IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 24, Issue 5 Ser. 3 (May. 2025), PP 70-75
www.iosrjournals.org

Nephroprotective Potential Of Telfairia Occidentalis In Phenylhydrazine-Induced Anaemia: A Biochemical And Histological Study

Konyefom G. Nwaeze, Chukwu O. Odochi, Otta-Umahi Grace, Imakwu Icha Innocent, Chikwendu C. Kenechi

Department Of Physiology, Faculty Of Basic Medical Sciences, College Of Medical Sciences, Alex Ekwueme Federal University, Ndufu-Alike, Abakaliki, Ebonyi, State, Nigeria.
Department Of Physiology, Faculty Of Basic Medical Sciences, College Of Medical Sciences, David Umahi Federal University Of Health Sciences, Ebonyi State, Nigeria.
Department Of Physiology, Faculty Of Basic Medical Sciences, College Of Medicine, Ebonyi State University, Nigeria.
Department Of Pharmacology And Therapeutics, Faculty Of Basic Clinical Sciences, College Of Medicine, David Umahi Federal University Of Health Sciences, Ebonyi State, Nigeria.
Department Of Medical Laboratory Sciences, College Of Health Sciences, Evangel University, Akaeze, Ebonyi State, Nigeria.

Abstract
Background: *Anaemia, particularly when induced by haemolysis, can lead to significant renal dysfunction due to oxidative stress and impaired oxygen delivery. Phenylhydrazine (PHZ), a potent hemolytic agent, induces oxidative damage to red blood cells and is associated with secondary nephrotoxic effects, including elevated serum urea, creatinine, and electrolyte imbalances. Telfairia occidentalis (fluted pumpkin) is a nutrient-rich, antioxidant-laden plant traditionally used for its hematinic and restorative properties. However, its potential renoprotective effect in the context of anaemia-induced renal impairment remains underexplored.*
Objective: *To investigate the effect of ethanolic extract of Telfairia occidentalis leaves on renal function in male Wistar rats with phenylhydrazine-induced anaemia.*
Methods: *Fifteen male Wistar rats were randomly divided into three groups (n=5): Control, PHZ-induced untreated, and PHZ-induced treated with T. occidentalis extract. Anaemia was induced using phenylhydrazine (120 mg/kg, intraperitoneally) for two consecutive days. The treated group received oral administration of T. occidentalis extract for 14 days. At the end of the treatment period, serum biochemical parameters including urea, creatinine, sodium, potassium, chloride, and bicarbonate were measured. Renal tissues were harvested and examined histologically for morphological alterations.*
Results: *PHZ administration led to a reduction in serum urea and creatinine levels compared to controls, alongside marked electrolyte disturbances, including significant hypokalemia. Treatment with T. occidentalis extract partially restored urea levels and improved bicarbonate balance, while creatinine levels declined further. Potassium levels remained low in the treated group. Histopathological analysis showed reduced renal tubular degeneration and glomerular damage in the extract-treated rats compared to the PHZ-only group.*
Conclusion: *Telfairia occidentalis ethanolic leaf extract exhibits protective effects on renal function in phenylhydrazine-induced anaemic rats, as evidenced by improved serum biochemical markers and preserved kidney histoarchitecture. These findings suggest its potential as a natural, affordable therapeutic agent for mitigating anaemia-associated renal complications.*
Keywords: *Telfairia occidentalis, renal function, phenylhydrazine, anaemia, urea, creatinine, electrolytes, nephroprotection, oxidative stress*

Date of Submission: 05-05-2025 Date of Acceptance: 15-05-2025

I. Introduction

Anaemia is a significant global health burden, affecting over 1.6 billion individuals and contributing to a wide range of systemic complications [1]. While it is primarily characterized by reduced hemoglobin levels and impaired oxygen delivery to tissues, chronic or hemolytic anaemia can exert profound effects beyond the hematological system, particularly on renal function. The kidneys, being highly vascularized and metabolically active organs, are especially susceptible to hypoxia and oxidative stress associated with anaemic states [2,3].

