

Research Article



Phytochemical Characterisation and Acute Oral Toxicity Evaluation of the Ethanolic Extract of *Swertia chirayita* In Female Wistar Rats

¹Shireen Shaikh*, ²Dr. Shailesh Tawade

¹Research Scholar, ²Assistant Professor

Department of Zoology, Patkar- Varde College, Goregaon (W), Mumbai-400102, University of Mumbai, India.

*Corresponding author's E-mail: shaikhshireen644@gmail.com

Received: 03-06-2026; Revised: 22-08-2026; Accepted: 27-08-2026; Published online: 20-09-2026.

ABSTRACT

Objective: To evaluate the acute oral toxicity of the ethanolic extract of *Swertia chirayita* in female albino Wistar rats.

Methods: Acute oral toxicity was assessed according to OECD Guideline 423. Female albino Wistar rats received the ethanolic extract orally at doses of 300 mg/kg and 2000 mg/kg body weight, while the control group received distilled water. Animals were observed for 14 days for mortality, clinical signs of toxicity, behavioural changes, and body weight variations. Gross necropsy was performed to examine pathological changes in vital organs.

Results: No mortality, clinical signs of toxicity, or behavioural abnormalities were observed in any treatment group throughout the study. Body weight increased normally and remained comparable to the control group. Gross examination of the brain, heart, liver, kidneys, spleen, and lungs revealed no treatment-related pathological changes. The oral LD₅₀ of the extract was estimated to be greater than 2000 mg/kg body weight according to OECD Guideline 423.

Conclusion: The ethanolic extract of *Swertia chirayita* exhibited a low order of acute toxicity and demonstrated a favourable acute safety profile following oral administration. These findings support its traditional medicinal use; however, further repeated-dose and chronic toxicity studies are warranted to establish its long-term safety.

Keywords: *Swertia chirayita*, Acute oral toxicity, Ethanolic extract, OECD 423, Wistar rats.

INTRODUCTION

Plant-based medicine is the oldest known form of healthcare and continues to serve as the primary treatment for up to 80% of the global population, particularly in developing countries ^{1,2}. In many of these regions, herbal remedies are commonly used as alternatives to modern pharmaceuticals, often regarded as safer due to their natural origin and long history of use. Reflecting this, the World Health Organisation³ has advocated for the integration of traditional medicine into national healthcare systems.

Despite their widespread use, herbal medicines contain bioactive compounds that can exert both therapeutic and toxic effects. Several reports have highlighted adverse reactions caused by long-term or improper use of herbal products ⁴. Thus, evaluating the safety of medicinal plants is essential to ensure their efficacy and minimise health risks.

Toxicology, a vital field within pharmacology, focuses on identifying and understanding the harmful effects of natural or synthetic substances ⁵. The toxic effects of phytochemicals can vary depending on the chemical composition and their interaction with biological membranes, tissues, or extracellular matrices ⁶. Acute toxicity studies, which estimate the median lethal dose (LD₅₀) following a single oral administration and observation over 14 days, are often the first step in evaluating plant safety in animal models ⁷.

Among the numerous medicinal plants in use, *Swertia chirayita* holds a notable place in Indian traditional medicine. Initially classified as *Gentiana chirayita* by Roxburgh in 1814, it is indigenous to the temperate Himalayan regions, found at altitudes between 1200 and 3000 meters, from Kashmir to Bhutan, and in the Khasi Hills ⁸. It is cultivated in sub-temperate climates ranging from 1500 to 2100 meters and is widely known by its vernacular names—chirata, chirayata, and kirata-tikta. This herb has been referenced in the *Charaka Samhita*, a foundational text of ancient Indian medicine, for its multifaceted therapeutic applications and is extensively used in Ayurvedic, Unani, and Siddha systems.

Swertia chirayita is a seasonal herbaceous plant (annual, biennial, or perennial) belonging to the Gentianaceae family, typically growing between 60 and 150 cm ⁹. It is rich in diverse bioactive constituents such as xanthenes (e.g., mangiferin, bellidifolin, swertianin), seco-iridoid glycosides (e.g., amarogentin, amaroswerin, sweroside, gentiopicroside), terpenoids, flavonoids, alkaloids, and lignans. The presence of chiratin and ophelic acid is responsible for its characteristic bitterness ¹⁰.

