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Effects of Phyllanthus Amarus Aqueous Extract on Haematological Parameters and Renal Morphology in an Adenine-Induced Rat Model of Chronic Kidney Disease

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ABSTRACT: Chronic kidney disease (CKD) is a progressive condition characterized by declining renal function and is frequently accompanied by secondary anaemia. Phyllanthus amarus is an important medicinal plant traditionally used for managing renal and urinary tract disorders. This study evaluated the effects of an aqueous extract of Phyllanthus amarus (PAAE) on haematological parameters and renal morphology in a Wistar rat model of CKD, with kidney changes assessed by both gross examination and histopathology following haematoxylin and eosin staining. Twenty-four adult male Wistar rats were randomly allocated to four groups (n = 6): normal control, adenine control (100 mg/kg body weight), adenine (100 mg/kg body weight) + PAAE (100 mg/kg body weight), and adenine (100 mg/kg body weight) + PAAE (200 mg/kg body weight). Treatments were administered orally once daily for 28 days. Adenine administration resulted in significant reductions in RBC count, haemoglobin concentration, and packed cell volume. Gross examination revealed pale and irregular kidneys, while histological assessment showed marked tubular dilation, crystal deposition, tubulointerstitial alterations, and epithelial degeneration. Co-administration of PAAE at both doses significantly improved RBC count, Hb concentration, and PCV compared with the adenine-treated group. Renal morphology also improved following PAAE treatment, with the 200 mg/kg dose producing greater restoration of the renal histological architecture. Overall, the findings support the nephroprotective and haematinic potential of Phyllanthus amarus in the experimental CKD model.

1. INTRODUCTION

Chronic kidney disease (CKD) is characterized by progressive damage to glomerular, tubular, and interstitial structures, ultimately resulting in a gradual loss of renal function, becoming one of the fastest-growing causes of death worldwide [1]. It is characterised by proteinuria, a reduced glomerular filtration rate, and progressive glomerular, tubular, and interstitial damage [2]. The main causes of CKD include hypertension, diabetes, advancing age, immune-related disease, glomerulonephritis, and hereditary kidney disorders, and the condition is closely linked to systemic inflammation and oxidative stress that worsen as renal function declines [3,4]. One of the earliest and most consistent systemic consequences of CKD is anaemia. As functioning

renal mass is lost, the peritubular fibroblasts that normally produce erythropoietin are damaged, and the resulting fall in erythropoietin output, together with a shortened red cell lifespan and a degree of iron-utilisation defect, produces a normocytic, normochromic anaemia that contributes substantially to the fatigue, reduced exercise tolerance, and poor quality of life reported by CKD patients [5].

The oral administration of adenine has been widely used as well as easily reproducible for inducing CKD in rodents. Upon systemic entry, adenine is converted into 2,8-dihydroxyadenine, which precipitates within the tubular epithelial cells, specifically localizing to the microvilli and the apical membrane of the cells. The process of this crystal deposition results in tubular degeneration, interstitial inflammation and fibrosis, similar to many of the biochemical and structural features of human CKD, establishing this as a standard model for pre-clinical renal disease studies [6,7].

Anaemia in CKD represents a critical complication. It strongly correlates with reduced exercise capacity, cognitive dysfunction, compromised cardiac function, and increased mortality. Managing this haematological deficit through erythropoiesis-stimulating agents (ESAs), iron supplementation, and novel therapeutics represents a vital pillar of chronic kidney disease care, running parallel to interventions aimed at arresting renal function loss. Consequently, any therapeutic agent capable of exerting dual efficacy by simultaneously stimulating erythropoiesis and mitigating structural renal damage holds profound clinical significance [8].

Conventional therapies offer effective management for CKD complications, yet their widespread utility is constrained by prohibitive costs, limited accessibility, and unfavourable side-effect profiles, sparking a shift toward botanical alternatives [8]. *Phyllanthus amarus* (also known as *Phyllanthus niruri*) is a small herbaceous plant of the family Euphorbiaceae, usually found in central and southern India and in many other tropical regions. It has long been utilized in traditional medication for jaundice, urogenital complaints, dysentery, dyspepsia, arthritis, hepatic disorders, and, notably, kidney and urinary stone disease, from which it derives its popular name, "stone-breaker." Phytochemical studies have identified lignans, flavonoids, alkaloids, tannins, and phenolic acids as its principal bioactive constituents, and pharmacological studies have documented antiviral, anticancer, anti-inflammatory, antioxidant, anti-amnesic, and hepatoprotective activities for the plant [9-14].

