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An acute and sub-acute toxicological assessment of *Reissantia indica* plant extract in male Sprague-Dawley rats: Hematological, serum biochemical and histopathology

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ABSTRACT

The traditional use of medicinal plants in Sub-Saharan health management lacks thorough toxicological evaluations, particularly concerning lethal dose levels. This study aims to assess the acute and sub-acute toxicity of *Reissantia indica* whole-plant extract (RIE) in male Sprague-Dawley rats, with a focal point on delineating its safety profile while exploring potential therapeutic applications.

RIE, obtained through precise cold maceration in 70 % ethanol, underwent rigorous analysis, revealing diverse secondary metabolites, including alkaloids, flavonoids, terpenoids, and glycosides. Renowned for antioxidant, anti-inflammatory, and anticancer properties, these compounds enhance RIE's pharmacological potential.

In the acute toxicity study, RIE was orally administered at 500 and 5000 mg/kg. Sub-acute toxicity involved oral administration of the extract at various doses (5, 50 and 500 mg/kg) over 28 days, with comprehensive assessments, including hematological, biochemical, and histopathological evaluations.

Results from the acute toxicity showed no mortality, suggesting a median lethal dose (LD50) exceeding 5000 mg/kg and indicating a substantial margin of safety. Sub-acute toxicity investigations, spanning 28 days revealed no significant changes in body and organ weights, hematological and biochemical parameters, or histopathological signs compared to the control group. Histological examination of kidney, liver, heart, and lung sections from treated animals showed no signs of degeneration.

Abbreviations: : RIE, *Reissantia indica* whole-plant extract; LD50, median lethal dose; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low-density lipoprotein; WBC, white blood cells; LYM, lympohocyte; RBC, red blood cells; HGB, haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCH, mean corpuscular haemaglobin; MCV, mean corpuscular volume; RDW, red cell distribution width; HCT, haematocrit; PLT, platelet; MPV, mean platelet volume; PDW, platelet distribution width; AST, aspartate amino transferase; ALT, alanine transaminase; ALP, alkaline phosphatase; SD, standard deviation; ANOVA, analysis of variance.

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This study, to our knowledge, pioneers a comprehensive investigation into the toxicity profile of *Reissantia indica's* whole-plant ethanolic extract, addressing a significant gap in existing literature on medicinal plant safety in the Sub-Saharan region.

Introduction

The profound tradition of utilizing medicinal plants to address various health concerns in the Sub-Saharan region reflects the deeply ingrained cultural practices within these societies [1]. This reliance on traditional healing practices, often considered a trusted form of therapy, has positioned herbal remedies as indispensable in addressing a spectrum of ailments. The prevalent perception that herbal remedies inherently lack adverse or toxic side effects due to their natural origins distinguishes them from synthetic drugs used in conventional medicine [2].

Reissantia indica, (Willd) N. Hallé, a member of the Celastraceae family, holds substantial medicinal significance, particularly in the western regions of Africa, including Ghana and Nigeria, as well as in central African nations such as Chad, Congo, and Guinea [3]. Boasting heights of up to 12 m, the plant features a distinctive greyish-to-yellowish-greenish stem and thrives in a variety of habitats, including damp or dry forests, woodlands, riverbanks, and occasionally stony ground. Despite its global recognition and extensive ethnomedicinal applications, research on the safety profile of *Reissantia indica's* whole-plant ethanolic extract (RIE) remains limited. The plant's extract, widely used in herbal preparations, especially in Ghana for respiratory ailments and wound healing, has shown promising activities against breast cancer progression [4]. Phytochemical analyses reveal a diverse array of compounds in RIE, including alkaloids, flavonoids, terpenoids, triterpenoids, glycosides, tannins, coumarins, and saponins, contributing to its antioxidant, anti-inflammatory, nephroprotective, and anticancerous activities [5]. Notably, the inclusion of saponins is suggested to play a role in safeguarding the plant against diseases and herbivores [6].

This study employs male Sprague-Dawley rats for the administration of RIE, chosen for their docile nature, ease of handling, and well-characterized physiology, providing a relevant mammalian model to investigate the acute and sub-acute oral toxicities of RIE. While acknowledging the differences between rat and human anatomy and metabolism, the use of male Sprague-Dawley rats allows for valuable insights into potential biological activities and responses to RIE, contributing to our understanding of its safety profile.

To the best of our knowledge, this study represents the first comprehensive investigation into the toxicity profile of *Reissantia indica's* whole-plant ethanolic extract. Despite the extensive documentation of its biological and pharmacological applications in the literature [4,5,7,8], this research aims to address the existing gap and systematically evaluate the safety profile of RIE through contemporary methodologies in acute and sub-acute toxicity assessments.

Materials and methods

Plant collection and extraction

The entire plant specimens for this study were gathered from the Bunso Eco Park situated in the Eastern region of Ghana. After collection, the plant was meticulously identified and authenticated as *Reissantia indica* (KNUST/HM1/2017/L039) at the herbarium of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Ghana. The *Reissantia indica* plant samples underwent a cleaning process by rising with deionized water, were air-dried at 38 °C for 72 h, pulverized, and homogenized (Polymix® PX-MFC 90D, Switzerland). A quantity of 750 g of the homogenized product was precisely weighed and subjected to cold maceration using 2 L of 70 % v/v ethanol in water. The resultant ethanolic extract was processed using a rotary evaporator (R-210, BUCHI, Switzerland) followed by air-drying (for 48 h at 38 °C) and desiccation. This resulting product was preserved as a stock solution, serving as the foundation for all subsequent preparations. The *Reissantia indica* plant extract obtained was finally administered orally, using gavage at the respective doses for the acute and subacute toxicity studies.

