

Original Research Article

Sub-chronic toxicity evaluation of Ethanol whole Plant Extract of *Nelsonia gracilis* in Rats

Danjuma Mallam*, Ja'afar M. Sa'id, Esther P. Baduku, Asma'u B. Ismail-J, Safiya S. Sambo, Maryam A. Lawal, Kenneth B. David

¹Department of Pharmacology & Toxicology, Faculty of Pharmaceutical Sciences, Kaduna State University, Kaduna, Nigeria.

*For correspondence: Email: mallandanjuma@gmail.com +2348024337937

Sent for review: 10 June 2025

Revised accepted: 03 February 2026

Abstract

Purpose: The aim of this research work is to assess the phytochemicals and to conduct the toxicological profile of ethanol whole plant extract of *Nelsonia gracilis* in Wistar rats.

Methods: The whole plant of *Nelsonia gracilis* was extracted with 70% v/v ethanol and was evaporated to dryness at 40°C. The ethanol extract was screened for plants' chemicals by the methods described by Sofowora and Edeoga. The lethal dose was determined by OECD 425 Guidelines. Toxicity assessment involved the sub-chronic (28 days) studies with twenty Wistar rats. Group I, was given Distilled water (D/W 1 mL/kg), Groups II, III and IV were given graded doses (250, 500 and 1000 mg/kg) of *Nelsonia gracilis* respectively. On day 29, the rats were sacrificed using diethylether and blood was collected for biochemical, renal and hematological assessments.

Results: The following (Flavonoids, Glycosides, Alkaloids, Terpenoids, Steroids, Saponins, and Tannins) plants' chemicals were found to be present. The lethal dose of 5000 mg/kg did not show any signs of toxicity or mortality. The sub-chronic study demonstrated no significant changes in biochemical, renal or hematological parameters across all groups. However, the levels of sodium ion (in renal function) significantly decreased at 500 mg/kg (31.24±9.26) compared to the control.

Conclusion: The findings demonstrated that the ethanol whole plant of *Nelsonia gracilis* is safe. However, it should be used with caution for long period of time.

Keywords: Lethal dose, *Nelsonia gracilis*, Phytochemical, Sub-chronic, Toxicity

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Drug Research is indexed by Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

© 2026 The authors. This work is licensed under the Creative Commons Attribution 4.0 International License Trop J Drug Res, January 2026; 3(1): 352 - 356

INTRODUCTION

Medicinal plants have long been integral to traditional medicine systems worldwide, providing therapeutic remedies for various ailments. Medicinal herbs or plants contain substances from one or more organs of the plant used for therapeutic purposes or their secondary metabolites are used for the synthesis of other drugs. Among these, *Nelsonia gracilis*, a herbaceous plant from Acanthaceae family, is commonly used in folk medicine, particularly in tropical areas.¹ It is employed for the treatment of inflammatory disease conditions, antimicrobial, and analgesic properties, which have made it a staple in managing conditions such as infections, pain, and fever.¹ However, despite its widespread application, scientific data on its safety and toxicity are limited. The liver and kidneys are vital organs responsible for detoxification, metabolism, and excretion of foreign substances, including plant-derived compounds. These organs are particularly susceptible to toxicological damage caused by prolonged exposure to certain phytochemicals.² Toxicity studies are therefore critical to understanding the potential toxic profile of herbal plants. Chronic or sub-chronic toxicity studies assess the effects of repeated exposure to a substance over a moderate duration. These studies are essential for identifying potential risks associated with medicinal plants and establishing safe dosage ranges. They involve evaluating biochemical markers and histological changes in key organs, providing a comprehensive understanding of a plant's impact on health. While *Nelsonia gracilis* has been known to possess therapeutic benefits, its pharmacological properties are likely mediated by bioactive compounds such as flavonoids, alkaloids, and saponins.³ These phytochemicals, though beneficial, may also exert toxic effects depending on their concentration and the host's physiological response. Consequently, rigorous evaluation of its safety profile is necessary before integrating it into mainstream medicine or recommending its prolonged use. Several studies on other medicinal plants have highlighted the dual nature of phytochemicals, which can be both therapeutic and toxic.⁴ For instance, excessive consumption of certain plant extracts has been linked to hepatotoxicity and nephrotoxicity. This duality underscores the need for detailed toxicological assessments of plants like *Nelsonia gracilis* to ensure their safe application in traditional and modern healthcare settings. The growing interest in natural remedies has prompted increased usage of medicinal plants without adequate regulatory oversight or scientific validation. This trend raises concerns about potential health risks, particularly for individuals relying heavily on herbal remedies as an alternative to conventional medicine. The present study focuses on *Nelsonia gracilis*, aiming to bridge the knowledge gap regarding its safety profile. The toxicological properties of *Nelsonia gracilis* will not only enhance its therapeutic application but also protect users from potential harm. This aligns with the broader goal of