Traditionally, this plant has been employed as an anthelmintic, antidiarrheal, blood purifier, digestive aid, carminative, expectorant, febrifuge, antiperiodic, and laxative ¹¹. Its pharmacological efficacy also extends to treating flatulence, skin ailments, malaria, gout, and bronchial asthma. Additionally, it demonstrates antimalarial, antidiabetic, hepatoprotective, anti-



inflammatory, antioxidant, anticancer, antimicrobial, and central nervous system (CNS) stimulant activities¹². Although the entire plant possesses medicinal value, the roots are especially potent, with the leaves and stems also showing significant biological activity in several studies¹³.

Swertia chirayita has shown promising pharmacological properties; however, its safety profile remains inadequately explored. Given its widespread traditional use, it is essential to conduct standardised toxicological assessments to ensure its safe use in therapeutic applications¹⁴.

This study highlights the importance of evaluating the acute oral toxicity of the ethanolic extract of *Swertia chirayita* in albino Wistar rats following OECD guidelines 423¹⁵. The findings of this study will contribute to the safety assessment of this medicinal plant and provide scientific evidence supporting its traditional use.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

The whole dried plant of *Swertia chirayita* was procured from Anant Ayurveda Bhandara, Goregaon, Mumbai, India. The plant was identified and authenticated by the botanist Dr. Kamlakar H. Patil, Department of Botany, Shivaji University. A voucher specimen number (SSAK 001) was prepared and deposited in the herbarium of the department.

Preparation of Ethanolic Extract

The dried plant material was powdered and stored. 95% ethanol was used to macerate the weighed powder, and the mixture was agitated in a shaker for 24 hours to facilitate the extraction of phytoconstituents. The extract was subsequently filtered using Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator at controlled temperature and pressure to afford crude ethanolic extract. The concentrate was further evaporated and dried by a hot water bath at 75°C. Lastly, the final extract was stored in the refrigerator and was ready for the acute toxicity test.

Preliminary Phytochemical Screening

The ethanolic extract was screened for the presence of major phytochemical constituents, including flavonoids, tannins, phenolic compounds, terpenoids, saponins, and glycosides, using standard qualitative tests based on characteristic colour changes and precipitation reactions. All analyses were performed in triplicate. The screening methods were adopted from previously published procedures with minor modifications.

Quantification of Total Flavonoid and Phenolic Content

Total flavonoid content (TFC) and total phenolic content (TPC) were estimated using the aluminium chloride colourimetric assay and the Folin–Ciocalteu method, respectively, following established protocols. The results were expressed as mg quercetin equivalents (QE)/g extract and mg gallic acid equivalents (GAE)/g extract, respectively.

Experimental animals

An acute oral toxicity test was performed to determine the LD50 of the ethanolic extract of *Swertia chirayita*. Experiments were carried out using healthy female albino Wistar rats aged 8-12 weeks and weighing 230-300 g. The animals were housed under standard laboratory conditions. The study was conducted at SCITESLA Private Limited in Mumbai, Maharashtra, India, in accordance with OECD guidelines 423. The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC; Approval No. SCI/IAEC/2024-25/167) and conducted in compliance with CPCSEA guidelines¹⁶.

Experimental Design

All the animals were weighed, and their health was verified. The animals selected for the study were nulliparous and non-pregnant. They were randomly divided into three groups, each comprising four female Rats. Animals were allowed an acclimatisation period of 7 days to the laboratory conditions before initiation of the study. Each animal was assigned to a cage and was numbered for identification. All groups of rats fasted overnight before administration. Following the fasting period, all animals were weighed, and the doses were calculated based on their body weight. The ethanolic extract of the plant was then administered orally at the doses of 300 mg/kg (group I) and 2000 mg/kg (group II) body weight of rats in the test groups. Animals in the control group (group III) received distilled water. All treatments were administered orally using an appropriate gavage technique.

Housing and Feeding Conditions

The animals were housed in polypropylene cages with bedding of autoclaved corn cob. The animal room was maintained at a temperature of 20 ± 3°C with relative humidity ranging from 50% to 70%. A 12-hour light and 12-hour dark cycle was maintained throughout the study period. The animals were fed a standard laboratory pellet diet, and drinking water was provided ad libitum except during the fasting period before dosing.

