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Assessment of Cyclophosphamide-Induced Nephrotoxicity in a Preclinical Model and Evaluation of the Protective Efficacy of Sylvestroside I via Modulation of Pro-Inflammatory Cytokine Pathways

Hazim Y. Saeed[1], Mohammed Kareem Jabbar[2], Asawer Hasan Najm[3] , Alaa Sabeeh Alkhazali[3] and Ghadeer Sabah Bustani[4*]

1. Medical Laboratory Techniques Department, College of Health and Medical Technique, Al-Mustaqbal University, Babylon 51001, Iraq.

2. Alkafeel university

3. Al-Bayan University , Baghdad, Iraq

4. Al-Zahrawi University college

5. The Islamic University, Najaf, Iraq.

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*Corresponding Author E-Mail:

Bustani@iunajaf.edu.iq

ABSTRACT

The search for natural bioactive compounds capable of mitigating the side effects of pharmaceutical agents is a significant frontier in food science and functional nutrition. Cyclophosphamide (CYP), while a critical drug, induces nephrotoxicity primarily through its metabolite acrolein, provoking oxidative damage and inflammatory cytokine dysregulation. This study investigated the protective efficacy of Sylvestroside I (SYL), a secoiridoid glycoside of potential botanical origin, against CYP-induced renal injury in a preclinical model. Forty male Wistar rats were allocated into four groups: Control, CYP-only (200 mg/kg, i.p.), SYL-only (50 mg/kg/day, oral), and CYP+SYL. After a 30-day experimental period, renal function was assessed via serum creatinine and blood urea nitrogen (BUN). Oxidative stress was evaluated by measuring malondialdehyde (MDA) and total antioxidant capacity (TAC). Pro-inflammatory (TNF-α, IL-6) and protective (IL-22) cytokines were quantified, and renal histopathology was examined. CYP administration significantly impaired renal function, elevated oxidative stress (increased MDA, decreased TAC), disrupted cytokine balance (elevated TNF-α and IL-6, reduced IL-22), and caused severe histopathological damage. Sylvestroside I co-treatment markedly attenuated these effects, normalizing renal biomarkers, enhancing antioxidant status, restoring cytokine levels, and preserving renal tissue architecture. The results demonstrate that Sylvestroside I exerts potent renoprotective effects against CYP-induced toxicity, primarily through antioxidant and anti-inflammatory mechanisms. These findings position Sylvestroside I as a promising candidate for further development within the context of functional ingredients or nutraceutical strategies aimed at supporting renal health and reducing chemical-induced tissue injury.

1-Introduction

Cyclophosphamide is a nitrogen mustard alkylating agent, which has been used clinically widely to provide treatment for malignant diseases, such as lymphomas, breast cancer, and leukemias, moreover used for autoimmune diseases as systemic lupus erythematosus and rheumatoid arthritis. While the therapeutical application of cyclophosphamide is wide, the clinical utility has been decided in a big way by the dose-limiting toxicities, and nephrotoxicity has been evidently at the forefront. Kidneys are very sensitive to xenobiotic-triggered damage because these organs are involved in purification of the blood and concentrating metabolites that are toxic. Furthermore, the cyclophosphamide undergoes metabolism during the metabolic process, it metabolizes to the active species phosphoramide mustard, the molecule responsible for cytotoxicity, and the very reactive metabolite acrolein that triggers oxidative stress, lipid peroxidation, and DNA lesions in renal tissue (Ayza et al., 2022; Mombeini et al., 2022). The pathogenesis of cyclophosphamide nephrotoxicity involves a multi-factorial process involving oxidative stress, inflammation, and apoptosis. Experimental studies have found that cyclophosphamide induction leading to a rise in serum creatinine and blood urea nitrogen to be consistent with impaired glomerular filtration and renal impairment (Ghobadi et al., 2017a; Selvakumar et al., 2006; Yu et al., 2021).

