ORIGINAL ARTICLE

Eurycomanone standardized *Eurycoma longifolia* Extract Effects on Macrophage Marker Expression in Subtotal Nephrectomized Rat

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ABSTRACT

Introduction: *Eurycoma longifolia* is a medicinal plant known for its anti-inflammatory properties, which may modulate chronic kidney disease (CKD) progression via macrophage-related mechanisms. However, its nephroprotective potential in a subtotal nephrectomy model has not been clearly established. This study aimed to evaluate the effects of *Eurycoma longifolia* extract standardized to 8% eurycomanone (ESEL) as a nephroprotective agent in a rat model of CKD. **Methods:** Twenty male rats (10–14 weeks old) were randomly divided into five groups: (1) SHAM control, (2) untreated CKD, and CKD groups treated with ESEL extract at doses of (3) 32.5 mg/kg BW, (4) 65 mg/kg BW, and (5) 130 mg/kg BW. All treatments were administered for 10 weeks following subtotal nephrectomy. **Results:** ESEL extract reduced albuminuria across all treatment groups compared to the untreated CKD group. The 65 mg/kg BW group showed the most significant improvement in creatinine clearance and the greatest inhibition of COX-2 expression, suggesting strong anti-inflammatory effects. In contrast, the 130 mg/kg BW group exhibited increased glomerulosclerosis and upregulated CD-11b mRNA expression, indicating potential toxicity. Notably, CD-206 mRNA expression—a marker of M2 macrophages—was significantly elevated only in the 32.5 and 65 mg/kg BW groups, supporting a dose-dependent immunomodulatory effect. **Conclusion:** ESEL extract demonstrates dose-dependent nephroprotective effects in a CKD rat model, primarily through the enhancement of M2 macrophage activity. The 65 mg/kg BW dose appears most effective, while higher doses may lead to adverse renal effects.
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Keywords: *Eurycoma longifolia*, chronic kidney disease, nephroprotective, Macrophage M2

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INTRODUCTION

Chronic kidney disease (CKD) is characterized by progressive renal inflammation, fibrosis, and structural damage to renal tubules and vasculature. Macrophages play a central role in the progression of kidney failure; however, their precise contribution—whether protective or detrimental—remains complex and context-dependent. Tissue macrophages are functionally classified into three phenotypes: inflammatory (M1), wound-healing (M2), and fibrolytic macrophages (1,2,3). In early CKD, kidney injury or infection activates pro-inflammatory M1 macrophages, which release cytokines that exacerbate tissue damage. This is followed by a compensatory response involving M2c macrophages, which facilitate clearance of apoptotic cells and promote epithelial and vascular repair. When these reparative efforts fail to restore tissue integrity, macrophages may shift toward the M2a phenotype, which contributes to fibrogenesis via profibrotic signaling. Ultimately, fibrolytic macrophages are recruited to limit excessive fibrosis and support matrix remodeling (4,5). Given their dynamic roles, modulating macrophage polarization represents a promising therapeutic target in CKD (4,5).

Eurycoma longifolia has demonstrated potential in modulating macrophage activity (6). In vitro studies using RAW 264.7 macrophage-like cells revealed that extracts of *Eurycoma longifolia* suppress the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), key markers of

inflammation. In vivo, the extract improved survival rates in lipopolysaccharide (LPS)-induced septic shock models (3). One of its bioactive compounds, 7-methoxy-(9H-β-carbolin-1-il)-(E)-1-propenoic acid (7-MCPA), has been shown to exert anti-inflammatory effects through inhibition of the Nrf2/HO-1 pathway and suppression of NF-κB activation in macrophages challenged with LPS (7).

In addition to its immunomodulatory properties, *Eurycoma longifolia* exhibits renal protective effects. In paracetamol-induced nephropathy models, it has been shown to reduce serum creatinine and urea levels, decrease albuminuria, and improve creatinine clearance (8). Building on this evidence, the present study investigates whether *Eurycoma longifolia* extract standardized to eurycomanone (ESEL) can provide nephroprotection in a rat model of CKD, potentially through modulation of macrophage polarization.

MATERIALS AND METHODS

Experimental animal model

This study used twenty male Wistar rats (8–10 weeks old, weighing 150–200 grams) obtained from the Integrated Research and Testing Laboratory (LPPT) at Universitas Gadjah Mada. Only male rats were used to avoid potential confounding effects of hormonal variations associated with the female estrous cycle, which can influence renal physiology, inflammatory responses, and gene expression profiles.

