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RESEARCH

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Preliminary sub-acute toxicological assessment of methanol leaves extract of *Culcasia angolensis* (Araceae) in Wistar rats

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Abstract

Background: The plant *Culcasia angolensis* (Araceae) has diverse ethnomedicinal uses, including the management of rheumatic pain, healing of cuts, dislocations, and bruises. Despite its potential therapeutic uses, the toxicity profile of *Culcasia angolensis* has not been evaluated. This study assessed the sub-acute toxicity effects of *Culcasia angolensis* leaves extract (CAE). The phytochemical determination of the CAE was conducted as per the standard protocols. The median lethal dose (LD_{50}) was determined using the Organization for Economic Cooperation and Development (OECD) 423 guideline. Besides, the sub-acute toxic effects of the CAE (125, 250, and 500 mg/kg) were investigated following administration of the CAE daily for 28 consecutive days as per the OECD 407 guideline. The weekly body weights were recorded. The animals were euthanized on the 29th day, and blood samples were obtained for haematological and biochemical investigations. The heart, kidney, liver, and lungs were collected for histological examinations. Besides, the relative organ weights (ROW) were determined.

Results: The CAE contains cardiac glycosides, alkaloids, tannins, flavonoids, steroids, saponins, and terpenoids. The oral LD_{50} was above 5 g/kg. There was a remarkable decline in the weekly body weight at all the CAE doses. The CAE increased the lymphocytes, aspartate transaminase, and urea. However, the levels of alanine transaminase and alkaline phosphatase were elevated remarkably. The histological studies did not reveal any serious organs abnormalities.

Conclusion: The CAE is relatively safe on acute administration. However, it may be slightly toxic on sub-acute administration, especially to the liver and kidney.

Keywords: Biochemical parameters, Weekly body weight, *Culcasia angolensis*, Haematological parameters, Relative organ weight, Sub-acute toxicity

Background

The plant-derived products have been used for nutritional and therapeutic purposes to manage various pathological conditions and form the major source of medicinal products (Jiménez-estrada et al. 2013; Miekus et al. 2020). About 80% of the global population use plants products as a basis for their primary health needs, particularly in developing countries (Ahmad et al. 2021a, b). Several scientific studies have reported promising therapeutic properties of medicinal plants that could guide drug development and discovery (Anand et al. 2019; Bernardini et al. 2018). The urgent requirement to discover new drugs, global attention in herbal products, and the high price of orthodox medications result in an upsurge in the utilization of herbal preparations from traditional practice (Ouedraogo et al. 2012). Besides, there has been a general perception that medicinal plants are without adverse effects (Seremet et al. 2018). However, many of them have been reported to be toxic (Kharchoufa et al. 2018; Nasri and Shirzad 2013; Ndhlala et al. 2013). Besides, limited data exist on the clinical and

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safety profile of many medicinal plants (Zhou et al. 2013). Therefore, it is essential to document scientific information on the toxic concerns of herbal preparations, including the plant *Culcasia angolensis* to increase confidence in therapeutic use and to discover effective medicinal products (Ahmad et al. 2021a, b; Ukwuani et al. 2012).

The plant *Culcasia angolensis* is a part of the family Araceae. It is a robust forest climber tree with thick and tough stems (about 6 cm in diameter) that grow more than 30 m in length and attaches to its host by clasping roots (Burkill 1985). The plant is widely available in tropical African nations, including Sierra Leone, Cameroon, and Angola. The whole plant is harvested from the wild environment for use in traditional medicine. The *Culcasia angolensis* leaves have been in use against menstrual problems, pain, and inflammation in Africa, including Nigeria (Bown 2000). However, safety information on the plant is not available in the literature. Therefore, this research was intended to check the safety profile of *Culcasia angolensis* leaves extract (CAE) after the acute and sub-acute administration via oral route to stimulate more research and discover novel, effective, and safe medicinal compounds.

Methods

Plant collection and authentication

The *Culcasia angolensis* was obtained from Ngaring Nok, Jaba Local Government of Kaduna State, Nigeria, in April 2020 and authenticated by Mallam Namadi Sanusi at the Herbarium section of the Botany Department, Faculty of Life Sciences, Ahmadu Bello University (ABU), Nigeria. The comparison of the plant was with a specimen previously kept at the herbarium. The voucher sample number was 01676.

Laboratory animals

Both genders of adult Wistar rats (160–200 g) employed for the experiment were obtained from the laboratory animal section of Pharmacology and Therapeutics Department, ABU, Zaria, Nigeria. The animals were kept in clean, dried, adequately ventilated cages with sufficient and standard laboratory feed (Vital feed, Jos, Nigeria) and water provision adequately. They were kept at optimum laboratory conditions (temperature $22 \pm 3\ ^{0}C$, relative humidity 30–70% with 12 h light and 12 h dark). The animals were acclimatized for two weeks to the laboratory environment before the experimental work commenced. The permission to conduct the experiment was given by ABU Ethical Committee on Animal Use and Care Research Policy (ABUCAUC) with a permission number (ABUCAUC/2019/006) as per the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. At the end of the experiment, the rats were anaesthetized with chloroform and euthanized by cervical dislocation, after which they were appropriately buried in accordance with the University guide of disposing the remains of experimental animals.

Plant extraction

The *Culcasia angolensis* leaves were shade-dried with frequent weighing until a constant weight was achieved and powdered into fine particles with mortar and pestle. The dried and finely powdered leaves (1500 g) were extracted with methanol ($70\%^{v/v}$) using Soxhlet apparatus for 72 h. The CAE was concentrated at reduced pressure at a temperature of 45 °C on a water bath and kept in a closed container tightly. The percentage yield of the extract was calculated as follows:

$$\text{Percentage yield (\%)} = \frac{\text{Weight of the } \textit{Culcasia angolensis} \text{ leaves extract (g)}}{\text{Weight of the dried and powdered } \textit{Culcasia angolensis} \text{ leaves (g)}} \times 100.$$

Phytochemical investigation

The phytochemical analysis to determine the secondary metabolites present in CAE was carried out as per the method previously reported by Sofowora (1993).

Acute toxicity

The acute toxic actions of the CAE were evaluated in rats as per the Organization of Economic Co-operation and Development (OECD) 423 guideline (OECD 2001). The median lethal dose (LD_{50}) following oral administration was evaluated in nulliparous and non-pregnant female rats. Two groups with three rats were fasted before extract administration. (Food was withheld overnight for rats and 3 h for mice with the provision of water sufficiently.) In the first phase, 2000 mg/kg of the CAE was administered to each rat and observed for 48 h for any sign of toxicity and mortality. In the second phase, 5000 mg/kg of the CAE was administered to the rats and checked for signs of adverse effects once every 30 min within the first 4 h and subsequently for 14 consecutive days.

