

Original Article

FOLLOWING OECD GLP GUIDELINES: PRELIMINARY IN VIVO SAFETY ASSESSMENT OF *BRUCEA JAVANICA* FRUIT AQUEOUS EXTRACT IN SPRAGUE DAWLEY RATS

Bee Ping Teh¹, Nor Syaidatul Akmal Mohd Yousof^{1*}, Umi Rubiah Sastu@Zakaria¹, Siti Zubaidah Che Lem², Azlina Zulkapli², Inirah Che Ishak², Tavinraj Rajendran¹, Bazilah Jusoh¹, Nurul Syahira Shabudin¹, Dalnitha Lea Nicol¹, Norzahirah Ahmad¹

1. Herbal Medicine Research Centre, Institute for Medical Research, National Institutes of Health, Ministry of Health Malaysia, 40170 Shah Alam, Selangor, Malaysia

2. Special Resource Centre, Institute for Medical Research, National Institutes of Health, Ministry of Health Malaysia, 50588 Kuala Lumpur, Malaysia

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***Corresponding author:**

Nor Syaidatul Akmal Mohd

Yousof,

syaidatul.akmal@moh.gov.my,

Tel: +603-33627997

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ABSTRACT

This study evaluates the acute toxicity of *Brucea javanica* fruit following a single oral administration in female Sprague Dawley rats, with clinical assessments conducted over 14 days, in line with the Organisation for Economic Co-operation and Development Guideline for the Testing of Chemicals, Section 4, Test No. 420: Acute Oral Toxicity - Fixed Dose Procedure. The chemical profile of the *B. javanica* raw material used in this study was investigated, and its authenticity was confirmed with bruceine A detection. Two doses, 300 mg/kg and 2,000 mg/kg body weight (BW), were tested, with five rats receiving 300 mg/kg BW and one rat receiving 2,000 mg/kg BW, which displayed clinical signs of toxicity. Rats given 300 mg/kg BW had increased body weight at the end of the observation period, along with increased food intake. Macroscopic evaluations revealed no significant abnormalities except a dilated uterine horn (n = 3) and whitish discolouration on the liver's caudate lobe. Histological evaluation revealed mild, dose-dependent lesions in the liver and ileum at both 300 and 2,000 mg/kg BW, whereas moderate lesions were observed in the kidneys, duodenum, and jejunum were confined to the 2,000 mg/kg BW group. Gastrointestinal lesions in the 300 mg/kg BW rats were likely due to fasting-related erosion, and liver and kidney lesions may be associated with physiological changes during euthanasia. Further investigation into the mortality of the rat given 2,000 mg/kg BW dose revealed slightly pronounced organ lesions. The current result indicates the median lethal dose (LD₅₀) value for the freeze-dried aqueous extract of *B. javanica* fruits is likely below 2,000 mg/kg BW. Follow-up studies with prolonged exposure and repeated dosing are warranted to fully characterise the toxicity of this medicinal plant.

KEYWORDS: *Brucea javanica*, melada pahit, acute toxicity, OECD GLP, median lethal dose (LD₅₀)

INTRODUCTION

In recent years, there has been a resurgence in interest in herbal medicine, driven by a global shift toward natural and holistic approaches to health and wellness. The use of plant-based remedies is deeply embedded in the cultural practices and traditions of various societies, often passed down through generations (1, 2). In recent years, there has been a resurgence in interest in herbal medicine, driven by a global shift toward natural and holistic approaches to health and wellness. Research has demonstrated that many herbal medicines possess pharmacological properties that can complement conventional treatments, offering patients a broader range of therapeutic options (3, 4). Furthermore, the integration of herbal medicine into primary healthcare can contribute to more sustainable health practices, reduce healthcare costs, and alleviate the burden on healthcare systems (5). As patients increasingly seek alternative therapies, it becomes imperative to validate the safety and efficacy of herbal remedies to ensure informed and evidence-based usage.

B. javanica, belonging to the Simaroubaceae family, is a tropical fruit-bearing plant indigenous to Southeast Asia. The plant is characterised by its small, bitter fruits, which have been traditionally utilised in folk medicine for various ailments. Phytochemical analyses of *B. javanica* have revealed a diverse array of therapeutic bioactive compounds. For instance, research by Subeki et al. (6) identified quassinoids such as bruceine A and bruceantinol, which exhibit potent anti-dysenteric properties, reinforcing the plant's role in traditional dysentery management (7). In another study, Guo et al. (8) highlighted the presence of quassinoids that demonstrated anti-malarial activities, suggesting potential protective effects against oxidative stress. Furthermore, NoorShahida et al. (9) reported the presence of bruceines E and D with hypoglycaemic properties. Literature also documented traditional uses of *B. javanica* for treating colic, fever, body ache, scurf, ringworm, boils, and centipede bites, underscoring its significance in ethnomedicine (7). Efficacy studies have indicated that extracts from *B. javanica* possess anti-tumour effects (10–15).