Phytochemical analysis

Phytochemical screening of both extract and powder specimens of RIE involved specific tests for various compounds: flavonoids, tannins (ferric chloride test), phenolic compounds (ferric chloride test), saponins (Froth test), sterols, triterpenoids (Salkowski test), alkaloids (Mayer and Dragendorff's test), coumarins (sodium hydroxide test), anthraquinones (Borntrager's test), and cardiac glycosides (Kedde reagent test). These screenings followed established standard procedures [9–14].

Volatile compounds profiling

A Pegasus gas chromatography (GC) system coupled to a high-resolution time-of-flight mass spectrometry (MS) setup (GC—HRTOF-MS) from LECO Corporation, St Joseph, MI, USA, was utilized. The initial column temperature was set at 40 °C for 5 min, followed by a gradual increase at a rate of 2 °C/min to reach 70 °C, then further elevated at 10 °C/min to reach 230 °C within a total run time of 40 min.

Helium gas was employed to carry one microliter of the injected samples at a rate of 1 mL/min (inlet: 250 °C, transfer line: 225 °C). The MS data acquisition followed the recommended rate and system extraction frequency. An air flow rate of 80 mL/min was

maintained throughout the process. Subsequently, data analysis was performed using ChromaTOF® software.

Identification of significant metabolites was carried out by comparing the mass spectra obtained—utilizing mass-to-charge ratios *(m/z)*, retention times (RT), and peak areas—with those of standard compounds, referencing the National Institute of Standards and Technology (NIST).

Experimental animals

Fifty-six (56) male Sprague-Dawley rats aged 6–8 weeks and weighing 150–200 g, were sourced from the Department of Animal Experimentation (DAE) at the Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Accra. Before selection, a thorough examination ensured that only robust and healthy specimens were chosen for the experiments. The rats were group-housed, with nine individuals in each stainless-steel cage measuring 34 cm × 47 cm × 18 cm, and provided with softwood shavings as bedding. They received a standard commercial pellet diet (AGRIMAT, Kumasi), had access to water ad libitum, and were maintained under optimal laboratory conditions. These conditions included a controlled temperature of 22 ± 2 °C, relative humidity ranging between 60 and 70 %, and a 12 h light-dark cycle, ensuring suitable acclimatization. All animal procedures and techniques in this study adhered to the guidelines set by the Noguchi Institute Animal Care and Use Committee (NIACUC) and the National Institute of Health Guidelines for the Care and Use of Laboratory Animals [15]. The Noguchi Institutional Animal Care and Use Committee (NIACUC) approved all experimental procedures (ID: 2018-02-2V.3). Out of the 56 rats used in the entire experiment, 18 were designated for the acute toxicity test, and 38 for the sub-acute toxicity test. In accordance with the guidelines provided by the Organization for Economic Cooperation and Development (OECD) numbers 425 and 407, slight modifications were made to assess the acute and sub-acute oral toxicities of RIE in male Sprague-Dawley rats.

Acute oral toxicity study

In accordance with OECD guideline 425 [16], the Sprague-Dawley rats were randomly allocated into three groups, each comprising five rats. The control groups ($n = 4$/group,) received only 10 mL/kg distilled water while RIE was administered in a single dose of 500 or 5000 mg/kg dissolved in 70 % ethanol via oral gavage to five rats ($n = 10$) that had been fasted overnight. After the singular administration of both the vehicle control and the RIE, the Sprague-Dawley rats were periodically weighed and examined every 15 min over the subsequent 3 h to observe potential clinical signs of toxicity. Monitoring encompassed the observation of clinical toxic symptoms, including alterations in movement, salivation, mydriasis, respiratory patterns, piloerection, stool frequency and consistency, and mortality, spanning a 48 h period, thereby facilitating the determination of the lethal dose (LD50). The rodents were continuously monitored and observed daily for the subsequent 14 days to detect any persisting toxidromes and monitor mortality. At the conclusion of the study period on the 15th day, the rats underwent an overnight fasting period and were humanely euthanized using isoflurane. The rats were anesthetized followed by blood collection by cardiac puncture. The liver, kidneys, heart, brain, spleen, and lungs were excised, weighed, and examined for histopathological defects, except for the brain and spleen.

Sub-acute oral toxicity study

This study adhered to the OECD guideline 407 with minor adaptations [17]. A total of thirty-six animals, weighing between 150 and 200 g, were randomly allocated into four experimental groups, each comprising nine [9] animals. For a duration of 28 days, three groups received daily doses of the plant extract at concentrations of 5, 50, or 500 mg/kg, administered via oral gavage and diluted in distilled water. Concurrently, one group was administered 10 mL/mg of distilled water, serving as the control. Throughout the experiment, observations and daily recordings were conducted for parameters such as body weight, water intake, food consumption, behavioral aspects, and potential signs of toxicity.

Upon completion of the treatment period, animals underwent an overnight fasting period, followed by anesthesia. Blood collection was performed through cardiac puncture for subsequent hematological and biochemical analyses. After euthanasia, target organs were collected for further analysis.

Relative organ weight

On the 29th day, animals were euthanized following an overnight fast. Organs including the liver, lungs, kidneys, brain, heart, and spleen were meticulously dissected and weighed in grams. The relative organ weight for each animal was computed using the formula: Relative organ weight (%) = 100 × absolute organ weight (g)/ body weight (g). This calculation provided a standardized measure to assess the proportional weight of each organ in relation to the body weight.

