RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2026.088

ScienceAsia 52 (5): 2026: ID 2026088: **1–9**

Unveiling the safety of medicinal okra: Acute toxicity investigation of a single dose methanolic extract of *Abelmoschus esculentus* and its derived flavonoids in rats

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Received 1 May 2026, Accepted 25 Sep 2026
Available online 18 Oct 2026

ABSTRACT: *Abelmoschus esculentus* (okra) is valued for its nutritional and medicinal benefits, particularly its flavonoid constituents with significant pharmacological effects. Despite its traditional use, systematic evaluation of the toxicity profile of *A. esculentus* and its bioactive compounds when administered at higher doses remains limited. Present study addresses this gap by evaluating the acute oral toxicity screening of methanolic extract of *A. esculentus* and its two major flavonoid constituents to support dose selection for subsequent biological activity studies. Two major compounds were identified, isolated using UHPLC techniques and further confirmed through LCMS/MS analysis. Male Sprague Dawley rats were randomly assigned to four groups and orally administered a single dose of 300 mg/kg of either compound A (rutin), B (quercitrin), the methanolic extract, or a vehicle control. Rats were monitored over a 14-day period for signs of toxicity, including changes in body weight, food and water intake, physical, behavioural, sensory responses, and organ weights. No mortality or treatment-related adverse effects were observed. No significant differences were observed in metabolic, behavioural, or sensory parameters across all groups. Relative vital organs weight demonstrated a non-significant change, although a slight increase in spleen weight was noticed in the methanolic extract group, it remained within physiological limits. These findings suggest that methanolic extract of *A. esculentus* and its major constituents are well tolerated at the tested dose of 300 mg/kg, supporting their potential safety as guidance for dosage in therapeutics. Further investigation involving chronic toxicity and mechanistic evaluations are warranted to confirm long-term safety.

KEYWORDS: *Abelmoschus esculentus*, flavonoids, methanolic extract, acute toxicity

INTRODUCTION

Natural products and plant-based compounds have long been explored for their therapeutic potential, offering bioactive constituents with diverse pharmacological benefits [1]. Toxicity assessments are crucial to determine the potential risks of natural products, ensuring their safe application in medicine and nutraceuticals [2]. Acute toxicity studies are fundamental in identifying possible adverse effects following short-term exposure and establishing safe dosage ranges [3].

Abelmoschus esculentus, commonly known as okra is widely consumed staple vegetable known for its nutritional and medicinal properties. Rich in bioactive compounds such as flavonoids, polysaccharides, and phenolic acid compounds, *A. esculentus* has been traditionally used for its antioxidant, anti-inflammatory, and hypoglycemic effects [4]. Among its key constituents, rutin and quercitrin have been identified as major flavonoids contributing to its pharmacological activities [4, 5]. Despite these benefits, comprehensive toxicity evaluations of *A. esculentus* and its major flavonoids remain limited, particularly regarding their acute oral toxicity profile.

Given the increasing interest in *A. esculentus* as a functional food and medicinal plant, it is imperative to conduct preliminary acute oral toxicity screening to support dose selection for downstream biological activities evaluation. This study isolated and identified major flavonoids in okra and investigated the acute oral toxicity of the treatment samples. A modified methodology from the dose-threshold principles outlined in Organisation or Economic Co-operation and Development (OECD) acute oral toxicity guidelines. The assessment included observations of changes in the body weight, food and water consumption, physical and behavioural responses, sensory function, and variations in organ weight. The findings from this study contribute to the knowledge on preliminary toxicity profile and toxicological data of *A. esculentus* and its isolated flavonoids, thereby supporting its potential applications and guiding dosage for therapeutic usage in medicine and health-related industries.

MATERIALS AND METHODS

Sample collection and identification

Young whole *A. esculentus* fruits were obtained from a vegetable farm in Rawang, Selangor, Malaysia. The sample was outsourced to the Biodiversity Unit, In-

stitute of Biosciences (IBS), Universiti Putra Malaysia (UPM), for identification and authentication by a plant taxonomist. The voucher number for the plant was MFI 0248/22.