Sub-acute toxicity investigation

The Organization for Economic Co-operation and Development (OECD) test guideline 407 was used (OECD 2008). Twenty-four rats (both genders) were categorized into four groups, with 3 males and 3 female rats per group. (The males were separated from the females.) The rats were orally treated daily with CAE (125, 250, and 500 mg/kg) and distilled water (1 ml/kg) for 28 days. The weekly body weight was determined, and signs of harmful effects and deaths were monitored. On day 29, they

were deprived of food with free access to water for 24 h and euthanized (inhaled chloroform). The blood samples were obtained from each rat through the cardiac puncture into ethylenediaminetetraacetic acid (EDTA) and plain containers for haematological and biochemical investigations, respectively. Some organs (liver, kidney, heart, and lung) were dissected out free from adjoining supportive tissues, gently rinsed in normal saline, blotted with filter paper, and weighed. The relative organ weight (ROW) of the organs was determined using the following relation:

$$\mathrm{ROW} = \frac{\text{Absolute organ weight (g)}}{\text{Final body weight of the animal (g)}} \times 100.$$

Haematological analysis

The haematological investigation was conducted to determine any possible changes in the levels of haematocrit (HCT), platelet count (PLT), red blood cell (RBC) count, haemoglobin (HB), white blood cell (WBC) count, monocytes (MON), lymphocytes (LYMPH), neutrophils (NTP), and eosinophils (ENP) (OECD 2008).

Biochemical analysis

The non-heparinized blood samples were stored at room temperature for 1 h to clot and centrifuged at 3000 revolutions per minute for 10 min. The plasma obtained was used to determine any changes in the biochemical biomarkers, including alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST), total protein, albumin urea, and creatinine (OECD 2008).

Histopathology

The liver, kidneys, lung, and heart of each animal were fixed in 10% formalin. The sections of the organs were cut 4–5 µm with rotary microtone, stained with haematoxylin and eosin, and analysed at a magnification of 250× for any histopathological changes by a consultant histopathologist.

Statistics

All the values were displayed as mean values ± SEM in figures and tables. One-way analysis of variance (ANOVA) was used to analyse the ROW, biochemical, and haematological parameters, whereas repeated measure ANOVA was employed to analyse the weekly body weight. Dunnett's post hoc test was employed to compare the means. The $p \leq 0.05$ values were taken as significant.

Results

Percentage yield

A sticky-black solid residue weighing 107.6 g with a mild smell was obtained from a 1500 g crude plant of *Culcasia angolensis* powdered sample representing 7.2% $^{w}/_{w}$ as the percentage yield.

Phytochemical constituents

Preliminary phytochemical determination of CAE showed cardiac glycosides, triterpenes, tannins, flavonoids, alkaloids, saponins, and steroids.

Acute toxicity study

Acute oral toxicity results showed that the CAE has no adverse effect in rats. Besides, no mortality was observed at the dose levels tested. Therefore, the oral LD_{50} of the CAE could be above 5000 mg/kg.

Weekly body weight

The CAE significantly declined the rats' body weight at all the doses tested. Figure 1 displays the results of the 28-day oral administration of CAE on the weekly body weight.

Relative organ weight

The CAE produced no remarkable change in the ROW of the selected organs at all the doses as shown in Table 1.

Haematological parameters

There were no significant alterations in the levels of HCT, HGB RBC, PLT, WBC, MON, ENP, and NTP in relation to the control class. On the contrary, a remarkable elevation in lymphocytes was detected at 500 mg/kg as shown in Table 2.

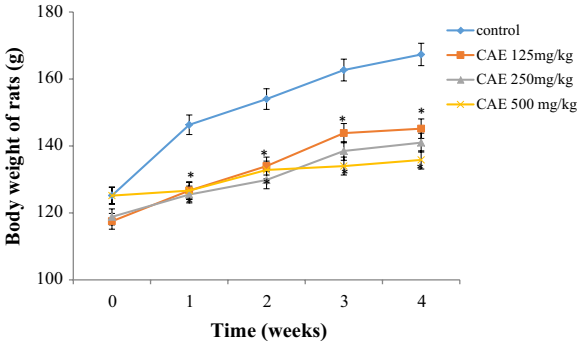

Fig. 1 Weekly body weight of rats following 28-day repeated administration *Culcasia angolensis* leaves extract (CAE). The values were displayed as mean ± SEM; $^{*}p \leq 0.05$ in relation to the control group (repeated measure ANOVA followed by Dunnett's post hoc test, $n = 6$, *CAE Culcasia angolensis* leaves extract)

Table 1 Relative organ weight of rats following 28-day repeated administration of *Culcasia angolensis* leaves extract (CAE)

Organs	Treatment groups (mg/kg)			
	DW (1 ml/kg)	CAE (125)	CAE (250)	CAE (500)
Liver	7.56 ± 0.64	7.71 ± 0.22	7.68 ± 0.09	7.54 ± 0.14
Kidney	0.74 ± 0.45	0.77 ± 0.78	0.81 ± 0.92	0.76 ± 0.07
Lungs	2.29 ± 0.04	2.33 ± 0.03	2.38 ± 0.04	2.27 ± 0.06
Heart	0.68 ± 4.42	0.70 ± 9.81	0.72 ± 4.62	0.75 ± 9.72

The values were documented as mean ± SEM (one-way ANOVA followed by Dunnett's post hoc test)

DW distilled water, *p.o* per oral, *CAE Culcasia angolensis* leaves extract, $n = 6$

Table 2 Haematological parameters of rats following 28-day repeated administration of *Culcasia angolensis* leaves extract (CAE)

Parameters	Treatment (mg/kg)			
	DW (1 ml/kg)	CAE (125)	CAE (250)	CAE (500)
WBC ($\times 10^9$/L)	3.85 ± 0.27	4.07 ± 0.22	4.27 ± 0.10	3.85 ± 0.15
HGB (g/dL)	12.72 ± 0.33	12.62 ± 0.97	11.15 ± 0.69	11.12 ± 0.51
RBC ($\times 10^6$/µL)	5.85 ± 0.17	5.98 ± 0.15	5.70 ± 0.14	5.93 ± 0.19
PLT ($\times 10^3$/µL)	7.20 ± 0.10	7.18 ± 0.10	7.18 ± 0.11	7.08 ± 0.06
HCT (%)	37.16 ± 1.86	36.67 ± 3.18	35.00 ± 1.97	35.17 ± 1.30
MON (%)	2.33 ± 0.56	1.67 ± 0.21	1.50 ± 0.34	2.00 ± 0.37
LYMPH (%)	78.17 ± 1.17	77.67 ± 0.72	78.50 ± 0.99	81.33 ± 1.38*
ENP (%)	2.17 ± 0.30	1.66 ± 0.33	2.00 ± 0.36	1.83 ± 0.31
NTP (%)	17.33 ± 2.08	19.00 ± 0.97	18.00 ± 1.15	15.00 ± 0.97

The values were tabulated as mean ± SEM, $*p \leq 0.05$ in relation to the control group (one-way ANOVA followed by Dunnett's post hoc test), $n = 6$

DW distilled water, *CAE Culcasia angolensis* leaves extract, *WBC* white blood cell, *RBC* red blood cells, *HGB* haemoglobin, *HCT* haematocrit, *PLT* platelets, *LYMP* lymphocytes, *MON* monocytes, *ENP* EOSINOPHILS, *NTP* neutrophils

Hepatic parameters

The ALT was remarkably reduced at 250 and 500 mg/kg. Besides, ALP was reduced in the category that received the 250 mg/kg of the CAE. However, there was an elevation of AST in relation to the control group. No alteration was observed in the protein and albumin levels. The effects of the CAE on hepatic parameters are displayed in Table 3.

