International Journal of Agricultural Technology 2026 Vol. 22(1):287-306
DOI: https://doi.org/10.63369/ijat.2026.22.1.287-306
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Evaluation of acute and subacute toxicity of *Argyreia acuta* Lour. leaf extract in mice

Nhung, T. T. P. and Quoc, L. P. T.*

Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Ho Chi Minh City, Vietnam.

Nhung, T. T. P. and Quoc, L. P. T. (2026). Evaluation of acute and subacute toxicity of *Argyreia acuta* Lour. leaf extract in mice. International Journal of Agricultural Technology 22(1):287-306.

Abstract This study evaluated the acute and subacute oral toxicity of *Argyreia acuta* Lour. leaf ethanol extract (EEAC) in Swiss albino mice at different doses. In the acute study (14 days), the highest dose (5000 mg/kg) increased relative liver (4.25% vs. 3.83% in controls), heart (0.88% vs. 0.44%), and kidney weights (1.66% vs. 1.13%; $p < 0.05$), and was accompanied by a temporary reduction in food (6.58 ± 0.05 g) and water intake (5.59 ± 0.04 mL). In the subacute study (28 days), EEAC at 500 mg/kg produced similar dose-related trends and increased RBC (9.36×10^6 vs. 8.63×10^6 cells/mm^3), WBC (4.06×10^3 vs. 3.35×10^3 cells/mm^3), and total protein (7.12 vs. 6.49 g/dL; $p < 0.05$). While liver function remained stable and renal changes were mild and reversible, urinary indices suggested metabolic adaptation. Histopathology showed no severe toxicity. Notably, parameters in satellite groups returned to control ranges by day 42 (acute) and day 56 (subacute). Overall, high-dose EEAC induced transient physiological changes without evidence of long-term toxicity under the tested conditions.

Keywords: Acute toxicity, Ethanol extract, Subacute toxicity, Swiss albino mice

Introduction

Argyreia acuta Lour. (Convolvulaceae) is a climbing plant widely distributed in tropical and subtropical regions, including India, Southeast Asia, and parts of Africa. The species is recognized by its heart-shaped leaves with wavy dark-green margins and showy flowers that attract pollinators (Gunadasa *et al*., 2024). In traditional medicine, *A. acuta* is valued as a medicinal resource, and its therapeutic use is generally linked to bioactive constituents present across different plant parts (Li *et al*., 2021). The leaves are commonly prepared for topical use to relieve pain and swelling, and for oral use in gastrointestinal, respiratory, and skin-related complaints (Zhang *et al*., 2020). Roots and flowers have also been used for conditions such as asthma, pneumonia, and infections (Hu *et al*., 2018). Phytochemical work on the leaves reports major groups such

*Corresponding Author: Quoc, L. P. T.; Email: lephamtanquoc@iuh.edu.vn

as flavonoids, saponins, alkaloids, and phenolics, which are associated with anti-inflammatory, antioxidant, and antimicrobial activities (Li *et al*., 2021). Similar pharmacological potential has been described for other *Argyreia* species, supporting the broader therapeutic relevance of the genus (Yin *et al*., 2015; Wang *et al*., 2016; Yu *et al*., 2017; Kashyap *et al*., 2020; Lalan *et al*., 2015).

Despite growing evidence on efficacy, systematic toxicity data for *A. acuta* remain limited, particularly from controlled animal studies. This gap is important because herbal preparations may cause adverse effects, interact with conventional drugs, or present quality-related safety issues depending on composition and dose (Wang *et al*., 2023). Toxicity assessment, therefore, remains a key step for defining safe exposure ranges and supporting responsible development of plant-derived products (Amorim *et al*., 2024). In this context, the present study investigated the acute and subacute oral toxicity of EEAC in mice and assessed the reversibility of any changes using satellite recovery groups.

Materials and methods

Collection of plant material and extract preparation

Argyreia acuta Lour. leaves were collected in February 2024 from Son Tra District, Quang Ngai Province, Vietnam. A voucher specimen (AC120224VST) was deposited at the Biotechnology Laboratory, Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City. Leaves were inspected, washed, and shade-dried for three days to reduce light exposure and preserve constituents. The dried material was ground using a Retsch GM 200 grinder (Retsch GmbH, Germany) and stored under dry conditions until extraction.

One hundred grams of leaf powder were macerated in 1000 mL ethanol (1:10, w/v) at room temperature for 24–72 h with intermittent stirring. After filtration through No. 4 filter paper, the filtrate was concentrated at 40 °C using a rotary evaporator (Buchi, Büchi Labortechnik AG, Switzerland). The extraction yield was 27%, and the resulting ethanol extract of *A. acuta* leaves (EEAC) was stored in dark containers at 4 °C.

Phytochemical analysis of Argyreia acuta extract

Qualitative assays (colorimetric/precipitation reactions) were used to screen for tannins, flavonoids, terpenoids, polyphenols, saponins, steroids, alkaloids, and cardiac glycosides (Nhung and Quoc, 2025). Total polyphenol

content (TPC) was determined by the Folin–Ciocalteu method (absorbance at 765 nm), and total flavonoid content (TFC) was measured using the $AlCl_3$ method (absorbance at 415 nm) (Tran *et al*., 2023a)

Animal experiments

Swiss albino mice (30–32 g) were obtained from the Pasteur Institute of Ho Chi Minh City, Vietnam. Animals were acclimated for seven days and housed under controlled conditions (24–26 °C; 55–60% humidity; 12 h light/12 h dark), with pellet diet and filtered water available ad libitum. All procedures followed the principles of the 3Rs and humane experimental technique (Hubrecht and Carter, 2019).

Experimental designs

Acute oral toxicity. Acute toxicity was conducted according to OECD Test Guideline 420 (2001) and WHO guidelines (2008). Twenty-four mice were randomized into four groups (n = 6): control (distilled water, 5 mL/kg b.w.) and EEAC-treated groups (1000, 3000, and 5000 mg/kg b.w.). Clinical signs were monitored intensively for 6 h after dosing and then daily for 14 days. Two satellite groups (control and 5000 mg/kg) were treated for 14 days and observed for an additional 28 days to evaluate recovery (Nhung and Quoc, 2024a). Body weight, food/water intake, urine parameters, and mortality were recorded weekly. At study end, mice were euthanized by CO_2 inhalation for gross examination and histopathology of heart, liver, and kidneys.

Subacute toxicity. The 28-day study followed WHO recommendations (2008), with reference to OECD Test Guideline 470 (2022) as cited in the original protocol. Mice were assigned to four groups (n = 6): control (distilled water, 5 mL/kg b.w.) and EEAC at 100, 300, or 500 mg/kg b.w., administered once daily for 28 days. Two satellite groups (control and 500 mg/kg) were observed for 28 additional days without EEAC to assess reversibility (Nhung and Quoc, 2024a). Body weight, food/water intake, urine parameters, and clinical/behavioral observations were recorded throughout. On day 28, animals were euthanized for hematological, biochemical, and histopathological evaluations; satellite groups were sampled after the recovery period using the same endpoints.

Evaluation of body weight and relative organ weight

According to Tran and Tran (2021), body weight was recorded weekly using a Sartorius electronic balance (Germany) with 0.01 g precision, and body weight gain was calculated by:

$$\text{Body weight gain (g)} = \frac{\text{Final body weight (g)} - \text{Initial body weight (g)}}{\text{Initial body weight (g)}} \quad \text{(Eq. 1)}$$

Following euthanasia, the heart, liver, and kidneys were excised and weighed (absolute organ weight). Relative organ weight (ROW) was determined using:

$$\text{Relative organ weight (\%)} = \frac{\text{Organ weight (g)}}{\text{Body weight (g)}} \times 100 \quad \text{(Eq. 2)}$$

Assessment of weekly food and water consumption

Food intake (g/day) was calculated from the difference between the food provided and remaining food (accounting for spillage). Water intake (mL/day) was measured similarly using graduated bottles (Nhung and Quoc, 2023a).