Despite the promising therapeutic potential of *B. javanica*, research gaps regarding its safety assessment for human use remain. Although safety data has been reported using various protocols (9, 16, 17), safety assessment of *B. javanica* using standardised protocols and in accordance with the Organisation for Economic Co-operation and Development (OECD) Good Laboratory Practice (GLP) principles has not been reported. Such protocols are essential to provide reliable data on the safety and efficacy of herbal products (18). These guidelines promote a systematic approach to the testing of chemicals, including herbal medicines, ensuring that studies are conducted with rigor and transparency. Conducting safety assessments not only protects consumers but also helps in building trust in herbal products within the healthcare community. Additionally, regulatory agencies, such as the National Pharmaceutical Regulatory Agency (NPRA) in Malaysia, require robust safety data to make informed decisions regarding the approval and use of herbal medicines in clinical practice (19). This study aims to assess the acute toxicity of aqueous extract *B. javanica* fruit through a single oral administration in female Sprague Dawley rats, following the OECD GLP test guideline. By investigating the potential toxic effects and establishing a median lethal dose (LD₅₀) value, this research will add to the safety profile of *B. javanica*.

MATERIALS AND METHODS

Chemicals and Reagents

The chemical standard bruceine A (CAS No.: 25514-31-2) was purchased from TargetMol (Boston, MA, USA). Solvents such as chloroform, methanol, 4-methoxybenzaldehyde, concentrated sulfuric acid, and acetic acid were obtained from Merck & Co., Inc. (Darmstadt, Germany). Phosphate-buffered saline

tablets were purchased from Sigma-Aldrich (St. Louis, MO, USA), 10% neutral buffered formalin was obtained from Leica (Richmond, IL, USA), and isoflurane was acquired from Baxter (Deerfield, IL, USA).

Chemical Identification of the Plant

Chemical identification of the plant was obtained using the high-performance thin layer chromatography (HPTLC) and high-performance liquid chromatography (HPLC) techniques. The identity of *B. javanica* plant used in this study was verified using bruceine A as the chemical marker, as it is one of the most abundant and chemically distinct quassinoids in the plant and thus serves as a representative constituent for authenticity and quality control (20). The chemical standard solution (bruceine A) was prepared at a concentration of 0.2 mg/mL in methanol. For the sample solution preparation, 1.5 g of the dried ground powder was mixed with 20 mL of water and sonicated for 15 min at room temperature. After sonication, the sample solution was filtered through Whatman No. 1 filter paper and allowed to evaporate to dryness. The filtrate was reconstituted with methanol and filtered through a 0.22 µm syringe filter. The sample solution was used for the chemical identification analysis.

HPTLC chromatography was performed on 10 × 10 cm HPTLC silica gel 60 F₂₅₄ plates. Standard and sample solutions (5 µL each) were separately applied to the plate as 8 mm wide bands with an automatic TLC applicator (Linomat-V, CAMAG, Muttentz, Switzerland), 8 mm from the bottom. The mobile phase consisted of chloroform:methanol (9:1 v/v). The plate was developed in a 10 × 10 twin glass chamber saturated with the mobile phase at room temperature up to a height of 8 cm. After development, the plates were air-dried, and derivatisation of the chromatogram was conducted by dipping the plate in anisaldehyde reagent (1.0 mL of 4-methoxybenzaldehyde in 170 mL of methanol added with 10 mL of concentrated sulphuric acid and 20 mL of acetic acid), followed by heating at 105 °C for 5 min or until the colour of the zones became visible. The plates were observed under a CAMAG UV cabinet at 254 nm (before derivatisation) and under UV 366 nm (after derivatisation).