A growing body of experimental work supports a protective part for Phyllanthus species in kidney as well as in the blood. Ogunmoyole and colleagues reported that *Phyllanthus amarus* extract restored deranged biochemical parameters in a rat model of combined hepatotoxicity and nephrotoxicity [15]. Reddy and colleagues demonstrated nephroprotective activity of a methanolic extract of *Phyllanthus niruri* [16]. Nwankpa and colleagues showed that *Phyllanthus amarus* leaf extract improved haematological parameters deranged by Salmonella typhi infection in albino rats, indicating that the plant can support red cell parameters even outside the setting of primary renal disease [17]. In the specific context of the adenine model, restoration of erythropoietin-driven erythropoiesis, as shown for Angelica sinensis polysaccharide, has been proposed as one route through which plant-derived agents can correct CKD-associated anaemia [18].

Despite this background, the therapeutic efficacy of *Phyllanthus amarus* aqueous extract (PAAE) on the haematological profile and renal morphology, as confirmed by both gross examination and H&E histopathological evaluation, in the adenine-induced CKD model has not been specifically documented. The current study was therefore carried out to fill this gap, evaluating the effect of PAAE at two oral doses on red cell indices, gross renal appearance, and microscopic kidney architecture in adenine-induced CKD in Wistar rats, as part of a wider investigation into the renal and neuroprotective potential of this plant.

2. MATERIALS AND METHODS

2.1 Ethical clearance

Ethical clearance for the animal experiment was obtained from the Institutional Animal Ethics Committee (IAEC; Approval No. YU/IAEC/25/2020). All experimental procedures involving the animals were conducted in accordance with the institutional requirements for animal welfare and research.

2.2 Experimental animals

Twenty-four healthy male Wistar albino rats, aged 9–10 weeks and weighing 250–300 g, were obtained from Liveon Biolabs Pvt. Ltd., Tumakuru, Karnataka, India (CPCSEA registration No. 1610/RO-Bi-Bt/S/2012/CPCSEA). The animals were maintained in polypropylene cages under controlled laboratory conditions, with a 12-h light/dark cycle. Standard pellet feed

and drinking water were provided ad libitum throughout the experimental period. Prior to treatment, the rats were acclimatized to the laboratory conditions for seven days.

2.3 Chemicals

Adenine was obtained from Durga Laboratory, Mangalore, Karnataka was used to induce chronic kidney disease in the experimental animals.

2.4 Plant material and preparation of *Phyllanthus amarus* aqueous extract (PAAE)

Leaves of *Phyllanthus amarus* were collected, taxonomically identified, and authenticated by a botanist. A voucher specimen (No. 9749) was deposited at the Pilikula Herbarium for future reference. The collected leaves were shade-dried and then coarsely powdered. An aqueous extract was prepared from 400 g of the powdered material using a Soxhlet apparatus, following the method described previously [19]. The extract obtained was concentrated in a water bath maintained below 60°C, giving a yield of 19% w/w. The dried extract was subsequently reconstituted in distilled water to the required concentration and freshly prepared for administration on each day of the experiment.

2.5 Experimental design and grouping

The animals were randomly assigned to four groups, each consisting of six rats, and received their respective treatments once daily throughout the 28-day study period.

Group I: Normal control (Distilled water)

Group II: Adenine control (100 mg/kg body weight, orally)

Group III: Adenine (100 mg/kg body weight, orally) + PAAE (100 mg/kg body weight, orally)

Group IV: Adenine (100 mg/kg body weight, orally) + PAAE (200 mg/kg body weight, orally)

2.6 Sample collection

At the end of the 28-day treatment period, the rats were anesthetized, and blood samples were collected through cardiac puncture into EDTA-containing tubes for subsequent haematological analysis. The animals were then humanely euthanized by cervical dislocation. Both kidneys were promptly excised and rinsed with ice-cold saline to remove adhering blood and tissue debris. The organs were photographed to document their gross morphology and subsequently fixed in 10% neutral buffered formalin for histopathological examination.

2.7 Haematological analysis

Haematological assessment of the collected whole blood was performed using an automated analyser. The analysis included determination of RBC count, Hb concentration, and PCV, along with the erythrocyte indices MCV, MCH, and MCHC.

2.8 Gross morphological examination of the kidney

Following dissection, both kidneys from each animal were examined for size, colour, and surface texture. Representative kidneys from each experimental group were photographed under identical conditions to document gross morphological changes.