Rats were housed in standard polypropylene cages with wood bedding under 12 h light/dark cycles, temperature 22–25°C, 50–70% humidity, with free access to standard pellet diet and water. Animals were acclimatized for 7 days prior to the procedure. All procedures complied with ethical guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada (KE/FK/0097/EC/2020).

The sample size for each group was n = 4, totaling 20 animals across five experimental groups. This number was selected to minimize animal use while ensuring adequate statistical power, particularly for detecting molecular differences such as gene expression. Furthermore, based on the E value formula (E = total number of animals – total number of groups), this study yielded an E value of 15, which falls within the recommended range of 10–20 for preliminary experimental designs (9). Thus, the sample size was deemed ethically and scientifically appropriate for a preclinical exploratory study.

Eurycomanone standardized *Eurycoma longifolia* extract

Eurycomanone standardized extract was obtained from Javaplant. Standardized extracts are certain extracts that have non-specific and specific parameters. The non-specific parameters are ash content, metal content, water content and microbial contamination. The specific parameter is Eurycomanone itself. Briefly, *Eurycoma longifolia* (Pasak Bumi) were extracted using the Quadran Percolation System (QPS), a continuous multi-stage extraction technology employing four interconnected percolators operating in cyclic 24-hour phases over 7 days. This system optimizes solvent penetration and bioactive compound yield while minimizing thermal degradation. The solvent mixture was circulated dynamically to ensure complete extraction of eurycomanone and related quassinoids. The crude extract was further purified via ion-exchange chromatography to isolate eurycomanone-enriched fractions. The extract used contains 8% Eurycomanone contained in Pasak Bumi.

Study design

The CKD model was established using a 5/6 subtotal nephrectomy procedure performed in two stages. In the first stage, a total left uninephrectomy was carried out. One week later, the second surgery involved removing approximately two-thirds of the right kidney. Therapeutic intervention began one week after the second surgery.
There were five groups of treatment, the SHAM group, the untreated CKD group (UNTREATED), the CKD group given 32.5 mg/kg BW of the ESEL extract (DOSE 1), the CKD group given 65 mg/kg BW of the ESEL extract (DOSE 2), and the CKD group given 130 mg/kg BW of the ESEL extract (DOSE 3). The treatment duration was ten weeks started after subtotal nephrectomy surgery.

The doses of *Eurycoma longifolia* extract used in this study were adapted from previous study which showed that the anti-inflammatory effects of *Eurycoma longifolia* extract was at 200, 400, and 800 mg/kg BW using an extract containing 1.3% eurycomanone. In our study, we used a standardized extract containing 8% eurycomanone. To match the active compound content, the equivalent doses were calculated proportionally, resulting in 32.5, 65, and 130 mg/kg BW (10).

Creatinine clearance analysis

Urine was collected over 24 h using individual metabolic cages. Samples were stored in clean, labeled tubes at -20°C prior to biochemical analysis. Examination of creatinine levels in urine and blood at the end of treatment were conducted at the Department of Clinical Pathology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada.

Albuminuria analysis

Albumin levels in urine were examined at the Department of Clinical Pathology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada using 24-hour tamping urine samples.

Analysis of glomeruloslerosis Score and tubular damage
The kidney removed at the end of the treatment was

then made into paraffin blocks for histological analysis with Hematoxylin and Eosin (HE) staining. Kidney samples were analyzed at the Department of Anatomical Pathology, Faculty of Veterinary Medicine, Gadjah Mada University. We evaluated 20 glomeruli per section per rat for glomerulosclerosis using a 0–4 scale under blinded conditions. Tubular injury was graded similarly. A score of 0 indicates no kidney damage, a score of 1 indicates kidney damage 1-24%, a score of 2 indicates kidney damage 25-49%, a score of 3 indicates kidney damage 50-74%, and a score of 4 indicates kidney damage 75-100%. Examiners were blinded to group assignment to reduce observer bias.