Hematological and biochemical analysis

Blood was collected by retro-orbital puncture. Hematology samples were placed in EDTA tubes and analyzed using a Mindray BC-5300 Vet analyzer (Shenzhen, China). Serum was obtained from clot activator tubes by centrifugation (3500 rpm, 10 min) and analyzed using a BX-3010 biochemical analyzer (Sysmex, Kobe, Japan).

Urine analysis

Urine was collected over 16 h in metabolic cages (water allowed; food withheld to avoid contamination) (Nhung and Quoc, 2023b). Samples were analyzed for pH, specific gravity, ketones, and ions using a Urisys 1100 urine analyzer (Roche Diagnostics, Switzerland).

Organ histopathology evaluation

Organs were rinsed with 0.9% saline and fixed in 10% phosphate-buffered formalin for ≥24 h. Tissues were embedded in paraffin, sectioned at 4–5 µm (Leica RM2235, Germany), stained with H&E, and examined under a light microscope (Olympus CX43, Japan) for pathological changes (e.g., inflammation, necrosis, fibrosis).

Statistical analysis

Data are presented as mean ± SD and analyzed using one-way ANOVA, with $p < 0.05$ considered significant. Analyses were performed using Statgraphics Centurion.

Results

Qualitative and quantitative phytochemical analysis of bioactive compounds in extracts

Phytochemical screening of the ethanol extract from *A. acuta* leaves (EEAC) revealed multiple bioactive compounds, including tannins, flavonoids, terpenoids, polyphenols, saponins, steroids, and alkaloids, but no cardiac glycosides. The total polyphenol and flavonoid contents were 71.57 ± 1.81 mg GAE/g and 43.64 ± 1.46 mg QE/g, respectively, indicating notable antioxidant potential (Table 1).

Table 1. Phytochemical screening and quantification of ethanol extract from *Argyreia acuta* leaves

Phytoconstituents	Test	Observation	Present in EEAC	Quantification of phytochemicals
Tannins	2 mL EEAC + 2 mL H_2O + 2-3 drops $FeCl_3$ (5%)	Green precipitate	+	NT
Flavonoids	1 mL EEAC + 1 mL $Pb(OAc)_4$ (10%)	Yellow coloration	+	43.64 ± 1.46 (mg QE/g)
Terpenoids	2 mL EEAC + 2 mL $(CH_3CO)_2O$ + 2-3 drops conc. H_2SO_4	Deep red coloration	+	NT
Polyphenol	2 mL EEAC + 2 mL $FeCl_3$	Bluish-green appearance	+	71.57 ± 1.81 (mg GAE/g)
Saponins	5 mL EEAC + 5 mL H_2O + heat	Froth appears	+	NT
Steroids	2 mL EEAC + 2 mL $CHCl_3$ + 2 mL H_2SO_4 (conc.)	The reddish-brown ring at the junction	+	NT
Cardiac glycosides	2 mL EEAC + 2 mL $CHCl_3$ + 2mL CH_3COOH	Violet to Blue to Green coloration	-	-
Alkaloids	2 mL EEAC + a few drops of Hager's reagent	Yellow precipitate	+	NT

Note: Phytochemicals in EEAC are (+) present, (-) absent, and (NT) not tested.

Body weight and organ weight changes in toxicity studies

The effects of EEAC on mouse body weight gain in acute (14 days) and subacute (28 days) toxicity studies are summarized in Table 2. Control groups consistently showed higher weight gains, whereas EEAC-treated groups exhibited dose-dependent reductions. In the acute study, the EEAC5000 group had the lowest weight gain (18.03 ± 0.06 g vs. 25.00 ± 0.27 g in controls, $p < 0.05$), but satellite groups recovered by day 42 (34.63 ± 0.15 g, $p < 0.05$). Similarly, in the subacute study, higher EEAC doses reduced weight gain (EEAC500: 17.91 ± 0.07 g vs. 26.18 ± 0.24 g in controls, $p < 0.05$), yet satellite groups normalized by day 56 (35.01 ± 0.16 g, $p < 0.05$).

In both the acute (Figure 1A) and sub-acute (Figure 1B) toxicity studies of EEAC in mice, the relative organ weights of the liver, heart, and kidney increased dose-dependently ($p < 0.05$). In the acute toxicity study, after 14 days, liver weight increased from 3.83% (control) to 4.25% (EEAC5000), heart weight from 0.44% to 0.88%, and kidney weight from 1.13% to 1.66%. By day 42, liver and kidney weights in the satellite groups decreased to 3.46–3.48% and 1.04–1.06%, respectively, indicating a potential recovery following EEAC discontinuation. Similarly, in the sub-acute toxicity study, after 28 days, liver weight increased from 3.90% (control) to 4.25% (EEAC500), heart weight from 0.53% to 0.88%, and kidney weight from 1.33% to 1.66%. By day 56, liver and kidney weights in the satellite groups decreased to 3.49–3.51% and 1.21–1.23%, respectively, approaching control levels.

Table 2. Changes in body weight of mice in acute and sub-acute toxicity assessments of EEAC

Acute toxicity	**"0"**	**7 days**	**14 days**	**42 days**
Control group	0.00 ± 0.00^{a}	15.01 ± 0.26^{f}	25.00 ± 0.27^{e}	-
EEAC1000 group	0.00 ± 0.00^{a}	10.81 ± 0.11^{d}	22.03 ± 0.09^{d}	-
EEAC3000 group	0.00 ± 0.00^{a}	10.04 ± 0.09^{c}	20.06 ± 0.07^{c}	-
EEAC5000 group	0.00 ± 0.00^{a}	8.00 ± 0.08^{a}	18.03 ± 0.06^{b}	-
Satellite control group	0.00 ± 0.00^{a}	14.65 ± 0.01^{e}	24.63 ± 0.02^{e}	34.61 ± 0.20^{A}
Satellite5000 group	0.00 ± 0.00^{a}	7.24 ± 0.16^{b}	16.23 ± 0.20^{a}	34.63 ± 0.15^{A}
Sub-acute toxicity	**"0"**	**14 days**	**28 days**	**56 days**
Control group	0.00 ± 0.00^{a}	16.09 ± 0.22^{e}	26.18 ± 0.24^{e}	-
EEAC100 group	0.00 ± 0.00^{a}	11.08 ± 0.09^{d}	21.96 ± 0.07^{d}	-
EEAC300 group	0.00 ± 0.00^{a}	10.06 ± 0.09^{c}	19.95 ± 0.08^{c}	-
EEAC500 group	0.00 ± 0.00^{a}	8.00 ± 0.08^{b}	17.91 ± 0.07^{b}	-
Satellite control group	0.00 ± 0.00^{a}	14.99 ± 0.01^{e}	24.98 ± 0.01^{e}	34.93 ± 0.20^{A}
Satellite500 group	0.00 ± 0.00^{a}	5.97 ± 0.14^{a}	16.01 ± 0.17^{a}	35.01 ± 0.16^{A}

Note: Values are expressed as Mean ± SD, letters (a, b, c, d, e, f, and A) represent the difference between groups ($p < 0.05$)