2.9 Histopathological examination

For histopathological examination, kidney tissues collected from the experimental groups were initially immersed in 10% neutral buffered formalin for 48 h. Following fixation, the tissues underwent dehydration using ascending concentrations of ethanol and were then processed with xylene as the clearing agent. The cleared specimens were infiltrated and embedded in paraffin wax using an automated tissue processor. Sections measuring 5 µm in thickness were prepared from the paraffin-embedded tissues with a rotary microtome and transferred onto glass slides. The sections were subsequently subjected to haematoxylin and eosin (H&E) staining. After staining, the slides were examined using a light microscope (Olympus, Tokyo, Japan), and representative images were captured for subsequent histopathological assessment.

2.10 Statistical analysis

The results for each experimental group (n = 6) were summarized as mean ± SD. Statistical comparisons among the groups were performed using one-way analysis of variance (ANOVA), with Tukey's HSD test applied for post hoc multiple comparisons. Differences were considered statistically significant when the p-value was below 0.05.

3. RESULTS

3.1 Effect of PAAE on haematological parameters

The erythrocytic parameters recorded for the different experimental groups are presented in Table 1. Compared with the normal control (Group I), the adenine-treated group (Group II) showed a significant decline in RBC count, Hb concentration, and PCV. The corresponding values were 5.83 ± 0.41 × 10^{12}/L, 10.17 ± 1.20 g/dL, and 36.83 ± 6.34% in Group II, compared with 8.83 ± 0.41 × 10^{12}/L, 14.33 ± 0.52 g/dL, and 46.83 ± 1.80%, respectively, in Group I. The differences were highly significant for RBC count and Hb concentration ($p < 0.001$) and significant for PCV ($p < 0.01$). In contrast, the values of MCV, MCH, and MCHC did not differ significantly between the adenine-treated and normal control groups. Taken together, the reduction in RBC count, Hb, and PCV in the absence of appreciable changes in erythrocyte indices indicates a normocytic, normochromic pattern of anaemia, consistent with the haematological abnormalities commonly associated with CKD.

Treatment with PAAE improved the red cell profile in both treatment groups. In Group III, which received adenine together with PAAE at 100 mg/kg, RBC count increased to 8.67 ± 0.52 × 10^{12}/L, while Hb concentration and PCV reached 14.00 ± 0.90 g/dL and 45.83 ± 3.19%, respectively. These values differed significantly from those observed in the adenine control group, with $p < 0.001$ for RBC count and Hb concentration and $p < 0.01$ for PCV. A similar response was noted in Group IV following administration of PAAE at 200 mg/kg. The RBC count, Hb concentration, and PCV in this group were 8.83 ± 0.41 × 10^{12}/L, 13.67 ± 0.52 g/dL, and 46.00 ± 2.37%, respectively, and were significantly higher than those of Group II ($p < 0.001$ for RBC count and Hb; $p < 0.01$ for PCV). When the PAAE-treated groups were compared with the normal control, none of the measured red cell parameters showed a significant difference, suggesting substantial restoration of the haematological profile. No statistically significant differences were detected in MCV, MCH, or MCHC across the experimental groups.

Table 1: Effect of 28-day treatment with PAAE on haematological parameters

Group	Treatment	RBC (10^{12}/L)	Hb (g/dL)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
I	Normal (Distilled water)	8.83 ± 0.41	14.33 ± 0.52	46.83 ± 1.80	55.68 ± 4.18	16.50 ± 1.05	29.67 ± 1.51
II	Adenine control (100 mg/kg b.w.)	5.83 ± 0.41 ^{a***}	10.17 ± 1.20 ^{a***}	36.83 ± 6.34 ^{a**}	53.67 ± 3.56	16.17 ± 0.76	30.00 ± 1.10
III	Adenine (100 mg/kg) + PAAE (100 mg/kg b.w.)	8.67 ± 0.52 ^{b***}	14.00 ± 0.90 ^{b***}	45.83 ± 3.19 ^{b**}	52.17 ± 2.23	15.84 ± 0.99	30.00 ± 0.64
IV	Adenine (100 mg/kg) + PAAE (200 mg/kg b.w.)	8.83 ± 0.41 ^{b***}	13.67 ± 0.52 ^{b***}	46.00 ± 2.37 ^{b**}	54.00 ± 1.10	16.17 ± 0.76	29.50 ± 0.84

Data are presented as mean ± SD for six animals in each group (n = 6). Superscript "a" denotes a significant difference from the normal control, whereas "b" denotes a significant difference from the adenine control. Significance levels are indicated by *, **, and ***, corresponding to $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. Differences among groups were analysed by one-way ANOVA followed by Tukey's HSD multiple-comparison test. RBC, red blood cell; Hb, haemoglobin; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; PAAE, *Phyllanthus amarus* aqueous extract.