Clinical Observation

Following administration of the test substance, the animals underwent 30-minute observations, which continued regularly during the initial 24-hour period, with a specific emphasis on the 6-hour mark, and were subsequently conducted daily for a total of 14 days. Clinical evaluations involved assessments of behaviour alteration, posture, locomotor activity, skin and fur condition, respiratory pattern, autonomic responses, mucous membranes, and neurological functions. Particular attention was paid to the occurrence of tremors, convulsions, salivation, diarrhoea, lethargy, sleep disturbances, coma, or any other signs suggestive of toxicity.

Body Weight Assessment

Individual body weights of animals were recorded shortly before the test substance was administered and at least



weekly thereafter. Weight changes were calculated and recorded. At the end of the test, surviving animals were weighed for general health status and potential treatment-related toxicity. The body weight of each animal was recorded on day 0, day 7 and day 14. Mean body weights for each group were calculated and compared.

Mortality Assessment

Animals were observed for toxic reactions and mortality throughout the 14 days. The time and occurrence of any observed deaths would have been recorded and considered during toxicity classification.

Gross Necropsy Examination

After the observation period, surviving animals were humanely euthanised and subjected to gross necropsy. Each animal's gross pathological alterations were noted. Vital organs, including the brain, heart, liver, kidneys, spleen, and lungs, were carefully examined for any visible abnormalities such as changes in size, colour, texture, haemorrhage, congestion, or other pathological alterations.

Statistical Analysis

Body weight data are expressed as mean $\pm$ SEM ($n = 4$). Percentage body weight changes relative to day 0 were calculated for each animal to account for baseline variation between groups. Group means (absolute body weight and percentage change) were compared using one-way ANOVA followed by Tukey's post hoc test, with $p < 0.05$ considered statistically significant. All statistical analyses were performed using GraphPad Prism software.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

Qualitative phytochemical analysis of the ethanolic extract demonstrated the presence of flavonoids, phenolic compounds, tannins, saponins, and terpenoids, whereas cardiac glycosides were not detected (Table 1).

Quantitative Phytochemical Analysis

Quantitative analysis showed that the ethanolic extract contained 144.60 ± 5.20 mg QE/g extract of total flavonoids and 90.40 ± 4.35 mg GAE/g extract of total phenolic compounds (Table 2).

Effect of Ethanolic Extract of *Swertia chirayita* on Body Weight

The body weight was measured on days 0, 7, and 14. One-way ANOVA revealed a statistically significant difference in absolute body weight between groups at each time point (Day 0: $F(2,9) = 25.61$, $p < 0.001$; Day 7: $F(2,9) = 16.08$, $p = 0.001$; Day 14: $F(2,9) = 8.88$, $p = 0.007$); however, this difference was already present at day 0, before any dosing, and most likely reflects unequal distribution of animals at randomisation rather than a treatment-related effect. When expressed as percentage body weight gain from day 0 to day 14, all groups showed comparable, progressive growth (Control: 6.4%; 300 mg/kg: 6.2%; 2000 mg/kg: 9.6%; $p > 0.05$), indicating that administration of the extract did not adversely affect normal growth or physiological development during the 14-day observation period (Table 3).

Table 1: Preliminary phytochemical screening of the ethanolic extract of *Swertia chirayita*

Phytochemical constituent	Test employed	Observation	Status
Flavonoids	Alkaline reagent test	Yellow coloration	Present (+)
Phenolic compounds	Ferric chloride test	Bluish-black coloration	Present (+)
Tannins	Ferric chloride test	Greenish-black colouration	Present (+)
Saponins	Froth test	Persistent froth formation	Present (+)
Terpenoids	Salkowski test	Reddish-brown interface	Present (+)
Cardiac glycosides	Keller–Killiani test	No brown ring observed	Absent (–)

(+) indicates presence; (–) indicates absence. Qualitative screening was performed using standard phytochemical methods, each in triplicate.

Table 2: Quantitative estimation of total flavonoid and phenolic content in the ethanolic extract of *Swertia chirayita*

Parameter	Content (Mean $\pm$ SD)
Total flavonoid content (mg QE/g extract)	144.60 ± 5.20
Total phenolic content (mg GAE/g extract)	90.40 ± 4.35

Values are expressed as Mean $\pm$ SD ($n = 3$), QE- quercetin equivalent; GAE, gallic acid equivalent.