Kidney parameters

The result showed a remarkable elevation in the plasma urea level in the group that received 500 mg/kg of the CAE related to the normal group. On the contrary, no remarkable change in the creatinine levels and serum electrolytes (sodium, potassium, chlorine, and bicarbonate) was observed as shown in Table 4.

Table 3 Hepatic parameters of rats following 28-day repeated administration of *Culcasia angolensis* leaves extract (CAE)

Parameters	Treatment (mg/kg)			
	DW (1 ml/kg)	CAE (125)	CAE (250)	CAE (500)
ALT (IU/L)	26.2 ± 4.11	27.2 ± 7.20	15.1 ± 1.57*	16.3 ± 2.07*
AST (IU/L)	37.67 ± 0.92	41.68 ± 0.58*	44.80 ± 1.67*	59.66 ± 2.28*
ALP (IU/L)	22.76 ± 3.38	19.68 ± 4.32	15.80 ± 3.24*	19.21 ± 4.03
TP (mg/dL)	6.54 ± 0.24	6.69 ± 0.32	6.44 ± 0.30	6.93 ± 0.34
ALB (mg/dL)	3.14 ± 0.07	3.17 ± 0.08	3.08 ± 0.06	3.03 ± 0.09

The values were tabulated as mean ± SEM, $*p \leq 0.05$ in relation to control group (one-way ANOVA followed by Dunnett's post hoc test), $n = 6$

DW distilled water, *ALT* alanine transaminase, *AST* aspartate transaminase, *ALP* alkaline phosphatase, *TP* total protein, *ALB* albumin, *CAE Culcasia angolensis* leaves extract

Histopathology

There were no histopathological alterations in the hepatic tissue of the group treated with 125 mg/kg of the CAE. In contrast, the groups that received the extract at 250 and 500 mg/kg showed moderate hepatic necrosis (HN) (Fig. 2). Besides, the groups that received the lowest (125 mg/kg) and highest (500 mg/kg) doses revealed slight tubular necrosis (TN), whereas lymphocyte hyperplasia (LH) was observed at 250 mg/kg (Fig. 3). However, no histopathological abnormalities were observed in the heart muscles of rats in all the treated categories (Fig. 4). Slight alveoli congestion (AC) was observed in the categories that received the lower doses (125 and 250 mg/kg), whereas the CAE (500 mg/kg) revealed nuclei hardening and pyknosis (HP) (Fig. 5).

Discussion

The herbal products have been used in a traditional practice because they have chemical agents with potential therapeutic actions for treating various human diseases (Hosseinzadeh et al. 2015). However, experimental investigations have shown that some of the herbal products are toxic, which necessitates the need to examine the toxicological effect of plants with medicinal values (Ndhlala et al. 2013). In fact, international regulatory agencies such as Food and Drug Administration (FDA) have encouraged taking effective strategies against the use of herbal products with no scientific and toxicological information (De Smet 2004; Kale et al. 2019).

Table 4 Kidney parameters of rats following 28-day repeated administration of *Culcasia angolensis* leaves extract (CAE)

Parameters	Treatment group (mg/kg)			
	DW (1 ml/kg)	CAE (125)	CAE (250)	CAE (500)
Urea (mmol/L)	32.2 ± 1.22	30.80 ± 0.89	31.66 ± 2.70	38.40 ± 1.82*
Creatinine (mmol/L)	0.80 ± 0.05	0.85 ± 0.11	0.88 ± 0.12	0.90 ± 0.60
Sodium (mmol/L)	220.32 ± 7.44	221.56 ± 2.99	225.08 ± 6.91	222.98 ± 3.24
Potassium (mmol/L)	7.90 ± 0.10	6.76 ± 1.20	7.30 ± 0.36	6.60 ± 2.27
Chloride (mmol/L)	96.67 ± 5.39	98.67 ± 5.26	87.83 ± 3.02	89.40 ± 1.50
Bicarbonate (mmol/L)	88.8 ± 3.30	88.60 ± 2.11	94.50 ± 3.71	89.84 ± 1.82

The values were tabulated as mean ± SEM; *$p \leq 0.05$ compared to control group (one-way ANOVA followed by Dunnett's post hoc test), $n = 6$

DW distilled water, *CAE Culcasia angolensis* leaves extract

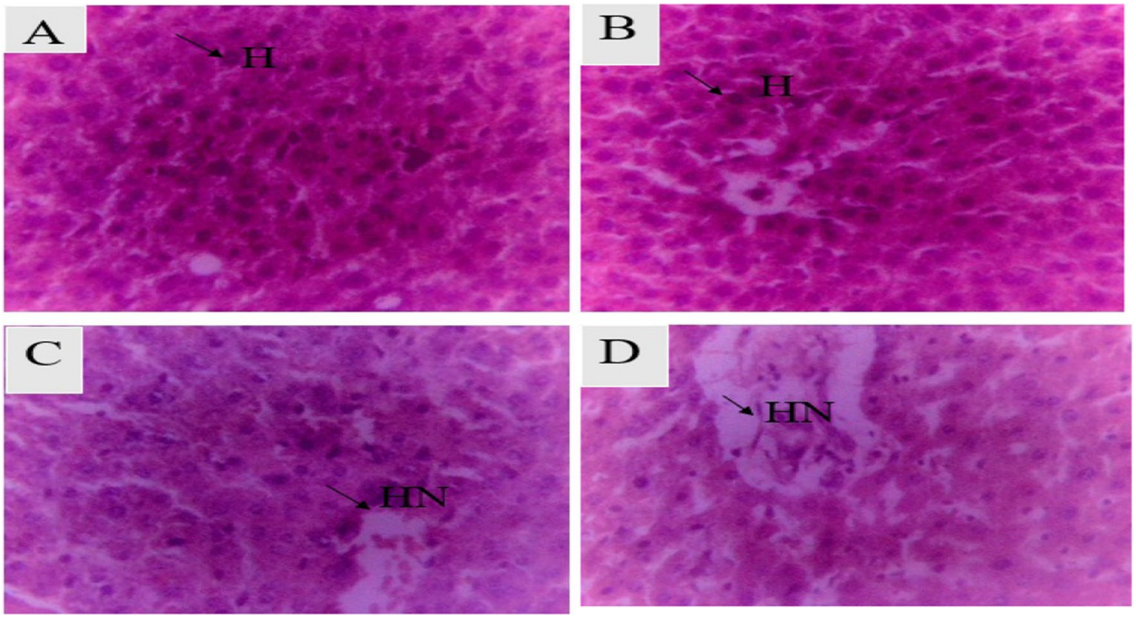

Fig. 2 Photomicrographs of liver sections of rats following 28-day oral administrations of *Culcasia angolensis* leaves extract (CAE) (haematoxylin and eosin-stained at ×250 magnification). **A** Control (distilled water); **B** CAE (125 mg/kg); **C** CAE (250 mg/kg); **D** CAE (500 mg/kg), H (normal hepatocytes); HN (moderate hepatocyte necrosis)

Plants possess bioactive chemicals that serve as lead in drug discovery (Susanto et al. 2017). Besides, documenting the safety data of medicinal plants is important for the subsequent pharmacological screening (Momin et al. 2014). The secondary metabolites' investigation in the current work shows that the CAE possesses cardiac glycosides, triterpenes, tannins, flavonoids, alkaloids, saponins, and steroids. Some of these phytochemical compounds have various pharmacological actions (Kpemissi et al. 2020). However, despite their promising therapeutic actions, they could have toxicity effects (Kpemissi et al. 2019). For instance, cardiac glycosides are associated with cardiovascular toxicity such as heart muscles lesions and arrhythmias (Botelho et al. 2018), tannins could cause mild hepatic and renal disturbances (Ekambaram et al. 2018), and flavonoids cause hepatic failure, haemolytic anaemia, hypoglycaemia (Galati and Brien 2004). Besides, alkaloids and saponins are associated with hepatic failure (Qin et al. 2009; Wiedenfeld 2011), and steroids have cardiovascular and hepatic toxicity as well as immune suppression effects (Amsterdam et al. 2010; Heming et al. 2018).