HPLC analysis was conducted using a Waters ACQUITY® Arc™ system (Waters, Milford, MA, USA) coupled to a diode array and UV-visible detector (Waters, Milford, MA, USA). Chromatography separation was carried out on a reverse phase C18 column (4.6 mm × 150 mm, 5 µm, Waters, Milford, MA, USA). Gradient elution was performed with 0.1% formic acid in water (as mobile phase A) and 0.1% formic acid in methanol (as B) at a flow rate of 1.2 mL/min. The initial condition (5% B) was held for 2 min, then increased to 60% B over 16 min, kept constant at 95% B for 3 min, and finally returned to the initial condition for a 2-min re-equilibration. The total run time was 25 min, with the column oven maintained at 35°C. The injection volume was 10 µL. The identification of the chemical marker peak was performed by comparison with the bruceine A standard.

In Vivo Acute Toxicity Assessment

a) Test Item

The matured fruits of *B. javanica* were collected and cleaned with tap water. After air drying, the fruit was ground into a powder form with a particle size of 0.355 mm. In order to mimic the traditional preparation, a water extraction method was used, where the dried fruit powder was subjected to hot water extraction (250 g dried powder in 2 L of distilled water at 80 °C for an hour), according to optimised in-house standard operating procedures. The resulting extract solution was concentrated by heating on a hot plate. The concentrated solution was stored inside a chiller at 4 °C for 24 hours before undergoing freeze-drying (5 days) to obtain the final test item in freeze-dried powder form. A voucher specimen of the plant was collected and deposited at the herbarium of Forest Research Institute Malaysia (specimen ID: PID 461119-24).

The freeze-dried powder was added to reverse osmosis water (vehicle) to achieve concentrations of 30 and 200 mg/mL. The test item was freshly prepared prior to administration. The volume given was adjusted to the body weight of the rats with the pre-determined dosing volume of 10 mL/kg body weight.

b) Experimental Animals

Six female rats (Sprague Dawley, 8–9 weeks, weighed between 160–200 g) were obtained from the Institut Farmaseutikal dan Nutraseutikal Malaysia (IPharm), 11700 Pulau Pinang, Malaysia.

The rats were housed in individually ventilated cages with corn-cob bedding (Biocob corncob, Biosys Corporation Pte Ltd., 111 North Bridge Road, #27-01 Peninsula Plaza, Singapore 179098) and were quarantined for 14 days to monitor their health. Following quarantine, they were acclimatised to laboratory conditions and human handling for five days before formulation administration. The experimental room was maintained at a temperature of 19–26 °C and humidity at 35–65%, with a 12-hour light–dark cycle. The rats were provided with a standard rodent pellet diet (Specialty Feeds, 3150 Great Eastern Highway, Glen Forrest, Western Australia 6071) and ad libitum reverse osmosis water. To promote well-being, enrichment blocks (Aspen chips, LUFA-ITL GmbH, Tapvei Estonia OÜ, Paekna küla, Kiili vald, 75408 Harjumaa, Estonia) were supplied throughout the study.

The study complied with the test facility's standard operating procedures, Guidelines for ethical conduct in the care and use of animals for scientific purposes in the Ministry of Health (MOH) institutions and facilities (MOH-AG). All rat handling procedures were conducted in accordance with the Ministry of Health Malaysia's animal handling guidelines (21) and were approved by the Animal Care and Use Committee of the Ministry of Health (ACUC-MOH), Malaysia (ACUC number ACUC/KKM/02(1/2013)).

c) Animal Experimental Procedure

This study was conducted in accordance with test guideline 420 under the Organisation for Economic Cooperation and Development (OECD) principles of Good Laboratory Practice (22). The test item administration was performed via oral gavage using a ball-tipped intubation needle fitted to a syringe. All rats were fasted overnight and received a single dose of the formulation, then observed for 14 days.

The rats were randomly assigned to the sighting study group (n = 2) and the main study group (n = 4). Firstly, one rat in the sighting study group received 300 mg/kg body weight (BW) dose of the test item and was clinically observed. After determining the lack of acute response in the first rat, a second rat was administered 2,000 mg/kg BW dose and clinically observed. Lastly, all four rats in the main study group received the test item at 300 mg/kg BW dose and were clinically observed.

Body weight, as well as food and water consumption, were measured weekly. On the final day of observation, feed pellets were removed to allow for overnight fasting, and necropsy was performed on the following day. Each rat was euthanised using carbon dioxide gas, followed by gross pathology examinations conducted by a veterinarian, with any abnormalities recorded. The organs were then subjected to gross and histopathological examination. Abnormal organs were preserved in 10% formalin for histopathological analysis.

d) Parameters Measured During the Study

General Clinical Observation

The rats were monitored for clinical signs at regular intervals (every 0.5-hour, 1-hour, 2-hour, 3-hour, and 4-hour) after dosing and at least once daily. Observations, including changes in skin, fur, eyes, mucous membranes, behaviour patterns, tremors, salivation, diarrhoea, sleep, coma, and abnormalities in the oral cavity (e.g., tooth misalignment), were recorded over a 14-day period.