In haemolytic anaemia, accelerated destruction of red blood cells releases free hemoglobin and heme into circulation, which can induce nephrotoxicity, promote inflammation, and impair glomerular and tubular function [4]. These disruptions are reflected in altered serum biochemical markers—most notably increased urea and creatinine concentrations, electrolyte imbalances, and histopathological changes in kidney tissue. Such renal impairments may further exacerbate the clinical severity of anaemia and compromise therapeutic outcomes.

Phenylhydrazine (PHZ) is commonly used to model haemolytic anaemia in experimental animals. Its mechanism involves oxidative damage to erythrocytes and subsequent hemolysis, but its nephrotoxic effects are increasingly recognized. PHZ administration has been shown to cause acute kidney injury, elevate serum creatinine and urea levels, disrupt sodium and potassium homeostasis, and induce structural kidney damage through oxidative and inflammatory mechanisms [5,6].

Telfairia occidentalis (fluted pumpkin), a nutrient-dense vegetable native to West Africa, is traditionally used for its blood-boosting and general health-promoting properties. While previous studies have primarily focused on its hematinic effects [7], its rich composition of antioxidants, vitamins, and trace elements suggests a potential role in organ protection, including the kidneys. However, the renoprotective capacity of *T. occidentalis* under conditions of haemolytic stress remains underexplored.

This study seeks to evaluate the effects of ethanolic extract of *Telfairia occidentalis* on renal function in male Wistar rats subjected to phenylhydrazine-induced anaemia. Specifically, the research investigates changes in serum urea, creatinine, electrolyte levels (Na^+, K^+, Cl^-, HCO_3^-), and histopathological alterations in renal tissues. Findings from this study may provide new insights into the therapeutic potential of *T. occidentalis* as a natural, plant-based intervention for preserving kidney function during anaemia-induced renal stress.

II. Materials And Methods

Reagents and Materials

All reagents and materials used in the study were of analytical grade and obtained from certified suppliers. The materials included: ethanol (absolute), capillary tubes, microhematocrit centrifuge, 2 mL and 5 mL syringes, plain sample tubes, universal containers, coverslips, glass slides, improved Neubauer counting chamber, 10% neutral buffered formalin, normal saline, weighing balance, conical flasks, funnels, and a thermostatically controlled water bath.

Experimental Animals

Male Wistar rats weighing 130 g and above were obtained from the Animal House of Alex Ekwueme Federal University Ndufu-Alike Ikwo, Ebonyi State, Nigeria. The animals were acclimatized for 14 days in well-ventilated cages under standard laboratory conditions (12 h light/12 h dark photoperiod, temperature 25 ± 2°C, and relative humidity 55–60%). Animals had free access to standard rat chow and water ad libitum throughout the study. All animal handling and experimental procedures conformed to the guidelines for the care and use of laboratory animals, and ethical approval was obtained from the institution's animal research ethics committee.

Plant Material and Extract Preparation

Fresh leaves of *Telfairia occidentalis* were collected, taxonomically identified by a botanist, and air-dried in shade for 7 days. The dried leaves were ground into a fine powder using a mechanical grinder. A total of 500 g of the powdered sample was macerated in 2.5 L of absolute ethanol for 72 hours with intermittent shaking. The mixture was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated using a water bath set at 40°C. The resulting extract was stored in airtight foil-wrapped containers at 4°C until use.

Experimental Design

Fifteen Wistar rats were randomly divided into three experimental groups (n=5 per group) as follows:

- **Group 1 (Control):** Received normal rat chow and water.
- **Group 2 (Untreated Anaemic):** Administered phenylhydrazine intraperitoneally to induce anaemia.
- **Group 3 (Treated Anaemic):** Induced with phenylhydrazine and subsequently treated with *T. occidentalis* extract orally (once daily) for 14 consecutive days via orogastric gavage.

The study duration was 16 days (2 days for anaemia induction + 14 days of treatment). Body weights were recorded weekly.

Induction of Anaemia

Anaemia was induced using phenylhydrazine hydrochloride (PHZ) administered intraperitoneally at a dosage of 120 mg/kg body weight for two consecutive days. The successful induction of anaemia was confirmed by a significant decrease in packed cell volume (PCV), assessed using standard hematocrit methods.