Inflammatory cytokines are no less important in cyclophosphamide induced renal lesions. TNF-α is one of such pro-inflammatory cytokines to initiate the down-stream signaling cascades to apoptosis and necrosis of tubular cells in the kidneys (Ghobadi et al., 2017b; Kara, 2024; Mombeini et al., 2022). Similarly, IL-6 has been found to have a role in the facilitation of the enhancement in inflammation and the induction in leukocyte recruitment to renal parenchyma, thus leading to tubular degeneration and glomerular atrophy (Abdelfattah-Hassan et al., 2019; Hu, H. C. et al., 2022; Sonigo et al., 2019). On the other hand, IL-22 has been found to have a protective one as a cytokine by initiating the induction in the signaling pathway JAK2/STAT3 to induce cell survival, repair, and regeneration in the model of nephritis caused by cyclophosphamide (Dong, Y. et al., 2021). The interaction among the above cytokines thus determines the direction in tissue destruction or repair in the model of nephritis caused by cyclophosphamide.

There have been recent efforts to investigate the activity of the natural antioxidants and plant molecules as adjuvant therapies to combat cyclophosphamide nephrotoxicity. The phytoconstituents berberine, thymoquinone, and phenolic fractions have been found to show nephroprotection by down-regulation of oxidative stress and modulation of pro-inflammatory cytokines and thus mediate histological and functional repair in animal models (Abd-Alla et al., 2024; Aladaileh et al., 2021; Bokhary et al., 2022). Of special interest here is Sylvestroside I, a secoiridoid glycoside, inasmuch as it has been found to be a molecule of interest because it possesses very good antioxidant and anti-inflammatory activity. Although not the center of as rigorous a study as other phytoconstituents, there are hints in the light of structurally akin secoiridoids that Sylvestroside I has the potential to provide renoprotection by radical scavenging, recovery of enzymic antioxidant activity, and control over the response in the cytokines (Ayza et al., 2022).

Therefore, the present study was designed to evaluate the histopathological and biochemical alterations associated with cyclophosphamide induced nephritis and to investigate the renoprotective efficacy of Sylvestroside I. Special emphasis was placed on the modulation of inflammatory cytokines (TNF-α, IL-6, and IL-22), renal function markers (serum creatinine and

BUN), and oxidative stress indices (MDA and TAC). This comprehensive approach aims to provide mechanistic insights into the dual protective role of Sylvestroside I in ameliorating inflammation and oxidative stress while preserving renal architecture.

2-Material and methods

Ethics Approval:

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of The Islamic University, Najaf (2025-2-32; 432Is), following ethical guidelines for laboratory animal research.

Animals

Forty healthy male albino Wistar rats weighing between 150 and 200 g were used in this experiment. The animals were obtained from the Abu grab, Baghdad, Iraq. They were housed in standard international cages at the Animal House at Najaf, under controlled environmental conditions (temperature 25–30 °C). All rats were maintained on a standard pellet diet with free access to water throughout the study period.

Study design

The experiment was conducted on 40 healthy adults male Wistar rats randomly divided into four equal groups (n = 10 each) and observed for 30 days. Group I (CON) received a vehicle only and served as the control reference. Group II (CYP) the Cyclophosphamide was administered a single intraperitoneal dose of cyclophosphamide (200 mg/kg) on the first day of the experiment to induce nephrotoxicity (Al-Mousaw et al., 2022; Oyagbemi et al., 2016). Group III (SYL) received Sylvestroside I alone (50 mg/kg/day orally) to evaluate its independent effects. Group IV (CYP+SYL) was co-treated with cyclophosphamide (200 mg/kg, intraperitoneally) and Sylvestroside I (50 mg/kg/day orally) to assess the compound's protective role. At the end of the 30-day treatment period, blood samples were collected for biochemical analysis of serum creatinine, blood urea nitrogen (BUN), and total antioxidant capacity (TAC). In addition, kidney tissues were harvested for histopathological examination, assessment of oxidative stress markers such as malondialdehyde (MDA), and cytokine evaluation including TNF-α, IL-6, and IL-22.

Cyclophosphamide

The Cyclophosphamide was purchased from Al-Faiha Company-Najaf, Iraq.