integrating traditional medicinal plants into modern healthcare in a scientifically validated and safe manner.⁵

MATERIALS AND METHODS

Materials and Equipments

Nelsonia gracilis plant, Wistar rats, Distilled water, 70% Ethanol, Analytical weighing balance, Mortar and Pestle, evaporating dish, Thermostatic water cabinet HH-W420, Glass rod, Beaker, Syringe and needles, Hand gloves, Pipette, Spatula, Test tubes, Filter paper (Whatman paper No. 1), Masking tape.

Chemicals and Reagents

They were obtained from representatives of reputable chemical companies in Nigeria. The reagents and chemicals include: Distilled water, Ethanol absolute (99.7%) (JHD, Sci-Tech Co.LTD), Aspirin (Bond Chemical Ind. Ltd), Morphine, Dragendoff's reagent, concentrated sulphuric acid, concentrated hydrochloric acid, chloroform, glacial acetic acid, ferric chloride 10% sodium hydroxide solution, acetic anhydride.

Experimental Animals

Wistar rats were purchased from the Department of Pharmacology, Kaduna State University, Kaduna. Chikun feeds were used to feed them with adequate water based on ethical standards for animal use as accepted internationally.⁶ Ethical approval number KASU/AEC/2025/0003 for animal use was obtained from Directorate of Research and Development from the same institution.

Collection and identification of the plant

Nelsonia gracilis whole plant was collected in June, 2024 from Kufena, Kaduna state. It was identified by Mallam U.S. Gallah in the Herbarium Unit, Kaduna State University. A voucher number of KASU/BSH/16166 was given. The plant material was dried under shade. It was size-reduced into powder and was extracted using 6L of 70%v/v ethanol by cold maceration for 72 hrs followed by filtration and oven-dried at 400C. The concentrated extract was stored in a laboratory desiccator until ready for use. Solutions for administration were freshly prepared for each study using distilled water.

$$\% \text{ yield} = \frac{\text{weight of extract}}{\text{weight of dried plant material}} \times 100$$

Evaluation of phytochemicals of the plant extract

Evaluation of phytochemicals was carried out on the ethanol extract of *Nelsonia gracilis* using standard protocols.⁷⁻⁸

Lethal dose (LD50) study

Lethal dose study of *Nelsonia gracilis* extract according to OECD, 425 guidelines in rats. In this experiment, one rat was fasted 3 hrs before the administration of the extract. 2000 mg/kg fixed dose was given a rat that was fasted for 3 hours and was observed for 24 hours and for 14 days. Following survival after 24 hrs and for 14 days, an additional four rats were dosed with 5000 mg/kg and were observed for 48 hours and 14 days with periodical observations without any sign of toxicity or mortality.