Expression analysis of CD11, CD206, and COX-2 mRNA (Semiquantitative Reverse Transcriptase-PCR (RT-PCR))
Total RNA was extracted from kidney tissues using the Favorgen total RNA mini kit (Favorgen Biotech Corp., Taiwan) following the manufacturer's protocol. RNA concentration and purity were assessed spectrophotometrically, and complementary DNA (cDNA) was synthesized using the Smobio reverse transcriptase kit (Smobio, Taiwan). Gene expression analysis was performed by semiquantitative Reverse Transcriptase-PCR (RT-PCR) using specific primers for CD11 (M1 macrophage marker), CD206 (M2 macrophage marker), COX-2 (inflammatory marker), and GAPDH (housekeeping gene). The primer sequences used were as follows: GAPDH forward 5′-TGG GAA GCT GGT CAT CAA C-3′ and reverse 5′-GCT ACA ATT GGG ATG ATG TCG-3′; CD206 forward 5′-GAC AGA TAT GAA CAA GCA TTC C-3′ and reverse 5′-TGA ACA TCT GAG AGT CCT GTC-3′; CD11 forward 5′-GAA GCT CAC GTG CAT GTT GT-3′ and reverse 5′-GCA ACA ATT GGG ATG ATG TCG-3′; and COX-2 forward 5′-GAGCAGTTCCAGTATCAGAACG-3′ and reverse 5′-TGAGCAGTTGTTGTTGAGCAG-3′. PCR amplification was carried out under the following conditions: initial denaturation at 94°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 45 seconds, with a final extension at 72°C for 5 minutes. PCR products were separated on a 1.5% agarose gel and visualized using a GelDoc imaging system. Band intensity was analyzed using ImageJ software, and relative expression levels were normalized to GAPDH.

Data analysis
Data analysis was performed using Prism9 for MacOS software. The results obtained from the histological analysis, analysis of albuminuria 24, analysis of creatinine clearance, expression analysis of CD11 values, CD206 mRNA, and COX-2 were presented as mean ± standard error of measurement (SEM). Shapiro Wilk was used for normality analysis, then Oneway Anova and Post Hoc Tukey HSD were used to conclude differences in the mean values of different groups. The probability of $p < 0.05$ was considered statistically significant.

RESULTS

In this study, we evaluated the nephroprotective effects of ESEL in a rat model of chronic kidney disease. All outcomes of this study, including measurements of body weight, renal function, and gene expression, are summarized in Table 1 for an organized overview.

Table I: Effects of ESEL Treatment on Body Weight, Renal Function, Renal Histopathology, and Gene Expression

Group	SHAM	UNTREATED	DOSE 1	DOSE 2	DOSE 3
Body Weight (g)	301.33 ±9.61	323.55 ±21.69	317.6 ±7.44	286.43 ±21.81	252.93 ±34.42′
Albuminuria (mg/24h)	70.03 ±3.77	88.60 ±6.37	51.13 ±6.56	55.50 ±6.55	67.05 ±4.36
Creatinine Clearance (mg/dL)	62.92 ±9.42	21.47 ±4.08	28.16 ±4.82	40.25 ±11.82	19.30 ±2.65
Tubular Injury Score	0	40 ±21.60	38.75 ±25.29	12.5 ±6.45	41.25 ±25.29
Glomerulo-sclerosis Score	0	2.5 ±1.29	2.25 ±0.96	1.5 ±1.29	2.5 ±1.29
CD11/GAPDH	0.073 ±0.034	0.434 ±0.155	0.503 ±0.030	0.477 ±0.104	0.192 ±0.072
CD206/GAPDH	0.252 ±0.140	0.370 ±0.019	1.273 ±0.178	0.878 ±0.543	0.423 ±0.140
COX-2/GAPDH	0.03 ±0.06	0.08 ±0.008	0.01 ±0.003	0.015 ±0.004	0.22 ±0.035

At week 10 of treatment, animals receiving ESEL extract at 130 mg/kg BW showed significantly lower body weight compared to both SHAM (healthy controls) (p=0.01) and UNTREATED (5/6 nephrectomy controls) groups (p=0.001) (Figure 1). No significant weight differences were observed between SHAM, UNTREATED, and

