	0	0	0	0	
Treatment	Normal	5	5	5	5	5	5	Nil
	Not active	0	0	0	0	0	0	
	Died	0	0	0	0	0	0	

Body and Organ Weight Analysis

As shown in Table 3, there were no significant changes noted in the body weight of the mice. Gross examination during autopsy showed no apparent changes in organs of treated mice compared to the control. The relative organ weight of mice between control and treated groups also showed no significant differences (P > 0.05) (Table 4).

Table III: Body weight of control and treatment groups recorded during acute toxicity study

Group	Body weight (g)		
	Day 0	Day 7	Day 14
Control	29.7 ± 0.9	29.8 ± 1.2	29.8 ± 1.3
Treatment	29.3 ± 0.7	30.1 ± 1.3	30.1 ± 1.5

Values are expressed as mean ± Standard error mean. * P < 0.05 in relation to control.

Table IV: The relative organ weight of control and treatment groups at the end of the study

Relative organ weight (g)		
Organs	Control	*B. alba*
Heart	0.43 ± 0.02	0.44 ± 0.01
Lung	0.68 ± 0.04	0.69 ± 0.04
Liver	4.41 ± 0.09	4.47 ± 0.10
Left kidney	0.50 ± 0.02	0.48 ± 0.02
Right kidney	0.49 ± 0.02	0.48 ± 0.02
Spleen	0.50 ± 0.04	0.47 ± 0.01
Intestine	1.65 0.85	13.39 ±0.77

Biochemical Analysis

Table 5 shows the biochemical parameters in the mice serum induced with *B. alba* extract. There are no significant changes (P > 0.05) for the level of TBIL, ALT, ALP and AST in mice serum after the oral administration of *B. alba* extract.

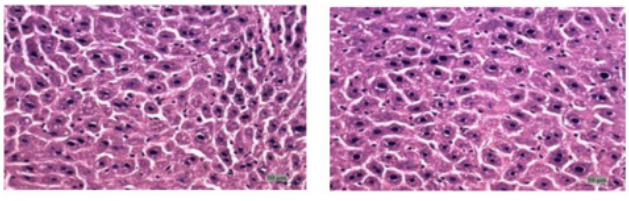
Table V: Serum TBIL, ALT, ALP and AST analysis

Groups	TBIL (µmol/L)	ALT (U/L)	ALP (U/L)	AST (U/L)
Control	0.7 ± 0.2	19.03 ± 3.3	77.2 ± 15.8	71.61 ± 7.82
Treatment	0.8 ± 0.3	19.08 ± 2.5	76.6 ± 13.4	70.72 ± 6.89

Values are expressed as mean ± Standard error mean. * P < 0.05 in relation to control.

Histopathology Analysis

The histopathology analysis on liver and kidney revealed no significant pathological alterations between the two groups (Figure 3 and 4). The microscopic examination of liver and kidney of treated mice showed no alteration in the structure and coordinations of cells or adverse effects compared to control mice when viewed under a light microscope using different magnifications. The density of Kupffer cells is similar and no signs of edema and hemorrhage lesion are observed in both groups. The liver histology shows normal hepatocytes and there was no local fatty degeneration, fibrosis or necrosis observed in the liver of treated mice. Meanwhile, kidney histology revealed normal appearance of glomeruli and capsules with no sign of acute tubular necrosis after the treatment with *B. alba* extract.



A B

Figure 3: Histological examination of liver (A) control and (B) treatment treatment at magnification of 40x

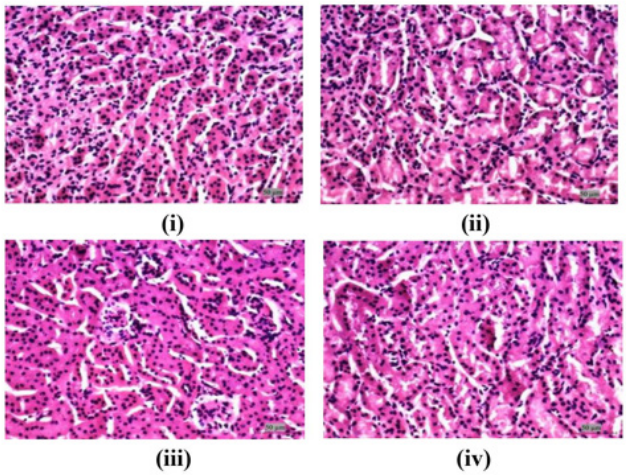


Figure 4: Histological examination of (A) right kidney (i) control and (ii) treatment and (B) left kidney (i) control and (ii) treatment at magnification of 40x.