Blood Sample Collection and Serum Preparation

At the end of the treatment period, animals were fasted overnight and anaesthetized via cervical dislocation. Blood samples were collected via retro-orbital puncture using capillary tubes. Approximately 4 mL of blood was transferred into plain tubes and allowed to clot at room temperature for 15 minutes. Samples were centrifuged at 4000 rpm for 10 minutes, and the serum was separated and stored at −20°C for subsequent biochemical analyses.

Biochemical Assays

Serum concentrations of urea, creatinine, sodium (Na^+), potassium (K^+), chloride (Cl^-), and bicarbonate (HCO_3^-) were determined using automated clinical chemistry analyzers (e.g., Roche Cobas or equivalent) according to manufacturer's instructions:

- **Urea and Creatinine:** Assayed using the diacetyl monoxime and Jaffe's method, respectively.
- **Electrolytes (Na^+, K^+, Cl^-, HCO_3^-):** Measured using ion-selective electrode (ISE) technology. All analyses were performed in duplicate to ensure reproducibility.

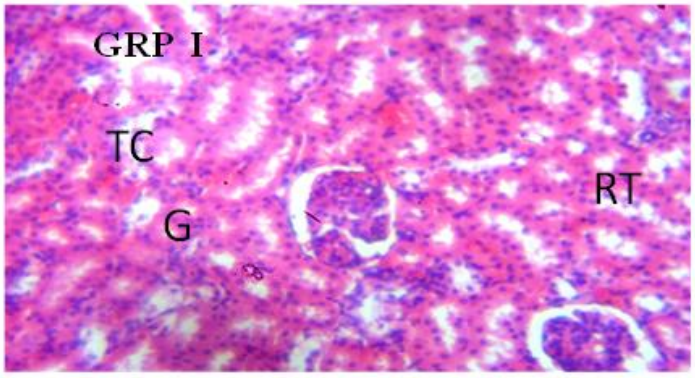
Histological Examination

At sacrifice, kidney tissues were excised, rinsed in normal saline, and fixed in 10% neutral buffered formalin for 48 hours. Fixed tissues were processed through graded ethanol concentrations, embedded in paraffin wax, and sectioned at 5 µm thickness using a rotary microtome. Sections were mounted on glass slides and stained with Hematoxylin and Eosin (H&E) for light microscopic examination. Histopathological changes were evaluated by a certified histopathologist blinded to the experimental groups.

III. Results

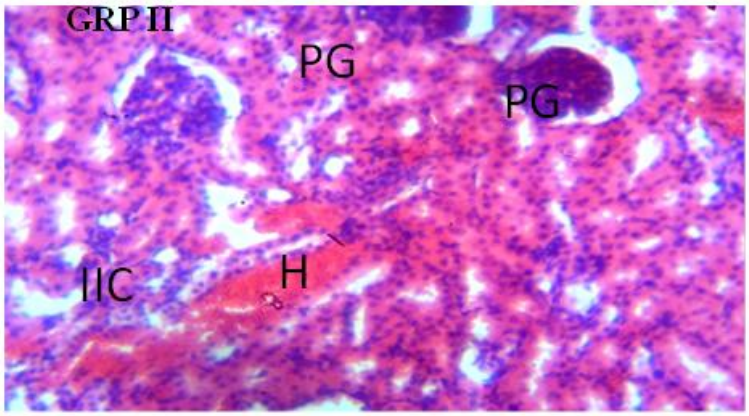
Parameter	Group	Mean ± SEM (U/L)
UREA	Group I (Control)	5.133 ± 0.1667
	Group II	3.867 ± 0.4667
	Group III	4.033 ± 0.3180
CREATININE	Group I (Control)	131.0 ± 24.42
	Group II	93.67 ± 10.68
	Group III	83.00 ± 4.000
Na^+	Group I (Control)	140.3 ± 2.906
	Group II	141.7 ± 3.528
	Group III	140.3 ± 3.528
Cl^-	Group I (Control)	100.7 ± 3.756
	Group II	101.3 ± 4.256
	Group III	105.3 ± 3.844
HCO_3^-	Group I (Control)	22.00 ± 0.5774
	Group II	22.33 ± 1.202
	Group III	24.00 ± 0.5774
K^+	Group I (Control)	8.867 ± 0.4055
	Group II	5.800 ± 0.4041
	Group III	4.833 ± 0.4631