The study of acute harmful effects of biological agents is utilized to check the toxic potential of bioactive agents following single-dose administration for the short term and is the starting point in determining the

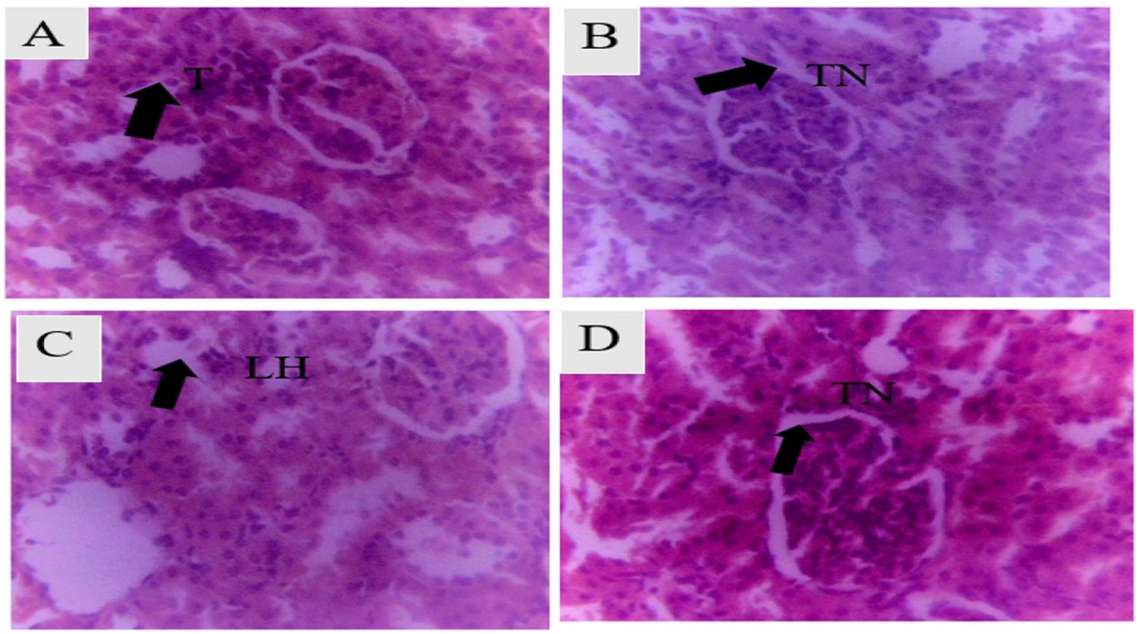

Fig. 3 Photomicrographs of kidney sections of rats following 28-day oral administrations of *Culcasia angolensis* leaves extract (CAE) (haematoxylin and eosin-stained at ×250 magnification). **A** Control (distilled water); **B** CAE (125 mg/kg); **C** CAE (250 mg/kg); **D** CAE (500 mg/kg), T (normal kidney tubules and glomerulus); TN (slight tubular necrosis); LH (lymphocyte hyperplasia)

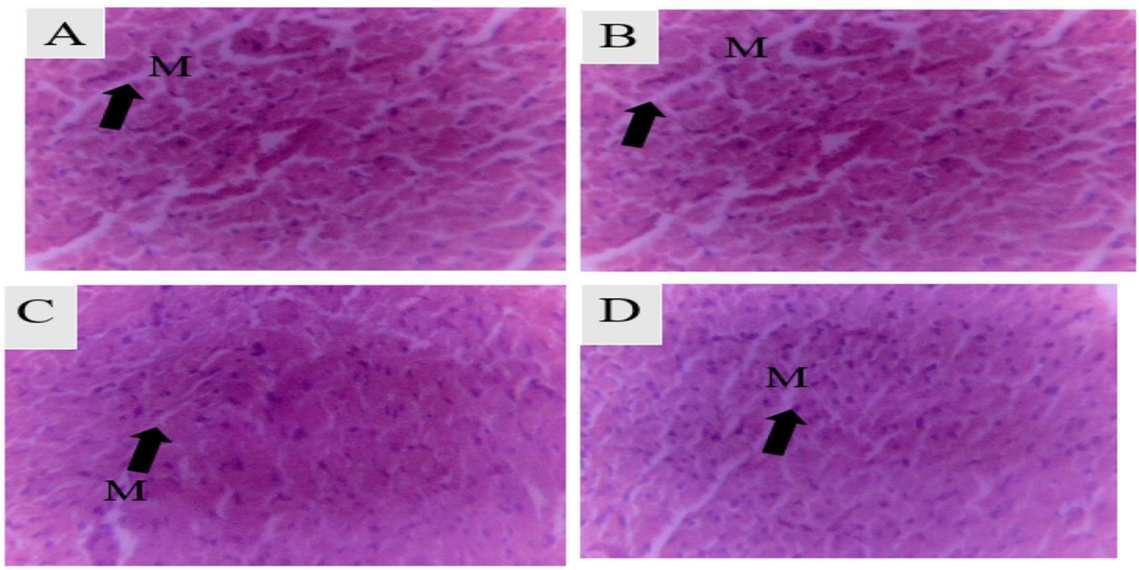

Fig. 4 Photomicrographs of heart sections of rats following 28-day oral administrations of *Culcasia angolensis* leaves extract (CAE) (haematoxylin and eosin-stained at ×250 magnification). **A** Control (distilled water); **B** CAE (125 mg/kg); **C** CAE (250 mg/kg); **D** CAE (500 mg/kg), M (normal cardiac muscles)

pharmacological actions of unknown agents (Kpemissi et al. 2020; Musila et al. 2017). Additionally, toxicity assessment plays an essential part in determining the LD50 of compounds (Ugwah-oguejiofor et al. 2019). Hence, the lack of obvious toxic signs and mortality by the CAE after the single administration showed that the LD_{50} could be above 5000 mg/kg. The result concurs with the report on hesperidin sourced from orange peel