DISCUSSION

Medicinal plants are widely used in promoting health, preventing and treating various diseases [21]. However the usage of medicinal plant extracts should not cause adverse effects on the host cells or interference in physiological pathways. According to National Cancer Institute (NCI) US, for the crude extract safety, IC50 value below 30 µg/ml was considered to be cytotoxic in an in vitro assay for incubation more than 48 h [22]. Cellular viability and proliferation is an essential characteristic of healthy growing cells. A decrease in the cell viability indicates cell death due to toxic effects of the test extracts or non-optimal culture condition while an increase in the cell viability indicates cell proliferation [23]. Treatment with *B. alba* extract did not cause significant reduction in cell viability with IC50 value more than 625 µg/ml indicating that it is non toxic.

Toxicity screening in medicinal plants is essential to assure the safety for the usage in clinical application. Acute toxicity study and histopathology are the commonly used toxicological analysis to evaluate the medicinal plants [18]. The acute toxicity study was carried out according to OECD guideline 420 [18] at a single dose of 2000 mg/kg body weight. The *B. alba* extract was administered to female ICR mice by oral route while water was given to the control mice. The clinical signs are one of the crucial observations to indicate the effects of toxicity in organs of the tested animals [24]. Administration of *B. alba* extract did not affect the food and water intake of the mice and no deleterious effects were shown. Thus, this shows that there was no disturbance in protein, carbohydrate and fat metabolism [25]. According to acute toxicity criteria set by Globally Harmonized Classification System (GHS, 2005), [26]when there is no injury or death occurred in tested animals at the highest dose (2000 mg/kg), it indicates that the extract was fit in class 5 (no or very low toxicity).

It is important to note the differences in body and organ weight of control and treated animals since the organs may reflect toxicity from the exposure of toxic substances [27]. The changes in body weight of mice are the indicators of adverse effects of tested extract and it is considered significant when the body weight loss is about 10% or higher from the initial weight [28,29]. Organ weight is an essential index of pathological and physiological status in treated animals. The relative organ weight is important to determine the possibilities of injuries. Heart, lung, liver, kidneys, spleen and intestine are the primary organs that are commonly affected due to metabolic reactions caused by the toxicants [30]. The results indicate that important organs such as heart, lung, liver, kidneys, spleen and intestine were not adversely affected with treatment of *B. alba*. Thus, this suggests that *B. alba* extract is virtually non toxic.

The biochemical liver function tests (LFT) was performed to diagnose liver disorders. The damages in liver cells cause the enzymes to leak out into the bloodstream, whereby they can be measured using blood tests. LFT was used to measure total bilirubin levels (TBIL), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) [31]. ALT and AST are indicators for liver injury and elevated levels of these enzymes indicate cellular leakage and destruction in functional integrity of cell membrane [32]. ALP is an indicator for pathological alteration in biliary stream and the elevated ALP level indicates infiltrative liver disease or chronic cholestasis, while increased TBIL shows hepatic disorder and failure [33]. Thus, it can be concluded that treatment with *B. alba* extract did not cause liver toxicity or damage. Liver and kidney are the most commonly affected organs during toxicity as they are involved in toxin metabolic breakdown and toxin filtration [34]. The consumption of *B. alba* extract is safe and does not cause any pathological effects especially in the liver and kidney. This study corroborates previous research supporting the safety of *B. alba*. While Chowdhury et al. [35] investigated the subchronic toxicity of carboxymethylated *B. alba* mucilage in Wistar rats, our study presents the first report on the acute toxicity of *B. alba* leaf extract. The absence of toxic effects at 2000 mg/kg suggests its safe use; however, further long-term studies are required to confirm its safety over extended periods. Additionally, the findings are in line with other commonly used medicinal plants, including Moringa oleifera [36], Ocimum sanctum [37], and Anredera cordifolia [38]. Similar to *B. alba*, these plants have demonstrated minimal toxic effects in acute and subchronic toxicity studies, further supporting the safety profile of *B. alba*. However, while Moringa oleifera and Ocimum sanctum have been studied extensively for long-term effects, *B. alba* requires further subchronic and chronic toxicity assessments to establish its comprehensive safety profile.The presence of bioactive compounds such as flavonoids, saponins,

and polyphenols may contribute to its non-toxic nature. Compared to other medicinal plants, *B. alba* demonstrates a favorable toxicity profile, as evidenced by histopathological and biochemical analyses. Its lack of cytotoxicity further supports its potential as a therapeutic agent. Additionally, comparisons between treatment and control groups have been expanded to highlight differences in biochemical markers, histopathological findings, and organ weight measurements, ensuring a more comprehensive evaluation.

