INFORMATICS JOURNALS

Research Article

Journal of Natural Remedies
DOI: 10.18311/jnr/2026/52769

eISSN: 2320-3358 | Vol 26(3), 2026 | pp. 661-672

Assessment of Nephroprotective Effect of Ethanolic Leaf Extract of *Rubus ellipticus* in Adenine-induced Chronic Kidney Disease in Wistar Albino Rats

Keranap James Ravichandran[1], Padmaja Sukumar[1], Rakeshkumar Kesavaraman Rajaram[1], Jane Betsy Isaac[2], Vivek Kumar Dhiman[3], Susmitha Kalaichelvan[3] and Vijayarangan Sivasamy[1]*

[1]Department of Pharmacology, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam - 603103, Tamil Nadu, India; drsvr1976@gmail.com

[2]Department of Pathology, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakkam - 603103, Tamil Nadu, India

[3]Centre for Herbal Pharmacology and Environmental Sustainability, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education, Kelambakam, - 603103, Tamil Nadu, India

Abstract

Background: Chronic Kidney Disease (CKD) is a progressive condition marked by persistent renal impairment and systemic consequences. Contemporary therapy strategies frequently encounter constraints due to undesirable effects and insufficient restoration of renal function. *Rubus ellipticus* is a medicinal plant abundant in bioactive phytochemicals; yet its nephroprotective efficacy has not been sufficiently assessed in experimental chronic kidney disease models. **Aim:** To evaluate the nephroprotective effect of ethanolic leaf extract of *R. ellipticus* in an adenine-induced CKD model using Wistar albino rats. **Methods:** Twenty-seven male Wistar albino rats were randomly allocated into five groups. Chronic kidney disease was produced in four groups with oral administration of adenine for a duration of 28 days. Treatment groups were administered either silymarin or *R. ellipticus* extract (200 and 400 mg/kg) through oral gavage. Physiological parameters, renal and hepatic biochemical indicators, haematological indices, and renal histology were assessed. The extract underwent phytochemical characterisation using GC-MS analysis. **Results:** GC-MS analysis identified eight principal phytochemical elements in the ethanolic extract. The administration of adenine led to substantial decreases in body weight, water consumption, and urine production, accompanied by increased blood urea nitrogen (BUN) (~33.4 mg/dL) and serum creatinine concentrations (~8.66 mg/dL), compared to the control group ($p< 0.05$). The *R. ellipticus* (400mg/kg) showed a significant reduction in BUN (~16.8mg/dL) and creatinine (~1.81 mg/dL), compared to CKD controls ($p<0.05$). Significant improvements in serum albumin (~3.6-4.1 g/dL), haemoglobin (>13 g/dL), and liver enzymes were also observed in this group ($p < 0.05$)in this group. **Conclusion:** The ethanolic leaf extract of *R. ellipticus* demonstrated dose-dependent nephroprotective benefits in adenine-induced CKD rats, with optimal efficacy noted at 400 mg/kg. These finding supports the potential of *R. ellipticus* as a supplementary treatment candidate for CKD, necessitating additional mechanistic and translational investigations. **Major Finding:** The ethanolic leaf extract of *R. ellipticus* shows significant dose-dependent nephroprotective effects, markedly reducing BUN and serum creatinine in adenine-induced CKD rats. These findings highlight its potential as a supportive therapeutic candidate in CKD.

Keywords: Chronic Kidney Disease, Nephroprotective Effect, *Rubus ellipticus*

Abbreviations: ALP - Alkaline Phosphatase; ALT - Alanine Transaminase; AST - Aspartate Transaminase; CKD - Chronic Kidney Disease; DBP - Dibutyl Phthalate; ESRD - End-Stage Renal Disease; GC-MS - Gas Chromatography-Mass Spectrometry; IAEC - Institute Animal and Ethics Committee; LFT - Liver Function Test; WHO - World Health Organisation.

(Article Chronicle: Article Received on: 24.10.2025; Revised on: 09.02.2026; Accepted on: 12.03.2026)

*Author for correspondence

1. Introduction

Chronic Kidney Disease (CKD) is an increasingly prevalent public health concern worldwide, impacting both developed and developing nations. A progressive and irreversible decline in renal function was the major characteristic of CKD, denoted by enduring damage to the renal parenchyma lasting over three months, with association of anatomical and functional abnormalities[1]. The CKD illness progresses through five clinical phases, starting with asymptomatic decline in kidney function, which leads to full renal failure or End-Stage Renal Disease (ESRD), which needs renal transplantation for survival[2]. As per World Health Organisation (WHO) data[3]. CKD is one of the top 10 causes of global health. Old age, sedentary life habits, and dietary modifications are associated with the rise of CKD[4]. Risk factors like diabetes mellitus, hypertension, obesity and cardiovascular disorders were found to increase the prevalence of CKD[4]. CKD often involves persistent inflammation, glomerular hypertension, oxidative stress, and tubulointerstitial fibrosis, which may lead to a gradual decline in renal function, along with histopathological alterations such as glomerulosclerosis, tubular atrophy, and interstitial inflammation and fibrosis. These alterations affect the kidney's normal capacity to sustain regulation of fluid and electrolyte equilibrium, management of blood pressure and its capacity to expel the metabolic waste, leading to uremia and systemic toxicity[5]. Anaemia, bone mineral abnormalities, cardiovascular dysfunction, vulnerability to infections, reduced quality of life, and longevity were the problems that the individual with CKD may encounter.

Currently, there has been a growing need for natural goods and plant-based medications as supplementary or alternative treatments for chronic illnesses, including renal problems. Historically, medicinal plants have served as a high-yielding supply of bioactive chemicals with therapeutic efficacy[6]. Their application in ancient medicine systems like Unani, Traditional Chinese Medicine, and Unani demonstrates their extensive pharmacological properties, including anti-inflammatory, antifibrotic, antioxidant and diuretic actions. Numerous research has demonstrated the effectiveness of herbal extracts in enhancing renal function, regulating oxidative stress indicators and safeguarding histoarchitecture in models of nephrotoxicity and CKD[7]. Phytochemicals of these plants are nephroprotective by alleviating oxidative damage, reducing inflammation, and inhibiting fibrosis. *R. ellipticus*, a spiny shrub from the family of Rosaceae commonly called the yellow Himalayan raspberry, is extensively found in the Himalayan areas of India, Nepal, Bhutan, and some portions of Southeast Asia[8]. Conventional use of *R. ellipticus* for several diseases, including diarrhoea, respiratory infections, wounds, inflammation, and microbial infections, is seen in traditional medicinal practices. Due to the presence of abundant phytochemicals, Ethnobotanical surveys and pharmacological investigations indicate that *R. ellipticus* has several medicinal properties[8]. Initial phytochemical analysis on *R. ellipticus* has evidently proved the presence of saponins, flavonoids, phenolics, alkaloids, tannins and terpenoids, in numerous regions of the plant, particularly in its leaves and fruits. These bioactive compounds are known to exhibit antioxidant, anti-inflammatory, antibacterial, hepatoprotective and anticancer properties. Several studies have found that leaf and fruit extract of *R. ellipticus* restricts the proliferation of harmful bacteria, such as "*Escherichia coli*"[9], "*Staphylococcus aureus*"[8,10], "*Pseudomonas aeruginosa*"[11,12] and "*Klebsiella pneumoniae*"[13]. Despite many positive attributes of *R. ellipticus,* there is only a few scientific evidences available, proving its renal protective effects, especially in models of CKD.