Extract preparation

The methanolic extract of *A. esculentus* fruit was prepared following a modified method from that previously described [4]. Freshly purchased fruits were washed, dried at 50 °C, and ground into a fine powder. A 30 g sample was extracted with 300 ml of 70% acidified methanol (0.1% hydrochloric acid, v/v) and sonicated for 15 min using an ultrasonic cleaner (Branson Ultrasonic Corporation, USA) at 42 kHz, 135 W. The mixture was then left at room temperature for 24 h and centrifuged at 4,000 × *g* for 10 min to obtain the supernatant. The residual material was re-extracted under the same condition, and the supernatants were combined. The pooled supernatant was concentrated using a Genevac EZ-2 Plus Personal Evaporator (Genevac Ltd., Ipswich, UK), and the dried residue was stored at −20 °C in the dark for further analysis.

Identification and isolation of major compounds from methanolic extracts of *A. esculentus*

Two major compounds were identified and isolated using the Agilent 1290 Infinity UHPLC System (Agilent Technologies, USA). Column C-18 was utilised with the mobile phases of (A) 1% acetic acid (v/v) and (B) 100% acetonitrile (v/v), with a gradient elution as follows: 0–2 min at 90% A, 2–20 min at 50% A, and 20–22 min returning to 90% A, followed by equilibration at 90% A for 3 min [6]. The compounds were identified by comparing their retention time to those of the standards rutin (Catalog No.: CFN99642) and quercitrin (Catalog No.: CFN98850), which were purchased from ChemFaces (Wuhan, China). Fractions of the two major compounds (compound A and compound B) were collected according to the retention time of the identified peaks. These compounds were then concentrated using a Genevac EZ-2 Ples Personal Evaporator (Genevac Ltd.). The isolated compounds were verified through analytical LCMS/MS analysis.

Characterisation of compounds isolated from methanolic extract of *A. esculentus* using LCMS/MS

For identification of isolated compound A (rutin) and B (quercitrin) from *A. esculentus* methanolic extract, LCMS/MS (Sciex Triple TOF 5600+ System, Canada) was employed. The LCMS/MS system used was reversed-phase C-18 column maintained at a temperature of 37 °C. The mobile phases for phenolic compound identification were: (A) acetonitrile (90:10, v/v) and (B) water, both containing 0.1% formic acid and 10 mM ammonium formate. The gradient elution in negative ion mode was performed as follows: 0–1 min 5–50% A; 1–3.5 min 50–80% A; 3.5–8.5 min 85–98% A; 8.5–9 min, 98–100% A and maintained 100% A for 3 min, followed by equilibration with 5% A for 3 min. While, in the positive mode, the mobile phase gradient was set as follows: 0–2 min 5–35% A; 2–5 min 35–45% A; 5–5.5 min 45–54% A; 5.5–6 min 54–67% A; 6–8.5 min 67–84% A; 8.5–14.5 min 84–90% A; 14.5–18 min 90–100% A; 18–18.5 min 100–5% A and kept at 5% A for 3 min [7]. The flow rate was maintained at 0.4 ml/min with an injective volume of 20 µl. The ESI source was operated in negative mode to produce $[M-H]^-$ ions under the following conditions: a vaporiser temperature of 450 °C, with a dry gas (nitrogen). The raw data was analysed using MS-DIAL software to facilitate the identification of precise compounds and its analytical measurement.

Animal and experimental design

Twenty male Sprague Dawley rats with 180–200 g body weight and aged 6–8 weeks were obtained from IBS, UPM. The animals were acclimatised for two weeks in the animal house facility of the Faculty of Medicine, Universiti Sultan Zainal Abidin (UniSZA). This experimental design was approved by the UniSZA Animal and Plant Research Ethics Committee (UAPREC), UniSZA, Malaysia, No. UAPREC/008/003 on 12th February 2025. Food and drinks were provided *ad libitumly*. Besides, the environment was kept under controlled condition with room temperature of 21 °C–23 °C and 12 h of light-dark cycle. Body weight was measured daily at 7.00 am [8].