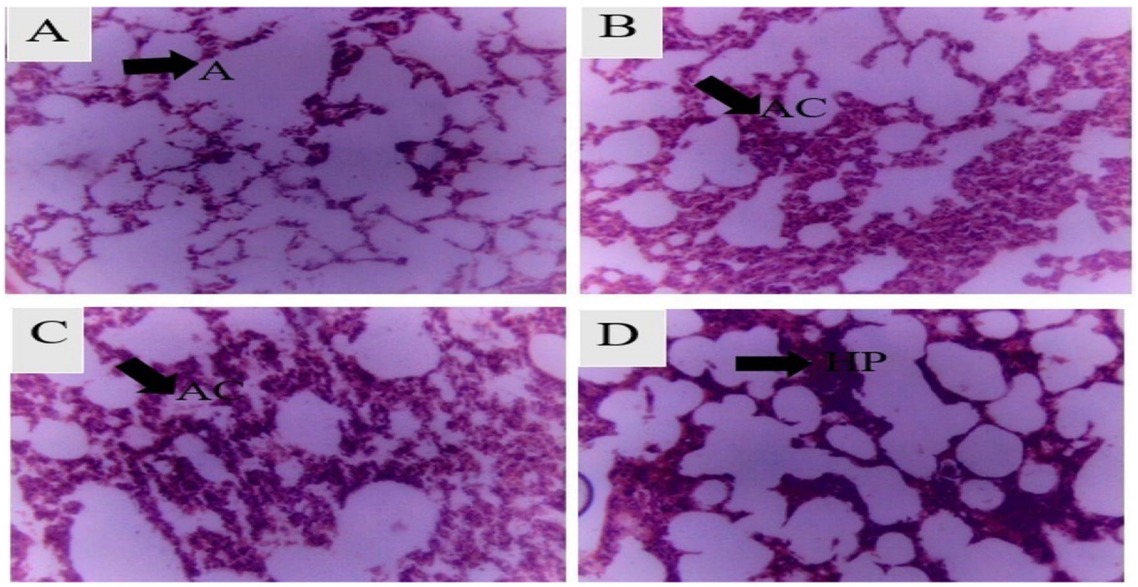

Fig. 5 Photomicrographs of lung sections of rats following 28-day oral administrations of *Culcasia angolensis* leaves extract (CAE) (haematoxylin and eosin-stained at ×250 magnification). **A** Control (distilled water); **B** CAE (125 mg/kg); **C** CAE (250 mg/kg); **D** CAE (500 mg/kg), A (normal lung alveoli); AC (slight alveoli congestion); HP (nuclei hardening and pyknosis)

(Li et al. 2019) and the leaves of *Combretum hypopilinum* (Ahmad et al. 2020a, b).

The determination of sub-acute harmful actions is used to document an information on the safety profile of chemical agents following 28-day repeated oral administration in rodents (Christapher et al. 2017). In addition, it shows the cumulative effects of the agents to certain tissues and organs (Loha et al. 2019). The parameters that are used in checking the long-term toxicological data of herbal products include animals' general behaviour, body weight, biochemical and haematological indices, and histopathological outcome (Jothy et al. 2011). Similar to the acute toxicity results in the current work, the CAE did not produce any clear signs of toxicity and death throughout the experimental period (28 days).

An alteration in the animals' body weight indicates harmful actions after being exposed to harmful agents due to fat accumulation, loss of appetite, and low caloric consumption (Prasanth et al. 2015). Likewise, reduction in organ weight expresses toxicity from toxic agents. The toxic effects of herbal preparations usually target key organs such as the kidney, liver, heart, and spleen (Unuofin et al. 2018). The body weight reduction caused by the CAE in this study could be attributed to loss of appetite, which may reduce food intake and interfered with nutrient absorption. Besides, the presence of phytocomponents such as saponins and tannins in the CAE could have produced antinutritional effects by interfering with nutrients absorption (Nguenang et al. 2020). The declined body weight in the current research is in line with the reduced ALP levels observed in the hepatic biomarkers which could have resulted in malnutrition, vitamins, and mineral deficiency due poor intestinal absorption (El Kabbaoui et al. 2017; Ray et al. 2017). Previous studies have shown that various plant extracts such as *Bridelia ferruginea* (Bakoma et al. 2013) and *Epigynum auritum* (Yang et al. 2019) reduced the animals' body weight. However, other plant extracts such as *Campomanesia velutina* (Araújo et al. 2017) and *Lycopersicon esculentum* (Nguenang et al. 2020) have no effect on body weight.

Several toxic agents target the haematopoietic system, which is a key indicator for health status (El Kabbaoui et al. 2017). An alteration in the blood parameters including RBC, HGB, HCT, MCH, MCV, MCHC is related to blood disorders, especially anaemia and heart-related diseases (Olorunnisola et al. 2012), whereas WBC including lymphocytes acts against infectious agents, inflammatory processes, and tissue injury (Hervé et al. 2020). The present result has shown that the CAE may not interfere with erythropoiesis and could be devoid of heart-related complications. The outcome concurs with the cardiac histology in the current experiments that revealed a lack of cardiotoxicity of the extract. Besides, the remarkable increase in the lymphocyte level shows that the CAE may contain biologically active agents that activate the immune system. The result corroborates with the work of Nguenang et al. (2020), which shows that the *Lycopersicon esculentum* leaves extract possesses immune-stimulatory actions.

The principal organ where the biotransformation of drugs and other bioactive agents takes place is in the liver (Nguenang et al. 2020). The ALT, AST, and ALP are liver biomarkers that determine the liver metabolic activities (El Kabbaoui et al. 2017). They are used to evaluate and manage hepatic disorders (Kim et al. 2012; Villela-nogueira et al. 2005). After a hepatic cellular injury, the levels of these enzymes increase in the serum due to changes in cell membrane permeability (Li et al. 2019), whereas their reduction is associated with chronic kidney disease that could complicate hepatic injury (Cavalcanti et al. 2012). The ALP is also important in evaluating biliary duct diseases, and its reduction is a result of vitamin C and B12 deficiency, malnutrition, hypothyroidism, hypophosphatasia, as well as magnesium and zinc deficiency (El Kabbaoui et al. 2017; Ray et al. 2017). Therefore, the dose-dependent elevation of the AST in the present study shows that the CAE could be hepatotoxic which is evident from the histopathological results of the liver in this research that indicates hepatic necrosis. Besides, the extract may adversely affect renal function as shown by the elevated urea levels and tubular necrosis from the outcomes of renal parameters and kidney histopathological examinations respectively. The findings have also shown that the CAE could cause malnutrition, mineral, and vitamin (C and B12) deficiency as a result of the reduced ALT and ALP, respectively. The malnutrition and vitamins and mineral deficiency produced by the CAE in the present study could be related to the reduction in the animals' body weight due to improper absorption. Previous research has shown the hepatic effects of *Psidium guajava* (Manekeng et al. 2019).

The kidney is responsible for maintaining vital physiological processes such as regulation of acid–base, electrolytes, and blood pressure (Bencheikh et al. 2021). Renal toxicity arises as a result of the inability of the kidney to sufficiently detoxify and remove toxicants (Kim and Moon 2012). Creatinine and urea are metabolic by-products removed from the body by glomerular filtration and used as an index for nephrotoxicity (Aprioku et al. 2014). In kidney disorder, the plasma urea levels increase due to the increased production, which is often used as a reliable indicator for assessing renal function (Oyagbemi et al. 2013). Therefore, the elevated plasma urea level at 500 mg/kg in this study shows that the CAE could lead to kidney damage due to enhanced urea production that exceeded its clearance. The outcome corroborates with the renal histopathology that shows tubular and glomerular necrosis. Other plant extracts such as *Terminalia schimperiana* (Awotunde et al. 2019), *Simarouba glauca* (Osagie-Eweka et al. 2021), and *Caralluma dalzielii* (Ugwah-oguejiofor et al. 2019) revealed a possible renal effect by remarkably increasing the plasma urea levels.