Table 3: Effect of *Swertia chirayita* Ethanolic Extract on the Body Weight of Rats During the 14-Day Acute Oral Toxicity Study

Group	Body Weight		
	Initial day	7 th day	14 th day
Control	261.75 ± 2.06	269.75 ± 2.06	278.5 ± 1.44
I	262 ± 1.47	270.5 ± 2.40	278.25 ± 2.29
II	243 ± 2.74	253.25 ± 2.78	266.25 ± 3.04

Group I-II ethanolic extracts with doses (I: 300 mg/kg; II: 2000 mg/kg). Values are expressed as mean $\pm$ SEM ($n = 4$). No treatment-related differences in body weight gain were observed during the study period.



Clinical observations and Mortality

All groups of animals showed neither any toxic effect nor any lethal effect and were free of any intoxicating signs throughout the observation period of 14 days, and thus were found to exhibit no toxic or clinical reactions. The present study shows that administration of ethanolic extract of *Swertia chirayita* at doses of 300mg/kg and 2000 mg/kg did not reveal any signs of toxicity or mortality in rats during the entire observation period. Animals showed normal behaviour, posture, locomotor activity, feeding habits, and grooming behaviour during the observation period.

Furthermore, no signs of tremors, convulsions, salivation, diarrhoea, lethargy, respiratory distress, sleep disturbances, coma, or other behavioural abnormalities were observed in any of the treated animals. No mortality occurred during the 14-day observation period (Table 4).

The absence of toxic manifestations following administration of the ethanolic extract of *Swertia chirayita* indicates that it was well tolerated at both tested doses. Therefore, the LD50 of the extract may be considered to be greater than 2000 mg/kg.

Table 4: Clinical Observations Following Oral Administration of Ethanolic Extract of *Swertia chirayita*

Observation Parameter	Control	300 mg/kg	2000 mg/kg
Mortality	None	None	None
Tremors	None	None	None
Convulsions	None	None	None
Salivation	None	None	None
Diarrhoea	None	None	None
Lethargy	None	None	None
Respiratory Distress	None	None	None
Behavioural Changes	None	None	None
Sleep Disturbances	None	None	None
Coma	None	None	None

Gross Necroscopy Examination

No gross abnormalities were observed in any of the vital organs—brain, heart, liver, kidneys, spleen, or lungs—of rats treated with ethanolic extract at 300 mg/kg and 2000 mg/kg.

Table 5: Gross Necropsy Observations of Vital Organs in Wistar Rats Administered Ethanolic Extract of *Swertia chirayita*

Organ	Control	300 mg/kg	2000 mg/kg
Brain	Normal	Normal	Normal
Heart	Normal	Normal	Normal
Liver	Normal	Normal	Normal
Kidneys	Normal	Normal	Normal
Spleen	Normal	Normal	Normal
Lungs	Normal	Normal	Normal

All organs appeared normal in size, colour, texture and morphology. No evidence of haemorrhage, congestion,

oedema, necrosis, enlargement, shrinkage, or other visible pathological alterations was observed in either treatment group. The findings were comparable to those observed in the control animals. These findings suggest that the extract did not cause visible organ toxicity at the tested doses (Table 5).

DISCUSSION

The use of medicinal plants as primary healthcare remedies has gained widespread popularity globally, especially in developing countries, due to the perception that natural sources are inherently safe. Bioactive compounds derived from herbal plants are commonly believed to be non-toxic and free from adverse health effects, which has led to their widespread use as over-the-counter (OTC) medications¹⁷. Plant-based drugs have played a significant role in managing chronic diseases and are increasingly preferred by researchers as alternatives to conventional allopathic pharmaceuticals¹⁸.

Toxicological evaluations are essential for assessing the safety of various products, including isolated compounds, compound mixtures, crude plant extracts, pesticides, pharmaceuticals, food additives, packaging materials, and their chemical constituents. The World Health Organisation (WHO) recognises medicinal herbs as a key source for the development of a wide range of therapeutic agents. Consequently, thorough scientific investigation is necessary to evaluate the pharmacological properties, safety profiles, and therapeutic efficacy of these medicinal plants⁶.