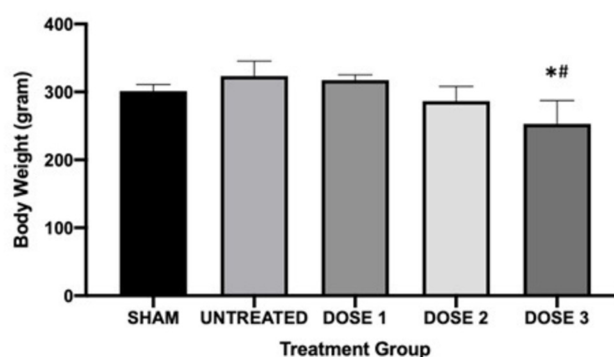

Figure 1: Body weight measurements at week 10 of treatment. *P < 0.05 vs. SHAM group; #P < 0.05 vs. UNTREATED group. Group definitions: SHAM: Healthy control rats (no nephrectomy), UNTREATED: 5/6 nephrectomy rats (no treatment), DOSE 1: 5/6 nephrectomy rats + ESEL (32.5 mg/kg body weight/day, weeks 2–12), DOSE 2: 5/6 nephrectomy rats + ESEL (65 mg/kg body weight/day, weeks 2–12), DOSE 3: 5/6 nephrectomy rats + ESEL (130 mg/kg body weight/day, weeks 2–12)

lower-dose ESEL groups (32.5 and 65 mg/kg BW).
Albuminuria was quantified through 24-hour urine collection. Compared to SHAM controls, rats with CKD exhibited significantly elevated urinary albumin excretion. Treatment with ESEL extract at doses of 32.5 and 65 mg/kg body weight significantly reduced albuminuria relative to untreated CKD controls ($p<0.05$). However, the highest dose (130 mg/kg) showed no significant reduction in albuminuria compared to untreated CKD animals. These results are presented in

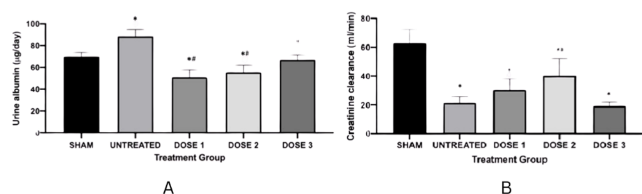

Figure 2: Renal function parameters: (A) Urinary albumin excretion and (B) Creatinine clearance.*P < 0.05 vs SHAM; #P < 0.05 vs UNTREATED. Experimental groups: SHAM: Healthy control rats (no nephrectomy), UNTREATED: 5/6 nephrectomy rats (no treatment), DOSE 1: 5/6 nephrectomy rats + ESEL (32.5 mg/kg body weight/day, weeks 2–12), DOSE 2: 5/6 nephrectomy rats + ESEL (65 mg/kg body weight/day, weeks 2–12), DOSE 3: 5/6 nephrectomy rats + ESEL (130 mg/kg body weight/day, weeks 2–12)

Figure 2A.
Renal function assessment revealed significantly reduced creatinine clearance in all CKD groups (both treated and untreated with ESEL extract) compared to SHAM controls (Figure 2B). Notably, the 65 mg/kg BW ESEL treatment group demonstrated partial renal function recovery, showing significantly higher creatinine clearance than untreated CKD animals ($p=0.002$).

Histopathological analysis showed significantly elevated glomerulosclerosis scores in both untreated CKD rats and those receiving the highest ESEL dose (130 mg/kg BW) compared to SHAM controls ($p=0.01$ and $p=0.02$

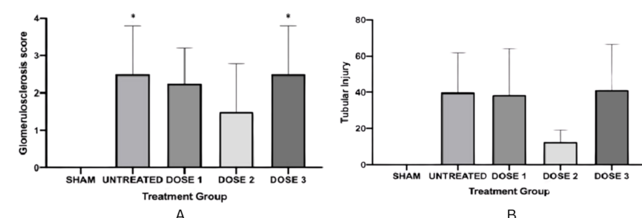

Figure 3: Renal histopathology: (A) Glomerulosclerosis score and (B) Tubular injury assessment. *P < 0.05 vs SHAM; #P < 0.05 vs UNTREATED. Experimental groups: SHAM: Healthy control rats (no nephrectomy), UNTREATED: 5/6 nephrectomy rats (no treatment), DOSE 1: 5/6 nephrectomy rats + ESEL (32.5 mg/kg body weight/day, weeks 2–12), DOSE 2: 5/6 nephrectomy rats + ESEL (65 mg/kg body weight/day, weeks 2–12), DOSE 3: 5/6 nephrectomy rats + ESEL (130 mg/kg body weight/day, weeks 2–12)

respectively) (Figure 3A).
There Quantitative analysis revealed no statistically significant differences in tubular injury scores among experimental groups (Figure 3B).