Sub-chronic Toxicity Study

The assessment of *Nelsonia gracilis* extract was carried out using the Organizational and Experimental Co-operation and Development (OECD) 424 Guidelines. Healthy Wistar animals were divided into four groups. Group I served as control which was given D/W 1 mL/kg. The three treated Groups were given graded doses of the extract every day (p.o) for 28 days. On day 29, the rats were fasted overnight and anesthetized using diethylether. The animals were sacrificed by cervical dislocation and the blood was collected through cardiac puncture and stored in an EDTA container. The samples were quickly analyzed for hematological indices. Biochemical analysis was carried out after 6000 rpm centrifuged at 4°C for 15 mins to obtain the serum which was stored at -20°C.

Blood analysis

An autoanalyzer (H1, Bayer Diagnostics) was used to analyze hematological parameters. The hematological parameters were determined using an autoanalyzer (System).

Liver Function Test

AST, ALP and ALT liver enzymes were analysed using blood samples that were centrifuged at 5000 rpm for 10 mins at 37 °C within 2 h using a Beckman Auto Analyzer (type Synchron LXi 725; Beckman Instruments, USA) with reagent kits provided by the manufacturer.⁹

Renal Function Test

The renal function evaluation of Serum creatinine, Blood Urea Nitrogen, Total Protein and Albumin were determined using commercial kits as described by the manufacture guidelines.

Statistical Analysis

Data was analysed, expressed as Mean \pm SEM. SPSS statistical tool using One-way ANOVA followed by Bonferroni post-hoc test. $p \leq 0.05$ was considered significant.

Table 2: The effect of *Nelsonia gracilis* whole plant extract administration on hematological parameters

Treatment (mg/kg)	WBC	LYMP	MID	GRA	RBC	HGB	HCT	PLT
D/W1 mL/kg	4.78 \pm 0.17	6.22 \pm 0.24	0.78 \pm 0.28	2.84 \pm 0.15	6.02 \pm 0.15	12.28 \pm 0.97	36.60 \pm 3.72	191.40 \pm 10.12
NGE 250	3.82 \pm 0.31	5.90 \pm 0.22	0.94 \pm 0.28	2.84 \pm 0.15	5.68 \pm 0.34	11.08 \pm 0.69	32.00 \pm 2.41	161.02 \pm 17.51
NGE 500	4.76 \pm 0.55	6.22 \pm 0.24	0.74 \pm 0.29	2.84 \pm 0.15	5.84 \pm 0.14	11.82 \pm 0.86	102.60 \pm 69.12	163.00 \pm 13.43
NGE 1000	4.92 \pm 0.32	6.10 \pm 0.31	0.74 \pm 0.29	2.84 \pm 0.15	5.90 \pm 0.23	12.28 \pm 0.67	36.00 \pm 1.67	183.02 \pm 10.97

Data were analyzed using one-way ANOVA, Values presented as mean \pm SEM, $p > 0.05$ as compared to D/W group, n=6, D/W distilled water, NGE *Nelsonia gracillis* extract, PCV packed cell volume, WBC white blood cells, LYMP lymphocytes, GRA granulocytes, MID cells, PLT platelet, RBC red blood cells, HGB hemoglobin.

Table 3: The effect of ethanolic whole plant extract of *Nelsonia gracilis* oral administration on liver enzymes

Treatment (mg/kg)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	TP (g/dL)	ALB(g/dL)
D/W 1 mL/kg	63.00 \pm 5.89	118.60 \pm 10.20	68.68 \pm 6.10	20.96 \pm 1.63	2.72 \pm 0.13
NGE 250	43.00 \pm 9.45	96.00 \pm 25.59	63.52 \pm 11.26	31.46 \pm 6.98	1.86 \pm 0.38
NGE 500	49.40 \pm 4.06	79.00 \pm 7.37	58.34 \pm 6.60	22.16 \pm 2.85	2.12 \pm 0.14
NGE 1000	45.00 \pm 5.18	138.00 \pm 17.29	58.82 \pm 4.33	26.44 \pm 2.05	2.84 \pm 0.21

Data were analyzed using one-way ANOVA, Values presented as mean \pm SEM, $p > 0.05$ as compared to D/W group. n=6, D/W distilled water, NGE = *Nelsonia gracillis* extract, ALP alkaline phosphatase, ALT alanine amino transferase, AST aspartate amino transferase, TP total protein, TB total bilirubin, ALB albumin.