In this research, we used an adenine-induced CKD model in Wistar albino rats to assess the possible nephroprotective effects of *R. ellipticus*[14]. The oral intake of adenine results in the deposition of 2,8-dihydroxyadenine crystals within renal tubules, leading to tubular blockage, interstitial inflammation, fibrosis, and progressive renal impairment. The current study evaluates the nephroprotective properties of the ethanolic leaf extract of R. ellipticus, which is expected to reduce renal damage and restore normal kidney architecture and function, likely due to its anti-inflammatory and antioxidant effects. This research could considerably progress the production of innovative novel plant-derived treatments for the prevention and management of CKD by confirming the therapeutic potential of *R. ellipticus* via scientific investigation. The study aimed to evaluate the nephroprotective effect of the ethanolic leaf extract of

R. ellipticus in an adenine-induced CKD model using Wistar albino rats.

2. Materials and Methods

2.1 Identification and Preparation of Plant Extract

The plant material was identified and authenticated by the Botanical Survey of India, High Altitude Western Himalayan Regional Centre, Nauni, Solan, Himachal Pradesh, India (Acc. No. BSI/HAWHRC/Tech./2024-25/664). Fresh *R. ellipticus* leaves were washed with water and dried in the shade for three days. The dried leaves were then made into a fine powder. A total of 100 g of powdered leaf material was extracted with 400 mL of ethanol in a Soxhlet apparatus for 72 h at room temperature. The crude extract obtained was filtered and stored at 4 °C for further analysis. Characterisation of the plant extract was carried out using GC-MS (Gas Chromatography-Mass Spectrometry)[15].

2.2 Phytochemical Screening

The preliminary phytochemical screening of *R. ellipticus* leaf extract was carried out using standard qualitative tests[16-30].

2.3 Antioxidant Activity (DPPH Reduction Assay)

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay was used to evaluate the antioxidant activity of the plant extract[31]. 1 mL of 0.4% DPPH solution was added to 1 mL of the test sample in a test tube, and the mixture was incubated in the dark for 30 min. The absorbance of the resultant solution was measured at 517 nm. The decrease in absorbance, relative to the control (blank), was used to calculate the radical scavenging activity, expressed as a percentage of DPPH inhibition. The IC50 (concentration of extracts resulting in 50 % reduction of the DPPH radical) values were calculated using linear regression analysis with GraphPad Prism software. The percentage of DPPH scavenging activity was calculated using the formula:

DPPH Scavenging activity (%) = Abs of control - Abs of sample/Abs of control x 100[32]

The experiment was performed with ascorbic acid as a standard antioxidant compound, and the AEAC (Ascorbic Acid Equivalent Antioxidant Capacity) was calculated for the test sample with the formula (IC_{50} of Ascorbic acid / IC_{50} of test sample) x 100[33].

2.4 Anti-inflammatory Activity (Egg Albumin Denaturation Assay)

The egg albumin denaturation method was used to demonstrate the anti-inflammatory activity. The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (fresh hen's egg), 2.8 mL of phosphate-buffered saline (PBS, pH 6.4), and 2 mL of the test sample at varying concentrations. An equal volume of double-distilled water was used as a control. The mixtures were incubated at 37 ± 2°C for 15 min and then heated at 70°C for 5 min. Following cooling, absorbance was assessed at 660 nm. Diclofenac sodium was used as the reference standard. The percentage inhibition of protein denaturation was calculated using the following formula[34]:

$$\% \text{ inhibition} = 100 \times [V_t / V_C - 1]$$

where,

V_t = absorbance of the test sample,

V_C = absorbance of control.

2.5 *In Vivo* Animal Study

2.5.1 *Study Design and Grouping*

This study was started after obtaining ethical clearance from the Institutional Animal Ethics Committee (IAEC 1/Proposal: 157/A. Lr:119/Dt20.02.2024) of Chettinad Academy of Research and Education, Kelambakkam, Tamil Nadu 6030103. The research utilised twenty-seven male Wistar albino rats, each weighing between 150 to 250 g. These animals were acclimatised to a 12-h light/dark cycle, regulated temperature (22 ± 2°C), and relative humidity (55 ± 5%). Throughout the trial, food and water were freely available to the animals. The rats were categorised into five groups (Groups I-V). In control (Group I), n is 3, whereas from Group II-V, the n value is 6.

2.5.2 *Induction of CKD*

CKD was induced experimentally in Groups II to V using adenine. Adenine at a concentration of 0.75%, dissolved in distilled water, and dosed at 40 mg per kg of body weight, was given once daily through oral gavage.

2.5.3 Treatment Administration

All treatments were given once daily through oral gavage for a duration of 28 days. In Group I, the normal control group, normal saline (10 ml/kg) was administered. Group II, the CKD control group, received adenine alone to establish the disease model. Group III, the standard treatment group, received adenine along with silymarin (200 mg/kg), the standard treatment for nephroprotection[35-37]. Groups IV and V received adenine in combination with *R. ellipticus* leaf extract of 200 mg/kg and 400 mg/kg, respectively.

2.5.4 Physiological and Biochemical Parameters

During the experiment, all animals were carefully observed for mortality and overall health. Physiological measures such as body weight, 24-h water consumption, and 24-h urine excretion were evaluated weekly. In addition, blood parameters such as serum creatinine, Blood Urea Nitrogen (BUN), serum total protein, and albumin concentrations were evaluated weekly. A Complete Blood Count (CBC) and Liver Function Tests (LFT) were recorded at baseline and at 28th day. Blood samples for these tests were obtained via retro-orbital puncture under appropriate anaesthesia. Urine specimens were collected using metabolic cages, which are specifically designed to quantify 24-h output.