3.2 Gross morphological findings of the kidney

Figure 1 displays the gross appearance of representative kidneys from each experimental group. Kidneys from the normal control group (Figure 1A) were smooth, reddish-brown, and bilaterally symmetrical. In contrast, the adenine control group (Figure 1B) showed distinctly paler, mottled kidneys with a rough surface and irregular shapes. This altered morphology reflects the characteristic crystal deposition and structural tissue damage of this model. Treatment with 100 mg/kg PAAE (Figure 1C) resulted in noticeable tissue preservation, showing reduced surface irregularities and less pallor. Furthermore, the kidneys from the 200 mg/kg PAAE group (Figure 1D) closely resembled the normal control organs in both colour and texture. Together, these structural improvements indicate that PAAE protects the renal architecture in a dose-dependent manner.

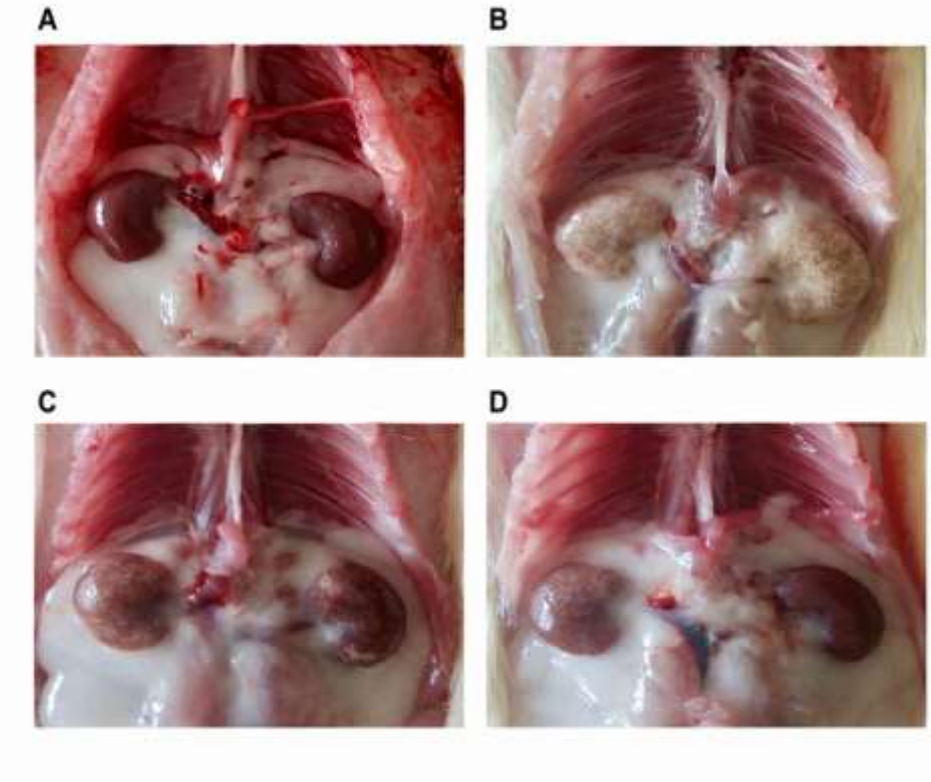

Figure 1. Gross appearance of representative kidneys across experimental groups. (A) Group I (Normal control): smooth, reddish-brown capsular surface. (B) Group II (Adenine control, 100 mg/kg): pale, roughened surface with a notable loss of normal architectural contour. (C) Group III (Adenine 100 mg/kg + PAAE 100 mg/kg): partial recovery of surface coloration and texture. (D) Group IV (Adenine 100 mg/kg + PAAE 200 mg/kg): near-normal gross morphology. PAAE: *Phyllanthus amarus* aqueous extract.

3.3 Histopathological Findings of the Kidney

Microscopic examination of the H&E-stained kidney sections demonstrated clear differences in renal morphology among the four experimental groups, involving the tubular, glomerular, and interstitial regions (Figure 2). The kidneys from the normal control animals (Figure 2A) maintained normal cortical and medullary architecture. The proximal and distal convoluted tubules displayed intact epithelial cells, clearly defined lumina, and preserved brush borders. The interstitial regions showed no evidence of inflammatory cell accumulation or tissue damage.

In contrast, substantial pathological changes were evident in the adenine control group (Figure 2B). Pale, acicular 2,8-dihydroxyadenine crystals were observed within the tubular lumina and represented a prominent feature of the renal lesions.

The tubules showed pronounced dilation together with marked degeneration of the epithelial lining, with areas of epithelial desquamation into the luminal spaces. In addition, the interstitium contained considerable inflammatory cell infiltration, indicating extensive renal tissue injury associated with the adenine-induced CKD model.