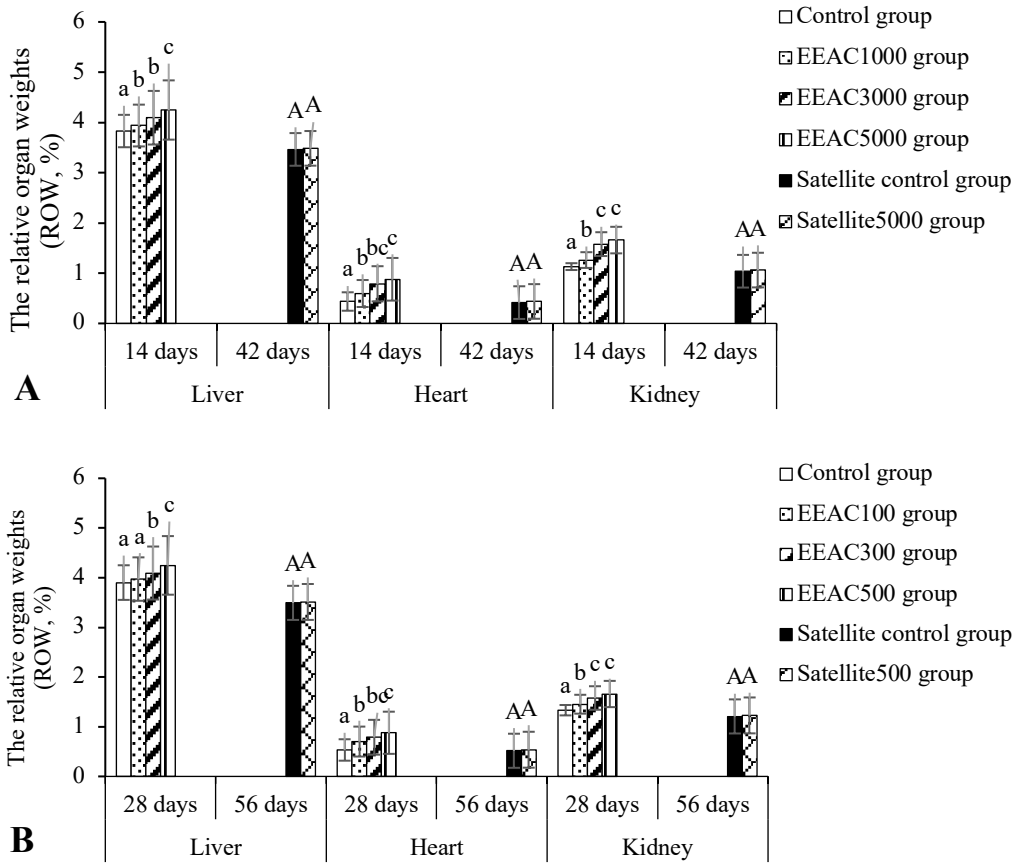

Figure 1. Relative organ weights (ROW) of mice in acute and sub-acute toxicity studies: (A) ROW in the acute toxicity study at 14 and 42 days. (B) ROW in the sub-acute toxicity study at 28 and 56 days. Data are presented as Mean ± SD and different letters (a, b, c, and A) denote statistically significant differences between groups ($p < 0.05$)

Assessment of food and water consumption in acute and sub-acute toxicity studies of EEAC

A dose-dependent decrease in food and water intake among mice treated with EEAC, most pronounced in the EEAC5000 (acute) and EEAC500 (subacute) groups is demonstrated in Tables 3 and 4. In acute studies, consumption declined significantly by days 7 - 14, while in subacute studies, reductions peaked around days 14 - 28. However, satellite groups recovered fully by days 42 and 56, respectively, returning to baseline levels (8.80 ± 0.11 g food, 8.57 ± 0.13 mL water, $p < 0.05$).

Table 3. Food consumption in acute and sub-acute toxicity studies of EEAC

Acute toxicity	"0"	7 days	14 days	42 days
Control group	4.76 ± 0.03^{a}	7.16 ± 0.09^{d}	8.03 ± 0.10^{d}	-
EEAC1000 group	5.38 ± 0.06^{d}	6.96 ± 0.08^{c}	7.91 ± 0.07^{c}	-
EEAC3000 group	5.16 ± 0.05^{c}	6.78 ± 0.07^{b}	7.78 ± 0.06^{b}	-
EEAC5000 group	4.98 ± 0.04^{b}	6.58 ± 0.05^{a}	7.58 ± 0.04^{a}	-
Satellite control group	5.59 ± 0.07^{e}	7.29 ± 0.10^{d}	8.17 ± 0.13^{d}	8.80 ± 0.11^{A}
Satellite5000 group	5.60 ± 0.08^{e}	6.64 ± 0.06^{a}	7.61 ± 0.05^{a}	8.78 ± 0.10^{A}
Sub-acute toxicity	**"0"**	**14 days**	**28 days**	**56 days**
Control group	4.55 ± 0.05^{a}	7.23 ± 0.09^{d}	8.11 ± 0.08^{c}	-
EEAC100 group	5.48 ± 0.10^{d}	6.98 ± 0.07^{c}	7.89 ± 0.06^{b}	-
EEAC300 group	5.09 ± 0.08^{c}	6.85 ± 0.06^{b}	7.82 ± 0.04^{b}	-
EEAC500 group	4.86 ± 0.07^{b}	6.68 ± 0.04^{a}	7.65 ± 0.02^{a}	-
Satellite control group	5.64 ± 0.12^{e}	7.29 ± 0.10^{d}	8.99 ± 0.09^{d}	8.99 ± 0.11^{A}
Satellite500 group	5.62 ± 0.11^{e}	6.74 ± 0.05^{a}	7.70 ± 0.03^{a}	8.97 ± 0.10^{A}

Note: Values are expressed as Mean ± SD, letters (a, b, c, d, e, and A) represent the difference between groups ($p < 0.05$)

Table 4. Water consumption in acute and sub-acute toxicity studies of EEAC

Acute toxicity	"0"	7 days	14 days	42 days
Control group	4.65 ± 0.04^{a}	6.16 ± 0.08^{d}	7.11 ± 0.09^{d}	-
EEAC1000 group	5.17 ± 0.07^{d}	5.92 ± 0.07^{c}	6.91 ± 0.08^{c}	-
EEAC3000 group	5.01 ± 0.06^{c}	5.73 ± 0.06^{b}	6.70 ± 0.05^{b}	-
EEAC5000 group	4.81 ± 0.05^{b}	5.59 ± 0.04^{a}	6.53 ± 0.03^{a}	-
Satellite control group	5.22 ± 0.09^{d}	6.22 ± 0.09^{d}	7.16 ± 0.12^{d}	8.57 ± 0.13^{A}
Satellite5000 group	5.20 ± 0.08^{d}	5.63 ± 0.05^{a}	6.56 ± 0.04^{a}	8.55 ± 0.12^{A}
Sub-acute toxicity	**"0"**	**14 days**	**28 days**	**56 days**
Control group	5.52 ± 0.06^{a}	7.11 ± 0.10^{d}	8.22 ± 0.09^{d}	-
EEAC100 group	6.17 ± 0.11^{d}	6.89 ± 0.08^{c}	8.05 ± 0.07^{c}	-
EEAC300 group	5.91 ± 0.09^{c}	6.64 ± 0.06^{b}	7.83 ± 0.05^{b}	-
EEAC500 group	5.69 ± 0.08^{b}	6.45 ± 0.04^{a}	7.63 ± 0.03^{a}	-
Satellite control group	6.23 ± 0.13^{d}	7.18 ± 0.11^{d}	8.26 ± 0.08^{d}	8.64 ± 0.08^{A}
Satellite500 group	6.21 ± 0.12^{d}	6.49 ± 0.05^{a}	7.68 ± 0.04^{a}	8.62 ± 0.07^{A}

Note: Values are expressed as Mean ± SD, letters (a, b, c, d, and A) represent the difference between groups ($p < 0.05$)

Evaluation of hematological and biochemical parameters in acute and sub-acute toxicity studies of EEAC

Hematological and biochemical parameters, RBC, WBC, and PLT counts increased dose-dependently, particularly in the EEAC5000 (acute) and EEAC500 (subacute) groups, are shown in Tables 5 and 6. In the acute phase, RBC, WBC, and PLT counts in the EEAC5000 group increased to 8.60×10^{6} cells/mm³, 2.68×10^{3} cells/mm³, and 712.8×10^{3} cells/mm³, respectively, compared to controls (8.20×10^{6} cells/mm³, 2.25×10^{3} cells/mm³, 631.34×10^{3} cells/mm³, $p < 0.05$). Similarly, in the subacute phase, RBC, WBC, and PLT

counts in the EEAC500 group reached 9.36 × 10^6 cells/mm^3, 4.06 × 10^3 cells/mm^3, and 715.94 × 10^3 cells/mm^3, respectively ($p < 0.05$). These values returned to baseline in satellite groups by day 42 (acute) and day 56 (subacute) ($p < 0.05$), confirming reversibility.