2.5.5 Histopathological Analysis

On day 28 after the completion of the study, all rats were humanely euthanised using high-dose halothane anaesthesia. After being extracted, the kidneys were preserved in 10% formalin for histological analysis. Tissue slices were processed, stained, and analysed microscopically for cellular changes.

3. Statistical Analyses

The data were expressed as mean ± standard deviation. GraphPad Prism 7.0 software (La Jolla, CA, USA) was used in analysing the data statistically. A one-way ANOVA and Tukey's multiple comparison test were employed for comparison between the groups. All results were considered statistically significant at $p<0.05$.

4. Result

4.1 Phytochemical Analysis

The phytochemical analysis of *R. ellipticus* leaf extract indicated the existence of phenols, flavonoids, tannins, glycosides, terpenoids, anthraquinones, alkaloids, carbohydrates and proteins.

4.2 GC-MS

After comparing the GC-MS chromatogram of ethanol leaf extracts from *R. ellipticus* with that of known bioactive compounds from the NIST library, eight peaks were identified. These peaks were identified by peak retention time, peak area (%), height (%), and mass spectral fragmentation patterns (Figure 1) (Table 1).

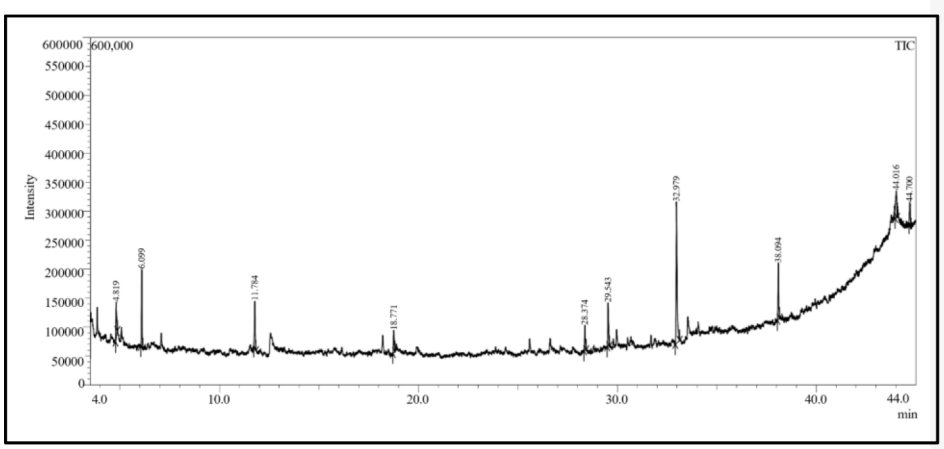


Figure 1. Representation of GCMS chromatogram showing phytoconstituents detected in ethanol leaf extract of *R. ellipticus*.

Table 1. Phytochemical constituents identified in the ethanol leaf extract of *R. ellipticus* using GC-MS

S. No.	Compound CID	Chemical name	Molecular Formula	Molecular Weight (g/mol)	Peak Area (%)
1	5366244	2-hexadecen-1-ol, 3,7,11,15-tetramethyl	$C_{20}H_{40}O$	296.5	32.65
2	12389	Tetradecane	$C_{14}H_{30}$	198.39	20.34
3	3026	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34	13.27
4	88669911	Hexane dioic acid, bis (2-ethylhex	$C_{27}H_{50}O_8$	502.7	10.01
5	91745395	Silane, dimethyl (docosyloxy)butoxy-	$C_{28}H_{60}O_2Si$	456.9	7.75
6	180.16	dl-Glyceraldehyde dimer	$C_6H_{12}O_6$	180.16	6.52
7	638072	Squalene	$C_{30}H_{50}$	410.7	4.97
8	11006	Hexadecane	$C_{16}H_{34}$	226.44	4.50

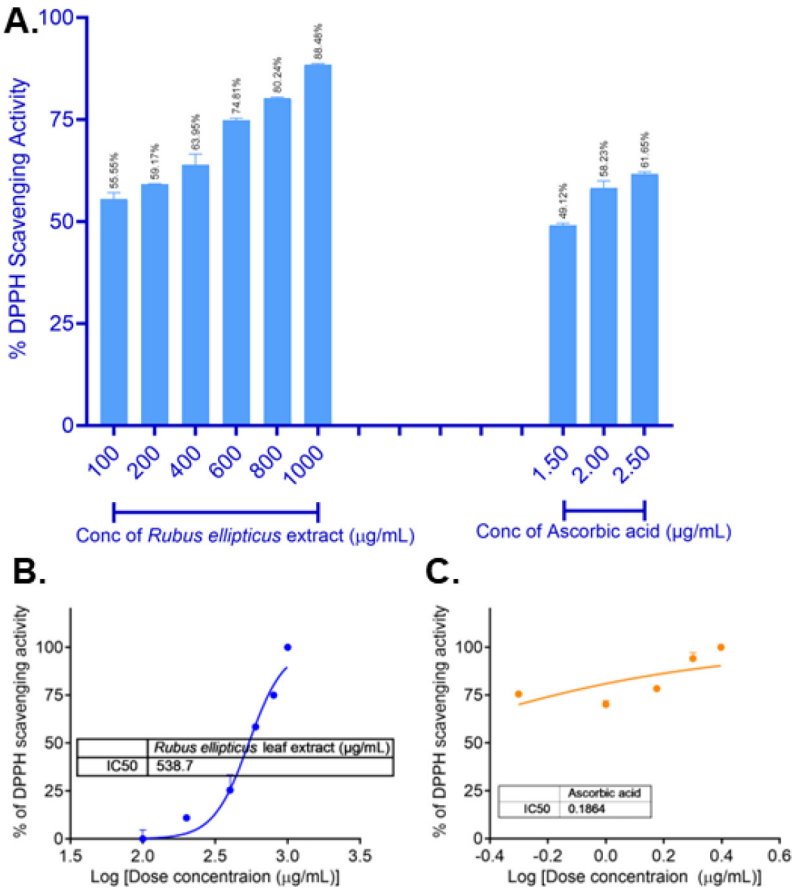


Figure 2. Evaluation of antioxidant activity of *R. ellipticus* leaf extract and Ascorbic acid; **A).** Dose-dependent DPPH radical scavenging activity (%); **B).** IC_{50} value of *R. ellipticus* leaf extract using DPPH assay; **C).** IC_{50} of Ascorbic acid extract using DPPH assay.

Phytochemicals in the plant extract were identified using preliminary phytochemical screening and GC-MS analysis, revealing substances with diverse biochemical characteristics.