Regarding inflammatory markers, CD-11 mRNA expression was significantly elevated in untreated CKD rats compared to SHAM controls ($p=0.01$). Notably, the highest ESEL dose (130 mg/kg BW) demonstrated anti-inflammatory effects, showing significantly reduced CD-11 mRNA expression versus untreated CKD animals ($p=0.0001$) (Figure 4A).

CD206 mRNA expression was significantly upregulated in CKD rats treated with ESEL at both 32.5 and 65 mg/kg BW compared to untreated CKD animals ($p=0.0001$ and

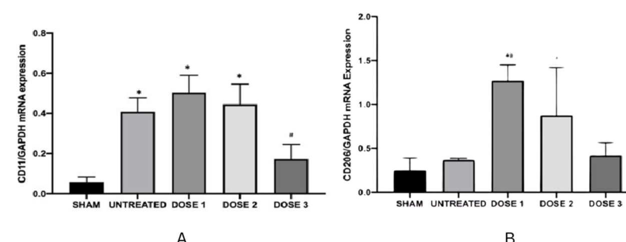

Figure 4: Macrophage polarization markers: (A) CD11/GAPDH and (B) CD206/GAPDH mRNA expression. *P < 0.05 vs SHAM; #P < 0.05 vs UNTREATED. Experimental groups: SHAM: Healthy control rats (no nephrectomy), UNTREATED: 5/6 nephrectomy rats (no treatment), DOSE 1: 5/6 nephrectomy rats + ESEL (32.5 mg/kg body weight/day, weeks 2–12), DOSE 2: 5/6 nephrectomy rats + ESEL (65 mg/kg body weight/day, weeks 2–12), DOSE 3: 5/6 nephrectomy rats + ESEL (130 mg/kg body weight/day, weeks 2–12)

$p=0.002$ respectively) (Figure 4B).

Regarding inflammatory markers, COX-2 expression showed a dose-dependent pattern, with the lowest levels observed in the 32.5 and 65 mg/kg BW ESEL groups (Figure 5).

Conversely, the highest dose (130 mg/kg BW) demonstrated elevated COX-2 expression compared to other groups. However, these differences did not reach statistical significance ($p>0.05$).

DISCUSSION

Albuminuria and Renal Function

This study showed that ESEL extract had a dose-dependent impact on renal function and proteinuria. Rats treated with ESEL at doses of 32.5 mg/kg BW (DOSE 1), 65 mg/kg BW (DOSE 2), and 130 mg/kg BW (DOSE 3) all showed reduced urinary albumin levels compared to the untreated CKD group (UNTREATED), with statistically significant differences ($p < 0.05$). The lowest albuminuria was found in DOSE 1 (51.13±6.56 mg/24h), followed by DOSE 2 (55.50±6.55 mg/24h), supporting the potential of ESEL to improve glomerular barrier integrity.

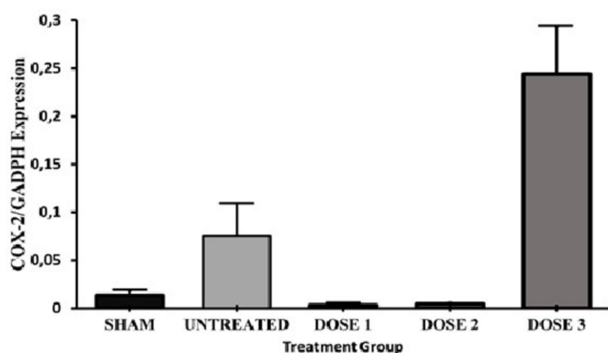

Figure 5: COX-2/GAPDH expression in experimental groups. No statistically significant differences were observed (P > 0.05 for all comparisons). Experimental groups: SHAM: Healthy control rats (no nephrectomy), UNTREATED: 5/6 nephrectomy rats (no treatment), DOSE 1: 5/6 nephrectomy rats + ESEL (32.5 mg/kg body weight/day, weeks 2–12), DOSE 2: 5/6 nephrectomy rats + ESEL (65 mg/kg body weight/day, weeks 2–12), DOSE 3: 5/6 nephrectomy rats + ESEL (130 mg/kg body weight/day, weeks 2–12)