The safety of plant extracts is primarily evaluated through acute oral toxicity analysis. Acute oral toxicity studies provide valuable information on the potential harmful effects of a substance after a single administration and are commonly used as an initial screening tool for safety evaluation. In the present study, the acute oral toxicity of the ethanolic extract of *Swertia chirayita* was investigated in female albino Wistar rats according to OECD Guideline 423. The findings demonstrated that oral administration of the extract at doses of 300 mg/kg and 2000 mg/kg did not produce mortality, behavioural abnormalities, or visible signs of toxicity during the 14-day observation period.

Changes in body weight are one of the most sensitive indicators of an animal's health. In this acute toxicity study, all animals exhibited normal and progressive body weight gain throughout the observation period. These findings suggest that the ethanolic extract of *Swertia chirayita* did not interfere with normal growth or induce systemic toxicity at the tested doses.

Clinical observations are a critical component of acute toxicity studies as they provide early evidence of toxic effects on the nervous, respiratory, and autonomic systems. No clinical signs of toxicity, including tremors, convulsions, salivation, diarrhoea, lethargy, respiratory distress, coma, or behavioural abnormalities, were observed in any treated animals during the study period. These findings indicate that the ethanolic extract of *Swertia chirayita* was well-tolerated at the tested doses.



Gross pathological examination revealed no visible abnormalities in the brain, heart, liver, kidneys, spleen, or lungs, suggesting the absence of treatment-related organ toxicity following acute oral administration.

The findings of the present study indicate that the ethanolic extract of *Swertia chirayita* possesses low acute oral toxicity, as evidenced by the absence of mortality, clinical signs of toxicity, and gross pathological changes at the tested doses. These results are consistent with previous studies reporting the safety of several medicinal plant extracts, such as *Pericampylus glaucus*¹⁸, *Moringa oleifera*¹⁹, *Echinops kebericho*²⁰, *Rhamnus prinoides*²¹, *Crassocephalum rubens*²², and *Aframomum melegueta*²³, evaluated according to OECD guidelines. Comparable findings have similarly been reported for *Reichardia picroide*²⁴, *Azadirachta indica*²⁵, *Erythrina senegalensis*²⁶, and *Alstonia scholaris*²⁷, among other traditionally used medicinal plants subjected to acute toxicity screening. Extracts of *Nasturtium officinale*²⁸, *Terminalia citrina*²⁹, *Carica papaya*³⁰, *Artemisia afra*³¹, and *Polygala fruticosa*³² have likewise been classified as practically non-toxic under similar OECD-based protocols. Further support comes from acute toxicity evaluations of *Nelsonia campestris*³³, *Salvia scutellarioides*³⁴, *Herniaria glabra*³⁵, *Cassia occidentalis*³⁶, and a panel of Nigerian ethnomedicinal plants³⁷, as well as a Moroccan herbal mixture³⁸ and java tea leaf extract³⁹, all of which reported an absence of mortality or major treatment-related toxicity at doses comparable to those used in the present study. The observed safety profile may be attributed to the presence of bioactive constituents such as xanthenes, seco-iridoid glycosides, flavonoids, terpenoids, and phenolic compounds, which are known for their diverse pharmacological activities^{10,12}. The findings support the traditional use of *Swertia chirayita* in Ayurvedic, Unani, and Siddha medicine. However, the present study was limited to acute toxicity assessment and did not include biochemical, haematological, or histopathological evaluations. In addition, the small group size (n = 4) resulted in an unequal distribution of baseline body weight across groups, which should be addressed in future studies through stratified randomisation or larger sample sizes. Therefore, further sub-acute, sub-chronic, and chronic toxicity studies are required to establish its long-term safety profile and therapeutic applicability.

CONCLUSION

The present study evaluated the acute oral toxicity of the ethanolic extract of *Swertia chirayita* in experimental animals and revealed no signs of toxicity, behavioural abnormalities, or mortality at the tested doses of 300 mg/kg and 2000 mg/kg body weight. Even at the higher dose of 2000 mg/kg, animals exhibited no adverse effects, indicating that the extract possesses a high safety margin. Based on the absence of mortality and treatment-related toxicity at 2000 mg/kg body weight, the oral LD50 of the ethanolic extract of *Swertia chirayita* is considered to be greater than 2000 mg/kg according to OECD Guideline 423.