RESULTS AND DISCUSSION

Nelsonia gracilis was found to be 4.97% yield with the weight of 45.69 g.

Qualitative phytochemical screening of ethanolic whole plant extract of *Nelsonia gracilis*

Alkaloids, Glycosides, Flavonoids, Terpenoids, Steroids, Saponins, and Tannins were found to be present (Table 1).

Table 1: The phytochemical constituents of the ethanolic whole plant extract of *Nelsonia gracilis* (Acanthaceae)

Phytochemical	Inference
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	+
Steroids	+
Terpenoids	+
Glycoside	+

KEY:

+ = Present

Acute Toxicity

Nelsonia gracilis ethanol extract demonstrated LD50 to be more than 5000 mg/kg, indicating the safety of the extract.

The effect of ethanolic whole plant extract of *Nelsonia gracilis* on hematological parameters

The treated groups did not show significant decrease in hematological parameters compared to the control as a result of administration of the ethanol extract of *Nelsonia gracilis* for 28 days (Table 2).

Effect of ethanolic whole plant extract of *Nelsonia gracilis* on hepatic enzymes

The treated groups did not show significant difference ($P>0.05$) in the levels of liver enzyme when compared to the control after 28-days repeated dosing of the extract (Table 3).

Effect of ethanolic whole plant extract of *Nelsonia gracilis* on hepatic parameters

The treated groups did not show significant difference in renal parameters compared to the control. However, the levels of sodium ion decrease significantly ($P<0.05$) at 500 mg/kg (31.24 ± 9.26) compared to the control (Table 4).

Table 4: Effect of the whole plant extract of *Nelsonia gracilis* oral administration on renal parameters and electrolytes

Treatment (mg/kg)	Urea (mmol/L)	Creatinine (μ mol/L)	Sodium (mmol/L)	Chloride (mmol/L)	Potassium (mmol/L)	Bicarbonate (mmol/L)
D/W 1 mL/kg	19.00 \pm 4.30	0.94 \pm 0.09	152.98 \pm 32.56	27.40 \pm 2.78	26.46 \pm 2.22	101.40 \pm 11.08
NGE 250	42.50 \pm 16.01	1.00 \pm 0.15	67.86 \pm 25.99	22.60 \pm 1.03	19.16 \pm 4.36	107.20 \pm 7.89
NGE 500	30.00 \pm 1.58	1.06 \pm 0.05	31.24 \pm 9.26*	26.80 \pm 3.85	23.02 \pm 4.08	98.40 \pm 4.33
NGE 1000	45.00 \pm 6.52	1.04 \pm 0.10	108.28 \pm 21.71	27.00 \pm 3.30	34.44 \pm 4.07	97.80 \pm 7.81

Data were analyzed using one-way ANOVA followed by Bonferroni post hoc test, Values presented as mean \pm SEM, * = $p<0.05$ as compared to D/W group. n=6, D/W distilled water, NGE = *Nelsonia gracilis* extract.