Acute oral toxicity screening in experimental animals

Acute oral toxicity was evaluated using a simplified screening method modified from the dose-threshold principles of OECD acute oral toxicity guidelines. The methanolic extract of *A. esculentus* fruits along with its flavonoid derivatives, rutin and quercitrin, were each administered orally at a single dose of 300 mg/kg. Healthy male rats (mentioned above) were randomly divided into four groups, with five animals in each group ($n = 5$). Group 1 served as the control group and received vehicle, 0.5% carboxymethyl cellulose (CMC) at 0.5 ml/kg. Group 2 was administered 300 mg/kg of methanolic extract of *A. esculentus* fruit, while Group 3 and 4 received 300 mg/kg of rutin and 300 mg/kg of quercitrin which were isolated from methanolic extract of *A. esculentus*, respectively. All treatments were administered orally by gavage. Toxicological and clinical observations were conducted after the administration of the extract at 1, 2, and 4 h, as well as daily for 14 days. Clinical signs indicative of toxic effects and any mortality among the rats were systematically recorded.

Physical changes assessment

The physical condition of the rats was monitored daily for 14 days post-treatment to evaluate potential signs of acute toxicity. Observations were conducted under

consistent environmental conditions to ensure accurate assessment [8]. The physical changes were monitored by observing the changes in eyes, fur, posture, faeces characteristics, and urine consistency.

Behavioural changes assessment

Behavioural changes in the rats were closely monitored daily for 14 days post-treatment to evaluate signs of acute toxicity. The evaluations were conducted in a quiet, well-lit environment to minimise external influences [9]. The behavioural changes observed include the grooming activity, gait, sleeping pattern, somatomotor activity, and salivation.

Sensory function test

Sensory function tests assessed auditory stimulation, visual reaction, and proprioceptive reactivity in Sprague Dawley rats over the 14-day monitoring period. A consistent sound source (hand clap) was introduced to evaluate auditory stimulation [10]. Observations were made for ear twitching, startle reflex, or freezing behaviour. The score for auditory stimulation was graded on a scale of 0–2 with the following criteria: 0 = no reaction, 1 = minimal response (slight ear or body twitch), and 2 = clear startle or movement directed towards the sound.

Visual reactions were assessed by moving a small object into the rat's visual field without generating air currents. The object's sudden presence within 20 cm of the rat allowed observation of defensive or attentional responses, such as head movement, retreating, or freezing [10]. The score for visual reaction was graded on the scale of 0–2 with the following criteria: 0 = no reaction, 1 = minor head or eye movement toward or away from the object, and 2 = significant response such as jumping or other defensive behaviour.

Proprioceptive reactivity was evaluated by testing paw placement reflexes and limb reorientation [10]. The forepaw was gently placed on its dorsum, and the time taken to correct the paw to its normal position was recorded. Hindlimb proprioception was assessed by gently lifting the rat and observing its ability to reorient and support its body when placed back on a flat surface. The score for proprioceptive reactivity was graded on a scale of 0–2 with the following criteria: 0 = no reaction, 1 = slow correction limb (<3 s), and 2 = immediate and smooth correction (<1 s).

Organ collection

At the end of the study, rats were euthanised using an intraperitoneal overdose of ketamine (350 mg/kg) and xylazine (40 mg/kg). The brain, heart, liver, kidney, testis, spleen, thymus and stomach were removed and weighed [11]. The relative organ was weighed using the following formula: Relative organ weight (%) = (Organ weight)/(Rat weight on the sacrifice day) × 100%.

Statistical analysis

The data obtained were tabulated as mean ± standard deviation (SD) ($n = 5$). The one-way ANOVA test in SPSS 22.0 for windows was used to determine the statistical significance. The $p < 0.05$, which was attained from ANOVA analysis using Tukey's post hoc test specified that the results are statistically significant.

RESULTS AND DISCUSSION

Identification of major compounds in methanolic extract of *A. esculentus*

Extraction yield of 100 g of young *A. esculentus* whole fruits was 12.94 ± 0.71 mg/g. Fig. 1 presents the UHPLC chromatograms of *A. esculentus* extract, highlighting its bioactive compounds. The chromatogram in Fig. 1A illustrates the separation of nine major compounds identified in the extract, emphasising the complexity and richness of its phytochemical profile. Fig. 1B demonstrates the overlapping chromatograms of the *A. esculentus* extract alongside mixed standards of rutin and quercitrin. This comparison reinforces the validity of our findings, as the retention times and UV absorption maxima (280 nm) of the detected compounds align precisely with those of the standards.