CONCLUSION

Cytotoxicity test revealed that *B. alba* leaf extract did not show significant inhibition towards Vero and WRL-68 cell lines with IC50 values more than 625 µg/ml after 72 h of treatment. The acute toxicity indicated that *B. alba* is non toxic when administered orally in ICR female mice at a single dose of 2000 mg/kg. No mortality or toxicity signs were observed in *B. alba* extract treated mice. The body and relative organ weight of mice showed no significant differences between control and treated group. The primary organs (heart, lung, liver, kidneys, spleen and intestine) were confirmed to be not affected with treatment of *B. alba*. The LFT of mice serum indicated no symptoms of liver injuries and damage due to normal levels of TBIL, ALT, ALP and AST. Histological examination of liver and kidney revealed no potential toxicity or structural damage. The result of in vivo acute toxicity in mice is in agreement with that of in vitro cytotoxicity against the normal liver and kidney cell lines which shows non toxic effect of *B. alba*. These findings highlight the safety usage of *B. alba* as an alternative therapeutics agent. Nonetheless, our study is limited by a small sample size and the absence of subchronic and chronic toxicity assessments. Future research should investigate dose-dependent effects and the molecular mechanisms underlying *B. alba*'s safety profile.

ACKNOWLEDGEMENT

This work is supported by UCSI Research Excellence & Innovation Grant (REIG-FAS-2022-044) from UCSI University, Kuala Lumpur, Malaysia.

REFERENCES

1. Rahmawati N, Indrian Mustofa F, Haryanti S, Subositi D, Widodo H, Yanti Marfuatush Sholikhah I, et al. Ethnopharmacology Study of Medicinal Plants Utilized for Hypercholesterolemia Treatment on Borneo Island of Indonesia. Jurnal Tumbuhan Obat Indonesia 2022;15:1–15. doi: 10.22435/jtoi.v15i1.5928
2. Mousavi SM, Hashemi SA, Behbudi G, Mazraedoost S, Omidifar N, Gholami A, et al. A Review on Health Benefits of Malva sylvestris L. Nutritional Compounds for Metabolites, Antioxidants, and Anti-Inflammatory, Anticancer, and Antimicrobial Applications. Evid Based Complement Alternat Med. 2021;2021:1–13. doi: 10.1155/2021/5548404.
3. Tran N, Pham B, Le L. Bioactive Compounds in Anti-Diabetic Plants: From Herbal Medicine to Modern Drug Discovery. Biology (Basel) 2020;9:252–82. doi: 10.3390/biology9090252.
4. Shaito A, Thuan DTB, Phu HT, Nguyen THD, Hasan H, Halabi S, et al. Herbal Medicine for Cardiovascular Diseases: Efficacy, Mechanisms, and Safety. Front Pharmacol 2020;11:1–32. doi: 10.3389/fphar.2020.00422.
5. Parasuraman S. Herbal Drug Discovery: Challenges and Perspectives. Curr Pharmacogenomics Person Med 2018;16:63–8. doi: 10.2174/1875692116666180419153313
6. Abdul W, Hajrah N, Sabir JamalSM, Al-Garni S, Sabir M, Kabli S, et al. Therapeutic role of Ricinus communis L. and its bioactive compounds in disease prevention and treatment. Asian Pac J Trop Med 2018;11:177–85. doi: 10.4103/1995-7645.228431
7. Okaiyeto K, Oguntibeju OO. African Herbal Medicines: Adverse Effects and Cytotoxic Potentials with Different Therapeutic Applications. Int J Environ Res Public Health 2021;18:5988–6007. doi: 10.3390/ijerph18115988
8. Bhavsar S, Chandel D. Cytotoxic and genotoxic effects of Kalanchoe pinnata (Lam.) Pers. fresh leaf juice in the cultured human blood lymphocytes. Drug Chem Toxicol 2022;45:360–6. doi: 10.1080/01480545.2019.1696814
9. Khoo YW, Tan HT, Khaw YS, Li S-F, Chong KP. First Report of Epicoccum sorghinum Causing Leaf Spot on Basella alba in Malaysia. Plant Dis 2023;107:939. doi: 10.1094/PDIS-02-22-0309-PDN.
10. Chaurasiya A, Pal RK, Verma PK, Katiyar A, . R, Kumar N. An updated review on Malabar spinach (Basella alba and Basella rubra) and their importance. J Pharmacogn Phytochem 2021;10:1201–7. Available from: www.phytojournal.com
11. Islam MdS, Rahi MdS, Jahangir CA, Rahman MH, Jerin I, Amin R, et al. In Vivo Anticancer Activity of Basella alba Leaf and Seed Extracts against Ehrlich's Ascites Carcinoma (EAC) Cell Line. Evid. Based Complement. Alternat. Med. 2018;2018:1–11. doi: 10.1155/2018/1537896.
12. Khanom B, Nahida U, Sultana Shapla S, Shahina Akter M, Farzana H, Zaman F, et al. Phytochemical and Pharmacological Review on Basella Alba L. Pharmacol. Onlin. 2019;1:4–48. Available from: www.pharmacologyonline.com
13. Kumari R, Goldar WA, Mondal S, Patra S, Bhattacharya S, Haldar PK. Protective effect of Basella alba leaf against diabetic nephropathy in rats. Adv. Tradit. Med. 2021;21:111–9. doi:

10.1007/s13596-020-00458-2
14. Acikgoz FE, Adiloglu S. A Review on a New Exotic Vegetable for Turkey: Malabar Spinach (Basella alba L. or Basella rubra L.). J. Hortic 2018;05:1–4. doi: 10.4172/2376-0354.1000239
15. Loha M, Mulu A, Abay SM, Ergete W, Geleta B. Acute and Subacute Toxicity of Methanol Extract of Syzygium guineense Leaves on the Histology of the Liver and Kidney and Biochemical Compositions of Blood in Rats. Evid. Based Complement. Alternat. Med. 2019;2019:1–15. doi: 10.1155/2019/5702159.
16. Vajrabhaya L, Korsuwannawong S. Cytotoxicity evaluation of a Thai herb using tetrazolium (MTT) and sulforhodamine B (SRB) assays. J Anal Sci Technol 2018;9:1–6. doi: 10.1186/s40543-018-0146-0
17. Jeenathunisa N, Rajan S. In vitro Cytotoxic studies of Saraca asoca bark extracts on HT-29 cancer cell line. Res J Pharm Technol 2021;14:42–6. doi:10.5958/0974-360X.2021.00008.1
18. Zainul Azlan N, Abd Ghafar N, Mohd Yusof YA, Makpol S. Toxicity study of Chlorella vulgaris water extract on female Sprague Dawley rats by using the Organization for Economic Cooperation and Development (OECD) Guideline 420. J Appl Phycol 2020;32:3063–75. doi: 10.1007/s10811-020-02195-0
19. Ugwah-Oguejiofor CJ, Okoli CO, Ugwah MO, Umaru ML, Ogbulie CS, Mshelia HE, et al. Acute and sub-acute toxicity of aqueous extract of aerial parts of Caralluma dalzielii N. E. Brown in mice and rats. Heliyon 2019;5:1–20. doi: 10.1016/j.heliyon.2019.e01179.
20. Balaji S, Ganesan KK. Acute and subacute toxicity evaluation of hydroalcoholic extract of Caryota urens leaves in Wistar rats. J Appl Pharm Sci 2020;10:121–8. https://doi.org/10.7324/JAPS.2020.104015.
21. Ahmad Khan MS, Ahmad I. Herbal Medicine. New Look to Phytomedicine, Elsevier; 2019, p. 3–13. doi: 10.1186/s40360-021-00522-w
22. Samra RM, Soliman AF, Zaki AA, El-Gendy AN, Hassan MA, Zaghloul AM. Chemical Composition, Antiviral and Cytotoxic Activities of Essential Oil from Cyperus rotundus Growing in Egypt: Evidence from Chemometrics Analysis. J. Essent. Oil-Bear. Plants 2020;23:648–59. doi: 10.1080/0972060X.2020.1823892
23. Mohammed FA, Elkady AI, Syed FQ, Mirza MB, Hakeem KR, Alkarim S. Anethum graveolens (dill) – A medicinal herb induces apoptosis and cell cycle arrest in HepG2 cell line. J Ethnopharmacol 2018;219:15–22. doi: 10.1016/j.jep.2018.03.008.
24. Fan Q, Zhao B, Wang C, Zhang J, Wu J, Wang T, et al. Subchronic Toxicity Studies of Cortex Dictamni Extracts in Mice and Its Potential Hepatotoxicity Mechanisms in Vitro. Molecules 2018;23:2486–505. doi: 10.3390/molecules23102486.
25. Xavier A, Rani SS, Shankar R, Nisha A, Sujith S, Uma R. Evaluation of acute oral toxicity of lemon grass oil and citral in albino rats. J Phytopharmacol 2022;11:281–5. doi: 10.31254/phyto.2022.11410
26. Mekonnen A, Tesfaye S, Christos SG, Dires K, Zenebe T, Zegeye N, et al. Evaluation of Skin Irritation and Acute and Subacute Oral Toxicity of Lavandula angustifolia Essential Oils in Rabbit and Mice. J Toxicol 2019;2019:1–8. doi: 10.1155/2019/5979546. 27.
27. Berhan M, Kassahun D, Ermias L, Mahlet A, Tizazu Z, Gezu F, et al. Acute skin irritation, acute and sub-acute oral toxicity studies of Rosmarinus officinalis essential oils in mice and rabbit. Afr J Pharm Pharmacol 2018;12:389–96. doi:10.5897/AJPP2018.4957
28. Al-Afifi NA, Alabsi AM, Bakri MM, Ramanathan A. Acute and sub-acute oral toxicity of Dracaena cinnabari resin methanol extract in rats. BMC Complement Altern Med 2018;18:1–14. doi: 10.1186/s12906-018-2110-3.
29. Shakya A, Chaudhary SK, Bhat HR, Ghosh SK. Acute and sub-chronic toxicity studies of Benincasa hispida (Thunb.) cogniaux fruit extract in rodents. Regul. Toxicol. Pharmacol. 2020;118:1–9. doi: 10.1016/j.yrtph.2020.104785.
30. Cedric Y. Acute Toxicity of Pentaclethra macrophylla and Psidium guajava Use as Antiprotozoan Medicinal Plants Feed Management in Pig Production and an Attempt for Improvement: A Case Study of Babadjou Locality in the Western Region of Cameroon View project antidiabetic effects of natural products. J Drug Discov Develop and Deliv 2020;6:1037–41. Available from: www.semanticscholar.com
31. Kharchoufa L, Bouhrim M, Bencheikh N, El Assri S, Amirou A, Yamani A, et al. Acute and Subacute Toxicity Studies of the Aqueous Extract from Haloxylon scoparium Pomel (Hammada scoparia (Pomel)) by Oral Administration in Rodents. Biomed Res Int 2020;2020:1–11. doi: 10.1155/2020/4020647.
32. Olayode OA, Daniyan MO, Olayiwola G. Biochemical, hematological and histopathological evaluation of the toxicity potential of the leaf extract of Stachytarpheta cayennensis in rats. J