The extract may therefore be classified under GHS Category 5 or unclassified depending on regulatory interpretation.

The absence of clinical toxicity symptoms and pathological changes further supports the non-toxic nature of the extract at the administered doses. These findings align with the traditional use of *Swertia chirayita* in herbal medicine and reinforce its potential as a safe natural remedy. However, while the acute toxicity results are promising, further studies are recommended to establish a comprehensive safety profile and support the therapeutic application of *Swertia chirayita* in modern medicine.

ACKNOWLEDGEMENT

The authors would like to thank the Zoology department, Patkar-Varde College, Mumbai, India, for their support. They would also like to express their gratitude to Dr Chaudari & members of the Laboratory of SCITESLA PRIVATE LIMITED, Mumbai, Maharashtra, India, for their support. Lastly, the authors would like to thank their family and colleagues for their encouragement.

AUTHORS' CONTRIBUTIONS

All authors contributed to the conception and design of the study, data collection, analysis and interpretation of results, manuscript preparation, and approved the final version of the manuscript.

ETHICAL APPROVAL

The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC; Approval No. SCI/IAEC/2024-25/167) in accordance with CPCSEA guidelines for the care and use of laboratory animals¹⁶.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Santos JA, Arruda A, Cardoso CaL, Vieira MDC, Piccinelli AC, De Santana Aquino DF, Kassuya CaL, Arena AC. Subacute and reproductive oral toxicity assessment of the hydroethanolic extract of *Jacaranda decurrens* roots in adult male rats. Evidence-based Complementary and Alternative Medicine. 2013;13:1-6. DOI: 10.1155/2013/414821.
2. Loha M, Mulu A, Abay SM, Ergete W, Geleta B. 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. Evidence-based Complementary and Alternative Medicine. 2019;2019:1-15. DOI: 10.1155/2019/5702159.
3. World Health Organization. International Health Regulations (2005). 3rd ed. Geneva: World Health Organization; 2016.
4. Ezeja MI, Anaga AO, Asuzu IU. Acute and sub-chronic toxicity profile of methanol leaf extract of *Gouania longipetala* in rats. Journal of Ethnopharmacology. 2014;151(3):1155-1164. DOI: 10.1016/j.jep.2013.12.034.