A. esculentus known as superfood, is gaining recognition for its high nutritional value and therapeutic effects supported by scientific evidence. Despite its widespread use in culinary and traditional medicine, pharmacological research remains preliminary, primarily focusing on crude or fractionated extracts to isolate active compounds that contribute to its pharmacological benefits. Phenolic compounds, especially flavonoids, are regarded as one of the most important bioactive compounds in *A. esculentus* fruit [4]. Methanol was used to extract *A. esculentus* fruits based on previous study, which demonstrated a higher yield of phenolic content from *A. esculentus* [4]. A review also reported that methanolic extracts of *A. esculentus* exhibited significant antioxidant and biological activities compared to aqueous and ethanolic extracts [12]. In this study, two major flavonoids, rutin and quercitrin, were identified from the methanolic extract using UHPLC techniques. Identifying rutin and quercitrin within the extract defines the potential pharmacological significance of these flavonoids, which are known for their antioxidant and anti-inflammatory properties [13].

Characterisation of isolated compounds from methanolic extract of *A. esculentus*

The identities of the isolated compounds were further supported by LC-MS/MS analysis, with the isolated rutin and quercitrin showing $[M-H]^-$ ions at m/z 609.12 and 445.07, respectively, consistent with their corresponding reference standards (609.13 and 445.10). These values were determined through

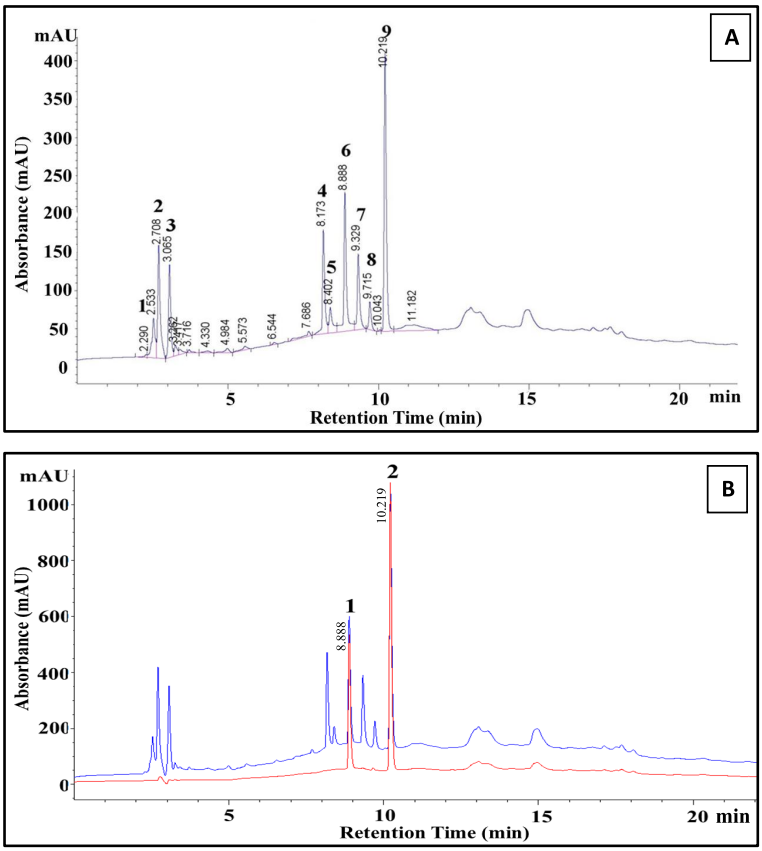


Fig. 1 Ultra-high performance liquid chromatograms of *A. esculentus* extract (A). The chromatograms of mixed standards, rutin (1) and quercitrin (2) overlapped on the *A. esculentus* extracts chromatograms (B).

comparative analysis with the MS spectrum. A precise match was observed between the standards of rutin and quercitrin and the compounds isolated from *A. esculentus* extracts in this study. This strong correlation confirms the identification of these isolated compounds. The consistency in m/z values indicates that the structural integrity of the isolated compounds was maintained, strengthening their potential bioactive qualities and allowing for further exploration into their pharmaceutical uses [14]. UHPLC analysis demonstrated chromatographic purities of 97.58% for rutin and 99.08% for quercitrin. These values exceed the >95% purity criterion reported for isolated natural products [15], supporting the suitability of both compounds as relatively well-defined isolated test compounds for subsequent biological evaluation.