Blood sampling procedure

Blood samples were collected utilizing the cardiac puncture technique to ensure precision. The collected blood was partitioned into two segments for optimal analysis. One portion was collected in non-heparinized plain bottles, while the other was collected in heparinized bottles. To obtain serum and plasma, the blood samples underwent centrifugation at 3000 g for 10 min, utilizing a bench centrifuge (SBS-LZ-6000HS, Expondo GmbH, Germany). The resulting serum was skillfully transferred into fresh plain sample bottles, setting the stage for subsequent comprehensive hematological and biochemical analyses.

Table 1
Compounds identified in ethanolic extract of *Reissantia indica* extract GC–MS analysis.

S/N	Compound	Chemical formula	Ontology	RT	Area (%)	Molecular Weight
1	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	Cyclic methylsiloxane	6.34	21.1	444
2	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	Cyclic methylsiloxane	8.57	15.08	519
3	2-Tridecanone	$C_{13}H_{26}O$	Methyl ketone	8.99	9.8	198
4	2- Pentadecanone	$C_{15}H_{30}O$	Methyl ketone	9.69	11.2	226
5	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane	$C_{18}H_{52}O_7Si_7$	Fatty acid ester	10.7	14.11	577
6	3-[3-Bromophenyl]-7-chloro-3,4-dihydro-10-hydroxy-1,9 (2H,10H)-acridinedione	$C_{19}H_{13}BrClNO_3$	Acridinone alkaloids	13.5	12.48	418
7	5à-Cholestan-2-one, oxime	$C_{27}H_{47}NO$	oxime	14.5	3.5	401
8	5,14,23-Octadecatrien-14,15-diol	$C_{28}H_{52}O_2$	Fatty alcohol	26.2	12.48	420

Hematological analysis

A systematic approach was adopted for hematological analysis. Prior to analysis, the blood samples collected after the 28-day period underwent gentle homogenization on an automatic bioshaker to ensure uniformity. The samples were processed using the advanced automated Hematological analyzer (KX-2IN, Sysmex Corporation, Japan).

The Hematological analyzer played a pivotal role in conducting a comprehensive examination, encompassing a wide array of parameters crucial for a thorough understanding of blood composition. Evaluated parameters included the red blood cell (RBC) count, shedding light on the quantity of these essential blood components. Furthermore, levels of hemoglobin (HGB), values of hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were scrutinized, offering a nuanced perspective on the cellular characteristics of blood.

The assessment extended to the identification of reticulocytes (Reti), platelet (PLT) count, and white blood cell (WBC) count, providing valuable insights into the body's immune response and overall blood health. The WBC differential count involved a detailed analysis of neutrophils (NEU), lymphocytes (LYM), monocytes (MONO), eosinophils (EOS), and basophils (BASO), presenting a comprehensive profile of the various types of white blood cells. This detailed examination aimed to enhance our understanding of the hematological aspects, contributing to a more comprehensive interpretation of the study's findings.

Biochemical analysis

The serum from the collected blood samples underwent analysis using the Selectra Junior version 04 autoanalyzer (Vital Scientific Bv, Netherlands). This advanced autoanalyzer facilitated a thorough biochemical analysis encompassing renal function (urea, creatinine, potassium, and sodium), lipid profile (total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) cholesterol), and liver function test (total protein, albumin, globulin, direct, indirect and total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) enzyme assays).

Histopathological studies

Kidney, liver, heart, and lung tissues extracted from each treatment group were subjected to histopathological examinations. Following fixation in 10 % formalin, the tissues were dehydrated and expertly mounted in paraffin blocks. Subsequent sectioning at 5–7 μm was followed by routine histopathology using hematoxylin-eosin staining.

Statistical analysis

All statistical analyses were conducted with GraphPad Prism Version 9.0.1 (GraphPad Software, San Diego, CA, USA). The presentation of data adhered to a format of mean ± S.E.M ($n = 5$). Significance for body weight, hematology, clinical chemistry, and organ weight analyses was considered at $P \leq 0.05$.

Results and discussions

Phytochemical screening, GC–MS analysis and pharmacological implications

The initial inquiry of this study aimed to identify the phytochemicals present in RIE. This preliminary examination of the RIE indicated the presence of various essential phytochemicals, including alkaloids, tannins, terpenes, steroids, flavonoids, and coumarins. These constituents are recognized in modern pharmacology and traditional medicine for their role in managing various ailments [18, 19]. Alkaloids, flavonoids, terpenes and steroids have been observed with plant extracts that possess anti-inflammatory features [6,8,

Table 1a
The effects of RIE (5, 50, and 500 mg/kg) on the weights of major organs (g) isolated from rats in a 28-day toxicity study.

Organ weight (g)	Control	5 mg/kg	50 mg/kg	500 mg/kg	*p*-value
Heart	0.96 ± 0.07	0.65 ± 0.07	0.81 ± 0.08	0.79 ± 0.09	0.08
Liver	7.73 ± 0.17	6.43 ± 0.49	7.35 ± 0.49	7.29 ± 0.29	0.15
Kidney 1	0.81 ± 0.06	0.61 ± 0.04	0.73 ± 0.04	0.68 ± 0.04	0.09
Kidney 2	0.81 ± 0.02	0.60 ± 0.03	0.70 ± 0.5	0.70 ± 0.03	0.06
Lung	1.78 ± 0.09	1.26 ± 0.12	0.96 ± 0.21	1.29 ± 0.12	0.06
Spleen	0.78 ± 0.03	0.73 ± 0.09	0.64 ± 0.11	0.70 ± 0.10	0.71
Brain	1.61 ± 0.09	1.45 ± 0.08	1.5 ± 0.08	1.63 ± 0.05	0.3

Data are mean ± S.E.M. ($n = 5$). There was no significant difference whereas $p \leq 0.05$ was considered significant when compared to the control group (One-way ANOVA).