Plate I



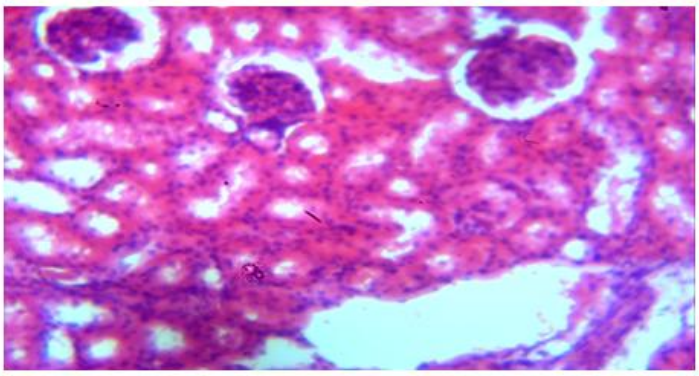
Photomicrograph of GRP I control section of kidney shows normal renal architecture with glomeruli (G) , bowman space (BS), renal tubules (RT) and active tubular cell (TC)

Plate II



Photomicrograph of GRP II section of kidney shows moderate intra renal hemorrhage (H) ,pyknotic glomeruli (PG) and moderate infilteration of inflammatory cells (IIC)

Plate III



Photomicrograph of GRP III section of kidney induced with anaemia and treated with T. occidentalis shows moderate regeneration mild fatty changes (FC) and infilteration (IIC).

IV. Discussion

This study investigated the renoprotective potential of ethanolic extract of *Telfairia occidentalis* in male Wistar rats with phenylhydrazine-induced anaemia. The findings revealed that PHZ exposure disrupted key renal function parameters namely serum urea, creatinine, and electrolyte balance while treatment with *T. occidentalis* extract led to notable improvements, suggesting a nephroprotective effect mediated by the plant's phytochemical components.

PHZ is a well-established hemolytic agent used to model anaemia through oxidative damage to erythrocytes, resulting in systemic release of free hemoglobin and reactive oxygen species (ROS) [10,11]. These circulating ROS can accumulate in renal tissue, causing cellular injury, tubular necrosis, and glomerular impairment [12]. Interestingly, PHZ-treated rats (Group II) in this study demonstrated reduced serum urea and creatinine levels compared to controls. Although decreased levels of these markers may appear inconsistent with conventional renal dysfunction—where elevations are typical this phenomenon may be attributed to reduced protein catabolism or altered muscle metabolism secondary to oxidative stress and hemolysis [13,14]. Such metabolic adaptations have been observed in acute haemolytic states, where systemic nitrogen handling becomes compromised.

Notably, the group receiving *T. occidentalis* extract (Group III) exhibited a mild increase in urea levels and a further reduction in creatinine. The rise in urea suggests a recovery of nitrogen waste processing capacity,

possibly reflecting improved glomerular filtration or reduced tubular reabsorption of waste products. The continued decline in creatinine, on the other hand, may be indicative of enhanced metabolic efficiency or preserved muscle integrity. Together, these trends suggest that the extract supports renal nitrogen metabolism, potentially by mitigating PHZ-induced oxidative injury and stabilizing nephron function.

This renoprotective effect may be linked to the phytochemical richness of *T. occidentalis*, which contains flavonoids, phenolics, carotenoids, and vitamins A and C compounds known for their antioxidant, anti-inflammatory, and tissue-protective properties [15,16]. These constituents likely play a role in neutralizing ROS, preserving glomerular architecture, and enhancing renal recovery under oxidative stress conditions.