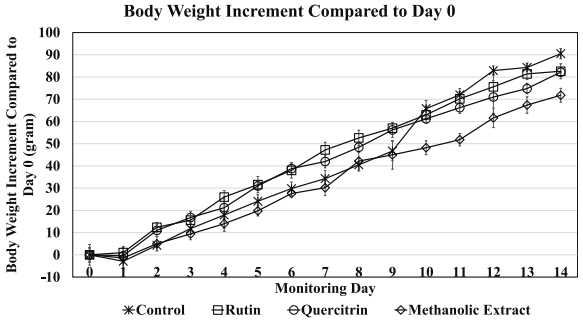


Fig. 2 Effect of 300 mg/kg of rutin, quercitrin and methanolic extract of *A. esculentus* on body weight increment compared to control ($n = 5$ rats per group).

Acute oral toxicity assessment

The mortality and morbidity of the rats were monitored for 14 days. Orally treated rats with 300 mg/kg of methanolic extract, rutin, and quercitrin showed no mortality or toxic symptoms. A selected dose of 300 mg/kg body weight was chosen as preliminary screening dose using a simplified protocol inspired by the dose-threshold approach described in OECD 423 acute oral toxicity guidelines [16]. Male rats were utilised in this acute toxicity studies to minimise variability with consistent pharmacokinetics, as previous studies demonstrated female rats exhibit higher drug exposure and hepatic and renal distribution relative to male rats [17].

Although multiple studies report on toxicity of

rutin and quercitrin, it is included in this study to understand the impacts of extraction methods on stability and bioactivity of isolated compounds, thereby affecting their therapeutic potential. A single and same dose (300 mg/kg) of methanolic extract, rutin and quercitrin were chosen to facilitate standardised comparison of their acute toxicity tolerability dose prior to the selection of dose levels for subsequent biological activity evaluation [18]. The doses of isolated rutin and quercitrin were not intended to replicate their concentrations in the methanolic extract, as the isolated compounds may exhibit different bioavailability and biological responses from those observed within the complex plant matrix. Therefore, the isolated compounds were evaluated at 300 mg/kg independently, to characterise their individual acute toxicity responses under the same experimental conditions.

No significant differences ($p < 0.05$) in body weight of treatment group compared to control group was observed throughout the treatment period. The daily body weight increment (Fig. 2) was comparable to those of the control groups throughout the 14-day observation.

Body weight is a crucial parameter in acute toxicity studies, as significant weight loss may indicate systemic toxicity, appetite suppression, or metabolic impairment. This study shows no significant differences in body weight among the control and treated groups, suggesting that the methanolic extract of *A. esculentus* and its major constituents (rutin and quercitrin) did not alter feeding behaviour or metabolic function. This finding was consistent with the previous studies on different plant extracts and isolated constituents [18]. Rutin [19] and quercitrin [20] were reported to induce body weight gain in rats treated with 2,000 mg/kg of respective compounds, within the normal physiological limits.

The food and water consumption of all rats in the control group, as well as those treated with 300 mg/kg of methanolic extract of *A. esculentus*, rutin, and quercitrin, demonstrated consistent food consumption, ranging from 17–25 g of pellets per day, and stable water intake between 20 and 30 ml of tap water throughout the 14 days of observation. No significant difference in mean daily food and water intake between the control and treated group rats. Consistent mean food and water intake by rats throughout the study period suggests that methanolic extract of *A. esculentus*, rutin and quercitrin were well-tolerated and did not affect behaviour and metabolic process in the treated rats. These findings aligned with previous report, that food and water intake are strongly correlated to body weight, nutritional outcomes, and metabolic balance [21]. Furthermore, the stable body weight observed throughout the 14 days supports the toxicity profile of *A. esculentus* and its constituents, as severe toxicity often manifests as weight reduction due to impaired digestion, absorption, or energy metabolism [21]. However, the body weight increment in methanolic extract-treated group exhibited a numerically lower body weight increment, while the difference was not statistically significant compared with other groups. This finding aligns with previous study conducted by Sarkar et al [22], non-significant changes in body weight gain indicates that observed variation reflects no major systemic toxicity effects or metabolic disruptions.