Biochemical analysis revealed increased total protein (EEAC5000: 6.21 g/dL vs. control: 5.48 g/dL; EEAC500: 7.12 g/dL vs. control: 6.49 g/dL, $p < 0.05$), triglycerides (EEAC5000: 131.7 mg/dL vs. control: 130.53 mg/dL; EEAC500: 134.03 mg/dL vs. control: 132.85 mg/dL, $p < 0.05$), and glucose (EEAC5000: 67.86 mmol/L vs. control: 66.48 mmol/L; EEAC500: 69.58 mmol/L vs. control: 68.68 mmol/L, $p < 0.05$), suggesting metabolic adjustments. Liver function markers (AST, ALT, ALP) remained within normal limits ($p > 0.05$), while kidney function markers (urea and BUN) showed mild, reversible increases (EEAC5000: BUN 14.87 mg% vs. control: 13.88 mg%; EEAC500: BUN 15.98 mg% vs. control: 14.96 mg%, $p < 0.05$), indicating transient renal adaptations.

Table 5. Hematological and biochemical parameters in acute toxicity study of EEAC

Acute toxicity	Control group	EEAC1000 group	EEAC3000 group	EEAC5000 group	Satellite control group	Satellite5000 group
	14 days	14 days	14 days	14 days	42 days	42 days
RBC (×10^6 cells/mm^3)	8.20 ± 0.11^{a}	8.35 ± 0.21^{ab}	8.46 ± 0.23^{ab}	8.60 ± 0.26^{b}	8.22 ± 0.14^{A}	8.24 ± 0.15^{A}
WBC (×10^3 cells/mm^3)	2.25 ± 0.17^{a}	2.41 ± 0.22^{ab}	2.54 ± 0.25^{bc}	2.68 ± 0.29^{c}	2.28 ± 0.19^{A}	2.29 ± 0.20^{A}
PLT (×10^3 cells/mm^3)	631.34 ± 14.22^{a}	666.03 ± 24.25^{b}	690.65 ± 30.05^{c}	712.8 ± 32.19^{d}	633.29 ± 17.46^{A}	633.43 ± 18.76^{A}
Protein (g/dL)	5.48 ± 0.19^{a}	5.62 ± 0.13^{a}	5.96 ± 0.17^{b}	6.21 ± 0.23^{b}	5.51 ± 0.11^{A}	5.53 ± 0.12^{A}
Triglyceride (mg/dL)	130.53 ± 2.15^{a}	130.98 ± 2.16^{b}	131.38 ± 2.39^{c}	131.7 ± 3.41^{d}	130.57 ± 2.52^{A}	130.56 ± 2.61^{A}
Glucose (mol/L)	66.48 ± 0.36^{a}	66.94 ± 0.42^{b}	67.47 ± 0.35^{c}	67.86 ± 0.21^{d}	66.5 ± 0.38^{A}	66.52 ± 0.29^{A}
AST (U/L)	95.64 ± 0.10^{a}	96.02 ± 0.22^{b}	96.26 ± 0.24^{a}	96.85 ± 0.32^{c}	95.67 ± 0.16^{A}	95.71 ± 0.17^{A}
ALT (U/L)	71.52 ± 0.25^{a}	72.16 ± 0.32^{b}	71.74 ± 0.35^{a}	73.64 ± 0.37^{c}	71.54 ± 0.27^{A}	71.56 ± 0.28^{A}
ALP (U/L)	123.09 ± 2.11^{a}	123.22 ± 2.29^{a}	123.71 ± 3.12^{b}	124.12 ± 3.46^{c}	123.11 ± 2.23^{A}	123.13 ± 2.44^{A}
Urea (mg/dL)	12.75 ± 0.15^{a}	13.19 ± 0.16^{b}	13.47 ± 0.21^{c}	13.95 ± 0.24^{d}	12.76 ± 0.18^{A}	12.77 ± 0.11^{A}
BUN (mg%)	13.88 ± 0.13^{a}	14.19 ± 0.21^{b}	14.44 ± 0.27^{b}	14.87 ± 0.31^{c}	13.91 ± 0.15^{A}	13.87 ± 0.14^{A}

Note: Values are expressed as Mean ± SD, letters (a, b, c, d, and A) represent the difference between groups ($p < 0.05$)

Table 6. Hematological and biochemical parameters in sub-acute toxicity study of EEAC

Sub-acute toxicity	Control group	EEAC100 group	EEAC300 group	EEAC500 group	Satellite control group	Satellite500 group
	28 days	28 days	28 days	28 days	56 days	56 days
RBC ($\times 10^6$ cells/mm^3)	8.63 ± 0.12^{a}	8.85 ± 0.21^{ab}	9.11 ± 0.24^{bc}	9.36 ± 0.27^{c}	8.65 ± 0.15^{A}	8.67 ± 0.16^{A}
WBC ($\times 10^3$ cells/mm^3)	3.35 ± 0.18^{a}	3.53 ± 0.23^{a}	3.82 ± 0.26^{b}	4.06 ± 0.30^{b}	3.38 ± 0.20^{A}	3.39 ± 0.21^{A}
PLT ($\times 10^3$ cells/mm^3)	642.72 ± 16.25^{a}	669.21 ± 26.47^{b}	707.03 ± 31.23^{c}	715.94 ± 34.56^{d}	643.79 ± 19.49^{A}	643.83 ± 20.74^{A}
Protein (g/dL)	6.49 ± 0.11^{a}	6.81 ± 0.15^{b}	6.97 ± 0.19^{bc}	7.12 ± 0.25^{c}	6.52 ± 0.13^{A}	6.54 ± 0.14^{A}
Triglyceride (mg/dL)	132.85 ± 2.17^{a}	133.17 ± 2.75^{b}	133.61 ± 3.12^{c}	134.03 ± 3.22^{d}	132.88 ± 2.33^{A}	132.91 ± 2.52^{A}
Glucose (mol/L)	68.68 ± 0.57^{a}	69.03 ± 0.43^{b}	69.33 ± 0.66^{c}	69.58 ± 0.41^{c}	68.71 ± 0.59^{A}	68.72 ± 0.71^{A}
AST (U/L)	96.68 ± 0.53^{a}	97.05 ± 0.25^{b}	97.45 ± 0.27^{c}	97.93 ± 0.35^{d}	96.69 ± 0.88^{A}	96.73 ± 0.79^{A}
ALT (U/L)	74.61 ± 0.26^{a}	75.17 ± 0.33^{b}	75.37 ± 0.36^{b}	75.91 ± 0.38^{c}	74.63 ± 0.28^{A}	74.64 ± 0.29^{A}
ALP (U/L)	125.12 ± 2.62^{a}	125.31 ± 3.17^{a}	125.64 ± 3.44^{b}	126.15 ± 3.27^{c}	125.14 ± 2.64^{A}	125.17 ± 2.75^{A}
Urea (mg/dL)	13.81 ± 0.16^{a}	14.26 ± 0.17^{b}	14.43 ± 0.21^{b}	14.72 ± 0.24^{c}	13.83 ± 0.11^{A}	13.84 ± 0.12^{A}
BUN (mg%)	14.96 ± 0.15^{a}	15.26 ± 0.24^{b}	15.58 ± 0.25^{c}	15.98 ± 0.33^{d}	14.98 ± 0.17^{A}	15.01 ± 0.18^{A}