Nelsonia gracilis extract's lethal dose was more than 5,000 mg/kg suggesting its safety when administered orally. Acute toxicity study is normally conducted to determine the range of doses that could be toxic to the animal and also guides in the choice of the doses of the extract for the study.¹⁰ All the doses that were used in this experiment were below 30% of the estimated LD₅₀ which have been previously reported to be safe in ethnopharmacological studies.¹⁰ *Nelsonia gracilis* (Acanthaceae), is a plant that is traditionally used in various parts of Africa and Asia for the treatment of pain, fever, and inflammatory conditions.¹¹ Many herbal plants are used for medicinal properties, but their toxicity profiles are often not well-studied or scientifically validated. While traditional use suggests benefits, there is often a lack of rigorous research on potential adverse effects and safe dosages, making it risky to use them without proper scientific evidence.¹² Many traditional remedies have not undergone the rigorous testing that pharmaceutical drugs do, so the long-term effects or side effects are not always known.¹³ Causes of adverse reactions or effects of medicinal plants can be divided into "direct" and "indirect" reasons. Adverse reactions that occur at normal therapeutic dosage are termed direct reasons of adverse reactions while the indirect adverse reactions are associated with herbal medicines that occur from contamination of products with toxic metals, adulteration, misidentification, substitution of herbal ingredients, or improperly processed or prepared products.¹⁴ Research is essential to ensure that the potential benefits of herbal medicine are not outweighed by adverse effects, which can range from mild side effects to severe toxicity.¹² Sub-chronic toxicity study showed no significant effect on majority of the biochemical parameters and hematological markers, with the exception of the decrease in sodium level at 500 mg/kg extract-treated group compared to the control which is indicative of hyponatremia. The sodium levels in the extract-treated groups showed an inconsistent trend which may be due to an effect on sodium retention. Another study carried out on another specie of the genus *Nelsonia*, *Nelsonia canescens* showed no toxicity to the liver and kidney and was considered to be relatively safe.¹⁵ Similarly, another study carried out on *Justicia schimperiana* (Acanthaceae) which is from the same family as *Nelsonia gracilis*, showed its relative safety.¹⁶ Some phytochemicals can cause toxicity, especially in high doses

or when consumed as isolated supplements rather than from whole foods. While phytochemicals in whole foods are generally considered safe, potential risks include liver toxicity from high levels of green tea catechins or hormonal imbalances from excessive soy isoflavones. Some individual phytochemicals are naturally toxic (phytotoxins) or act as antinutrients, and even beneficial compounds like polyphenols can become pro-oxidants at very high intakes.¹⁷ There are health benefits associated with phytochemicals yet their toxic profile need to be determined scientifically. Phytochemical toxicity arises from diverse mechanisms. Reactive oxygen species generated during metabolism can induce oxidative stress, damaging cellular components. Some compounds interfere with enzymes, disrupting essential biochemical pathways. Accumulation of toxic metabolites, particularly in cases of excessive consumption, can lead to adverse effects. Additionally, interactions between phytochemicals and medications can potentiate toxicity. Understanding these mechanisms is crucial for risk assessment and management. By elucidating the fine balance between beneficial effects and potential harm, researchers and health practitioners can guide safe consumption and harness the positive attributes of phytochemicals while minimizing the risks.¹⁸

CONCLUSION

The oral lethal dose (LD₅₀) of the ethanol whole plant extract of *Nelsonia gracilis* was estimated to be more than 5000 mg/kg while the sub-chronic toxicity study showed no significant changes in the hematological and biochemical parameters compared to the control group suggesting that the extract is relatively safe in both acute and sub-chronic oral use. However, it should be used with caution for long period of time.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS DECLARATION

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

ACKNOWLEDGEMENT

Authors are very thankful to the Technologists of the Department of Pharmacology and Toxicology, Kaduna State University for their inputs in the course of the laboratory work.