Physical changes

Daily monitoring exhibited comparable physical changes in all treatments of *A. esculentus* and control groups. The physical observations of the treated animals over the 14 days indicate no apparent signs of acute toxicity associated with the tested compounds. Table 1 summarises the physical changes observed for all groups.

The absence of discharge, swelling, or inflammation in the eyes, coupled with normal pupillary responses, suggests no adverse ocular effects [23]. The integrity of the ocular structures is crucial in toxicity assessments, as systemic toxicity often manifests in conjunctival redness, excessive lacrimation, or corneal clouding. Similarly, the fur remained smooth and evenly distributed without indications of alopecia, matting, or external parasitic infections [24]. Toxic exposures commonly affect skin and fur condition, but the lack of such signs suggests that the administered compounds did not induce dermatological or metabolic stress. Additionally, normal posture and movement across the groups indicate the absence of musculoskeletal or neurological dysfunction. These findings suggest that neither the methanolic extract of *A. esculentus* nor its major flavonoids disrupted neuromuscular coordination or general physiological stability [25].

Moreover, treated rats exhibited normal stool consistency and urine characteristics, suggesting the extract and its constituents did not impair digestive or renal health by remaining gastrointestinal and renal function. No faecal mucus or foul odour was observed, indicating normal gut microbial activity [26]. Normal urine appearance indicates no signs of abnormalities in urine or metabolic disturbance. Kidney function is critical parameter in toxicity assessments, often leads to changes in urine appearance, including cloudiness, dark discolouration, or the presence of the protein and red blood cells. Notably, there was no abnormal odour, which may occur in the presence of toxic waste products [27].

Piloerection is indicated as physiological response to stress, pain or autonomic nervous system activation. The lack of this response suggests that the tested compound did not trigger excessive sympathetic nervous system activation or thermoregulatory imbalances [28]. These observations reinforce the toxicity profile of *A. esculentus* methanolic extract, rutin, and

Table 1 Summary of the physical changes observed in all control and treated group rats.

Parameter	Observation
Eyes	Eyes appeared clear and free from discharge, swelling, or redness, with no signs of opacity, cloudiness, or inflammation.
Lacrimation	No excessive lacrimation, swelling, redness, dryness, or evidence of crusting or staining around the eyes.
Fur	Fur was smooth, evenly distributed, and free of any signs of matting, alopecia (hair loss), discoloration, excessive grooming, or fur plucking.
Posture	Normal posture without signs of hunching, stiffness, or abnormal positioning observed.
Faeces	Faecal pellets were of normal appearance, size, shape, colour, and consistency, with no evidence of diarrhoea, mucus, abnormal discoloration, or irregularity.
Urine	Urine appeared clear to pale yellow, with no apparent abnormal colour or odour.
Piloerection	No abnormal piloerection was observed; the fur remained normally positioned against the body.

Table 2 Summary of the behavioural changes observed in all control and treated group rats.

Parameter	Observation
Grooming behaviour	Normal grooming, without excessive grooming.
Itching or scratching behaviour	No abnormal or persistent behaviours related to itching or scratching.
Gait	Smooth and coordinated movements without limping, dragging of limbs, or ataxia.
Sleeping pattern	Rest predominantly during daylight hours with no excessive lethargy.
Salivation	No abnormal salivation, drooling or frothy saliva.
Somatomotor activity	Normal exploration, reactivity to stimuli, and appropriate responses to handling.
Bizarre behaviour	No repetitive circling, head bobbing, or unprovoked aggression.
Coma	Alert and responsive.
Death	No fatalities recorded.

quercitrin, as no abnormalities were detected in key physical indicators of toxicity. Similar observations were reported in previous acute toxicity studies of methanolic extract of *A. esculentus* [29], rutin [19], and quercitrin [20], where rats treated with 2,000 mg/kg maintained a healthy physical appearance. The absence of distress-related behaviours or external manifestations of systemic toxicity highlights the tolerability of the administered doses. These findings align with the body weight results, further corroborating the non-toxic nature of the tested compounds under the condition of this study.