Note: Values are expressed as Mean ± SD, letters (a, b, c, d, and A) represent the difference between groups ($p < 0.05$)

Evaluation of urinary biomarkers in acute and sub-acute toxicity studies of EEAC

The urinary biomarkers are presented in Table 7. Higher doses led to increased specific gravity, urinary pH, ketones, urobilinogen, Na^+, and Cl^- ($p < 0.05$), indicating potential metabolic and renal adaptations. In the acute phase, specific gravity increased to 1.08 ± 0.18 in the EEAC5000 group (vs. control: 1.06 ± 0.13, $p < 0.05$), while in the subacute phase, it reached 1.12 ± 0.21 in the EEAC500 group (vs. control: 1.06 ± 0.14, $p < 0.05$). Urinary pH also increased, with the highest values recorded in EEAC5000 (acute: 7.89 ± 0.13) and EEAC500 (subacute: 8.02 ± 0.15, $p < 0.05$), suggesting metabolic shifts affecting acid-base balance. Ketone levels rose dose-dependently, reaching 0.98 ± 0.22 mg/mL (EEAC5000, acute) and 0.87 ± 0.24 mg/mL (EEAC500, subacute) compared to controls (0.83 ± 0.18 mg/mL, $p < 0.05$), suggesting increased fat metabolism or altered glucose utilization. Urobilinogen levels also

increased, particularly in EEAC5000 (acute: 33.39 ± 1.3 mg/mL) and EEAC500 (subacute: 38.49 ± 1.4 mg/mL, $p < 0.05$) vs. controls (30.81 ± 0.15 mg/mL), indicating possible liver involvement or bilirubin metabolism changes. Electrolyte analysis showed significant increases in Na^+ and Cl^- levels. In the acute phase, Na^+ reached 79.88 ± 0.16 mmol/L (EEAC5000) vs. 71.59 ± 0.18 mmol/L (control, $p < 0.05$), while Cl^- increased to 79.87 ± 0.21 mmol/L (vs. control: 71.37 ± 0.26 mmol/L, $p < 0.05$). Similarly, in the subacute phase, Na^+ and Cl^- levels in the EEAC500 group were 74.63 ± 0.17 mmol/L and 84.92 ± 0.27 mmol/L, respectively ($p < 0.05$). K^+ levels remained stable, suggesting no severe electrolyte imbalances.

Table 7. Urinary biomarkers in acute and sub-acute toxicity studies of EEAC

Acute toxicity	Control group	EEAC1000 group	EEAC3000 group	EEAC5000 group	Satellite control group	Satellite5000 group
	14 days	14 days	14 days	14 days	42 days	42 days
Specific gravity	1.06 ± 0.13^{a}	1.07 ± 0.16^{b}	1.07 ± 0.17^{a}	1.08 ± 0.18^{b}	1.07 ± 0.14^{A}	1.07 ± 0.15^{A}
pH	7.64 ± 0.17^{a}	7.77 ± 0.17^{b}	7.81 ± 0.12^{c}	7.89 ± 0.13^{d}	7.67 ± 0.08^{A}	7.68 ± 0.09^{A}
Ketone (mg/mL)	0.83 ± 0.18^{a}	0.86 ± 0.21^{ab}	0.92 ± 0.24^{b}	0.98 ± 0.22^{c}	0.86 ± 0.19^{A}	0.87 ± 0.21^{A}
Urobilinogen (mg/mL)	30.81 ± 0.15^{a}	31.34 ± 0.18^{d}	31.86 ± 0.11^{b}	33.39 ± 0.13^{c}	30.85 ± 0.16^{A}	30.81 ± 0.15^{A}
Na^+ (mmol/L)	71.59 ± 0.18^{a}	72.78 ± 0.13^{c}	74.17 ± 0.14^{b}	79.88 ± 0.15^{d}	71.67 ± 0.11^{A}	71.74 ± 0.11^{A}
K^+ (mmol/L)	45.03 ± 0.23^{a}	46.24 ± 0.27^{b}	47.83 ± 0.31^{c}	49.61 ± 0.29^{d}	45.01± 0.21^{A}	45.03 ± 0.22^{A}
Cl^- (mmol/L)	71.37 ± 0.26^{a}	76.63 ± 0.22^{c}	79.87 ± 0.18^{d}	74.16 ± 0.21^{b}	71.45 ± 0.18^{A}	71.46 ± 0.22^{A}
Sub-acute toxicity	**Control group**	**EEAC100 group**	**EEAC300 group**	**EEAC500 group**	**Satellite control group**	**Satellite500 group**
	28 days	28 days	28 days	28 days	56 days	56 days
Specific gravity	1.06 ± 0.14^{a}	1.08 ± 0.17^{b}	1.09 ± 0.19^{c}	1.12 ± 0.21^{d}	1.07 ± 0.15^{A}	1.07 ± 0.16^{A}
pH	7.79 ± 0.09^{a}	7.95 ± 0.13^{c}	7.88 ± 0.12^{b}	8.02 ± 0.15^{d}	7.81 ± 0.12^{A}	7.84 ± 0.11^{A}
Ketone (mg/mL)	0.81 ± 0.21^{a}	0.84 ± 0.23^{ab}	0.91 ± 0.26^{c}	0.87 ± 0.24^{bc}	0.81 ± 0.21^{A}	0.83 ± 0.22^{A}
Urobilinogen (mg/mL)	34.37 ± 0.06^{a}	35.74 ± 0.11^{b}	37.12 ± 0.13^{c}	38.49 ± 0.14^{d}	34.65 ± 0.08^{A}	34.61 ± 0.07^{A}
Na^+ (mmol/L)	66.64 ± 0.12^{a}	71.97 ± 0.15^{c}	69.31 ± 0.14^{b}	74.63 ± 0.17^{d}	67.04 ± 0.12^{A}	67.06 ± 0.13^{A}
K^+ (mmol/L)	1.06 ± 0.14^{a}	1.08 ± 0.17^{b}	1.09 ± 0.19^{c}	1.12 ± 0.21^{d}	1.07 ± 0.15^{A}	1.07 ± 0.16^{A}
Cl^- (mmol/L)	7.79 ± 0.09^{a}	7.95 ± 0.13^{c}	7.88 ± 0.12^{b}	8.02 ± 0.15^{d}	7.81 ± 0.12^{A}	7.84 ± 0.11^{A}

Note: Values are expressed as Mean ± SD, letters (a, b, c, d, and A) represent the difference between groups ($p < 0.05$)

Macroscopic and histopathological analysis of organs in EEAC toxicity studies

The organ morphology from control and EEAC-treated groups during acute and subacute toxicity studies are compared as seen in Figure 2. Control organs (Figure 2a, 2b, 2c) showed no abnormalities, whereas high-dose acute treatment (EEAC5000) produced mild changes such as pale liver coloration, irregular surfaces, and slight kidney congestion (Figure 2d, 2e, 2f), suggesting metabolic stress. These alterations resolved in the Satellite5000 group (Figure 2g, 2h, 2i). In the subacute study, the EEAC500 group (Figure 2k, 2m, 2n) exhibited moderate liver congestion and minor kidney discoloration, which were less pronounced than in the high-dose acute group and largely reversed in Satellite500 (Figure 2o, 2p, 2q).