20]. This means they may act independently or in synergy to elicit the observed biological activities.

The relevance of alkaloids, flavonoids, and glycosides in pharmaceuticals is noteworthy, considering their presence in drugs used for malaria, diabetes, cancer, and cardiac dysfunction treatments [21,22]. Terpenes and tannins, known for their anticancer, antimicrobial, antifungal, antiviral, and anti-inflammatory properties, are crucial to drug manufacturing [23,24]. Sterols have demonstrated anticancer properties *in vivo* [25], while coumarins exhibit anticoagulant characteristics as a phenolic compound [26]. The presence of these phytochemicals in RIE suggests their involvement in its anti-inflammatory and anti-cancer properties.

Gas Chromatography analysis revealed the dominance of Cyclohexasiloxane, dodecamethyl- (D6) in RIE, comprising 21.1 % of the total content (Table 1). A recent study has associated D6 with enhanced antioxidant responses [27], indicating its potential role in preventing free radical damage linked to cancer development [28]. This suggest that the detected secondary metabolites observed in RIE may contribute to their antimicrobial properties [28,29]. The observed variation in chemical composition might be attributed to environmental factors like topography [30].

Acute toxicity evaluation

The assessment of the acute toxic impact of the aqueous extract followed the guidelines outlined in OECD 425 [16], utilizing a limit test dose of 5000 mg/kg. Following oral administration of the RIE doses of 500 and 5000 mg/kg, no observable treatment-related toxic symptoms or mortality occurred. A thorough monitoring of changes in movement, salivation, mydriasis, respiratory pattern, piloerection, frequency and consistency of stool, temperature, and mortality revealed no discernible changes in both the extract-treated animals and the control group. This comprehensive observation spanned a short period initially 3 h, extended over 24 h and continued on a daily basis. The findings indicated the safety of the extract at doses of 500 and 5000 mg/kg, with the median lethal dose (LD50) exceeding 5000 mg/kg emphasizing the lack of apparent adverse effects.

The selection of doses, 500 and 5000 mg/kg, in our study was based on considerations of potential human exposure and traditional use, aiming to align with real-world scenarios. These doses were chosen to cover a range that reflects both typical exposure levels and higher concentrations that might be encountered in specific situations. The lower dose of 500 mg/kg, adhering to the OECD guidelines, enables the identification of toxicity signs without severe effects, crucial for safety assessments; conversely, the higher dose of 5000 mg/kg, assesses toxic effects under significantly elevated exposure, relevant for evaluating risks in high-dose exposures in various scenarios.

RIE is commonly used as traditional medicine traditionally used for managing a respiratory distress, wounds and inflammation [5,8]. The use of various plant extracts in the regions of Ghana occurs without meticulous consideration of dosage or concentration, potentially leading to toxic side effects [31]. Oral intake of such extracts may be detrimental to vital organs few days after administration. Following the acute toxicity study for RIE, this study showed no mortality in Sprague Dawley rats used in the experiment. A single oral administration of RIE (500 and 5000 mg/kg) revealed no behavioral or physiological impairment in the study rats. Thus, there were no significant alterations in respiratory pattern among rats administered a single dose of RIE (500 or 5000 mg/kg) compared to the control group. Further scrutiny of internal organs after the 12-day experimental period revealed no discernible changes when juxtaposed with the control group (data not shown). The absence of animal fatalities led to the conclusion that the LD50 of RIE among male Sprague Dawley rats must exceed 5000 mg/kg, categorizing the tested substance as of low toxicity, falling into Category 5 of the OECD guideline 425 [16]. In line with similar studies, our findings indicate that many plant extracts possessing anti-inflammatory properties, commonly used for ailment management, demonstrate non-toxicity, particularly under acute toxicity assessments [32,33].

Sub-acute toxicity evaluation

All administered doses of RIE (5, 50, 500 mg/kg) throughout the sub-acute study exhibited no alterations or abnormal reactions in salivation, movement, mydriasis, piloerection, frequency, and consistency of stool in rats. There were no observable changes in respiratory patterns relative to the control group following the oral administration of each respective dose. These findings highlight the plant extract's well-tolerated nature, affirming its extensive application in herbal medicine.

Conducting the sub-acute toxicity assessment in accordance with OECD guideline 407 [17], the administered doses (5, 50, 500 mg/kg) ensured the survival of animals over the entire 28-day period (Table 1a). Upon evaluating the internal organs of the rats after

Table 2
Assessment of RIE on some serum biochemical parameters in sub-acute toxicity study.