- Body Weight

Body weights were measured on Day 0-1, Day 7-8, and Day 14-15.

- Food and Water Intake

Food and water consumption were measured and recorded on the same days as body weight.

- Histopathology Examination

Histopathological lesions were analysed through systematic microscopic examination, focusing on inflammatory, circulatory, and cellular changes. Lesions were first described, and their extent was then evaluated according to established in-house criteria, adapted from Shackelford et al. (23) (**Table 1**). All slides were examined under single-blinded conditions by a veterinary pathologist.

Table 1. Assessment of the severity of histopathological changes in the examined organs.

Severity	Criteria
Minimal	Affecting less than 1% of the organ section.
Mild lesion	Presence of the lesion involving between 1% and 20% of the organ section
Moderate lesion	Presence of the lesion involving between 21% and 60% of the organ section
Severe lesion	Presence of the lesion involving more than 60% of the organ section

e) Statistical Analysis

All collected data were recorded, analysed, and summarised using Excel (Version 2411 Build 16.0.18227.20082, USA) and GraphPad prism (version 7.00 for Windows, GraphPad Software, La Jolla, CA, USA). The Kaplan-Meier plot was used to depict percentage survival (**Figures 2(a) and 2(b)**), while the mean and standard deviations were calculated for the results presented in **Figure 2(c)**.

RESULTS

Chemical Identification

The HPTLC and HPLC analyses confirmed the presence of bruceine A in the aqueous extract of *B. javanica* dried fruit powder. In the TLC chromatogram, both the bruceine A standard and the extract showed a band at retention factor (R_f) of 0.47, visible under UV light at 254 nm before derivatisation and under UV light at 366 nm after derivatisation with anisaldehyde reagent. Similarly, HPLC analysis revealed a peak at a retention time (t_r) of 19.846 minutes for the bruceine A standard, with a corresponding peak in the extract at t_r = 19.840 minutes. The UV spectrum further confirmed the similarity between the bruceine A standard and the extract, validating the presence of bruceine A in the aqueous extract (**Figure 1**).

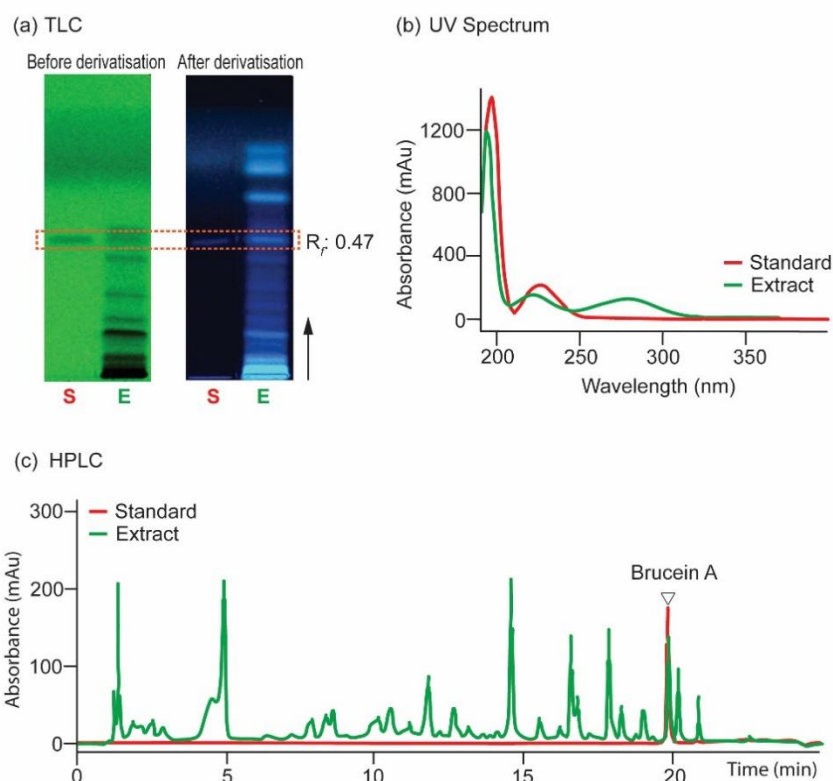


Figure 1. (a) HPTLC chromatogram of bruceine A (S) at R_f : 0.47 and aqueous extract of *B. javanica* dried fruits powder (E) observed under UV at 254 nm before derivatisation and UV at 366 nm after derivatisation with anisaldehyde reagent; (b) Overlay UV spectrum of bruceine A standard solution (0.2 mg/mL) and aqueous extract of *B. javanica* dried fruits powder; and (c) Overlay HPLC chromatogram of bruceine A standard solution (0.2 mg/mL) at t_r = 19.846 min and aqueous extract of *B. javanica* dried fruit powder showing peak corresponding to bruceine A standard solution at t_r = 19.840 min.