In the Adenine + PAAE 100 mg/kg group (Figure 2C), histopathological damage was significantly less severe than that observed in the adenine control cohort. Notable preservation of the tubular architecture was evident, characterized by reduced tubular dilation and a decrease in cellular debris within the lumens. Furthermore, intraluminal crystal deposits were completely absent, and interstitial inflammation was markedly attenuated despite remaining present residually.

In the adenine + PAAE 200 mg/kg group (Figure 2D), the kidney sections showed substantial recovery of the renal architecture. Most renal tubules displayed preserved epithelial lining and patent lumina, while the cortical arrangement appeared similar to that observed in the normal control group. No crystalline deposits were observed in the examined sections. The interstitial areas showed only minimal inflammatory cell infiltration. Overall, the histological findings suggest a pronounced protective effect of the higher PAAE dose on renal tissue, which was consistent with the gross morphological findings.

In summary, the gross morphological and microscopic H&E evaluations reveal a consistent and complementary pattern. The progressive pathological and structural changes induced by adenine are markedly reduced in the kidneys of rats treated with PAAE. This profound tissue protection is evident across both treatment dosages, confirming that the extract effectively halts renal architectural degradation.

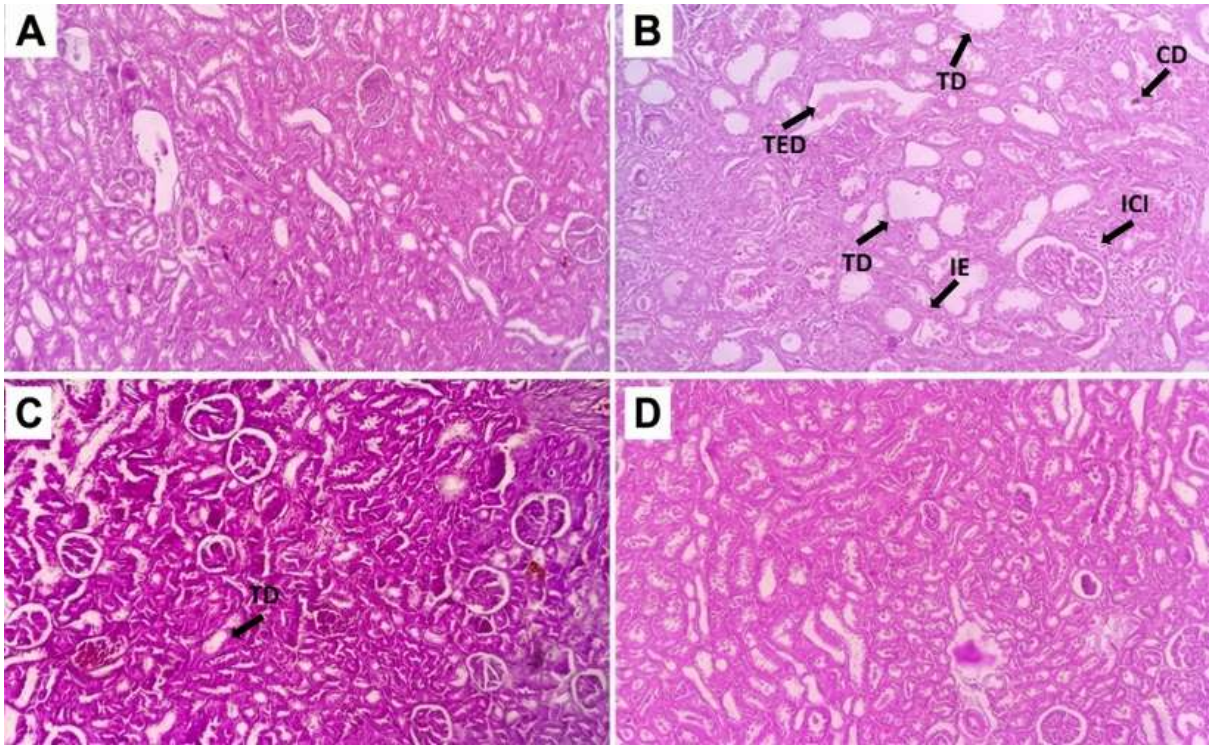

Figure 2. Histopathological examination of kidney tissue sections stained with H&E (100×). (A) Normal control group: intact tubular and glomerular architecture with well-defined brush borders. (B) Adenine control group: widespread structural changes characterized by prominent TD, 2,8-DHA CD, TED, and marked TIC with secondary IE and ICI. (C) Adenine + PAAE 100 mg/kg group: partial restoration of tubular morphology alongside a notable reduction in TD and TIC. (D) Adenine + PAAE 200 mg/kg group: near-normal renal histology with minimal residual pathology. Abbreviations: TD, tubular dilation; 2,8-DHA, 2,8-dihydroxyadenine; CD, crystal deposits; TED, tubular epithelial degeneration; TIC, tubulointerstitial changes; IE, interstitial edema; ICI, inflammatory cell infiltration; PAAE, *Phyllanthus amarus* aqueous extract; H&E, haematoxylin and eosin.