Parameter	Control	RIE 5 mg/kg	RIE 50 mg/kg	RIE 500 mg/kg	*p*-value
Total protein (gL^{-1})	6.9 ± 0.20	7.20 ± 0.41	7.32 ± 0.52	7.36 ± 0.52	0.87
Albumin (gL^{-1})	3.51 ± 0.14	3.49 ± 0.19	3.87 ± 0.31	4.15 ± 0.19	0.13
Globulins (gL^{-1})	3.39 ± 0.21	3.87 ± 0.22	3.45 ± 0.35	3.21 ± 0.47	0.56
Total bilirubin ($\mu molL^{-1}$)	2.21 ± 0.63	2.92 ± 0.45	2.76 ± 0.17	3.67 ± 1.43	0.66
Direct bilirubin ($\mu molL^{-1)}$	0.86 ± 0.14	0.67 ± 0.11	0.83 ± 0.13	2.15 ± 0.99	0.19
Indirect bilirubin (μmolL-1)	1.35 ± 0.58	2.25 ± 0.43	2.14 ± 0.28	2.32 ± 0.64	0.51
AST (UL^{-1})	326.36 ± 74.63	456.26 ± 100.5	429.66 ± 92.10	380.62 ± 102.7	0.77
ALT (UL^{-1})	95.06 ± 14.68	122.16 ± 24.82	126.88 ± 21.78	112.48 ± 18.04	0.7
ALP (UL^{-1})	601.94 ± 83.70	714.4 ± 169.6	515.56 ± 43.48	569.32 ± 20.05	0.54

AST – Aspartate amino transferase, ALT – Alanine transaminase, ALP – Alkaline phosphatase. Data are mean ± S.E.M. ($n = 5$). $p \leq 0.05$ was considered significant when compared to the control group (One-way ANOVA).

Table 3
The lipid profile of Sprague-Dawley rats after 28-day oral administration of RIE (5, 50, and 500 mg/kg).

Parameters	Vehicle	5 mg/kg	50 mg/kg	500 mg/kg	*p*-value
Total cholesterol	1.89 ± 0.17	1.97 ± 0.36	2.46 ± 0.30	2.43 ± 0.19	0.32
Triglycerides	1.19 ± 0.31	1.97 ± 0.36	2.46 ± 0.30	2.43 ± 0.19	0.03*
HDL	0.49 ± 0.07	0.83 ± 0.11	0.71 ± 0.04	0.87 ± 0.09	0.02*
LDL	0.84 ± 0.07	1.3 ± 0.37	0.98 ± 0.28	0.9 ± 0.17	0.58
VLDL	0.54 ± 0.14	0.44 ± 0.06	0.73 ± 0.07	0.66 ± 0.10	0.21

RIE – *Reissantia indica* extract; SD – Standard deviation; ANOVA – Analysis of variance; HDL – High density lipoprotein; LDL – Low density lipoprotein; VLDL – Very low-density lipoprotein. Data are mean±SEM ($n = 5$). $P \leq 0.05$*; compared to the vehicle group (one-way ANOVA followed by a Dunnett's multiple comparison test.

this duration, no significant changes were observed when compared with the control group. Changes in organ weight could be indicative of physiological responses or potential toxicity, and they are often examined as part of toxicity studies to assess the impact of substances on organ health. This consistency in results between the acute and sub-acute stages suggests that RIE maintains a favorable safety profile for at least 28 days. No signs of toxicity were observed in the group treated with the extract, showcasing a comparable outcome to the control group and reinforcing the safety of RIE under the conditions tested.

Biochemical marker assessment

A detailed examination of biochemical markers, especially those linked to liver function, indicated no significant alterations upon RIE administration. Parameters including total protein, albumin, globulin, direct and indirect bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) remained unchanged. The non-alteration of these markers suggests the safety of RIE within the studied dosage range recording no significant levels (Table 2).

Albumin, primarily synthesized in the liver, plays a crucial role as a transporter for medicines and other substances within the body, while also preventing blood leakage in blood vessels. Fluctuations in serum albumin levels can denote varying health conditions—higher levels are associated with dehydration, while lower levels could indicate severe liver diseases, intestinal malabsorption syndromes, nephrotic syndrome, and protein-calorie malnutrition [34].

The consistency of albumin levels, along with the unchanged globulins and bilirubin parameters following RIE administration, is indicative of the extract's lack of influence on these key markers [34,35]. The unaltered indirect bilirubin levels, which denotes the phase where bilirubin attaches to albumin for transportation to the liver, also align with the extract's negligible impact on liver health parameters [36].

Elevated levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are commonly used markers in assessing liver diseases. The absence of significant changes in these markers further supports the safety dosage of RIE, underscoring its lack of adverse influence on liver functionality.

Additionally, a lipid profile examination was conducted to assess various lipid abnormalities such as total cholesterol, triglycerides, low and high-density lipoproteins. In particular, the study observed that HDL (high-density lipoprotein) and triglycerides were positively regulated following RIE administration, and this effect was statistically significant (Table 3).

This observation is primarily beneficial as an increase in HDL levels can be associated with a reduced risk of cardiovascular diseases and is attributed to its role in mitigating fatty acid buildup. The RIE's positive effect on HDL levels suggests a potential in curbing lipid-related abnormalities.

Several factors may contribute to the increase in triglyceride levels in response to RIE. The extract may contain constituents influencing lipid metabolism, with certain plant compounds known to impact triglyceride synthesis and breakdown [37]. Herbal extracts, including RIE, could induce inflammatory responses, thereby influencing lipid metabolism. Such interactions are well-documented in the literature, as inflammation is closely associated with alterations in lipid profiles [38,39]. Future research

Table 4
Effects of *Reissantia indica* extract on the kidneys of Sprague-Dawley rats.