5. Loomis TA, Hayes AW. Loomis's Essentials of Toxicology. 4th ed. San Diego: Academic Press; 1996.
6. Subha D, Natesan Geetha N. Evaluation of acute toxicity of the methanolic extract of *Tanacetum parthenium* L. in albino wistar rats. *Journal of Scientific and Innovative Research*. 2017;6(3):113-115. DOI: 10.31254/jsir.2017.6307.
7. Alelign T, Chalchisa D, Fekadu N, Solomon D, Sisay T, Debella A, Petros B. Evaluation of acute and sub-acute toxicity of selected traditional antiurolithiatic medicinal plant extracts in Wistar albino rats. *Toxicology Reports*. 2020;7:1356-1365. DOI: 10.1016/j.toxrep.2020.10.001.
8. Latif A, Rehman S. Standardization of a herbal medicine- *Swertia chirayita* Linn. *Pharmacophore*. 2014;5(1):98-108.
9. Nayak K, Vailanka MS, Sheeba E. Antibacterial activity of different extracts of the medicinal plant *Swertia chirata*. *International Journal of Current Microbiology and Applied Sciences*. 2015;4(7):889-894.
10. Swati K, Bhatt V, Sendri N, Bhatt P, Bhandari P. *Swertia chirayita*: a comprehensive review on traditional uses, phytochemistry, quality assessment and pharmacology. *Journal of Ethnopharmacology*. 2022;300:115714. DOI: 10.1016/j.jep.2022.115714.
11. Padhan JK. Understanding biosynthesis of major chemical constituents in *Swertia chirayita* [PhD dissertation]. Jaypee University of Information Technology; 2018.
12. Aleem A, Kabir HMD. Review on *Swertia chirayita* as traditional uses to its phytochemistry and pharmacological activity. *Journal of Drug Delivery and Therapeutics*. 2018;8(5-s):73-78. DOI: 10.22270/jddt.v8i5-s.1957.
13. Tabassum S, Mahmood S, Hanif J, Hina M, Uzair B. An overview of the medicinal importance of *Swertia chirayita*. *International Journal of Applied Science and Technology*. 2012;2(1):205-210.
14. Kumar V, Van Staden J. A review of *Swertia chirayita* (Gentianaceae) as a traditional medicinal plant. *Frontiers in Pharmacology*. 2016;6:308. DOI: 10.3389/fphar.2015.00308.
15. Organisation for Economic Co-operation and Development. Test No. 423: Acute Oral Toxicity - Acute Toxic Class Method. Paris: OECD Publishing; 2001.
16. Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). CPCSEA guidelines for laboratory animal facility. *Indian Journal of Pharmacology*. 2003;35(4):257-274.
17. Vaghasiya Y, Shukla V, Chanda S. Acute oral toxicity study of *Pluchea arguta* Boiss extract in mice. *Journal of Pharmacology and Toxicology*. 2011;6(2):113-123. DOI: 10.3923/jpt.2011.113.123.
18. Kifayatullah M, Mustafa MS, Sengupta P, Sarker MMR, Das A, Das SK. Evaluation of the acute and sub-acute toxicity of the ethanolic extract of *Pericampylus glaucus* (Lam.) Merr. in BALB/c mice. *Journal of Acute Disease*. 2015;4(4):309-315. DOI: 10.1016/j.joad.2015.06.010.
19. Awodele O, Oreagba IA, Odoma S, Da Silva JAT, Osunkalu VO. Toxicological evaluation of the aqueous leaf extract of *Moringa oleifera* Lam. (Moringaceae). *Journal of Ethnopharmacology*. 2011;139(2):330-336. DOI: 10.1016/j.jep.2011.10.008.
20. Deyno S, Abebe A, Tola MA, Hymete A, Bazira J, Makonnen E, Alele PE. Acute and sub-acute toxicity of *Echinops kebericho* decoction in rats. *BMC Complementary Medicine and Therapies*. 2020;20(1):5. DOI: 10.1186/s12906-019-2794-z.
21. Abebe MS. Acute and subacute toxicity of *Rhamnus prinoides* leaves on histopathology of liver, kidney, and brain tissues, and biochemical profile of rats. *Journal of Toxicology*. 2023;2023:1-7. DOI: 10.1155/2023/3105615.
22. Adewale OB, Onasanya A, Anadozie SO, Abu MF, Akintan IA, Ogbale CJ, Olayide II, Afolabi OB, Jaiesimi KF, Ajiboye BO, Fadaka AO. Evaluation of acute and subacute toxicity of aqueous extract of *Crassocephalum rubens* leaves in rats. *Journal of Ethnopharmacology*. 2016;188:153-158. DOI: 10.1016/j.jep.2016.05.003.
23. Akpanabiatu MI, Ekpo ND, Ufot UF, Udoh NM, Akpan EJ, Etuk EU. Acute toxicity, biochemical and haematological study of *Aframomum melegueta* seed oil in male Wistar albino rats. *Journal of Ethnopharmacology*. 2013;150(2):590-594. DOI: 10.1016/j.jep.2013.09.006.
24. Aouachria S, Boumerfeg S, Benslama A, Benbacha F, Guemmez T, Khennouf S, Arrar L, Baghiani A. Acute, sub-acute toxicity and antioxidant activities (in vitro and in vivo) of *Reichardia picroide* crude extract. *Journal of Ethnopharmacology*. 2017;208:105-116. DOI: 10.1016/j.jep.2017.06.028.
25. Ashafa AOT, Orekoya LO, Yakubu MT. Toxicity profile of ethanolic extract of *Azadirachta indica* stem bark in male Wistar rats. *Asian Pacific Journal of Tropical Biomedicine*. 2012;2(10):811-817. DOI: 10.1016/S2221-1691(12)60234-2.
26. Atsamo AD, Nguelefack TB, Datté JY, Kamanyi A. Acute and subchronic oral toxicity assessment of the aqueous extract from the stem bark of *Erythrina senegalensis* DC (Fabaceae) in rodents. *Journal of Ethnopharmacology*. 2011;134(3):697-702. DOI: 10.1016/j.jep.2011.01.023.
27. Baliga M. The evaluation of the acute toxicity and long-term safety of hydroalcoholic extract of *Sapthaparna* (*Alstonia scholaris*) in mice and rats. *Toxicology Letters*. 2004;151(2):317-326. DOI: 10.1016/j.toxlet.2004.01.015.
28. Clemente M, Miguel M, Felipe K, Gribner C, Moura P, Rigoni A, Fernandes L, Carvalho J, Hartmann I, Piltz M, Henneberg R, Montrucchio D, Miguel O. Acute and sub-acute oral toxicity studies of standardized extract of *Nasturtium officinale* in Wistar rats. *Regulatory Toxicology and Pharmacology*. 2019;108:104443. DOI: 10.1016/j.yrtph.2019.104443.
29. Das N, Goshwami D, Hasan MS, Raihan SZ. Evaluation of acute and subacute toxicity induced by methanol extract of *Terminalia citrina* leaves in Sprague Dawley rats. *Journal of Acute Disease*. 2015;4(4):316-321. DOI: 10.1016/j.joad.2015.05.001.
30. Halim SZ, Abdullah NR, Afzan A, Rashid BAA, Jantan I, Ismail Z. Acute toxicity study of *Carica papaya* leaf extract in Sprague Dawley rats. *Journal of Medicinal Plants Research*. 2011;5(10):1867-1872. DOI: 10.5897/JMPR.9000043.
31. Mukinda J, Syce J. Acute and chronic toxicity of the aqueous extract of *Artemisia afra* in rodents. *Journal of Ethnopharmacology*. 2007;112(1):138-144. DOI: 10.1016/j.jep.2007.02.011.
32. Mukinda JT, Eagles PFK. Acute and sub-chronic oral toxicity profiles of the aqueous extract of *Polygala fruticosa* in female