4. DISCUSSION

The findings demonstrate that 28 days of oral adenine administration produced a significant normocytic, normochromic anaemia in Wistar rats, characterized by reductions in RBC count, Hb concentration, and PCV while MCV, MCH, and MCHC remained unchanged. Co-administration of *Phyllanthus amarus* aqueous extract markedly improved these haematological alterations, with the greater response observed at the 200 mg/kg dose. Gross and microscopic examination of the kidneys following H&E staining showed corresponding evidence of renal injury. Adenine administration resulted in visible pallor and surface irregularities of the kidneys, which were confirmed histologically by pronounced tubular degeneration, crystal deposition, and interstitial alterations. PAAE treatment substantially attenuated the renal lesions, and the most pronounced histological recovery was observed in animals receiving 200 mg/kg. At this dose, the renal architecture showed marked improvement and was closer to the appearance of the normal control kidneys.

Anaemia changes observed here is consistent with the pattern typically reported in both clinical and experimental CKD. As renal parenchyma is progressively destroyed by the crystal-induced tubular injury that characterises the adenine model, the peritubular fibroblasts responsible for erythropoietin synthesis are lost along with functioning nephrons, and the resulting fall in erythropoietin drive is a major contributor to the anaemia of CKD, independent of iron status or red cell morphology, which explains why cell size and haemoglobin concentration per cell (MCV, MCH, MCHC) remained unaffected while total red cell mass and haemoglobin fell [5,6]. A comparable mechanism has been invoked to explain the correction of CKD-associated anaemia by a plant-derived polysaccharide from Angelica sinensis, which improved red cell parameters in an adenine model largely by restoring erythropoietin production and iron availability [18]. The present findings indicate that PAAE may act through a comparable pathway, where mitigating ongoing tubulointerstitial injury preserves the erythropoietin-producing capacity of the renal tissue.

The histopathological findings in the present study deserve particular attention, as they provide a mechanistic window into the changes that underlie both the renal morphological observations and the haematological abnormalities. The presence of acicular crystal deposits within tubular lumens in the adenine group confirms the expected mechanism of adenine nephrotoxicity: conversion of adenine to 2,8-dihydroxyadenine followed by tubular precipitation [6,7]. The accompanying tubular degeneration, cellular desquamation, and tubular dilation represent a combination of direct crystal induced injury, secondary inflammation, and progressive tubulo-interstitial changes, which together reduce the number of functioning nephrons and suppress erythropoietin output, thus establishing the link between the structural renal damage and the haematological changes observed. PAAE treatment produced a pronounced, dose-related reduction in these histopathological features, achieving maximum efficacy at the 200 mg/kg dose. This structural preservation aligns with the established antioxidant and anti-inflammatory property of the plant [9-14].

Alternatively, the observed haematological improvement may stem from a more direct hematopoietic or antioxidant action within the bone marrow or on circulating erythrocytes. This possibility is supported by the work of Nwankpa and colleagues, who reported that *Phyllanthus amarus* leaf extract improved haematological parameters that had been deranged by Salmonella typhi infection in albino rats, a model in which anaemia arises largely from infection related haemolysis and marrow suppression rather than from renal erythropoietin deficiency [17].The fact that *Phyllanthus amarus* improves red cell parameters across at least two mechanistically distinct models of anaemia proposes that the plant might have a generalised protective influence on erythropoiesis, possibly mediated by its antioxidant phytoconstituents, which could limit oxidative damage to developing red cells and to the marrow microenvironment in addition to any renal-specific action.

The gross and microscopic renal findings in the current study are reliable with previous reports of nephroprotective activity for Phyllanthus species across different experimental models. Ogunmoyole and colleagues showed that *Phyllanthus amarus* extract restored deranged biochemical parameters in a combined model of hepatotoxicity and nephrotoxicity [15]. Adeneye and Benebo reported that aqueous leaf and seed extracts of *Phyllanthus amarus* protected against gentamicin- and acetaminophen-induced nephrotoxicity in rats [20]. Hashim et al. demonstrated that ethanolic *Phyllanthus amarus* leaf extract markedly reduced histopathological evidence of tubular degeneration, glomerular shrinkage, and haemorrhage in a sodium arsenite model of nephrotoxicity, restoring a near-normal microscopic architecture at the tissue level [21]. The present study adds to this body of evidence by demonstrating, for the first time, a similar structural protection in the adenine CKD model, with the important advantage that the adenine model more closely reproduces the progressive interstitial and tubular disease of clinical CKD than acute toxicant models.