Parameters	Vehicle	5 mg/kg	50 mg/kg	500 mg/kg	*p*-value
Urea	7.03 ± 0.55	5.474 ± 0.43	6.26 ± 0.47	6.392 ± 0.44	1.81
Creatinine	44.73 ± 2.08	30.40 ± 1.77	35.79 ± 3.23	47.79 ± 0.03	0.07
Potassium	7.318 ± 0.25	6.83 ± 0.35	6.452 ± 0.13	6.78 ± 0.15	0.12
Sodium	137.5 ± 0.69	136.0 ± 0.50	137.5 ± 0.67	137.0 ± 1.33	0.55
Chloride	100.6 ± 0.12	101.5 ± 0.55	100.5 ± 0.28	100.7 ± 0.31	0.22

RIE – *Reissantia indica* extract. Data are mean±SEM ($n = 5$). $P \leq 0.05$*; compared to the vehicle group (one-way ANOVA followed by a Dunnett's multiple comparison test.

Table 5
Effects of the aqueous extract of *Reissantia indica* extract on hematological parameters.

Parameter	Vehicle	5 mg/kg	50 mg/kg	500 mg/kg	*p*-value
WBC (10^3 μL^{-1})	3.90 ± 0.90	6.236 ± 1.22	5.89 ± 1.07	5.8 ± 0.89	0.40
LYM (%)	69.79 ± 8.47	82.24 ± 3.26	73.416 ± 16.10	90.66 ± 0.54	0.41
RBC (10^3 μL^{-1})	8.77 ± 0.47	8.40 ± 0.31	8.88 ± 0.39	8.07 ± 0.22	0.35
HGB (g dL^{-1})	14.64 ± 0.99	12.02 ± 0.47	13.48 ± 0.96	13.4 ± 0.11	0.13
MCHC (gdL^{-1})	34.84 ± 2.01	38.82 ± 1.81	38.94 ± 1.47	39.64 ± 0.33	0.16
MCH(pg)	17.36 ± 0.4	16.60 ± 0.53	17.62 ± 0.60	17.18 ± 0.22	0.47
MCV (fl)	52.16 ± 0.94	52.9 ± 0.77	53.04 ± 0.95	53.58 ± 0.95	0.83
RDW_SD (fl)	13.04 ± 0.40	13.44 ± 0.16	13.52 ± 0.12	13.8 ± 0.13	0.19
RDW_CV (%)	30.25 ± 0.25	32.32 ± 0.60	33.92 ± 0.59	33.32 ± 1.06	0.32
HCT (%)	51.08 ± 1.56	50.06 ± 0.56	51.32 ± 0.56	51.16 ± 0.0.64	0.77
PLT (10^3 μL^{-1})	1012 ± 131.7	1301.4 ± 240.1	1032 ± 116.8	1087.4 ± 24.41	0.52
MPV (fl)	6.46 ± 0.12	6.76 ± 0.09	6.72 ± 0.18	6.72 ± 0.11	0.36
PDW (fl)	11.98 ± 2.42	11.3 ± 0.32	12.8 ± 0.54	11.42 ± 0.05	0.06

RIE – *Reissantia indica* extract; WBC – White blood cells; LYM – Lympohocyte; RBC – Red blood cells; HGB – Haemoglobin; MCHC – Mean corpuscular haemoglobin concentration; MCH – Mean corpuscular haemaglobin; MCV – Mean corpuscular volume; RDW – Red cell distribution width; HCT – Haematocrit; PLT – Platelet; MPV – Mean platelet volume; PDW – Platelet distribution width; Data are mean±SEM ($n = 5$). $P \leq 0.05$*; compared to the vehicle group (one-way ANOVA followed by a Dunnett's multiple comparison test.

should identify specific RIE compounds responsible for lipid effects and explore temporal dynamics

Renal function and hematological parameters

In investigating the health and performance of the kidneys after RIE administration, the renal function test focused on substances including urea, creatinine, and electrolytes (potassium, sodium, and chloride). The results indicated no significant deviation from normal levels for all the measured parameters (Table 4).

Serum creatinine, known to be a more precise indicator of renal function than urea, is instrumental in determining potential kidney dysfunction [40]. Elevated serum creatinine levels or inefficient clearance can signify irregular glomerular filtration rates, which could potentially lead to acute kidney injury. Furthermore, urea, a by-product released by the liver, increases in conditions where renal clearance decreases, indicating acute or chronic renal failure [41]. The evaluation of electrolytes, which includes the measurement of Na^+, K^+, Cl^-, levels, helps assess the overall activity and function of the kidneys since changes in the concentrations of these electrolytes may indicate renal injury [42]. The absence of significant changes observed in the aforementioned indices implies that the administration of RIE did not exhibit any nephrotoxicity or influence the function of the kidneys. This further support the safety of RIE within the dosage levels (Table 4).

Hematological parameters measured the effects of the extract on the blood and blood-forming tissues. This study recorded no significant difference between the vehicle-treated group and the RIE (5, 50 and 500 mg/kg) with regards to measurements of various blood cells including white blood cells, lymphocytes, and platelets (Table 5).

Assessing hematological parameters is crucial for determining an individual's health status, offering valuable insights into various physiological processes and potential impacts on blood-related aspects [43]. These parameters serve as essential indicators, shedding light on the overall well-being and potential effects of herbal remedies on the circulatory system. This study did not record any noticeable change in hematological parameters providing evidence that the plant extract is nontoxic.