4.3 Antioxidant Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay was used to evaluate the antioxidant activity of the plant extract (Figure 2). The extract

showed a concentration-dependent increase in radical scavenging activity, with values of 55.65%, 59.71%, 63.96%, 74.61%, 80.25%, and 88.46% at concentrations of 100, 200, 400, 600, 800, and 1000 µg/mL, respectively (Figure 2A). Hence, the more effective neutralisation of free radicals is seen at higher concentrations. The IC_{50} of *R. ellipticus* extract was 538.7 µg/mL (Figure 2B), while the IC_{50} of ascorbic acid was 0.1864 µg/mL (Figure 2C). Thus, the quantifiable antioxidant capacity was demonstrated by *R. ellipticus* extract. The ascorbic acid equivalent Antioxidant Capacity (AEAC) was 0.0346%, showing its ability to stabilise free radicals and to donate electrons. Compared with ascorbic acid, the extract's potency is considerably weaker, although significant scavenging activity at higher concentrations suggests the presence of bioactive phytochemicals with antioxidant potential that are dose-dependent.

4.4 Anti-inflammatory Activity

The anti-inflammatory activity of *R. ellipticus* leaf extract was tested using the egg albumin denaturation experiment, and it was compared with the standard drug diclofenac (Figure 3). A concentration-dependent increase in protein denaturation inhibition was observed. At concentrations of 100, 200, 300, 400, and 500 µg/mL, the extract exhibited 9%, 32%, 46%, 67%, and 80% inhibition, respectively. In contrast, diclofenac demonstrated markedly higher inhibition at the same concentrations, with 35%, 75%, 77%, 87%, and 93% inhibition, respectively. Statistical analysis

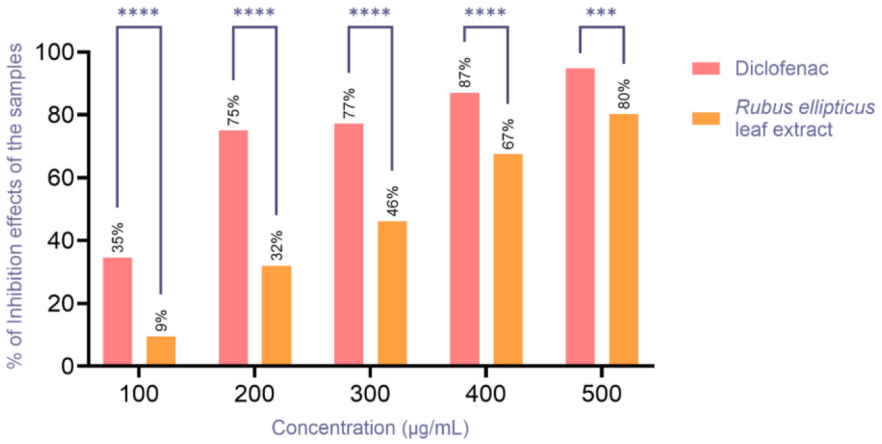


Figure 3. Evaluation of anti-inflammatory activity of *R. ellipticus* leaf extract compared with diclofenac using egg albumin assay.

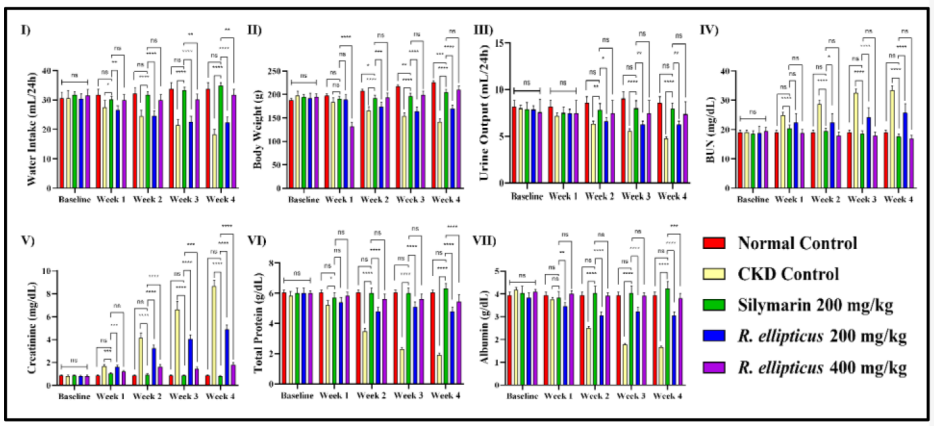


Figure 4. Physiological and biochemical analysis, (I) water intake, (II) body weight, (III) urine output level, (IV) BUN level, (V) creatinine level, (VI) total protein level and (VII) albumin level. Values are presented as mean (n=6). ****p<0.0005, ***p<0.005, ***p<0.05 and ns is for non-significant.

revealed a highly significant difference in inhibition between the extract and diclofenac at 100-400 µg/mL and a moderately significant difference at 500 µg/mL. The results show that at lower concentrations, the anti-inflammatory action of *R. ellipticus* leaf extract is weaker than that of diclofenac. However, at greater concentrations, it becomes almost as effective as the standard medicine.

4.5 *In Vivo* Animal Study

4.5.1 Physiological and Biochemical Parameters

A clear progression was observed in responses of physiological and biochemical parameters across the treatment groups over four weeks (Figure 4). Water intake, body weight, and urine output remained stable or increased slightly throughout the study period, indicating normal physiology. There was progressive deterioration in water intake (~30 ml from baseline to ~16-20 ml/day by week 4), along with a concurrent decrease in urine output, and a decrease in body weight was observed in the CKD control group. A dose-dependent effect was observed with Silymarin and R. ellipticus on these parameters. Biochemically, blood urea nitrogen (BUN) increased sharply in the CKD group (up to ~35 mg/dL and 9 mg/dL, respectively) from baseline to week 4 ($p<0.05$), while Silymarin and *Rubus*-treated groups, especially the 400 mg/kg, maintained near-normal levels. Serum creatinine levels also increased from baseline to week 4 in the CKD group ($p<0.05$). Meanwhile, silymarin showed no significant differences in creatinine levels from baseline to week 4. The low dose *R. ellipticus* (200mg/kg) elicited a profound increase in serum creatinine levels when compared to the silymarin and high dose (400mg/kg) group ($p<0.05$). In the *R. ellipticus* 400 mg/kg group, the serum creatinine levels at week 4 were significantly lower when compared with the CKD control. At week 4, BUN levels remained below 20 mg/dL in the 400 mg/kg *R. ellipticus*, and creatinine levels were approximately 1.5-2 mg/dL. A similar protective trend was observed in serum total protein and albumin levels, which declined significantly in CKD induced rats (albumin reduced to ~1.6 g/dL), indicating protein loss. Conversely, *Rubus* 400 mg/kg groups preserved serum protein (~5.1-6.1 g/dL) and albumin (~3.6-4.1 g/dL) ($p<0.05$), demonstrating effective attenuation of renal dysfunction and systemic protein depletion.