Acute Toxicity Findings

a) Clinical Observation

Sighting study and main study rats receiving 300 mg/kg BW dose recorded a 100 percent survival rate (**Figure 2 (a)** and **Figure 2 (b)**). One rat receiving 2,000 mg/kg BW in the sighting study died after almost approximately 8-hour of post-dosing (right panel, **Figure 2 (a) (ii)**).

Approximately 2 hours post-dosing, the rat experienced mild diarrhoea. At approximately 3 hours post-dosing, the rat showed additional signs of lethargy, piloerection, sunken and watery eyes, and maintained a hunched back posture. At approximately 8 hours post-dosing, the rat died (**Figure 2**). Following this observation, the main study was conducted 300 mg/kg BW dose.

Apart from bite marks found on the tail of three rats receiving 300 mg/kg BW dose possibly caused by excess grooming behaviour, there were no clinical signs of toxicity that could be attributed to treatment at the 300 mg/kg BW dose. No abnormalities were detected with regard to changes in skin, fur, eyes & mucous membranes, behaviour pattern, tremors, salivation, diarrhoea, sleep & coma, and

abnormality of the oral cavity (such as misalignment of teeth), and were noted as normal at all observation intervals.

b) Body Weight

The rats' weekly mean body weights were 200.85 ± 16.59 g and 219.74 ± 21.88 g, showing a consistent increase in body weight over the 14-day period. Similarly, the weekly mean food intakes were 105.03 ± 10.58 g and 113.67 ± 16.44 g, with food consumption increased each week throughout the same period. In contrast, the weekly mean water intakes were 195.0 ± 33.4 mL and 178.6 ± 43.3 mL, indicating a gradual decrease in water intake over the 14 days (**Figure 2(c)**). Changes in body weight, food intake, and water intake were not statistically tested due to the absence of a control group for comparison.

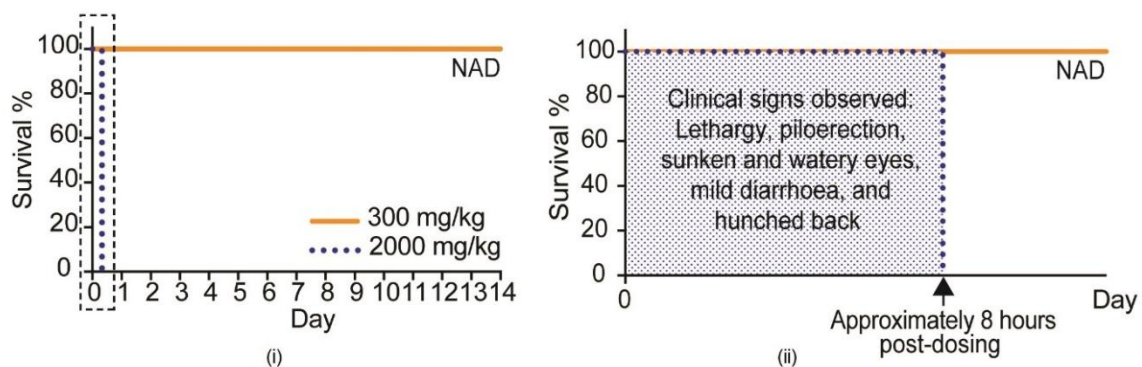
c) Gross Examination

The macroscopic evaluation of all organs revealed no abnormality, except the one rat administered with 300 mg/kg BW dose had a caudate lobe of liver (right side) with the presence of white discolouration on the lobe edges. The other three rats in the main study had dilated uterine horns bilaterally with prominent blood vessels. The gross pathology findings are presented in **Figure 3a**.