Behavioural changes

The behavioural changes were monitored daily for 14 days. Continuous monitoring reveals insignificant differences between the *A. esculentus* extract and its constituents compared to the control group, with no apparent treatment-related abnormalities. Table 2 summarises the behavioural changes observed for all treated rats.

Grooming as a key indicator of normal behaviour, is a vital self-maintenance activity in rodents. Its continued presence suggests healthy neurological and physiological function. No rats exhibited excessive scratching or itching, indicating that the compounds did not cause skin irritation, allergic reactions, or other dermatological problems [30]. Toxic substances often induce histaminergic responses or hypersensitivity reactions, leading to persistent scratching or piloerection, but such signs were not observed in any treatment group in this study.

Toxic effects on the nervous system often result in tremors, stiffness, or movement difficulties [31], none of which were reported in this study by all the tested compounds. Likewise, normal sleep patterns and maintained rest-activity cycle in the treated animals suggest the absence of systemic toxicity, metabolic stress, or neurological depression [31].

Similarly, salivation remained normal among all treated groups signifies the absence of toxicity symptom resulting from oral mucosa irritation or metabolic disturbances [32]. Moreover, the rats exhibited appropriate somatomotor activity, a critical indicator of general health and neurological integrity, with absence of hypoactivity or hyperactivity. This suggests that the administered compounds did not cause sedation or overstimulation [33]. Additionally, no abnormal bizarre behaviour indicative of neurobehavioural toxicity was observed in the treated rats. Throughout the observation period, all animals remained alert and responsive, and confirms the absence of serious neurological impairment or life-threatening toxicity [34]. The absence of mortality is a fundamental indicator in acute toxicity studies, and these findings strongly suggest that *A. esculentus* methanolic extract and its major bioactive compounds did not elicit acute toxic effects at the tested concentrations. The current findings are further supported by previous acute toxicity studies of methanolic extract of *A. esculentus* [29], rutin [19], and quercitrin [20], in which rats treated with 2,000 mg/kg reported to demonstrate normal behavioural activities.

Sensory function

The auditory, visual and proprioceptive changes were monitored over a 14-day observation period. All treat-

Table 3 Effect of 300 mg/kg of rutin, quercitrin, and methanolic extract of *A. esculentus* on relative organ weight (%) compared to control. Data are shown as mean percentage ± standard deviation (SD) ($n = 5$ rats per group).

Treatment	Brain	Heart	Liver	Right kidney	Right testis	Spleen	Thymus	Stomach
Control	0.58 ± 0.07	0.50 ± 0.20	3.66 ± 0.42	0.71 ± 0.08	0.97 ± 0.38	0.23 ± 0.09	0.18 ± 0.07	0.50 ± 0.12
Rutin	0.70 ± 0.07	0.40 ± 0.04	4.07 ± 0.31	0.83 ± 0.04	1.11 ± 0.13	0.24 ± 0.01	0.23 ± 0.05	0.62 ± 0.04
Quercitrin	0.69 ± 0.05	0.37 ± 0.03	4.03 ± 0.31	0.83 ± 0.08	1.04 ± 0.15	0.29 ± 0.03	0.24 ± 0.07	0.61 ± 0.05
Methanolic extract	0.70 ± 0.10	0.39 ± 0.02	4.04 ± 0.43	0.83 ± 0.06	0.93 ± 0.31	0.34 ± 0.06*	0.21 ± 0.04	0.61 ± 0.10

* Represent significant differences ($p < 0.05$) compared to control treatment.