In CKD control rats, there was a marked decline in RBC count (mean drop from ~8.3 to ~6.0 $x10^6/\mu L$) and haemoglobin concentration (falling from ~16 g/dL to ~11.4 g/dL), consistent with CKD-induced anaemia. This effect is partly recovered with the treatment of Silymarin and *Rubus* (400 mg/kg), which maintains RBC counts (>7.3) and haemoglobin levels (>13g/dL) at week 4 ($p< 0.05$). A sharp increase in the WBC levels in CKD rats indicates inflammatory responses, whereas

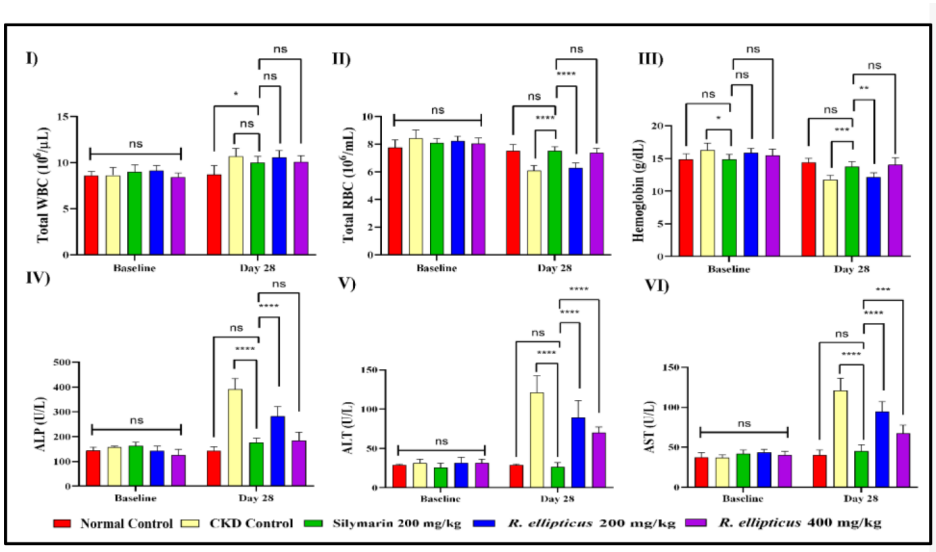


Figure 5. Complete blood count analysis and liver function test, (I) total WBC count, (II) total RBC count, (III) haemoglobin level, (IV) ALP level, (V) ALT level and (VI) AST level. Values are presented as mean (n=6). ****p<0.0005, **p<0.05 and ns is for non-significant.

a moderate elevation in WBC count in treatment groups suggests inflammatory benefits. A progressive elevation of AST from baseline reaching 150 U/L and ALT reaching 146 U/L, and ALP elevation to 430 U/L at the 28th day shows hepatic stress in the CKD group. A modest reduction in these enzymes was seen in high dose *Rubus* group to a similar extent in the silymarin group (AST ~54-81 U/L, ALT ~64-81 U/L, ALP ~126-217 U/L at 28th day) ($p<0.05$). *Rubus* 200 mg/kg showed intermediate reduction in enzymes compared with high dose *Rubus* group and the silymarin group (Figure 5). These findings suggest the reno-hepatoprotective effect of *R. ellipticus*, particularly at higher doses.

4.6 Histopathology Analysis

A significant histopathological change is seen among the experimental groups (Figure 6). On microscopy, the renal architecture shows normocellular glomeruli, intact cuboidal epithelium in the renal tubules, unobstructed capillaries with no evidence of inflammation, fibrosis or cast formation is seen in the normal control group (Group I). On the contrary, widespread glomerulosclerosis, flattening of tubular epithelium with substantial interstitial oedema along with interstitial lymphocytic infiltration (green arrow) and extensive tubular necrosis (blue arrow) were seen in the CKD control group (Group II). Also, there were many proteinaceous casts seen inside the tubular lumens (red arrow), indicating tubular damage. In the Silymarin group (Group III), most glomeruli showed a pattern similar to the normal control group, with the tubular epithelium showing areas of regeneration, but there was also visibility of certain areas of acute tubular necrosis (blue arrow) and interstitial inflammation (green arrow) along with proteinaceous casts (red arrow), suggesting a partial recovery of tubular function. In low dose *Rubus* group, a partial recovery was seen (Occasional glomerulosclerosis, vacuolar degeneration, persistent interstitial inflammation (green arrow), scattered proteinaceous cast (red arrow) in the tubular epithelium, localised interstitial fibrosis). More evidently, in high dose *R. ellipticus* extract group (400 mg/kg) demonstrated substantial architectural preservation. Most glomeruli retained normal morphology, with only focal sclerotic changes. The tubular epithelium appeared largely intact, and interstitial fibrosis was markedly reduced compared to lower-dose treatment. Proteinaceous casts were infrequent (red arrow), and interstitial inflammation (green arrow) was mild and localised, indicating significant nephron repair and functional recovery. Collectively, these histopathological findings support

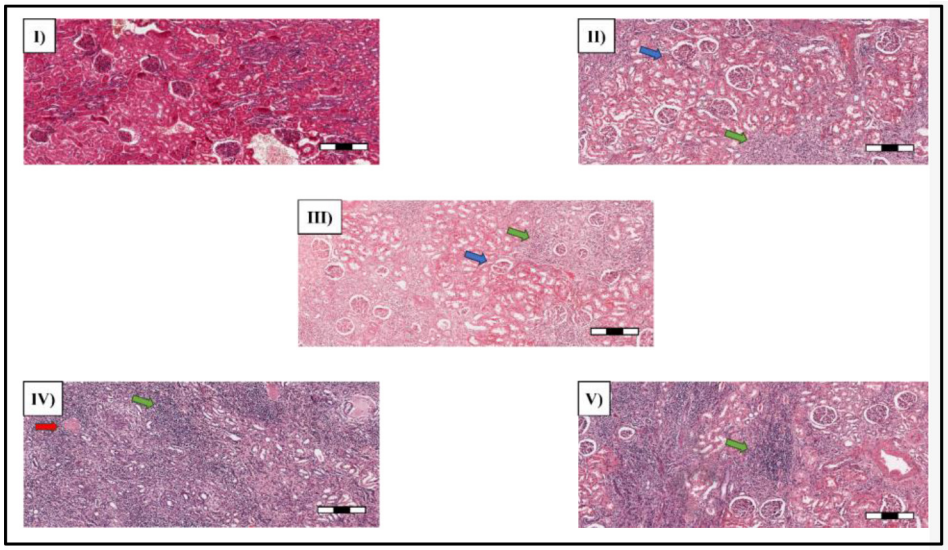


Figure 6. Histopathological analysis of the kidney in Wistar albino rats following treatment with ethanolic leaf extract of *R. ellipticus* for CKD. Each tissue section represents the micrograph of Hematoxylin and Eosin (H and E) staining from all experimental groups at 40× magnification. Blue arrow - Acute tubular necrosis changes; Green arrow - Interstitial inflammation; Red arrow - Cast.