The toxicological assessment of bioactive agents comprises histopathological examination of vital organs (Traesel et al. 2016). Hepatic necrosis is an essential marker for hepatotoxic studies. Hepatocytes necrosis usually arises due to inflammatory response, neutrophils, and mononuclear cells recruitment into the hepatic tissues. Besides, hepatic necrosis is accompanied by hepatic nuclear pyknosis and eosinophilic infiltration (Sharifudin et al. 2013). The slight hepatic necrosis observed in this work further showed the possible hepatotoxic actions of the CAE, which concurs with the elevated AST observed.

The slight tubular necrosis of the kidney observed at 250 and 500 mg/kg could be related to the delivery of harmful agents from the systemic circulation to the kidney and cause renal tubular system malfunction. It was reported that severe glomerular and renal tubular damage could impair renal tubular reabsorption, glomerular filtration, and electrolyte absorption which could be associated with the accumulated urea levels beyond the excretory ability of the kidney (Zhao et al. 2007). The alveoli congestion observed in the group administered with 125 and 500 mg/kg indicates that the CAE could impair oxygen diffusion and other gases across the alveoli epithelium and into the pulmonary circulation. The absence of histological alteration in the heart architecture shows that the CAE may not possess cardiotoxicity. Besides, the delivery of blood to the cardiac muscles may not be affected as shown by the non-interference of the extract with RBC production.

Conclusions

The outcome of the study has shown that the CAE could be relatively safe on acute administration. However, it may be slightly toxic on sub-acute administration, especially to the liver and kidney. Therefore, more research to determine the chronic toxicity effects of the plant should be conducted. Besides, traditional herbal practitioners should be educated on the possible harmful effects related to the long-term intake of *Culcasia angolensis*.

Abbreviations

ABU: Ahmadu Bello University; ABUCAUC: ABU Ethical Committee on Animal Use and Care Research Policy; ALP: Alkaline phosphatase; ALT: Alanine transaminase; ALT: Aspartate transaminase; ANOVA: One-way analysis of variance; ARRIVE: Animal Research: Reporting of In Vivo Experiments; CAE: *Culcasia angolensis* leaves extract; EDTA: Ethylenediaminetetraacetic acid; ENP: Eosinophils; HB: Haemoglobin; HCT: Haematocrit; LD50: Median lethal dose; LYMPH: Lymphocytes; MON: Monocytes; NTP: Neutrophils; OECD: Organization of Economic Co-operation and Development; PLT: Platelet; RBC: Red blood cell; ROW: Relative organ weight; WBC: White blood cell.

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Authors' contributions
IGA contributed to conceptualization, investigation, writing—original draft, and data analysis. MHA was involved in writing, review, and editing. All the authors read and approved the final manuscript.

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Availability of data and materials
The datasets generated during and/or analysed during the current study are available with the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The permission for the experiment was given by the Ahmadu Bello University Ethical Committee on Animal Use and Care Research Policy (permission number: ABUCAUC/2019/006) and carried out as per the Animal Research Reporting of In Vivo Experiments (ARRIVE) protocols.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References

Ahmad MH, Zezi AU, Anafi SB, Danraka RN, Alhassan Z (2020a) Evaluation of antidiarrhoeal activity of methanol extract of *Combretum hypopilinum* Diels (Combretaceae) leaves in mice. Adv Pharm J 5(2):54–61. https://doi.org/10.31024/apj.2020.5.2.3

Ahmad MH, Zezi AU, Bola AS, Alhassan Z, Mohammed M, Danraka RN (2020b) Mechanisms of antidiarrhoeal activity of methanol leaf extract of *Combretum hypopilinum* diels (Combretaceae): involvement of opioidergic and (α1 and β)-adrenergic pathways. J Ethnopharmacol 269:113750. https://doi.org/10.1016/j.jep.2020.113750

Ahmad MH, Jatau AI, Alshargi OY, Julde M, Mohammed M, Muhammad S, Mustapha S, Bala AA, Wada AS, Aminu M, Usman AM (2021a) Ethnopharmacological uses, phytochemistry, pharmacology, and toxicology of *Olax subscorpioidea* Oliv (Olacaceae): a review. Fut J Pharm Sci 7(115):1–13. https://doi.org/10.1186/s43094-021-00264-w

Ahmad MH, Jatau AI, Khalid GM, Alshargi OY (2021b) Traditional uses, phytochemistry, and pharmacological activities of *Cochlospermum tinctorium* A. Rich (Cochlospermaceae): a review. Fut J Pharm Sci 7(1):1–13. https://doi.org/10.1186/s43094-020-00168-1

Amsterdam JV, Opperhuizen A, Hartgens F (2010) Adverse health effects of anabolic–androgenic steroids. Regul Toxicol Pharmacol 57(1):117–123. https://doi.org/10.1016/j.yrtph.2010.02.001

Anand U, Jacobo-herrera N, Altemimi A, Lakhssassi N (2019) A comprehensive review on medicinal plants as antimicrobial therapeutics: potential avenues of biocompatible drug discovery. Metabolites 9(258):1–13

Aprioku JS, Nwidu LL, Amadi CN (2014) Evaluation of toxicological profile of ibuprofen in wistar albino rats. Am J Biomed Sci 6(1):32–40

Araújo MC, Barcellos NMS, Vieira PM, Gouveia TM, Guerra MO, Peters VM, Saúde-Guimarães DA (2017) Acute and sub chronic toxicity study of aqueous extract from the leaves and branches of *Campomanesia velutina* (Cambess) O. Berg J Ethnopharmacol 201:17–25. https://doi.org/10.1016/j.jep.2017.02.043

Awotunde OS, Adewoye SO, Dhanabal PS, Hawumba J (2019) Subacute toxicity study of aqueous root extract of *Terminalia schimperiana* in male Wistar rats. Toxicol Rep 6:825–832

Bakoma B, Berké B, Eklu-Gadegbeku K, Agbonon A, Aklikokou K, Gbeassor M, Creppy E, Moore N (2013) Acute and sub-chronic (28days) oral toxicity evaluation of hydroethanolic extract of *Bridelia ferruginea* Benth root bark in male rodent animals. Food Chem Toxicol 52:176–179. https://doi.org/10.1016/j.fct.2012.11.021

Bencheikh N, Bouhrim M, Kharchoufa L, Al Kamaly OM, Mechchate H, Es-Safi I et al (2021) The nephroprotective effect of *Zizyphus lotus* L.(desf.) fruits in a gentamicin-induced acute kidney injury model in rats: a biochemical and histopathological investigation. Molecules 26(16):4806

Bernardini S, Tiezzi A, Masci VL, Ovidi E (2018) Natural products for human health: an historical overview of the drug discovery approaches. Nat Prod Res 6419:1–25. https://doi.org/10.1080/14786419.2017.1356838

Botelho AFM, Pierezan F, Soto-blanco B, Melo MM (2018) A review of cardiac glycosides: structure, toxicokinetics, clinical signs, diagnosis and antineoplastic potential. Toxicon. https://doi.org/10.1016/j.toxicon.2018.11.429