ment groups consistently scored 2 for each sensory modality, indicating normal responsiveness throughout the study. The rats responded clearly to the clapping sounds in the auditory stimulation test characterised by ear twitching, head turning toward the sound source, and slight startle response without signs of hyperexcitability. This response suggests that tested compounds did not affect auditory perception or the associated neurological pathways [35]. The observed startle reflex represents an innate response mediated by subcortical neural circuits, further supporting the conclusion that both flavonoid compounds did not disrupt auditory-related neurophysiology [20]. In visual reaction, the rats consistently responded to moving objects presented within their visual field by orienting their heads and avoiding the stimulus. This observation indicates that visual acuity and sensorimotor integration remained intact throughout the study with unaffected central or peripheral nervous system [36]. As for proprioceptive reactivity, the rats took a short time for repositioning, suggesting the normal motor coordination and balance. Efficient proprioceptive reactivity relies on the integrity of both sensory input and motor output systems. Therefore, the observed responses indicate that these systems remained functionally intact. In contrast, delayed or impaired repositioning would suggest to be sign of neurotoxicity or sensory-motor integration deficit, which were not evident in this study [37]. These findings are consistent with previous studies reporting the neuroprotective effects of *A. esculentus* and flavonoids in preserving the functional integrity of the central and peripheral nervous systems [38].

Relative organ weight

All organs were collected and weighed. The organ weight assessment is a crucial indicator to evaluate pathological changes such as inflammation, atrophy, or hypertrophy due to toxic exposure [39]. In this study, administering the compounds insignificantly alter the weight of vital organs despite observable numerical variations, reinforcing the absence of acute toxicity [16]. All organ except spleen weights remained comparable across all groups, indicating that the tested compounds did not induce organ damage, fluid retention, or metabolic imbalances [39]. These findings may be attributed to inherent biological variability and the limited sample size, which potentially reduce the statistical power to determine moderate effects in acute oral toxicity screening of tested samples. Table 3 shows the relative organ weight (%) of collected vital organs as mean percentage ± SD.

The slight increase in spleen weight in the methanolic extract-treated group ($p < 0.05$) may suggest mild immune system activation or an adaptive physiological response rather than toxicity. The spleen plays a critical role in haematopoiesis and immune regulation, and a marginal increase in its weight may reflect enhanced immune cell proliferation or red blood cell turnover [40]. The slight increase in organ weight observed in this study contrasts with a previous study, in which methanolic extract of *A. esculentus* at 2,000 mg/kg did not adversely affect relative organ weight [29]. Importantly, the observed increase in spleen weight was not accompanied by other indicators of systemic toxicity, such as behavioural abnormalities, changes in body weight, or alterations in other organ indices. This absence of corroborative toxicological signs suggests that the splenic response is likely transient and non-adverse. However, absence of haematological and histological abnormalities indicating the biological significance of these findings inconclusive.

CONCLUSION

The acute oral toxicity study of methanolic extract of *A. esculentus* and its major constituents (rutin and quercitrin) demonstrated no significant toxic effects. Key physiological parameters, including body weight, food intake, and water consumption, remained stable throughout the 14-day experimental period, indicating no adverse effects on metabolisms or feeding behaviour. Furthermore, physical and behavioural observations showed no signs of neurological impairment, distress, or abnormal aggression. Sensory response assessments suggested no disruption of neural pathways and organ weight analysis showed no significant alterations in major vital organs. Despite these promising findings, the present study is limited to short-term toxicity evaluation with a relatively small sample size. Therefore, further studies are recommended to perform sub-acute and chronic toxicity studies with larger sample size, prioritising hematological, biochemical profiling, histopathological evaluations, and immunological assessments are recommended to elucidate the

underlying mechanisms and confirm the long-term safety of the methanolic extract of *A. esculentus* and its flavonoid derivatives, rutin and quercitrin. This will enable to determine a safer dosage range and advancing the fundamental understanding of the pharmacokinetic and metabolic behaviour of methanolic extract of *A. esculentus* and its major constituents.

Acknowledgements: This study is funded by Malaysian Ministry of Higher Education (MOHE) through Fundamental Research Grant Scheme (FRGS), (Grant Number: FRGS/1/2019/WAB11/UNISZA/03/1).

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