Regarding creatinine clearance, the highest improvement was observed in DOSE 2 (40.25±11.82 mg/dL), significantly higher than UNTREATED (21.47±4.08 mg/dL), indicating enhanced renal function at this dose. DOSE 1 also showed improvement (28.16±4.82 mg/dL), although less pronounced. Interestingly, the 130 mg/kg BW dose (DOSE 3) did not improve renal function and had clearance values (19.30±2.65 mg/dL) lower than both Groups I and II, suggesting a possible ESEL renal toxicity at higher doses. These findings were supported by lower body weight gain in DOSE 3 (33.95±20.03 g), which was significantly less than all other groups.

Macrophage Polarization: CD11/CD206

Macrophage plasticity plays a central role in the pathogenesis and resolution of chronic kidney disease (12). M1 macrophages contribute to acute inflammation and tissue injury, while M2 macrophages are involved in tissue repair, matrix remodeling, and fibrosis regulation (13,14). The balance between M1 and M2 macrophages is tightly regulated by the renal microenvironment and shifts over time during injury and healing (15). In the current study, CD11 (M1 marker) and CD206 (M2 marker) expression were evaluated to assess the polarization state of macrophages in the kidney. Our results showed that CD11 mRNA expression was significantly suppressed in the group treated with 130 mg/kg BW ESEL, while CD206 expression was elevated in the 32.5 and 65 mg/kg BW groups.

The reduced M1 activity and upregulated M2 markers reflect modulation of key inflammatory pathways, particularly NF-κB and Nrf2 (16). The NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) pathway is a master regulator of proinflammatory gene expression in macrophages, including TNF-α, IL-1β, and IL-6, all of which are implicated in CKD progression (17). Eurycomanone, the major active component in *Eurycoma longifolia*, has been shown in several in vitro and in vivo studies to inhibit NF-κB translocation, thereby downregulating inflammatory mediators (18). This supports the observed decrease in CD11 and COX-2 expression, particularly at the 65 mg/kg BW dose, where anti-inflammatory effects were most prominent.
Conversely, Nrf2 (nuclear factor erythroid 2–related factor 2) is a transcription factor that upregulates antioxidant enzymes and cytoprotective genes. Activation of Nrf2 has been associated with inhibition of M1 macrophage polarization, reduced oxidative stress, and renal protection in experimental kidney disease models (19). While this study did not directly measure Nrf2 activation, the increased CD206 expression and reduced oxidative damage markers observed at moderate ESEL doses suggest a potential upregulation of Nrf2-dependent mechanisms as part of the extract's protective effects. These may reflect enhanced macrophage plasticity and adaptive immune regulation, particularly relevant in chronic kidney injury (20).

COX-2 and Anti-inflammatory Mechanisms

Previous studies indicate that *Eurycoma longifolia* inhibits inflammatory mediators via NF-κB and Nrf2 signaling, reducing the expression of COX-2 and iNOS (10, 11, 18). In our study, COX-2 expression was lowest in the 65 mg/kg BW group, suggesting optimal anti-inflammatory effects at this dose. Meanwhile, COX-2 expression remained high in the 130 mg/kg BW group, and moderately elevated in the 32.5 mg/kg BW group, indicating a less effective or disrupted anti-inflammatory response.

COX-2 is known to mediate both inflammation and tissue repair. However, excessive inhibition of COX-2 may impair regenerative pathways and endothelial integrity in the kidney. Importantly, recent studies show that COX-2 suppression can inhibit M2 macrophage polarization, a critical step in resolving inflammation and promoting tissue repair (21). Furthermore, long-term inhibition of COX-2—such as that caused by NSAIDs—has been associated with reduced glomerular filtration, impaired tubular function, and increased risk of nephropathy (22). These mechanisms may explain the loss of renal benefit at the highest ESEL dose in our study.