Bown D (2000) Aroids: plants of the arum family. Timber Press; Portland, Oregon. http://tropical.theferns.info/viewtropical.php?id=Culcasia+angolensis

Burkill HM (1985) The useful plants of West Tropical Africa 2nd ed. vol 1 Royal Botanical Gardens Kew

Cavalcanti B, Pessoa E, Lopes DA (2012) The reduction of serum aminotransferase levels is proportional to the decline of the glomerular filtration rate in patients with chronic kidney disease. Clinics. https://doi.org/10.6061/clinics/2015(05)07

Christapher PV, Parasuraman S, Asmawi MZ, Murugaiyah V (2017) Acute and subchronic toxicity studies of methanol extract of *Polygonum minus* leaves in Sprague Dawley rats. Regul Toxicol Pharmacol. https://doi.org/10.1016/j.yrtph.2017.02.005

De Smet PA (2004) Health risks of herbal remedies: an update. Clin Pharmacol Ther 76(1):1–17. https://doi.org/10.1016/j.clpt.2004.03.005

Ekambaram SP, Perumal SS, Babu KB, Rajendran D (2018) Repeated oral dose toxicity study on hydrolyzable tannin rich fraction isolated from fruit pericarps of *Terminalia chebula* Retz in Wistar albino rats. Regul Toxicol Pharmacol. https://doi.org/10.1016/j.yrtph.2017.12.001

El Kabbaoui M, Chda A, El-Akhal J, Azdad O, Mejrhit N, Aarab L, Bencheikh R, Tazi A (2017) Acute and sub-chronic toxicity studies of the aqueous extract from leaves of *Cistus ladaniferus* L. in mice and rats. J Ethnopharmacol 209:147–156. https://doi.org/10.1016/j.jep.2017.07.029

Galati G, Brien PJO (2004) Potential toxicity of flavonoids and other dietary phenolics: significance for their chemopreventive and anticancer properties. Free Radic Biol Med 37(3):287–303. https://doi.org/10.1016/j.freeradbiomed.2004.04.034

Heming N, Sivanandamoorthy S, Meng P, Bounab R (2018) Immune effects of corticosteroids in sepsis. Front Immunol 9:1–11. https://doi.org/10.3389/fimmu.2018.01736

Hervé E, Nomane E, Goze B, Léandre K, Angoué K, Yapo P (2020) Effects of subacute oral administration of aqueous extract of *Macaranga barteri* Müll. Arg (Euphorbiaceae) leaf on anthropometric and haematological parameters in rats. Toxicol Res. https://doi.org/10.1007/s43188-020-00048-z

Hosseinzadeh S, Jafarikukhdan A, Hosseini A, Armand R (2015) The application of medicinal plants in traditional and modern medicine: a review of *Thymus vulgaris*. Int J Clini Med 6:635–642

Jiménez-estrada M, Velázquez-contreras C, Garibay-escobar A, Sierras-canchola D, Lapizco-vázquez R, Ortiz-sandoval C, Burgos-hernández A, Robles-zepeda RE (2013) In vitro antioxidant and antiproliferative activities of plants of the ethnopharmacopeia from northwest of Mexico. BMC Complement Altern Med. https://doi.org/10.1186/1472-6882-13-12

Jothy SL, Zakaria Z, Chen Y, Lau YL, Latha LY, Sasidharan S (2011) Acute oral toxicity of methanolic seed extract of *Cassia fistula* in mice. Molecules 16(6):5268–5282. https://doi.org/10.3390/molecules16065268

Kale OE, Awodele O, Akindele AJ (2019) Subacute and subchronic oral toxicity assessments of *Acridocarpus smeathmannii* (DC.) Guill. & Perr. root in Wistar rats. Toxicol Rep 6:161–175. https://doi.org/10.1016/j.toxrep.2019.01.005

Kharchoufa L, Merrouni IA, Yamani A, Elachouri M (2018) Profile on medicinal plants used by the people of North Eastern Morocco: toxicity concerns. Toxicon. https://doi.org/10.1016/j.toxicon.2018.09.003