Open Access

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited

REFERENCES

- Adekunle O. Toxicological studies of *Nelsonia canescens* ethanol extract: A focus on hepatotoxicity. *J Med Pls Res.* 2022;4:107-112
- Olubodun SO, Iyamu PE, Henry EU, Idris JA, Osemwengie TI. Toxicology of the renal indices of Wistar rats induced with leaf extracts of *Acalypha wilkesiana*. *J Bio Res and Biotech.* 2025;22(3):2522-2531. <https://doi.org/10.4314/> (Original work published 2024)
- Daniel AI, Gara TY, Atolagbe SO. *Nelsonia canescens* (Acanthaceae) aqueous extract and partitioned fractions ameliorates type-2 diabetes in alloxan-induced diabetic rats. *Futur J Pharm Sci.* 2022;8(53). <https://doi.org/10.1186/s43094-022-00445-1>
- Chiranthan N, Lertprasertsuke N, Srisook E, Srisook K. Acute and sub chronic oral toxicity assessment of extract from *Etlingera pavieana* rhizomes. *Toxicol Rep.* 2022; 9:1472-1483. Doi: 10.1016/j.toxrep.2022.07.005. PMID: 36518420; PMCID: PMC9742905.
- Chiranthan N, Lertprasertsuke N, Srisook E, Srisook K. Acute and subchronic oral toxicity assessment of extract from *Etlingera pavieana* rhizomes. *Tox Rep.* 2022;9:1472–1483. <https://doi.org/10.1016/j.toxrep.2022.07.005>
- National Institutes of Health Funding: FY1996-FY2025
- Sofowora A. Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd. 1993.
- Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. *Afri J Biotech.* 2005;4(7):685-688.
- Henry RJ, Chiamori N, Golub OJ, Berkman S. Revised spectrophotometric methods for the determination of glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, and lactic acid dehydrogenase. *Amer J Clin Pathol.* 1960;34:381–398.
- Clark JH. Denaturation changes in egg albumin with urea, radiation, and heat. *J Gen Physiol.* 1943;27(2): 101-111.
- Selina Wamucii. *Nelsonia Gracilis* Plant: Discover its Uses, Benefits, and Side Effects. Retrieved from 2022. <https://richfarmkenya.com/nelsonia-gracilis-plant/>
- Kavimani Subramanian, Divya Sankaramourthy, Mahalakshmi Gunasekaran, Chapter 18 - Toxicity Studies Related to Medicinal Plants, Editor(s): Subhash C. Mandal, Vivekananda Mandal, Tetsuya Konishi. *Nat Prod Drug Disc.* Elsevier. 2018;491-505. <https://doi.org/10.1016/B978-0-08-102081-4.00018-6>.
- Amitava Kabiraj, Rohitas Deshmukh. A review on Chinese herbal medicine used as carminative, *Pharmacol. Res Mod Chin Med.* 2024;100409, ISSN 2667-1425, <https://doi.org/10.1016/j.prmcm.2024.100409>.
- Zhang J, Onakpoya IJ, Posadzki P, Eddouks M. The safety of herbal medicine: from prejudice to evidence. Evidence-based complementary and alternative medicine: eCAM, 2015;316706. <https://doi.org/10.1155/2015/316706>. 2015.
- David BC, Tutuwa JA, Tadawus RH, Ogu EO, Ifraimu D, Sunday OG, Jesse PS, Agbu TD. Effect of Ethanolic Stem Extract of *Nelsonia Canescens* on Selected Biochemical Parameters in Male Wistar Rats Induced with Sodium Arsenite. *J Multidisc Sci.* 2024;2(1):110-127. <https://doi.org/10.58578/mikailays.v2i1.2777>.
- Feleke MK, Bekele T, Dessie G, Ayelgn T, Nigatu A, Jemere T, Alene AN. Effect of *Justicia schimperiana* (Acanthaceae) roots extract on blood glucose level and lipid profiles in streptozotocin-induced-diabetic-mice. *Metab Open.* 2024;21:100270. <https://doi.org/10.1016/j.metop.2024.100270>
- Ipek Süntar, Ömer Faruk Yakıncı, Chapter Six - Potential risks of phytonutrients associated with high-dose or long-term use, Editor(s): Seyed Mohammad Nabavi, Ipek Suntar, Davide Barreca, Haroon Khan, In Woodhead Publishing Series in Food Science, Technology and Nutrition, *Phytonutrients in Food*, Woodhead Publishing, 2020;137-155, ISBN 9780128153543, <https://doi.org/10.1016/B978-0-12-815354-3.00010-1>.
- Hossain MS, Wazed MA, Asha S, Amin MR, Shimul IM. Dietary Phytochemicals in Health and Disease: Mechanisms, Clinical Evidence, and Applications-A Comprehensive Review. *F Sci Nut.* 2025;13(3): e70101. <https://doi.org/10.1002/fsn.3.70101>