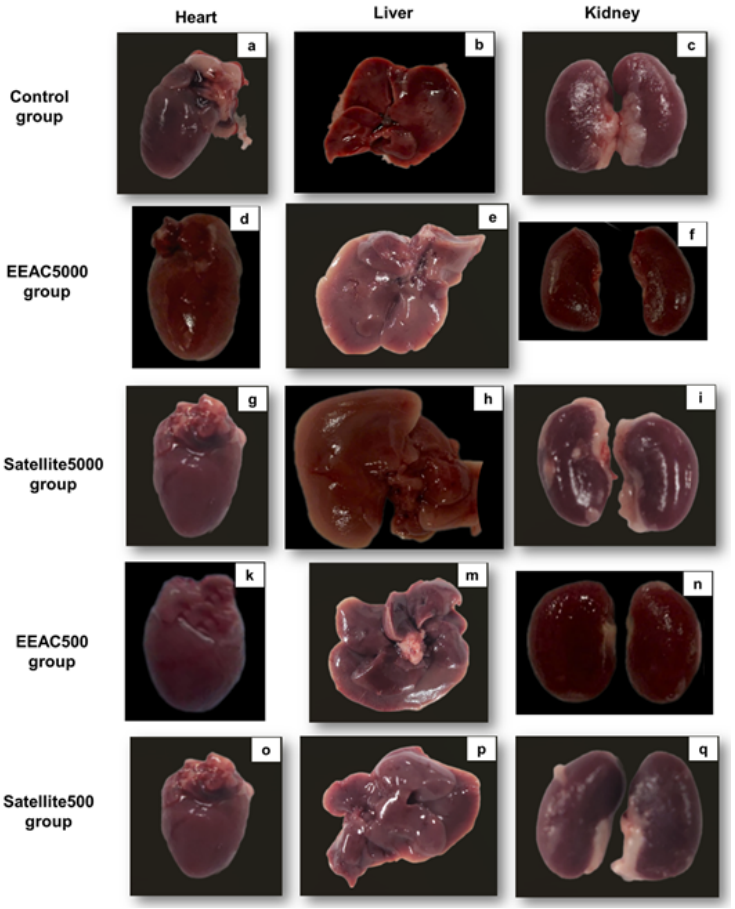

Figure 2. Macroscopic examination of the heart, liver, and kidney in acute and sub-acute toxicity studies

The microscopic findings are presented for acute and subacute studies, respectively (Figure 3A and 3B). Control tissues (Figure 3Aa, 3Ad, 3Ag; 3Ba, 3Bd, 3Bg) displayed normal architecture, including intact hepatocytes, organized cardiac myofibers, and healthy renal structures. High-dose acute exposure (EEAC5000) led to slight myocardial fiber disorganization, mild hepatic congestion, and subtle glomerular changes (Figure 3Ab, 3Ae, 3Ah), which recovered in Satellite5000 (Figure 3Ac, 3Af, 3Ai). Similarly, the subacute EEAC500 group (Figure 3Bb, 3Be, 3Bh) showed only minor cardiac and hepatic alterations that reversed in Satellite500 (Figure 3Bc, 3Bf, 3Bi).

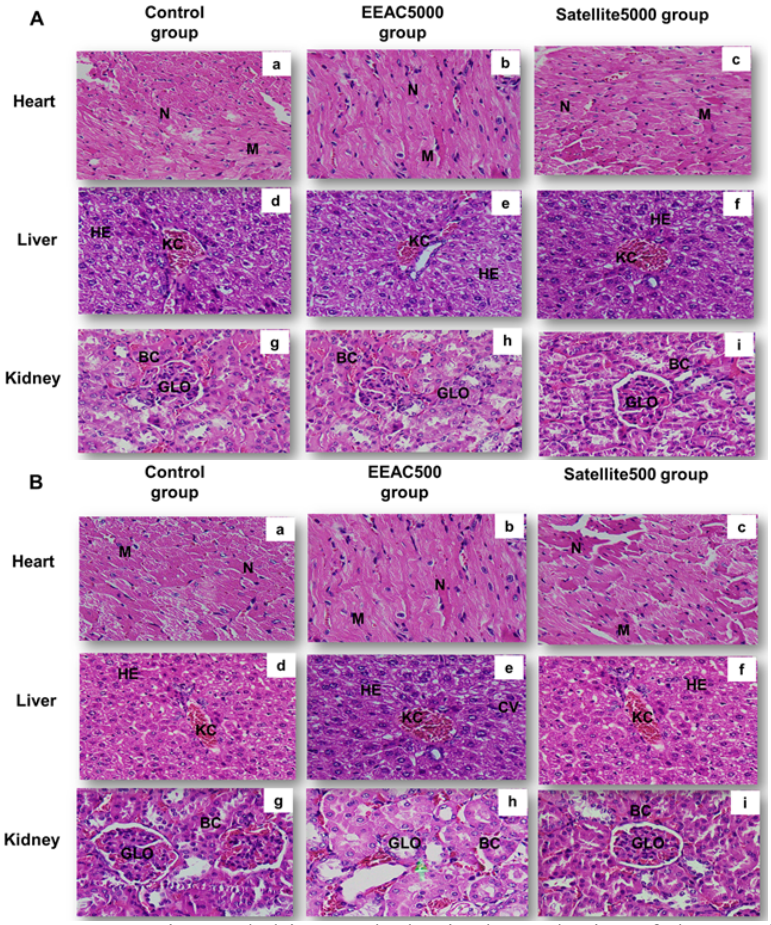

Figure 3. Macroscopic and histopathological analysis of heart, liver, and kidney in EEAC toxicity studies: (A) Acute toxicity study, (B) Sub-acute toxicity study. Histopathological analysis of the heart, liver, and kidney (Hematoxylin and Eosin staining, HE, magnification ×200). N - nucleus; M - myocardium; GLO - glomerulus; BC - Bowman's capsule; HE - hepatocytes; CV - central vein

Disscusion

EEAC contains multiple phytochemical groups that may underlie therapeutic activity but can also contribute to adverse effects when exposure is high (Mugale *et al*., 2024). For example, alkaloids may affect neural and cardiac function, saponins can produce hemolytic effects, and steroids/terpenoids may influence metabolic and endocrine pathways; tannins may reduce nutrient absorption (Kumar *et al*., 2023). Importantly, toxicity is not only compound-dependent but also shaped by concentration and interactions among constituents within the extract (Vaou *et al*., 2022). Excessive tannin intake may irritate the gastrointestinal tract and interfere with nutrient utilization (Zouine *et al*., 2024). These considerations highlight why dose–response characterization is essential before recommending medicinal use. Comparable phytochemical profiles and safety concerns have been reported in related species, reinforcing the need for careful toxicity evaluation (Goti and Dasgupta, 2024). In addition, an LD_{50} range of 2000–5000 mg/kg reported for another plant ethanol extract under GHS classification illustrates the relevance of preclinical safety screening before broader application (Subash *et al*., 2012).