Kim SY, Moon A (2012) Drug-induced nephrotoxicity and its biomarkers. Biomol Therap 20(3):268
Kim YJ, Jang BK, Kim ES, Park KS, Cho KB, Chung WJ, Hwang JS (2012) Rapid normalization of alanine aminotransferase predicts viral response during combined peginterferon and ribavirin treatment in chronic hepatitis C patients. Korean J Hepatol 18:41–47
Kpemissi M, Eklu-Gadegbeku K, Veerapur VP, Negru M, Taulescu M, Chandramohan V, Hiriyan J, Banakar SM, Thimmaiah NV, Suhas DS, Puneeth TA, Vijayakumar S, Metowogo K, Aklikokou K (2019) Nephroprotective activity of *Combretum micranthum* G. Don in cisplatin induced nephrotoxicity in rats: in-vitro, in-vivo and in-silico experiments. Biomed Pharmacother 116:108961. https://doi.org/10.1016/j.biopha.2019.108961
Kpemissi M, Metowogo K, Melila M, Veerapur VP, Negru M, Taulescu M, Potârniche A, Shivalingaiah D, Adinarayanashetty T, Vijayakumar S, Eklu-gadegbeku K, Aklikokou K (2020) Acute and subchronic oral toxicity assessments of *Combretum micranthum* (Combretaceae) in Wistar rats. Toxicol Rep 7:162–168. https://doi.org/10.1016/j.toxrep.2020.01.007
Li Y, Kandhare AD, Mukherjee AA, Bodhankar SL (2019) Acute and sub-chronic oral toxicity studies of hesperidin isolated from orange peel extract in Sprague Dawley rats. Regul Toxicol Pharmacol 105(1):77–85. https://doi.org/10.1016/j.yrtph.2019.04.001
Loha M, Mulu A, Abay SM, Ergete W, Geleta B (2019) Acute and subacute toxicity of methanol extract of *Syzygium guineense* leaves on the histology of the liver and kidney and biochemical compositions of blood in rats. Evid Based Complement Altern Med 5702159:1–15. https://doi.org/10.1155/2019/5702159
Manekeng HT, Mbaveng AT, Ntyam Mendo SA, Agokeng AJD (2019) Kuete V (2019) Evaluation of acute and subacute toxicities of *Psidium guajava* methanolic bark extract: a botanical with in vitro antiproliferative potential. Evid Based Complement Altern Med 6:66
Miekus N, Marszałek K, Podlacha M, Iqbal A, Puchalski C, Swiergiel AH (2020) Health benefits of plant-derived sulfur compounds. Molecules 25(3804):1–22. https://doi.org/10.3390/molecules25173804
Momin MAA, Bella SF, Rahman SMR, Rahman AY, Murshid GMM, Emran TB (2014) Phytopharmacological roots evaluation of ethanol extract of *Sida cordifolia* L. Asian Pac J Trop Biomed 4(1):18–24. https://doi.org/10.1016/S2221-1691(14)60202-1
Musila MN, Ngai DN, Mbiri JW, Njagi SM, Mbinda WM, Ngugi MP (2017) Acute and sub-chronic oral toxicity study of methanolic extract of *Caesalpinia volkensii* (Harms). J Drug Metab Toxicol 08(01):1–8. https://doi.org/10.4172/2157-7609.1000222
Nasri H, Shirzad H (2013) Toxicity and safety of medicinal plants. J HerbMed Pharmacol 2(2):21–22
Ndhlala AR, Ncube B, Okem A, Mulaudzi RB, Staden JV (2013) Toxicology of some important medicinal plants in southern Africa. Food Chem Toxicol J 62:609–621. https://doi.org/10.1016/j.fct.2013.09.027
Nguenang GS, Ntyam AS (2020) Kuete V (2020) Acute and subacute toxicity profiles of the methanol extract of *Lycopersicon esculentum* L. Leaves (Tomato), a botanical with promising in vitro anticancer potential. Evid Based Complement Altern Med 6:66
Olorunnisola OS, Bradley G, Afolayan AJ (2012) Acute and sub-chronic toxicity studies of methanolic extract of *Tulbaghia violacea* rhizomes in Wistar rats. Afr J Biotech 11(83):14934–14940. https://doi.org/10.5897/AJB12.1565
Organization for Economic Cooperation and Development (OECD) (2001) Guidelines for the testing of chemicals: health effect Test No. 423. Acute Oral Toxicity-Acute Toxic Class Method, Paris
Organization for Economic Cooperation and Development (OECD) (2008) Guidelines for the testing of chemicals: Guideline 407: repeated dose 28-day oral toxicity in rodents. Office of Economic and Community Development, Paris
Osagie-Eweka SE, Orhue NEJ, Omogbai EKI, Amaechina FC (2021) Oral acute and sub-chronic toxicity assessment of aqueous leaf extract of Simarouba glauca DC (Paradise tree). Toxicol Rep 8:239–247
Ouedraogo M, Baudoux T, Stévigny C, Nortier J, Colet J, Efferth T, Qu F, Zhou J, Chan K, Shaw D, Pelkonen O, Duez P (2012) Review of current and "omics" methods for assessing the toxicity (genotoxicity, teratogenicity and nephrotoxicity) of herbal medicines and mushrooms. J Ethnopharmacol 140(3):492–512. https://doi.org/10.1016/j.jep.2012.01.059
Oyagbemi AA, Omobowale TO, Azeez IO, Abiola JO, Adedokun RAM, Nottidge HO (2013) Toxicological evaluations of methanolic extract of *Moringa oleifera* leaves in liver and kidney of male Wistar rats. J Basic Clin Physiol Pharmacol 24(4):307–312. https://doi.org/10.1515/jbcpp-2012-0061
Prasanth KM, Suba V, Ramireddy B, Srinivasa BP (2015) Acute and subchronic oral toxicity assessment of the ethanolic extract of the root of *Oncoba spinosa* (Flacourtiaceae) in rodents. Trop J Pharm Res 14(10):1849–1855. https://doi.org/10.4314/tjpr.v14i10.16
Qin Y, Wu X, Huang W, Gong G, Li D, He Y, Zhao Y (2009) Acute toxicity and sub-chronic toxicity of steroidal saponins from Dioscorea zingiberensis C. H. Wright in rodents. J Ethnopharmacol J 126:543–550. https://doi.org/10.1016/j.jep.2009.08.047
Ray CS, Singh B, Jena I, Behera S, Ray S (2017) Low alkaline phosphatase (ALP) in adult population an indicator of zinc (zn) and magnesium (mg) deficiency. Curr Res Nutr Food Sci 5(3):347–352. https://doi.org/10.12944/CRNFSJ.5.3.20
Seremet OC, Olaru OT, Gutu CM, Nitulescu GM, Ilie M, Negres S, Zbarcea CE, Purdel CN, Spandidos DA, Tsatsakis AM, Coleman MD, Margina DM (2018) Toxicity of plant extracts containing pyrrolizidine alkaloids using alternative invertebrate models. Mol Med Report 17:7757–7763. https://doi.org/10.3892/mmr.2018.8795
Sharifudin SA, Fakurazi S, Hidayat MT, Hairuszah I, Aris M, Moklas M, Arulselvan P (2013) Therapeutic potential of *Moringa oleifera* extracts agains acetaminophen-induced hepatotoxicity in rats. Pharm Biol 51(3):279–288. https://doi.org/10.3109/13880209.2012.720993
Sofowora A (1993) Medicinal plants and traditional medicine in Africa (2nd ed). Spectrum Books Ltd.
Susanto DF, Aparamarta HW, Widjaja A, Gunawan S (2017) Identification of phytochemical compounds in *Calophyllum inophyllum* leaves. Asian Pac J Trop Biomed 7(9):1–9. https://doi.org/10.1016/j.apjtb.2017.08.001
Traesel GK, Menegati SELT, Santos AC, Souza RIC, Boas GRV, Justi PN, Kassuya CAL, Argandoña EJS, Oesterreich SA (2016) Oral acute and subchronic toxicity studies of the oil extracted from pequi (*Caryocar brasiliense*, Camb.) pulp in rats. Food Chem Toxicol 97:224–231. https://doi.org/10.1016/j.fct.2016.09.018
Ugwah-oguejiofor CJ, Okoli OC, Ugwah MO, Umaru ML, Ogbulie CS, Mshelia HE, Umar M, Njan AA (2019) Acute and sub-acute toxicity of aqueous extract of aerial parts of *Caralluma dalzielii* N. E. Brown in mice and rats. Heliyon 5(1):e01179. https://doi.org/10.1016/j.heliyon.2019.e01179
Ukwuani AN, Abubakar MG, Hassan SW, Agaie BM (2012) Toxicological Studies of Hydromethanolic Leaves Extract of *Grewia crenata*. Int J Pharm Sci Drug Res 4(4):245–249
Unuofin JO, Otunola GA, Afolayan AJ (2018) Evaluation of acute and subacute toxicity of whole-plant aqueous extract of *Vernonia mespilifolia* Less. in Wistar rats. J Integr Med. https://doi.org/10.1016/j.joim.2018.07.003
Villela-nogueira CA, Perez RM, de Segadas Soares JA, Moraes Coelho HS (2005) Gamma-glutamyl transferase (GGT) as an independent predictive factor of sustained virologic response in patients with hepatitis C treated with interferon-Alpha and Ribavirin. J Clin Gastroenterol 39(8):728–730
Wiedenfeld H (2011) Plants containing pyrrolizidine alkaloids: toxicity and problems. Food Addit Contamin A 28(3):37–41. https://doi.org/10.1080/19440049.2010.541288
Yang M, Wu Z, Wang Y, Kai G, Singor Njateng GS, Cai S, Cao J, Cheng G (2019) Acute and subacute toxicity evaluation of ethanol extract from aerial parts of *Epigynum auritum* in mice. Food Chem Toxicol 131:110534. https://doi.org/10.1016/j.fct.2019.05.042
Zhao Y, Zhai D, Chen X, Yang J, Song X, He H, Yu Q, Xing Y (2007) Ketoprofen glucuronidation and bile excretion in carbon tetrachloride and alpha-naphthylisothiocyanate induced hepatic injury rats. Toxicology 230(2–3):145–150. https://doi.org/10.1016/j.tox.2006.11.008
Zhou J, Ouedraogo M, Qu F, Duez P (2013) Potential genotoxicity of traditional chinese medicinal plants and phytochemicals: an overview. Phytother Res 27(12):1745–1755

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