The mechanisms proposed for this protective activity centre on the rich antioxidant and anti-inflammatory phytochemistry of the plant, including lignans, flavonoids, tannins, and phenolic acids, which are believed to scavenge reactive oxygen species and limit the pro-inflammatory cascade that accompanies tubular injury [9-14]. In addition, *Phyllanthus* species have long been reported to possess a mild diuretic action, with earlier work showing a significant increase in urinary excretion of sodium, potassium, and chloride, which could help promote clearance of the offending crystals from the tubular lumen and thereby reduce ongoing mechanical and inflammatory injury [22]. Together, these overlapping antioxidant, anti-inflammatory, and mildly diuretic properties provide a plausible, multi-pronged mechanistic explanation for the preservation of renal architecture and the improvement in red cell parameters seen with PAAE treatment in the present study.

Finally, anaemia and renal structural damage are not merely coincidental complications of CKD, but rather reciprocal comorbidities that exacerbate each other. The depletion of functional renal tissue severely compromises local oxygenation, which drives progressive renal fibrosis and worsens hypoxia-induced tubular inflammation. This tissue damage subsequently decreases erythropoietin production, further severe worsening the anaemia. By disrupting this destructive cycle, PAAE treatment achieved the concurrent improvements observed at both the haematological and renal morphological levels in the present study.

Although the present study includes detailed gross morphological and histopathological examinations of renal tissue, several limitations must be acknowledged. First, serum functional parameters were not analysed; evaluating markers related to renal function would have effectively complemented the structural findings. Additionally, while PAAE demonstrated positive effects on red blood cell indices, a comprehensive mechanism requires the direct measurement of erythropoietin levels, iron status, inflammatory cytokines, and oxidative stress markers in future investigations. Finally, employing specialized histological techniques, such as Masson's trichrome staining or immunohistochemical analyses, would provide deeper insights into the precise extent of renal fibrosis and tissue repair.

5. CONCLUSION

In the present study, adenine administration produced a significant normocytic, normochromic anaemia in Wistar rats, accompanied by pronounced renal injury. Histological examination demonstrated several features of renal damage, including tubular dilation and degeneration, crystal deposition, and interstitial changes. Co-treatment with the aqueous extract of *Phyllanthus amarus* substantially improved both the haematological abnormalities and renal histological changes, with the 200 mg/kg dose producing the greatest degree of recovery. The similar patterns observed in the gross morphological and microscopic findings further support the nephroprotective and haematological effects of *P. amarus* in the experimental CKD model. Overall, the findings provide experimental support for the traditional application of *P. amarus* in renal conditions and indicate its potential for further preclinical investigation.

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Conflict of Interest

The authors report no conflicts of interest related to this study.

REFERENCES

[1] Kok HM, Falke LL, Goldschmeding R, Nguyen TQ. Targeting CTGF, EGF and PDGF pathways to prevent progression of kidney disease. Nat Rev Nephrol. 2014;10:700-711.

[2] Stack AG, Casserly LF, Cronin CJ, Chernenko T, Cullen W, Hannigan A, Saran R, Johnson H, Browne G, Ferguson JP. Prevalence and variation of chronic kidney disease in the Irish Health System: initial findings from the National Kidney Disease Surveillance Programme. BMC Nephrol. 2014;15:185.