Our study's findings of reduced COX-2 expression and increased CD206 expression are consistent with the anti-inflammatory and pro-resolving effects of ESEL. These results suggest potential modulation of the Nrf2/NF-κB signaling pathways by ESEL. We did not directly measure the activation of these pathways, but our inference is based on the well-established roles of our selected downstream markers. The NF-κB pathway is a key regulator of pro-inflammatory responses, and its inhibition often leads to reduced expression of genes

like COX-2 (16,17). Conversely, the Nrf2 pathway is known for its role in upregulating antioxidant and anti-inflammatory genes, which can be linked to the shift toward M2 macrophage polarization as indicated by elevated CD206 expression (19). Therefore, our observed changes in these downstream markers provide a strong rationale for further investigation into ESEL's molecular mechanism through these specific pathways.

Dose-dependent Effects and Toxicity

Our findings emphasize the dose-dependent effects of ESEL. At doses of 32.5 and 65 mg/kg BW, ESEL demonstrated nephroprotective properties, as evidenced by improvements in albuminuria, creatinine clearance, and an upregulation of CD206 expression—suggesting activation of reparative (M2) macrophages. These beneficial effects align with the known anti-inflammatory properties of eurycomanone, a major bioactive constituent of *Eurycoma longifolia*.

However, at the higher dose (130 mg/kg BW), rats exhibited signs of possible toxicity: significantly reduced body weight gain, worsened glomerulosclerosis, suppressed CD206 expression, and impaired renal function. Although this data does not supported by other toxicity sign and symptoms, these findings suggest that high-dose ESEL may exert deleterious effects in CKD, despite its otherwise well-documented safety in healthy animals.

Although *Eurycoma longifolia* has shown a high safety margin in acute toxicity studies—reporting LD_{50} values ranging from 1000 to 3000 mg/kg BW in healthy rats (23)—these studies primarily assessed gross toxicological endpoints in healthy systems, not in the context of existing disease. Eurycomanone, the standardized component in our extract, may contribute to toxicity when administered at high doses in disease models like CKD, where renal metabolism and systemic clearance are already compromised.

Emerging evidence supports this concern. Study in vivo study in mice and brine shrimp shows that Eurycomanone is the most toxic compound of the extract of *Eurycoma longifolia* (24). Other study also showed that *Eurycoma longifolia* extract containing glycosaponins (40-65%) and eurycomanone (0.8-1.5%) has the potential to induce DNA damage (25)

This potential for sublethal but functional toxicity mirrors phenomena observed with NSAIDs, where excessive dosing disrupts prostaglandin-mediated repair, induces oxidative stress, and impairs renal recovery—despite therapeutic anti-inflammatory effects at lower doses (26, 27). It is plausible that eurycomanone may share similar toxicity profiles, particularly when the anti-inflammatory activity becomes excessive, suppressing essential immune-regenerative signaling such as M2 macrophage differentiation or COX-2–mediated repair. Thus, our results stress the importance of identifying an optimal therapeutic window for ESEL in CKD settings. Further studies are warranted to determine biochemical markers of systemic toxicity and assess long-term safety, particularly in vulnerable populations. Dose escalation beyond 65 mg/kg BW may negate therapeutic benefits and lead to adverse renal outcomes, particularly in chronic disease contexts where tissue repair requires a tightly regulated immune response.

This study provides valuable preliminary data on the nephroprotective effects of ESEL in a rat model of CKD. However, it is essential to acknowledge several limitations that should be addressed in future research. First, the small sample size (n=4 per group) restricts the statistical power and generalizability of our findings. Second, our use of semi-quantitative RT-PCR provides only a relative measure of gene expression and does not offer absolute quantification as would be possible with quantitative real-time PCR (qRT-PCR). Third, while our results are consistent with the modulation of specific molecular pathways, we did not perform a direct assessment of the Nrf2/NF-κB pathways. Finally, the toxicity assessment was limited to body weight and renal function markers; a more comprehensive safety evaluation would benefit from the inclusion of additional parameters such as liver enzymes or markers of oxidative stress.

CONCLUSION

This study demonstrates that *Eurycoma longifolia* extract standardized to 8% eurycomanone shows potential as a nephroprotective agent in CKD models. Specifically, doses of 32.5 and 65 mg/kg BW improved renal function parameters, reduced albuminuria, and enhanced CD206 expression, suggesting anti-inflammatory and reparative effects. In contrast, the highest dose (130 mg/kg BW) showed signs of functional toxicity. These findings support the potential use of *Eurycoma longifolia* at appropriate doses to mitigate kidney damage in chronic kidney disease.

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