the dose-dependent nephroprotective effects of *R. ellipticus* leaf extract, with the 400 mg/kg dose showing improvements in renal structure and function in this preclinical CKD model.

5. Discussion

This study provides supportive evidence of the nephroprotective effect of ethanolic leaf extract from *R. ellipticus*. A gradual decline in renal function, persistent inflammation, oxidative stress, anemia along with histopathological changes including glomerulosclerosis and tubulointerstitial fibrosis are also evidently seen in CKD. These findings were also seen in the adenine-induced chronic kidney disease animal model, which was used in the present study, to assess the nephroprotective effect[1,2].

In the present study, various phytochemical constituents were identified in *R. ellipticus* leaf extract, which may have a renal protective effect. 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl, a phytol derivative, has been found to contain notable antioxidant and anti-inflammatory properties, decreasing oxidative stress and inflammation[38,39]. Silane, dimethyl (docosyloxy) butoxy, a silane-based compound, may enhance cellular resilience by renal membrane stabilisation[40]. dl-Glyceraldehyde dimer, a sugar-based compound, that could play a role in influencing key metabolic pathways and in reducing the development of advanced glycation end-products, which are typically elevated in CKD and diabetic nephropathy[41]. Among the identified phytochemical constituents, squalene, a natural triterpene, serves as a potent antioxidant, which has the capacity of neutralising free radicals, enhancing mitochondrial efficiency, and modulating pro-inflammatory cytokine production, thereby offering potential to reduce CKD progression[42].

On administration of adenine, it was found that there was a reduction in body weight, urine output, and water consumption, along with increased levels of serum creatinine and blood urea nitrogen, which confirms compromise in renal function. Jager *et al.*[3], and Tungsanga *et al.*[4], also indicate deposition of 2,8-dihydroxyadenine crystals in renal tubules on administration of adenine leads to progressive nephron damage and systemic uremia. There was a reduction in these pathological changes observed in this current study on administration with *R. ellipticus* extract, especially at the high dose of 400 mg/kg. The extract restored physiological parameters and stabilised BUN and creatinine levels, similar to standard silymarin, within this experimental model[5].

In adenine administered groups, a notable increase in serum albumin and total protein levels, with other kidney function indicators were observed. In CKD, glomerular barrier impairment and tubulointerstitial injury were strongly associated with Proteinuria and hypoalbuminemia[43]. It was found that these markers were restored in the *R. ellipticus* group, particularly 400 mg/kg, which indicates both functional enhancement and potential preservation of glomerular structural integrity. The results of this current study align with research utilising plant extracts like *Curcuma longa* and *Phyllanthus niruri*, which have also shown enhancements in albumin levels and renal histology in CKD models[7,8]. In CKD, there was a decrease in erythropoietin level, leading to haematological changes like anaemia, and there was an increase in white blood cell counts, indicating an active systemic immunological response[44,45]. In the current study, on treatment with *R. ellipticus* hematological changes like anaemia and leukocytosis were successfully improved. The notable enhancements in RBC count, haemoglobin concentration, and adjusted WBC levels in the high-dose *R. ellipticus* group indicate a reduction in inflammation and potentially an activation of erythropoiesis.

In addition, there was an increased level of liver enzymes (AST, ALT, ALP) seen in CKD control rats, indicating a potential hepatorenal syndrome-like consequence. In the high dose *R. ellipticus* group, a significant decrease in these liver enzymes was observed, suggesting a secondary hepatoprotective effect of the extract, possibly via enhanced renal clearance and less systemic toxicity. Sharma *et al.*[46], who had done a study on *R. ellipticus* fruit extracts, on acetaminophen-induced nephrotoxicity in rats, found a similar nephroprotective activity confirmed by histopathological and biochemical observations[46]. Histopathological findings provide substantial structural evidence supporting the biochemical results. The untreated CKD group had significant glomerulosclerosis, tubular necrosis, and interstitial inflammation—hallmarks of chronic kidney disease. Treatment with *R. ellipticus* led to

significant architectural repair, characterised by less fibrosis, maintained glomeruli, and regenerated tubular epithelium, especially at the 400 mg/kg dose. Consequently, the current study indicates that *R. ellipticus* extract may function as a viable alternative or adjunct therapy for the treatment of chronic renal disease and its related problems.

There was a certain limitation in the present study, as it focused primarily only on biochemical, hematological and histopathological parameters. Investigating further on individual molecular mechanisms, such as specific signalling pathways involved in nephroprotection, would be an added advantage. Also, the study duration was limited to only 28 days, which may be critical for chronic conditions like CKD.

However, the findings of this study can be used for future research to evaluate the use of this extract along with other conventional nephroprotective drugs that may add better treatment benefits.

5. Conclusion

In conclusion, the ethanolic leaf extract of *R. ellipticus* demonstrated notable nephroprotective properties in adenine-induced chronic renal disease in Wistar albino rats. The medication significantly enhanced physiological parameters, decreased serum creatinine and BUN levels, maintained serum proteins, and alleviated anaemia and elevated liver enzymes associated with CKD. Histopathological study shows the protective effect, demonstrating significant repair of renal architecture, especially at the elevated dosage of 400 mg/kg. The findings indicate that *R. ellipticus* at higher doses possesses a significant therapeutic renoprotective effect in this experimental CKD model. Hence, this may be a promising natural agent for mitigating the course of chronic kidney disease, necessitating further research for clinical use.

6. Ethical Approval

This study was conducted after obtaining ethical clearance from the Institutional Animal Ethics Committee (IAEC 1 / Proposal: 157/A. Lr:119/ Dt20.02.2024) of Chettinad Academy of Research and Education.