[3] Cantaluppi V, Quercia AD, Dellepiane S, Ferrario S, Camussi G, Biancone L. Interaction between systemic inflammation and renal tubular epithelial cells. Nephrol Dial Transplant. 2014;29:2004-2011.
[4] Rubattu S, Mennuni S, Testa M, Mennuni M, Pierelli G, Pagliaro B, Gabriele E, Coluccia R, Autore C, Volpe M. Pathogenesis of chronic cardiorenal syndrome: is there a role for oxidative stress? Int J Mol Sci. 2013;14:23011-23032.
[5] Ikeda R, Imai Y, Maruyama W, Mizoguchi K. Systemic disorders of calcium dynamics in rats with adenine-induced renal failure: implication for chronic kidney disease-related complications. Nephrology (Carlton). 2010;15:54-62.
[6] Al Za'abi M, Al Busaidi M, Yasin J, Schupp N, Nemmar A, Ali BH. Development of a new model for the induction of chronic kidney disease via intraperitoneal adenine administration, and the effect of treatment with gum acacia thereon. Am J Transl Res. 2015;7:28-38.
[7] Ali BH, Al Zaabi A, Al Husseni I, Beegum S, Nemmar A. Motor and behavioral changes in rats with adenine-induced chronic renal failure: influence of acacia gum treatment. Exp Biol Med. 2011;236:107-112.
[8] Ali BH, Adham SA, Al Za'abi M, Waly MI, Yasin J, Nemmar A, Schupp N. Ameliorative effect of chrysin on adenine-induced chronic kidney disease in rats. PLoS One. 2015;10:e0125285.
[9] Calixto JB, Santos AR, Cechinel Filho V, Yunes RA. A review of the plants of the genus *Phyllanthus*: their chemistry, pharmacology, and therapeutic potential. Med Res Rev. 1998;18:225-258.
[10] Sripanidkulchai B, Tattawasart U, Laupatarakasem P, Vinitketkumneun U, Sripanidkulchai K, Furihata C, Matsushima T. Antimutagenic and anticarcinogenic effects of *Phyllanthus amarus*. Phytomedicine. 2002;9:26-32.
[11] Rajeshkumar NV, Joy KL, Kuttan G, Ramsewak RS, Nair MG, Kuttan R. Antitumour and anticarcinogenic activity of *Phyllanthus amarus* extract. J Ethnopharmacol. 2002;81:17-22.
[12] Bhattacharjee R, Sil PC. The protein fraction of *Phyllanthus niruri* plays a protective role against acetaminophen-induced hepatic disorder via its antioxidant properties. Phytother Res. 2006;20:595-601.
[13] Manjrekar AP, Jisha V, Bag PP, Adhikary B, Pai MM, Hegde A, Nandini M. Effect of *Phyllanthus niruri* Linn. treatment on liver, kidney and testes in CCl4-induced hepatotoxic rats. Indian J Exp Biol. 2008;46:514-520.
[14] Latha P, Chaitanya D, Rukkumani R. Protective effect of *Phyllanthus niruri* on alcohol and heated sunflower oil induced hyperlipidemia in Wistar rats. Toxicol Mech Methods. 2010;20:498-503.
[15] Ogunmoyole T, Awodooju M, Idowu S, Daramola O. *Phyllanthus amarus* extract restored deranged biochemical parameters in rat model of hepatotoxicity and nephrotoxicity. *Heliyon*. 2020;6:e05670.
[16] Reddy GS, Raparla LP, Reddy GR, Charan DV. Evaluation of nephroprotective activity of the methanolic extract of *Phyllanthus niruri* (Family-Euphorbiaceae). **Int J Pharm Phytopharmacol Res.** 2015;4(5):276–280
[17] Nwankpa P, Agomuo EN, Uloneme GC, Egwurugwu JN, Omeh YN, Nwakwuo GC. Effect of *Phyllanthus amarus* leaf extract on alterations of haematological parameters in *Salmonellae typhi* infested Wistar albino rats. **Sci Res Essays.** 2014;9(1):7–12. doi:10.5897/SRE2013.5611.
[18] Wang K, Wu J, Xu J, Gu S, Li Q, Cao P, Li M, Zhang Y, Zeng F. Correction of anemia in chronic kidney disease with *Angelica sinensis* polysaccharide via restoring EPO production and improving iron availability. *Front Pharmacol.* **2018**;9:803. doi:10.3389/fphar.2018.00803.
[19] Hegde KS, Bhat SS, Chandrashekhar S, Manikkoth S. Pre-clinical screening of indigenous medicinal plant *Phyllanthus amarus* for its analgesic activity. Int J Adv Biol Biomed Res. 2014 Sep;5(4):1194-1199.
[20] Adeneye AA, Benebo AS. Protective effect of the aqueous leaf and seed extract of *Phyllanthus amarus* on gentamicin and acetaminophen-induced nephrotoxic rats. J Ethnopharmacol. 2008;118:318-323.
[21] Hashim A, Prasanna KS, Nizamudeen AS. Effect of *Phyllanthus amarus* in sodium arsenite-induced tissue damage. Bangladesh J Pharmacol. 2022;17:79-83.
[22] Udupa AL, Sanjeeva, Benegal A, Prusty V, Kodancha GP, Satish Kumar MC, Bhat V, Ratnakar UP. Diuretic activity of *Phyllanthus niruri* (Linn.) in rats. **Health.** 2010;2(5):511–512.
[23] Bancroft JD, Gamble M. Theory and Practice of Histopathological Techniques. 8th ed. Philadelphia: Churchill Livingstone Elsevier; 2019.