In the present work, higher EEAC doses were associated with reduced body weight gain and increased relative organ weights in both acute (5000 mg/kg) and subacute (500 mg/kg) settings. Such changes can reflect physiological adaptation to xenobiotic exposure and are commonly used as early signals in toxicity screening (Tran and Tran, 2021). Reduced weight gain at higher doses may relate to a temporary metabolic burden linked to polyphenols/flavonoids, which are beneficial at moderate levels but may become stressful when administered in excess (Nhung and Quoc, 2024b). Increased liver, heart, and kidney weights can also occur as an adaptive response to bioactive constituents that influence lipid handling or organ workload (Tran *et al*., 2023b). Notably, biochemical findings and histopathology suggested that these effects were mild and reversible, consistent with recovery in satellite groups. Similar endpoints are routinely emphasized in herbal toxicology studies (Ugwah-Oguejiofor *et al*., 2019; Mekonen *et al*., 2022; Musa *et al*., 2019).

The dose-related decrease in food and water intake, especially at higher concentrations, likely reflects short-term appetite suppression, gastrointestinal discomfort, or metabolic adjustment rather than persistent toxicity, given the complete recovery observed in satellite groups (Nhung and Quoc, 2023a; Nhung and Quoc, 2024c). Polyphenols and flavonoids can modulate appetite and energy metabolism; however, higher doses may also shift redox balance

and reduce intake transiently (Nhung and Quoc, 2024d). Other constituents such as alkaloids, saponins, and tannins, may influence feeding behavior through effects on taste, digestion, or nutrient absorption (Yang and Ling, 2025). The concurrent reduction in water intake may reflect temporary changes in fluid regulation and hydration behavior (Perrier *et al*., 2020). Published work on related species reports variable effects on intake depending on extract composition and study duration (Kumar *et al*., 2011; Ping *et al*., 2013; Patil *et al*., 2012).

Hematological and biochemical shifts observed at higher doses also appear consistent with a reversible physiological response rather than overt pathology. Increases in RBC and WBC may reflect stimulation of erythropoiesis and immune modulation, which has been described for plant-derived flavonoids, alkaloids, and saponins (Roy *et al*., 2022; Saeed *et al*., 2024). Platelet increases may indicate an effect on hemostatic pathways and merit attention in longer-term studies (Sang *et al*., 2020). Mild elevations in metabolic indices (e.g., total protein, triglycerides, glucose) can occur as adaptive metabolic responses. Likewise, modest, reversible changes in renal markers (urea/BUN) are compatible with transient renal adaptation rather than sustained injury, particularly when values normalize after extract withdrawal (Nhung and Quoc, 2023a; Nhung and Quoc, 2023b).

Urinary biomarker changes further support an adaptive response pattern. Variations in specific gravity, pH, ketones, and electrolytes can reflect short-term shifts in renal handling of solutes and systemic metabolism (Tran and Tran, 2021; Nhung and Quoc, 2023c). Because urinary markers can detect early kidney stress before conventional serum indices, incorporating such endpoints strengthens safety interpretation (Vlasakova *et al*., 2014; Bonventre *et al*., 2010). In this study, the return of urinary parameters to baseline in satellite groups suggests that the observed changes were reversible and did not progress to persistent renal dysfunction.

Overall, EEAC at high doses produced mild, dose-dependent changes that resolved after treatment cessation, and histopathology did not reveal major lesions such as necrosis or fibrosis. Nonetheless, longer-duration studies (subchronic/chronic) remain necessary to exclude cumulative effects and to better define safety margins for extended use.

Acknowledgements

The authors are grateful to the Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Vietnam, for supporting this work.

Conflicts of interest

The authors declare no conflict of interest.

References

Amorim, A. M. B., Piochi, L. F., Gaspar, A. T., Preto, A. J., Rosário-Ferreira, N. and Moreira, I. S. (2024). Advancing drug safety in drug development: Bridging computational predictions for enhanced toxicity prediction. Chemical Research in Toxicology, 37:827-849.

Bonventre, J. V., Vaidya, V. S., Schmouder, R., Feig, P. and Dieterle, F. (2010). Next-generation biomarkers for detecting kidney toxicity. Nature Biotechnology, 28:436-440.

Goti, D. and Dasgupta, S. (2024). Phytochemical screening of stem extracts of *Argyreia nervosa*. Journal of Medicinal Plants Studies, 12:424-428.

Gunadasa, D. M. N. H., Jayasuriya, K. M. G. G., Baskin, J. M. and Baskin, C. C. (2024). Evolutionary reversal of physical dormancy to nondormancy: evidence from comparative seed morphoanatomy of *Argyreia* species (Convolvulaceae). AoB PLANTS, 16:033.

Hu, J. Y., Wu, X. H., Yin, Y. Q., Pan, J. T. and Yu, B. W. (2018). Three resin glycosides isolated from *Argyreia acuta*, including two isomers. Chinese Journal of Natural Medicines, 16:354-357.

Hubrecht, R. C. and Carter, E. (2019). The 3Rs and humane experimental technique: Implementing change. Animals, 9:754.

Kashyap, P., Ram, H., Shukla, S. D. and Kumar, S. (2020). Scopoletin: antiamyloidogenic, anticholinesterase, and neuroprotective potential of a natural compound present in *Argyreia speciosa* roots by *in vitro* and *in silico* study. Neuroscience Insights, 15:1-10.

Kumar, A., Nirmal, P., Kumar, M., Jose, A., Tomer, V., Oz, E., Proestos, C., Zeng, M., Elobeid, T., Sneha, K. and Oz, F. (2023). Major Phytochemicals: Recent advances in health benefits and extraction method. Molecules, 28:887.

Kumar, S., Alagawadi, K. R. and Rao, M. R. (2011). Effect of *Argyreia speciosa* root extract on cafeteria diet-induced obesity in rats. Indian Journal of Pharmacology, 43:163-167.

Lalan, B. K., Hiray, R. S. and Ghongane, B. B. (2015). Evaluation of analgesic and anti-inflammatory activity of extract of *Holoptelea integrifolia* and *Argyreia speciosa* in animal models. Journal of Clinical and Diagnostic Research, 9:1-4.

Li, Y., Zhu, X., Mo, L., Liu, C., Li, J., Li, B., Wei, J., Liao, G. and Lu, R. (2021). Chemical constituents of the folk medicinal plant *Argyreia acuta* Lour. and their anti-inflammatory activity. Natural Product Communications, 16:1-5.

Mekonen, K., Afework, M., Makonnen, E., Debela, A., Ergete, W. and Tolessa, T. (2022). Evaluation of acute and sub-acute toxicity of aqueous extracts of *Artemisia afra* leaves on brain, heart and suprarenal glands in swiss albino mice. Ethiopian Journal of Health Sciences, 30:981-990.

Mugale, M. N., Dev, K., More, B. S., Mishra, V. S., Washimkar, K. R., Singh, K., Maurya, R., Rath, S. K., Chattopadhyay, D. and Chattopadhyay, N. (2024). A comprehensive review on preclinical safety and toxicity of medicinal plants. Clinical Complementary Medicine and Pharmacology, 4:100129.

Musa, N. H. C., Zain, H. H. M., Ibrahim, H. and Jamil, N. N. M. (2019). Evaluation of acute and sub-acute oral toxicity effect of *Aquilaria malaccensis* leaves aqueous extract in male ICR Mice. Natural Product Sciences, 25:157-164.

Nhung, T. T. P. and Quoc, L. P. T. (2023a). Acute and sub-chronic toxicity studies of the ethanol extract of *Erythrina fusca* Lour. fruit via oral administration in mice. Acta Biologica Szegediensis, 67:111-122.

Nhung, T. T. P. and Quoc, L. P. T. (2023b). Assessment of toxic effects of *Hedyotis capitellata* Wall. leaves ethanol extract via biological assays in mice (*Mus musculus*). Malaysian Journal of Biochemistry and Molecular Biology, 26:122-134.

Nhung, T. T. P. and Quoc, L. P. T. (2023c). Protective effects of black shallot extract against acetaminophen-induced nephrotoxicity in mice. Acta Biologica Szegediensis, 67:177-185.