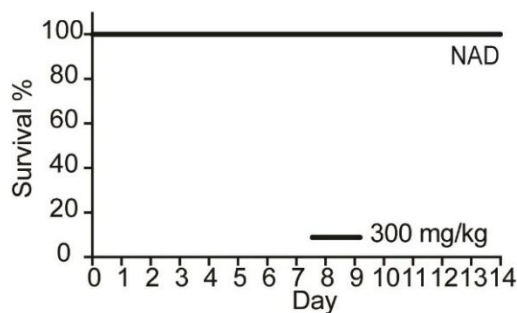
d) Histopathology

The observed lesions were likely dose-dependent. The administration of the test item at the dose level of 2,000 mg/kg BW resulted in mild changes in the gastrointestinal tract, notably erosion of villi tips and mild infiltration of inflammatory cells in the duodenum, jejunum, and ileum. Slightly more severe lesions were observed in the liver (congestion and hepatocytic vacuolation) and kidneys (congestion and glomerulonephritis) (**Figure 3b–h**) of the rats receiving the 2,000 mg/kg BW dose compared to those that received 300 mg/kg BW dose. Mild lesions in the gastrointestinal tract and liver are unlikely to cause clinical disease and should resolve once the causative agent is removed. However, prolonged exposure to the test item could increase the severity of these lesions.

(a) Sighting Study



(b) Main Study



(c) Weekly Monitoring

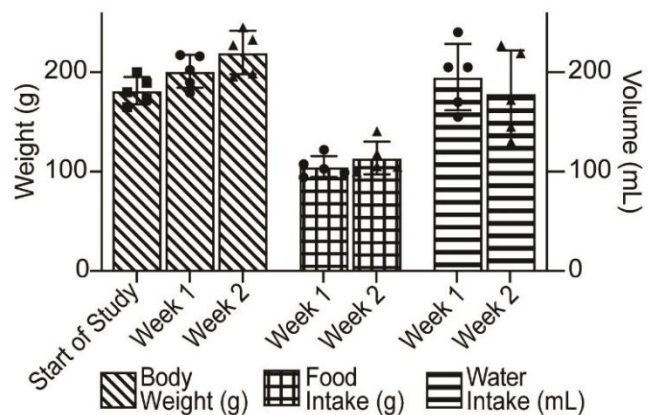


Figure 2. (a) Kaplan Meier percentage survival plot for the sighting study group. The regions bound by the dotted square is enlarged on the right panel. One rat receiving 2,000 mg/kg BW dose died approximately 8 hours post-dosing, while the rat receiving 300 mg/kg BW dose survived. (b) The Kaplan Meier percentage survival plot for the main study group indicated a 100% survival rate (NAD = No abnormality detected); and (c) Bar graphs of weekly mean rat body weights showed a steady increase over 14 days, with a corresponding increase in the weekly mean food intake. In contrast, the weekly mean water intake gradually decreased over the same period. The mean and standard deviations were calculated from rats receiving 300 mg/kg BW dose in the sighting study ($n = 1$) and main study group ($n = 4$).