7. Acknowledgement

We thank Chettinad Academy of Research and Education for their support in the conduct of this study.

8. References

1. Romagnani P, Remuzzi G, Glassock R, Levin A, Jager KJ, Tonelli M, Massy Z, *et al.* Chronic kidney disease. Nat Rev Dis Prim. 2017; 3(1):1-24. https://doi.org/10.1038/nrdp.2017.88 PMid:29168475
2. Martinusen D, Marin JG, Cheng E, Lau W, *et al.* Chronic kidney disease and end stage renal disease. Renal Med Clin Pharm: Springer; 2020. p. 45-115. https://doi.org/10.1007/978-3-030-37655-0_4
3. Jager KJ, Fraser SD. The ascending rank of chronic kidney disease in the global burden of disease study. Nephrol Dial Transplant. 2017; 32(suppl_2):ii121-ii8. https://doi.org/10.1093/ndt/gfw330 PMid:28201666
4. Tungsanga S, Bello AK. Prevention of chronic kidney disease and its complications in older adults. Drug Aging. 2024; 41(7):565-576. https://doi.org/10.1007/s40266-024-01128-7 PMid:38926293
5. Fetzer SJ. The renal system. Drain's PeriAnesthesia Nursing-E-Book: A critical care approach; 2022.
6. Javed R, Faraz M, Farid A, Selim S. Historical development of herbal medicine. Herbal Pharmacopeia: CRC Press; 2025. p. 19-35. https://doi.org/10.1201/9781003544418-2
7. Kausar MA, Parveen K, Siddiqui WA, Anwar S, Zahra A, Ali A, *et al.* Nephroprotective effects of polyherbal extract via attenuation of the severity of kidney dysfunction and oxidative damage in the diabetic experimental model. Cell Mol Bio. 2021; 67(4):42-55. https://doi.org/10.14715/cmb/2021.67.4.6 PMid:35809303
8. Kumar V, Sharma A, Sharma N, Saini R, Dev K, El-Shazly M, *et al.* A review of botany, traditional applications, phytochemistry, pharmacological applications, and toxicology of *Rubus ellipticus* Smith fruits. Naunyn-Schmiedeberg's Arch Pharmacol. 2024; 397(7):4483-4497. https://doi.org/10.1007/s00210-024-02964-5 PMid:38252298
9. Khanal LN, Sharma KR, Paudyal H, Parajuli K, Dahal B, Ganga GC, *et al.* Green synthesis of silver nanoparticles from root extracts of *Rubus ellipticus* Sm. and comparison of antioxidant and antibacterial activity. J Nanomat. 2022; 2022(1):1832587. https://doi.org/10.1155/2022/1832587
10. Sharma M, Pant S, Wani ZA. Himalayan yellow raspberry (*Rubus ellipticus* Sm.) potentially important medicinal plant for bioprospection. Vegetos. 2025:1-12. https://doi.org/10.1007/s42535-024-01156-z
11. Nautiyal V, Kumari S. Phytochemical composition, antibacterial activity, and metabolomic analysis of *Rubus*

elipticus Sm. leaf extracts. Vegetos. 2025:1-11. https://doi.org/10.1007/s42535-025-01205-1
12. Khalil S, Mehmood A, Khan MAR, Ahmad KS, Abasi F, Raffi M, *et al*. Antibacterial, antioxidant and photocatalytic activity of novel *Rubus ellipticus* leaf mediated silver nanoparticles. J Saudi Chem Soc. 2023; 27(1):101576. https://doi.org/10.1016/j.jscs.2022.101576
13. Kumari A, Prakash V, Gupta D, Kashyap L, Goyal R, Chopra H, *et al*. Identification and evaluation of antimicrobial and anti-arthritis activities of hydroethanolic extract of *Rubus ellipticus* leaves. Narra J. 2023; 3(3):e152.
14. Ali BH, Al Za"abi M, Adham SA, Yasin J, Nemmar A, Schupp N. Therapeutic effect of chrysin on adenine-induced chronic kidney disease in rats. Cell Physiol Biochem. 2016; 38(1):248-257. https://doi.org/10.1159/000438626 PMid:26784294
15. Nkogo L-FE, Mouendou MSM, Dumarçay S, Engonga PE, Gérardin P. Phytochemical study, FTIR and GC-MS characterization and evaluation of the antioxidant activity of *Letestua durissima* extracts. Forests. 2024; 15(3):429. https://doi.org/10.3390/f15030429
16. Pérez M, Dominguez-López I, Lamuela-Raventós RM. The chemistry behind the folin-ciocalteu method for the estimation of (poly) phenol content in food: Total phenolic intake in a mediterranean dietary pattern. J Agri Food Chem. 2023; 71(46):17543-17553. https://doi.org/10.1021/acs.jafc.3c04022 PMid:37948650 PMCid:PMC10682990
17. Omar GA, Mohammed LY. Physiochemical standardization and phytochemical screening of *Urtica dioica* L. leaves growing in Zakho, Kurdistan region, Iraq. Sci J Univ Zakho. 2023; 11(3):306-316. https://doi.org/10.25271/sjuoz.2023.11.3.1069
18. Box J. Investigation of the Folin-Ciocalteau phenol reagent for the determination of polyphenolic substances in natural waters. Water Res. 1983; 17(5):511-525. https://doi.org/10.1016/0043-1354(83)90111-2
19. Mahesh A, Ranganath M, Kumar DH. Enrichment of flavonoids from the methanolic extract of *Boerhaavia diffusa* roots by partitioning technique. Res J Chem Sci. 2013; 2231:606X.
20. Shaikh JR, Patil M. Qualitative tests for preliminary phytochemical screening: An overview. Int J Chem Stud. 2020; 8(2):603-608. https://doi.org/10.22271/chemi.2020.v8.i2i.8834
21. Sharma S, Kumar A, Singh P, *et al*. Preliminary phytochemical screening of bark extracts of *Ficus glomerata* (Gular), a lactiferous deciduous tree from Indian Origin; 2021.
22. Brinker AM, Seigler DS. Determination of cyanide and cyanogenic glycosides from plants. In: Linskens HF, Jackson JF, editors. Plant Toxin Analysis. Berlin, Heidelberg: Springer Berlin Heidelberg; 1992. p. 359-381. https://doi.org/10.1007/978-3-662-02783-7_15
23. Pradeep A, Dinesh M, Govindaraj A, *et al*. Phytochemical analysis of some important medicinal plants. Int J Biol Pharm Res. 2014; 5(1):48-50.
24. Rahimah SB, Djunaedi DD, Soeroto AY, *et al*. The phytochemical screening, total phenolic contents and antioxidant activities *in vitro* of white oyster mushroom (*Pleurotus Ostreatus*) preparations. Open Access Maced J Med Sci. 2019; 7(15):2404-2412. https://doi.org/10.3889/oamjms.2019.741 PMid:31666837 PMCid:PMC6814466
25. Egbuna C, Ifemeje JC, Maduako MC, Tijjani H, Udedi SC, Nwaka AC, *et al*. Phytochemical test methods: Qualitative, quantitative and proximate analysis. Phytochemistry: Apple Academic Press; 2018. p. 381-426. https://doi.org/10.1201/9780429426223-15
26. Hager H. Reaction auf Brucin. Zeitschrift für analytische Chemie. 1872; 11(1):201-202. https://doi.org/10.1007/BF01588432
27. Krishnaiah D, Devi T, Bono A, *et al*. Studies on phytochemical constituents of six Malaysian medicinal plants; 2009.
28. James O, William ML. Qualitative testing for carbohydrates. In: Neidig HA, editor. Modular Laboratory Programme in Chemistry. Pennsylvania: Chemical Education Resources; 1994. p. 1-12.
29. Benedict SR. The detection and estimation of reducing sugars. J Biol Chem. 1907; 3(2):101-117. https://doi.org/10.1016/S0021-9258(17)45999-0
30. Barbehenn RV. Measurement of protein in whole plant samples with ninhydrin. J Sci Food Agri. 1995; 69(3):353-359. https://doi.org/10.1002/jsfa.2740690312
31. Khang DT, Tien LTT, Men TT, Thuy NP. Potential of fermented fruit peel liquid in cosmetics as a skin care agent. Cosmetics. 2021; 8(2):33. https://doi.org/10.3390/cosmetics8020033
32. Gulcin İ, Alwasel SH. DPPH radical scavenging assay. Processes. 2023; 11(8):2248. https://doi.org/10.3390/pr11082248
33. Khan MSH, Rahman MH, Mosihuzzaman M, Rokeya B. Preliminary phytochemical screening and in vitro antioxidant capacity of some traditional medicinal plants of Bangladesh. Dhaka Univ J Pharm Sci. 2024; 23(2):205-209. https://doi.org/10.3329/dujps.v23i2.78577
34. Subalakshmi M, Priya V. *In-vitro* anti-inflammatory and antioxidant activity of n-chloropicolinamide. J Adv Sci Res. 2021; 12(02 Suppl 1):153-156.
35. Iqbal SM, Hussain L, Hussain M, Akram H, Asif M, Jamshed A, *et al*. Nephroprotective potential of a standardized extract of *Bambusa arundinacea*: *In vitro* and *in vivo* studies. ACS Omega. 2022; 7(21):18159-18167. https://doi.org/10.1021/acsomega.2c02047 PMid:35664584 PMCid:PMC9161425
36. Ghaznavi H, Mehrzadi S, Dormanesh B, *et al*. Comparison of the protective effects of melatonin and silymarin against gentamicin-induced nephrotoxicity in rats. Evid