Nhung, T. T. P. and Quoc, L. P. T. (2024a). Assessment of the acute and chronic toxicity studies of ethanol extract of *Blumea balsamifera* (L.) DC. leaves on murine models. Tropical Journal of Natural Product Research, 8:6224-6233.

Nhung, T. T. P. and Quoc, L. P. T. (2024b). Counteracting paracetamol-induced hepatotoxicity with black shallot extract: An animal model investigation. Tropical Journal of Natural Product Research, 8:5875-5880.

Nhung, T. T. P. and Quoc, L. P. T. (2024c). Potential anti-inflammatory effects of ethanol extract of *Caryota urens* Lour fruits on Freund's complete adjuvant-induced rheumatoid arthritis in mice. Tropical Journal of Natural Product Research, 8:6924-6931.

Nhung, T. T. P. and Quoc, L. P. T. (2024d). Ethanol extract of *Caryota urens* Lour fruits alleviates oxidative stress in a murine model of rheumatoid arthritis induced by Freund's complete adjuvant. Tropical Journal of Natural Product Research, 8:6948-6956.

Nhung, T. T. P. and Quoc, L. P. T. (2025). Effectiveness of antioxidants and resistance to diamondback moth infestation in green mustard plants using ethanol extracts from *Millettia pachyloba* Drake leaves: An *in vitr*o and *in vivo* evaluation. Caraka Tani: Journal of Sustainable Agriculture, 40:109-125.

OECD - Organisation for Economic Co-operation and Development. Test No. 420: Acute Oral Toxicity - Fixed Dose Procedure. (2001). OECD Guideline for Testing of Chemicals, Section 4. OECD Publishing, Paris.

OECD - Organisation for Economic Co-operation and Development. Test No. 470: Mammalian Erythrocyte Pig-a Gene Mutation Assay. (2022). OECD Guideline for the Testing of Chemicals, Section 4. OECD Publishing, Paris.

Patil, P. J. and Patil, V. R. (2012). Phytochemical and toxicological evaluation of *Acorus calamus* and *Argyreia speciosa* leaves extract. International Journal of Green Pharmacy, 8:1-4.

Perrier, E. T., Armstrong, L. E., Bottin, J. H., Clark, W. F., Dolci, A., Guelinckx, I., Iroz, A., Kavouras, S. A., Lang, F., Lieberman, H. R., Melander, O., Morin, C., Seksek, I., Stookey, J. D., Tack, I., Vanhaecke, T., Vecchio, M. and Péronnet, F. (2020). Hydration for health hypothesis: a narrative review of supporting evidence. European Journal of Nutrition, 60:1167-1180.

Ping, K. Y., Darah, I., Chen, Y., Sreeramanan, S. and Sasidharan, S. (2013). Acute and subchronic toxicity study of *Euphorbia hirta* L. methanol extract in rats. BioMed Research International, 2013:182064.

Roy, A., Khan, A., Ahmad, I., Alghamdi, S., Rajab, B. S., Babalghith, A. O., Alshahrani, M. Y., Islam, S. and Islam, M. R. (2022). Flavonoids a bioactive compound from medicinal plants and its therapeutic applications. BioMed Research International, 2022:5445291.

Saeed, M., Munawar, M., Bi, J. B., Ahmed, S., Ahmad, M. Z., Kamboh, A. A., Arain, M. A., Naveed, M. and Chen, H. (2024). Promising phytopharmacology, nutritional potential, health benefits, and traditional usage of *Tribulus terrestris* L. herb. Heliyon, 10:25549.

Sang, Y., Roest, M., Laat, B. D., Groot, P. G. D. and Huskens, D. (2020). Interplay between platelets and coagulation. Blood Review, 46:100733.

Subash, K. R., Muthulakshmi, B. G., Jagan, R. N. and Binoy, V. C. (2012). Phytochemical screening and acute toxicity study of ethanolic extract of *Alpinia galanga* in rodents. International Journal of Medical Research & Health Sciences, 2:93-100.

Tran, P. N. T. and Tran, T. T. N. (2021). Evaluation of acute and subchronic toxicity induced by the crude ethanol extract of *Plukenetia volubilis* Linneo leaves in swiss albino mice. BioMed Research International, 2021:1-13.

Tran, P. N. T., Nguyen, N. T. and Tran, G. B. (2023a). Protective effect of ethanol extract of *Coriolopsis aspera* fruiting bodies against adjuvant-induced arthritis mice. Nova Biotechnologica et Chimica, 22:1654.

Tran, T. P. N., Nguyen, T. T. and Tran, G. B. (2023b). Anti-arthritis effect of ethanol extract of Sacha inchi (*Plukenetia volubilis* L.) leaves against complete Freund's adjuvant-induced arthritis model in mice. Tropical Life Sciences Research, 34:237-257.

Ugwah-Oguejiofor, C. J., Okoli, C. O., Ugwah, M. O., Umaru, M. L., Ogbulie, C. S., Mshelia, H. E., Umar, M. and Njan, A. A. (2019). Acute and sub-acute toxicity of aqueous extract of aerial parts of *Caralluma dalzielii* N. E. Brown in mice and rats. Heliyon, 5:01179.

Vaou, N., Stavropoulou, E., Voidarou, C. C., Tsakris, Z., Rozos, G., Tsigalou, C. and Bezirtzoglou, E. (2022). Interactions between medical plant-derived bioactive compounds: Focus on antimicrobial combination effects. Antibiotics, 11:1014.

Vlasakova, K., Erdos, Z., Troth, S. P., McNulty, K., Chapeau-Campredon, V., Mokrzycki, N., Muniappa, N., Gu, Y. Z., Holder, D., Bailey, W. J., Sistare, F. D. and Glaab, W. E. (2014). Evaluation of the relative performance of 12 urinary biomarkers for renal safety across 22 rat sensitivity and specificity studies. Toxicological Sciences, 138:3-20.

Wang, H., Chen, Y., Wang, L., Liu, Q., Yang, S. and Wang, C. (2023). Advancing herbal medicine: enhancing product quality and safety through robust quality control practices. Frontiers in Pharmacology, 14:1265178.

Wang, L., Yan, Y. S., Cui, H. H., Yin, Y. Q., Pan, J. T. and Yu, B. W. (2016). Three new resin glycosides compounds from *Argyreia acuta* and their alpha-glucosidase inhibitory activity. Natural Product Research, 31:537-542.

WHO - World Health Organization. (2008). WHO Guidelines Approved by the Guidelines Review Committee. National Library of Medicine.

Yang, Y. and Ling, W. (2025). Health benefits and future research of phytochemicals: A literature review. Journal of Nutrition, 155:87-101.

Yin, Y. Q., Pan, J. T., Yu, B. W., Cui, H. H., Yan, Y. S. and Chen, Y. F. (2015). Two pentasaccharide resin glycosides from *Argyreia acuta*. Natural Product Research, 30:20-24.

Yu, B. W., Sun, J. J., Pan, J. T., Wu, X. H., Yin, Y. Q., Yan, Y. S. and Hu, J. Y. (2017). Four pentasaccharide resin glycosides from *Argyreia acuta*. Molecules, 22:440.

Zhang, R., Wei, J., Tang, C., Nong, B., Lu, G. and Wang, Y. (2020). Analysis on quality control of *Argyreia acuta* Lour. Medicinal Plant, 11:42-51.

Zouine, N., Ghachtouli, N. E., Abed, S. E. and Koraichi, S. I. (2024). A comprehensive review on medicinal plant extracts as antibacterial agents: Factors, mechanism insights and future prospects. Scientific African, 26:02395.

(Received: 8 February 2025, Revised: 6 January 2026, Accepted: 10 January 2026)