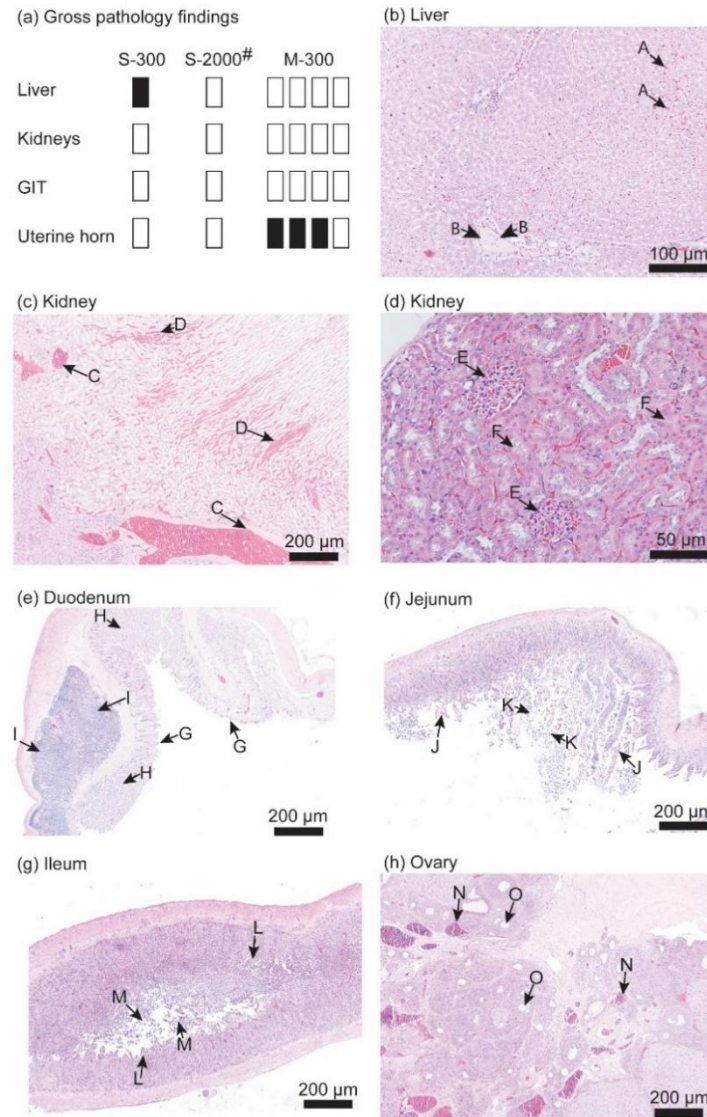


Figure 3. (a) Gross pathology findings from sighting study (S-300) and main study (M-300), which all rats receiving 300 mg/kg BW dose. Group S-2000# (a rat receiving 2,000 mg/kg BW dose) indicates no macroscopic abnormalities were observed but the organs were collected and subjected to histopathology due to mortality. (b)–(h): Organ lesions observed in rats treated with aqueous extract of *B. javanica* dried fruit powder: (b) Liver section from a rat administered a 2,000 mg/kg dose, showing a sinusoid congestion (arrows A) and thrombosis (arrows B) at 100x magnification; (c) Kidney section from a rat administered a 2,000 mg/kg dose, showing severe congestion of major blood vessels in the cortex (arrows C) and cortico-medullary junction (arrows D) at 40x magnification; (d) Kidney section at higher magnification from a rat administered a 2,000 mg/kg dose, displaying swollen, congested, hypercellular glomeruli filling nearly the entire Bowman's space (arrows E) and occluded convoluted tubules due to swollen epithelium (arrows F) at 200x magnification; (e) Duodenum section from a rat administered a 2,000 mg/kg dose, showing short villi (arrows G), numerous inflammatory cells (arrows H), and large gut-associated lymphoid tissue (arrows I) at 40x magnification; (f) Jejunum section from a rat administered a 2,000 mg/kg dose, showing short villi with denuded tips (arrows J) and cellular debris in the lumen (arrows K) at 40x magnification; (g) Ileum section from a rat administered a 2,000 mg/kg dose, displaying tall villi with denuded epithelium at the tip (arrows L) and cellular debris in the lumen (arrows M) at 40x magnification; and (h) Ovary section, similar in three rats administered a 300

mg/kg dose, showing congested blood vessels (arrows N) and numerous small follicles (arrows O) at 40x magnification. [Scale bars: a = 100 μ m; b, d, e, f, g = 200 μ m; c = 50 μ m]. Magnifications were selected to best demonstrate organ-specific lesions.

DISCUSSION

The present study provides insight into the safety profile of the freeze-dried powder of aqueous extract of *B. javanica* fruit. While tolerated at lower doses, adverse effects became evident at the highest tested dose of 2,000 mg/kg BW, indicating potential toxicity at this level. In this study, the observed pathogenesis is expected to follow the conventional route, with the ingested test item absorbed via the gastrointestinal tract, metabolised by the liver, and excreted through the kidneys. Accordingly, lesions in the gastrointestinal tract, liver, kidneys, or reproductive organs may reflect the effects of the test item.

However, at the lower dose of 300 mg/kg BW, gastrointestinal lesions could be influenced by the required pre-dosing fasting period, which can predispose rodents to gastric irritation and mucosal erosion. Fasting reduces gastric content and protective mucus secretion, thereby increasing the susceptibility of the stomach and intestines to mechanical and chemical injury. Similar findings have been reported in previous studies, where fasting-related gastric erosions were documented independent of test substance exposure (24–26). Thus, the lesions at this dose level may not be solely attributable to the test item but also to the fasting condition. Similarly, the liver and kidney lesions at this dose appear to stem from physiological changes during euthanasia rather than from the extract itself, as no clinical toxic symptoms were observed (27–29).