Histopathological evaluation

Histological studies serve as crucial benchmarks for identifying and characterizing pathological changes occurring in tissues and organs [44].The administration of RIE to the rat did not negatively affect the key vital organs of the organism namely kidney, liver, heart and the lung. Observations under this study reveal no glomerular and renal tubule abnormalities in the kidney after RIE (5, 50, and 500 mg/kg) administration. The presence of distinctive glomeruli and renal tubules were unaltered (Fig. 1). The various

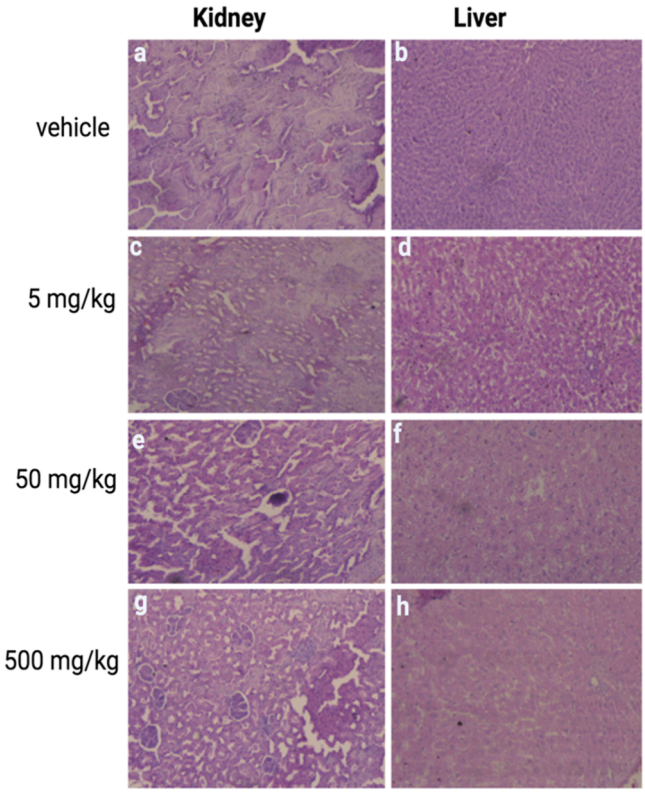

Fig. 1. Histological microphotograph of kidney and liver of male rats after 28-day exposure to *Reissantia indica* whole plant ethanolic extract (RIE). The figure shows histopathological examinations (a), kidney (b) and liver in control group; (c) kidney (f) liver in 5 mg/kg RIE group; (e) kidney (f) liver in 50 mg/kg RIE group and (g) kidney (h) liver in 500 mg/kg RIE group. Sections were hematoxylin-eosin stained (× 10).

administration of RIE administered to the liver and heart showed no disparity in normal morphological architecture hence no abnormalities were observed. The distributed hepatocyte and nuclei depicts a healthy liver with no associated harm. The liver of the organisms presented a normal sinusoid with no pathological findings in liver parenchyma. This suggest that, RIE in not capable to alter liver function at least 28-days of continuous administration. The observations here were further extended to the heart and as indicated no pathological change in myocytes in all cases was observed, the different dose administered notwithstanding. The effect of RIE on the lungs showed no abnormality in the alveolar epithelial cells indicating a sign of healthy lungs. This suggests no organ abnormalities regarding the *Reissantia indica* exposal hence having a safety profile. This is consistent with similar findings that observed no toxicity effect of plant extract mostly administered within Sub-Saharan to manage various ailments, suggestive of the fact that most of the plant extract used in traditional medicine against inflammation have little or no toxicity effects affecting the vital organs of animals at least in sub-acute toxicity studies. [20,45].

Our findings, demonstrating no significant adverse effects on vital organs, hematological, and biochemical indices in male Sprague-Dawley rats administered with *Reissantia indica* extract (RIE), provide substantial support for the safe traditional use of this plant. The lack of observed toxicity aligns with the longstanding ethnomedicinal applications of *Reissantia indica,* particularly in addressing respiratory distress, wounds, and inflammation. The agreement between traditional use and our experimental results reinforces the credibility of *Reissantia indica* as a herbal remedy in local healthcare practices. The favorable safety profile revealed in our toxicity studies opens new avenues for drug development. The presence of various secondary metabolites, including those recognized for their anticancer and antimicrobial activities, positions *Reissantia indica* as a promising candidate for pharmaceutical exploration. The absence of adverse effects on vital organs, coupled with the plant's ethnomedicinal history, suggests that the development of drugs derived from *Reissantia indica* may offer therapeutic solutions with reduced safety concerns. our study not only corroborates the safe traditional use of *Reissantia indica* but also highlights its potential in drug development. These findings contribute to the growing body of evidence supporting the integration of traditional herbal knowledge with contemporary scientific approaches for the development of safe and effective therapeutic interventions (Fig. 2)

While this study investigation presents the first ever reported acute and subacute toxicity of RIE, extending the studies conducted in the past [5,7,8], it's crucial to acknowledge certain limitations. First, the study primarily focused on male Sprague-Dawley rats, and gender-based differences in toxicity responses were not explored. The extrapolation of our findings to humans requires cautious consideration due to interspecies variations in metabolism and physiological responses.

Moreover, the study duration was limited to 28 days, providing insights into sub-acute toxicity. Longer-term studies, covering