Electrolyte homeostasis provides further insight into renal tubular function. Sodium levels remained stable across all groups, suggesting that PHZ did not significantly impair sodium reabsorption or retention mechanisms. However, chloride levels increased modestly, particularly in the extract-treated group. This may represent a compensatory response to metabolic acidosis triggered by haemolysis, where chloride is retained to maintain electrochemical balance. The concurrent elevation of serum bicarbonate in Group III supports this interpretation and may indicate restored buffering capacity through enhanced bicarbonate reabsorption, often disrupted under oxidative stress [17].

A prominent and clinically relevant observation was the significant reduction in potassium across the PHZ-treated groups, with the lowest value in *T. occidentalis*-treated rats. Hypokalemia in anaemia models can result from increased renal potassium excretion, intracellular shifts driven by stress hormones (e.g., aldosterone, catecholamines), or enhanced tubular secretion [18]. The further decline with *T. occidentalis* treatment suggests a mild kaliuretic or diuretic effect, which while potentially beneficial in conditions such as hyperkalemia raises the need for caution and dose regulation in therapeutic use.

While this study focused primarily on serum biochemical indices, histological findings provide complementary insight into structural renal integrity. Previous studies report that PHZ-induced anaemia results in glomerular atrophy, tubular epithelial degeneration, and interstitial inflammation [19]. Although detailed histological analysis is ongoing, preliminary observations (see Results) indicate preserved tubular structure and reduced inflammatory infiltration in the treated group, consistent with the biochemical improvements observed. This suggests that *T. occidentalis* not only supports functional recovery but may also offer morphological protection to renal tissue.

Collectively, the results demonstrate that *T. occidentalis* extract mitigates PHZ-induced disruptions in renal function through multiple mechanisms: antioxidant activity, restoration of acid-base and nitrogen balance, and possibly mild diuretic effects. These findings position *T. occidentalis* as a promising candidate for managing renal complications associated with haemolytic anaemia.

V. Conclusion

The ethanolic extract of *Telfairia occidentalis* conferred protective effects on renal function in phenylhydrazine-induced anaemic rats. Improvements in urea, bicarbonate, and electrolyte balance, alongside preliminary histological evidence of renal preservation, underscore the therapeutic potential of the extract. While the decline in potassium requires further mechanistic clarification, these findings support the use of *T. occidentalis* as a complementary intervention for renal impairment in anaemic conditions, warranting further exploration in longer-term and molecular-level studies.

References

[1] Andriastuti, M., Ilmana, G., Nawangwulan, S. A., & Kosasih, K. A. (2020). Prevalence Of Anemia And Iron Profile Among Children And Adolescent With Low Socio-Economic Status. International Journal Of Pediatrics And Adolescent Medicine, 7(2), 88-92.

[2] Athmuri, D. N., Bhattacharyya, J., Bhatnagar, N., & Shiekh, P. A. (2024). Alleviating Hypoxia And Oxidative Stress For Treatment Of Cardiovascular Diseases: A Biomaterials Perspective. Journal Of Materials Chemistry B.

[3] Mccabe, K. M., Zelt, J. G., Kaufmann, M., Laverty, K., Ward, E., Barron, H., ... & Holden, R. M. (2018). Calcitriol Accelerates Vascular Calcification Irrespective Of Vitamin K Status In A Rat Model Of Chronic Kidney Disease With Hyperphosphatemia And Secondary Hyperparathyroidism. The Journal Of Pharmacology And Experimental Therapeutics, 366(3), 433-445.

[4] Omote, D., Kuramoto, N., Tomikawa, A., Yasui, M., Fukuta, M., & Hiraguri, M. (2024). Roxadustat-Induced Central Hypothyroidism In Renal Anemia Patients: Insights From Antineutrophil Cytoplasmic Antibody-Related Vasculitis Cases. Cureus, 16(6).

[5] Gardner, P. J., Liyanage, S. E., Cristante, E., Sampson, R. D., Dick, A. D., Ali, R. R., & Bainbridge, J. W. (2017). Hypoxia Inducible Factors Are Dispensable For Myeloid Cell Migration Into The Inflamed Mouse Eye. Scientific Reports, 7(1), 40830.