In accordance with OECD Test Guideline 420 for single-dose acute oral toxicity, no control group was included in this study. This guideline is designed to identify an approximate lethal dose range using a fixed-dose procedure while minimising animal use; therefore, the study design focused on directly assessing the effects of the test substance rather than comparing them with a control baseline (22). This approach prioritises ethical considerations by reducing the number of animals required while still providing essential toxicity data. In accordance with this approach, the detailed clinical observations conducted post-dosing provide crucial toxicity assessment to identify the toxic dose level. Careful examination of the rats at the designated time intervals provided important acute assessment of the toxicity onset that otherwise could be missed. This approach coupled with gross and microscopic assessments could help to preliminarily identify targeted organs to be assessed in a longer duration study. One gross observation recorded (in the 300 mg/kg BW group) was the reddish colour of the uterine horn, which may be due to the female rats being in the oestrus phase of their reproductive cycle, where prominent blood vessels become visible (30). Vaginal smears investigation could be included in future studies to confirm this observation. However, it is important to note that control groups are essential in repeated dose studies, where the cumulative effects and long-term toxicity of a substance are evaluated over multiple administrations (22). Additionally, the body weight, food and water intake are non-invasive indicators that provide supportive context for interpreting treatment-related effects, complementing the pathological findings commonly used to monitor systemic toxicity.

In contrast to the minimal findings at 300 mg/kg BW, the limit dose of 2,000 mg/kg BW elicited detectable signs of toxicity. At approximately 2 hours post-dosing with the test item at 2,000 mg/kg, the rat exhibited mild diarrhoea, which may indicate gastrointestinal irritation or altered gut motility (31). By 3 hours, additional signs of lethargy, piloerection, sunken and watery eyes, and a hunched back posture emerged, reflecting systemic toxicity, thermoregulatory imbalance, dehydration, and pain or discomfort. The rat succumbed by 8 hours post-dosing, suggesting acute and severe

toxicological effects, potentially involving multi-organ failure or irreversible damage. According to Chen et al. (32), several metabolites were discovered in the *B. javanica* fruit. The fruit contains a broad spectrum of metabolites, with its toxicity predominantly attributed to water-soluble quassinoids. Among these metabolites, the potential contribution of bruceine A, particularly given its abundance in the extract, to the observed toxicity cannot be excluded. However, previous cell-based investigation showed that bruceine A exhibited low cytotoxicity (IC_{50} of $1251.32 \pm 0.16 \mu\text{g/mL}$) in normal cells, with cell viability remaining above 50% even at the upper tested concentrations, suggesting a favourable safety profile under these conditions (33). A limited in vivo data indicates that oral administration of bruceine A at 6.4 mg/kg/day for five days in dogs caused no clinical abnormalities, with haematological and biochemical parameters remaining within normal ranges (34). While these findings provide supportive context, direct cell-based concentrations cannot be accurately translated to in vivo exposure, and differences in test item, dosage and species limit direct comparability with the present study.

To better interpret the toxicological relevance of the present findings, extrapolation to the human equivalent dose (HED) is informative. Applying the standard 10-fold interspecies uncertainty factor (35, 36), the calculated HED for the observed-adverse-effect level (2,000 mg/kg) corresponds to is 32.26 mg/kg, equivalent to approximately 2.26 g of the formulation for a 70 kg adult. This amount is predicted to produce adverse effects, highlighting the importance of careful dose selection and the need for further safety evaluation.

Given that the extract contains a diverse array of bioactive metabolites, its overall effect reflects a balance between therapeutic potential and possible toxicity (32). Current investigation of the extract in its entirety provided valuable insight into the safety profile of the *B. javanica* fruit extract under physiological conditions. Evaluating whether the potential medical benefits justify the associated health risks requires a comprehensive analysis of various factors, including the nature of the benefits, the severity of the risks, and the context of the medical intervention. Therefore, further investigations, including pathological analysis, dose-response studies, and biochemical assessments, are recommended to determine the extract's toxicological mechanisms and establish a safe dose threshold.

CONCLUSION

A 2,000 mg/kg BW oral dose of *B. javanica* aqueous extract to Sprague Dawley rats induced toxic symptoms along with organ lesions, suggesting a LD_{50} of below 2,000 mg/kg. According to the Globally Harmonised System for Chemical Substances and Mixtures (GHS), the observed acute toxicity ranks this test item as Category 5. However, as this study involved only a single dose and a short observation period, a repeated dose study of longer duration is strongly recommended to assess the general toxicity of the extract more comprehensively.

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Investigation, methodology, writing – review & editing; Nurul Syahira Shabudin: Investigation, methodology, writing – review & editing; Dalnitha Lea Nicol: Investigation, methodology, writing – review & editing; Norzahirah Ahmad: Conceptualisation, data curation, formal analysis, investigation, methodology, project administration, resources, supervision, validation, visualisation, writing – review & editing.

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