Tradit Complement Med 2020;10:544–54. doi: 10.1016/j.jtcme.2019.05.001.
33. Ogidi OI, Ayibaene F-O, Oluwatoyin SO, Anani RO. Biochemical Study on the Effects of Ruzu Herbal Bitters Formulation on Wistar Albino Rats. Biomed J Sci Tech Res 2022;41:32434–9. doi: 10.26717/BJSTR.2022.41.006558
34. Nurul SAS, Hazilawati H, Mohd RS, Mohd FHR, Noordin MM, Norhaizan ME. Subacute Oral Toxicity Assessment of Ethanol Extract of Mariposa christia vespertilionis Leaves in Male Sprague Dawley Rats. Toxicol Res 2018;34:85–95. doi: 10.5487/TR.2018.34.2.085.
35. Chowdhury, M., De, P. K., Maji, H. S., & Das, D. Safety study of carboxymethylated Basella alba mucilage: a subchronic oral toxicity evaluation in Wistar albino rats. Journal of Applied Pharmaceutical Research, 12(2), 16-26 2024. doi. org/10.18231/j.joapr.2024.12.2.16.26.
36. Pop, O. L., Kerezsi, A. D., & Ciont, C. A comprehensive review of Moringa oleifera bioactive compounds—cytotoxicity evaluation and their encapsulation. Foods, 11(23), 3787 2022. doi.org/10.3390/foods11233787
37. Harsha, M., Kumar, K. M., Kagathur, S., & Amberkar, V. S. (2020). Effect of Ocimum sanctum extract on leukemic cell lines: A preliminary: in-vitro: study. Journal of Oral and Maxillofacial Pathology, 24(1), 93-98 2020. doi.10.4103/jomfp. JOMFP_181_19
38. Virginia, D. M., Saputra, R. P., & Setiawati, A. Selective cytotoxic activity of Anredera cordifolia leaves extract towards HeLa cells. Biotropia, 26(1), 33-39 2020. DOI: 10.11598/btb.2019.26.1.000