- mice and rats. *Journal of Ethnopharmacology*. 2010;128(1):236-240.
33. Olaniyan JM, Muhammad HL, Makun HA, Busari MB, Abdullah AS. Acute and sub-acute toxicity studies of aqueous and methanol extracts of *Nelsonia campestris* in rats. *Journal of Acute Disease*. 2015;5(1):62-70. DOI: 10.1016/j.joad.2015.08.006.
34. Ramírez JH, Palacios M, Tamayo O, Jaramillo R, Gutiérrez O. Acute and subacute toxicity of *Salvia scutellarioides* in mice and rats. *Journal of Ethnopharmacology*. 2006;109(2):348-353. DOI: 10.1016/j.jep.2006.07.040.
35. Rhiouani H, El-Hilaly J, Israili ZH, Lyoussi B. Acute and sub-chronic toxicity of an aqueous extract of the leaves of *Herniaria glabra* in rodents. *Journal of Ethnopharmacology*. 2008;118(3):378-386. DOI: 10.1016/j.jep.2008.05.009.
36. Silva MG, Aragão TP, Vasconcelos CF, Ferreira PA, Andrade BA, Costa IM, Costa-Silva JH, Wanderley AG, Lafayette SS. Acute and subacute toxicity of *Cassia occidentalis* L. stem and leaf in Wistar rats. *Journal of Ethnopharmacology*. 2011;136(2):341-346. DOI: 10.1016/j.jep.2011.04.070.
37. Tauheed AM, Mamman M, Ahmed A, Sani NA, Suleiman MM, Sulaiman MH, Balogun EO. Acute, sub-acute, sub-chronic and chronic toxicity studies of four important Nigerian ethnomedicinal plants in rats. *Clinical Phytoscience*. 2021;7(1):20. DOI: 10.1186/s40816-020-00244-2.
38. Chebaibi M, Bousta D, Chbani L, Zoubi YE, Touiti N, Achour S. Acute toxicity of plant mixture used in the traditional treatment of oedema and colic renal in Morocco. *Scientific African*. 2019;6:e00152. DOI: 10.1016/j.sciaf.2019.e00152.
39. Pariyani R, Ismail IS, Azam AA, Abas F, Shaari K, Sulaiman MR. Phytochemical screening and acute oral toxicity study of java tea leaf extracts. *BioMed Research International*. 2015;15:1-8. DOI: 10.1155/2015/74242

For any questions related to this article, please reach us at: globalresearchonline@rediffmail.com

New manuscripts for publication can be submitted at: submit@globalresearchonline.net and submit_ijpsrr@rediffmail.com