Based Complement Alternat Med. 2016; 21(4):NP49-NP55. https://doi.org/10.1177/2156587215621672 PMid:26703224
37. Hilmi SR, Dewan ZF, Kabir AN, Islam MM, Yusuf MA, Afreen KN, *et al*. Effect of silymarin on gentamicin induced nephrotoxicity in rats. Bangladesh J Infect Dis. 2023; 10(2):71-76. https://doi.org/10.3329/bjid.v10i2.70636
38. Dania OE, Dokunmu TM, Iweala EE. Comparism of bioactive components in hydroethanol extract of *Corchorus olitorius* and *Amaranthus hybridus* Leaves. Trop J Nat Prod Res. 2024; 8(8). https://doi.org/10.26538/tjnpr/v8i8.34
39. 39.Adam SA, Barthelomai IS, S SB, *et al*. The *in vitro* and *in silico* investigations on the α-amylase inhibitor derived from *Leptadenia lanceolata* (Poir.) Goyder leaf extract. Nat Prod Res. 2024:1-11.
40. Prabaharan J, Prabakaran M, Prabhakaran M, Abinaya K, Krishnan N, Karen DS, *et al*. Comparison on extracted metabolites from different regions grown *Murraya koenigii* and validation by antibacterial, antioxidant, and molecular docking studies. Biomass Convers Biorefin. 2023:1-29. https://doi.org/10.1007/s13399-023-04105-z
41. Barski OA, Tipparaju SM, Bhatnagar A. The aldo-keto reductase superfamily and its role in drug metabolism and detoxification. Drug metab Rev. 2008; 40(4):553-624. https://doi.org/10.1080/03602530802431439 PMid:18949601 PMCid:PMC2663408
42. Joshi RK. Bioactive usual and unusual triterpenoids derived from natural sources used in traditional medicine. Chem Biodiv. 2023; 20(2):e202200853. https://doi.org/10.1002/cbdv.202200853 PMid:36598091
43. Rabelink TJ, Heerspink HJL, de Zeeuw D. The pathophysiology of proteinuria. Chronic renal disease: Elsevier; 2015. p. 92-105. https://doi.org/10.1016/B978-0-12-411602-3.00009-3
44. Begum S, Latunde-Dada GO. Anemia of inflammation with an emphasis on chronic kidney disease. Nutrients. 2019; 11(10):2424. https://doi.org/10.3390/nu11102424 PMid:31614529 PMCid:PMC6835368
45. Atkinson MA, Warady BA. Anemia in chronic kidney disease. Ped Neph. 2018; 33(2):227-238. https://doi.org/10.1007/s00467-017-3663-y PMid:28412770
46. Sharma US, Kumar A, Hauz A. Therapeutic efficacy of *Rubus ellipticus* (Smith) fruits extracts in acute acetaminophen induced nephrotoxicity in rats. Pharmacologyonline. 2010; 3:514-524.

Author Contribution

Conceptualization: Vijayarangan Sivasamy; Formal analysis: Jane Betsy Isaac, Vivek Kumar Dhiman; Investigation: Keranap James Ravichandran; Resources: Susmitha Kalaichelvan; Data curation: Keranap James Ravichandran; Writing—original draft preparation: Keranap James Ravichandran; Writing—review and editing: Keranap James Ravichandran, Padmaja Sukumar, Rakeshkumar Kesavaraman Rajaram; Visualization: Rakeshkumar Kesavaraman Rajaram; Supervision: Vijayarangan Sivasamy. All authors have read and agreed to the published version of the manuscript.