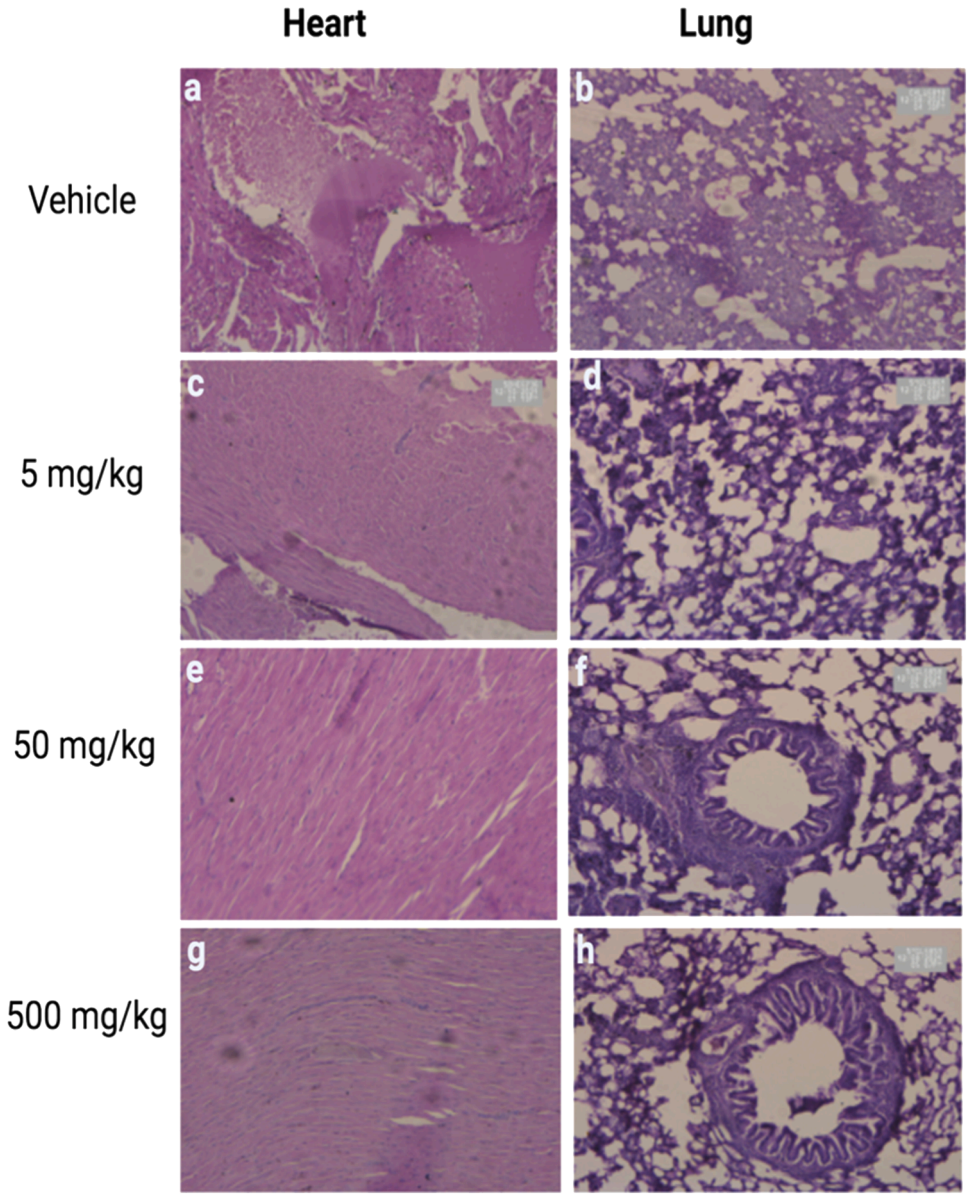

Fig. 2. Histological microphotograph of heart and lung of male rats after 28-day exposure to *Reissantia indica* whole plant ethanolic extract (RIE). The figure shows histopathological examinations (a), heart (b) and lung in control group; (c) heart (d) lung in 5 mg/kg RIE group; (e) heart (f) lung in 50 mg/kg RIE group and (g) heart (h) lung in 500 mg/kg RIE group. Sections were hematoxylin-eosin stained (× 10).

subchronic and chronic toxicity, are warranted for a more comprehensive safety evaluation. Our research did not encompass specific genotoxicity assessments, developmental toxicity, carcinogenicity, neurotoxicity, reproductive toxicity, among other aspects which could be considered in future studies. The study's generalization is also influenced by the specific conditions of our experimental setup, and caution is advised when applying the findings to diverse populations or varied environmental contexts.

Addressing these limitations in future research will contribute to a more nuanced understanding of the safety profile and potential applications of RIE.

Conclusion

In both acute and subacute toxicity studies, male Sprague-Dawley rats administered with *Reissantia indica* plant extract (RIE) exhibited no significant adverse effects on vital organs, hematological parameters, or biochemical indices. The LD50 estimation for the ethanolic extract surpassed 5000 mg/kg, indicating a substantial margin of safety and suggesting potential efficacy. The presence of various secondary metabolites in RIE, known for their anticancer and antimicrobial activities, did not elicit any observable impacts on

the kidneys, liver, heart, and lungs. This finding augurs well for the potential health benefits of RIE. To further enhance our understanding of its safety profile, future investigations should delve into subchronic and chronic toxicities, including studies on pregnant rats and fetuses, providing comprehensive insights into the applicability of this plant, particularly in managing respiratory distress and other health applications.

Ethical approval

The animal study was approved by Noguchi Institutional Animal Care and Use Committee (NIACUC) after application through application ID: 2018-02-2V.

Data availability

All data presented in this article are available with the authors

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CRediT authorship contribution statement

Emmanuel Owusu Amoateng: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Patrick Amoateng:** Conceptualization, Investigation, Validation, Formal analysis, Writing – review & editing, Supervision. **Paul Poku Sampene Ossei:** Methodology, Investigation. **Eric Asare Fenteng:** Methodology, Investigation, Writing – review & editing. **Isaac Kingsley Amponsah:** Methodology, Supervision, Investigation, Validation. **William Gilbert Ayibor:** Methodology, Writing – review & editing. **Samuel Adjei:** Methodology, Investigation, Validation, Supervision. **Tracy Narh-Bedu:** Methodology, Investigation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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