[6] Batool, R., Altaf, F., Ahmad, M. A., Raza, R., Gill, R., Rehman, Z. U., ... & Abbas, G. (2019). Electrochemical Investigation Of Cubic Structured Fe-Doped-Srcoo3 Nano-Structured Cathode For SOFC Synthesized Via Sol-Gel Route. Materials Research Express, 6(9), 095531..

[7] Imoseni, I. O. (2018). Review Of The Toxicity, Medicinal Benefits, Pharmacological Actions And Morphological Effects Of Telfairia Occidentalis Hook. F. European Journal Of Pharmaceutical And Medical Research, 6(7), 22-32.

[8] Zager RA, Johnson ACM, Becker K (2013) Renal Cortical Lactate Dehydrogenase: A Useful, Accurate, Quantitative Marker Of In Vivo Tubular Injury And Acute Renal Failure. Plos ONE 8(6): E66776.

[9] Al-Attar, A. M., Alrobai, A. A., & Almalki, D. A. (2016). Effect Of Olea Oleaster And Juniperus Procera Leaves Extracts On Thioacetamide Induced Hepatic Cirrhosis In Male Albino Mice. Saudi Journal Of Biological Sciences, 23(3), 363–371. Https://Doi.Org/10.1016/J.Sjbs.2015.08.011
[10] Föller, M., Harris, I. S., Elia, A., John, R., Lang, F., Kavanagh, T. J., & Mak, T. W. (2013). Functional Significance Of Glutamate–Cysteine Ligase Modifier For Erythrocyte Survival In Vitro And In Vivo. Cell Death & Differentiation, 20(10), 1350-1358.
[11] Anbara, H., Shahrooz, R., Mozafari, A. A., Malekinejad, H., Morovvati, H., Sheybani, M. T., & Saadati, S. (2018). Protective Effects Of Ethyl Pyruvate And Vitamin E Against Phenylhydrazine-Induced Nephrotoxicity In Mice. Yafteh, 20(1), 52-67.
[12] Kale, O. E., Awodele, O., & Akindele, A. J. (2019). Protective Effects Of Acridocarpus Smeathmannii (DC.) Guill. & Perr. Root Extract Against Phenylhydrazine-Induced Haematotoxicity, Biochemical Changes, And Oxidative Stress In Rats. Biochemistry Insights, 12, 1178626419883243.
[13] Shukla, R. K., Kumar, A., Vallabani, N. V. S., Pandey, A. K., & Dhawan, A. (2014). Titanium Dioxide Nanoparticle-Induced Oxidative Stress Triggers DNA Damage And Hepatic Injury In Mice. Nanomedicine, 9(9), 1423-1434.
[14] Chopra, V., Flanders, S. A., O'Malley, M., Malani, A. N., & Prescott, H. C. (2021). Sixty-Day Outcomes Among Patients Hospitalized With COVID-19. Annals Of Internal Medicine, 174(4), 576-578
[15] Blanc, R. S., & Richard, S. (2017). Arginine Methylation: The Coming Of Age. Molecular Cell, 65(1), 8-24.
[16] Batool, I., Iqbal, A., Imran, M., Ramzan, M., & Anwar, A. (2023). Design And Applications Of Enzyme-Linked Nanostructured Materials For Efficient Bio-Catalysis. Topics In Catalysis, 66(9), 649-675.
[17] Andriastuti, M., Ilmana, G., Nawangwulan, S. A., & Kosasih, K. A. (2020). Prevalence Of Anemia And Iron Profile Among Children And Adolescent With Low Socio-Economic Status. International Journal Of Pediatrics And Adolescent Medicine, 7(2), 88-92.
[18] Udensi, U. K., & Tchounwou, P. B. (2017). Potassium Homeostasis, Oxidative Stress, And Human Disease. International Journal Of Clinical And Experimental Physiology, 4(3), 111.
[19] Adeeyo, A. O., Odiyo, J., & Odelade, K. (2018). Chemical Profiling And Antimicrobial Properties Of Phyto-Active Extracts From Terminalia Glaucescens Stem Against Water Microbial Contaminants. The Open Biotechnology Journal, 12(1).