Sylvestroside I

Sylvestroside I (Cat. No. HY-N3030, purity 98%, CAS No. 71431-22-6) was obtained in purified form from MedChemExpress, USA (Figure 1; supplementary file). For experimental administration, Sylvestroside I was freshly suspended in 0.5% carboxymethylcellulose (CMC) aqueous solution, which served as the vehicle and ensured uniform oral delivery. The suspension was prepared daily to maintain stability and prevent compound degradation and was administered immediately after preparation. Rats in the treatment groups received Sylvestroside I orally at a dose of 50 mg/kg/day for 30 consecutive days.

Interleukin-6 (IL-6)

IL-6 concentrations were determined using a commercial Rat Interleukin-6 (IL-6) ELISA kit (SunLong Biotech Co., Ltd., China; Cat. No. SL0411Ra). This sandwich ELISA kit is intended for sensitive detection of IL-6 in rat serum, plasma, tissue homogenates, culture media, and other biological fluids. The assay has a detection range of 8–150 ng/L, with a sensitivity of 1.5 ng/L. Sample preparation was performed according to the manufacturer's instructions. Serum samples were obtained after clotting and centrifugation, while kidney tissues were homogenized in PBS (pH 7.4), followed by

centrifugation at 2,000–3,000 rpm for 20 minutes to collect the supernatant. Standards, controls, and prepared samples were added to antibody-coated wells, followed by incubation with an HRP-conjugated IL-6 antibody. After thorough washing, chromogen solutions A and B were introduced to develop color, and the reaction was terminated with stop solution. Absorbance was measured at 450 nm using a microplate reader, and IL-6 concentrations were calculated from a standard curve generated with known calibrators.

Interleukin-22 (IL-22)

IL-22 levels were measured using a commercial Rat Interleukin-22 (IL-22) ELISA kit (SunLong Biotech Co., Ltd., China; Cat. No. SL0790Ra). This sandwich ELISA utilizes microplate wells pre-coated with an IL-22-specific antibody to capture the target cytokine. Standards, controls, and prepared samples were added to the wells, followed by incubation with a horseradish peroxidase (HRP)-conjugated anti-IL-22 detection antibody. After washing, chromogenic substrates were introduced, and the reaction was terminated with stop solution, producing a measurable color change. Absorbance was recorded at 450 nm using a microplate reader. The assay provides a detection range of 3–200 pg/mL and a sensitivity of 0.6 pg/mL. IL-22 concentrations in serum and kidney tissue homogenates were calculated from a standard curve generated with known calibrators. Sample preparation and assay procedures were carried out strictly in accordance with the manufacturer's instructions

Tumor Necrosis Factor-Alpha (TNF-α)

The concentration of TNF-α was measured using a commercial ELISA kit (Melsin, China; Cat. No. EKRAT-0419), following the manufacturer's instructions. This assay is based on the enzyme-linked immunosorbent assay (ELISA) principle, which is widely applied in immunological studies to quantitatively detect proteins, peptides, and cytokines. Serum and kidney tissue homogenates were prepared and loaded into the antibody-coated wells, followed by incubation with specific detection antibodies and enzyme conjugates. After the addition of the substrate solution, colorimetric development was measured at the appropriate wavelength using a microplate reader. TNF-α concentrations were determined from a standard curve generated with known cytokine concentrations.

Total Antioxidant Capacity (TAC)

Total antioxidant capacity (TAC) was determined using the Total Antioxidant Capacity (T-AOC) Assay Kit (Solarbio Life Sciences, China; Cat. No. BC1315). Serum was separated from whole blood by centrifugation at 3000 × g for 10 minutes at 4 °C, and aliquots were stored at −80 °C until analysis. Before use, samples were thawed on ice and handled according to the manufacturer's protocol. Briefly, the provided extract solution was precooled at 2–8 °C and used for sample preparation. The standard solution (40 μmol/mL) was freshly prepared by dissolving 10 mg of the supplied powder in 0.9 mL of distilled water plus 20 μL concentrated H_2SO_4. A working reaction mixture was prepared immediately before use by combining Reagents I, II, and III in a 7:1:1 ratio. For each reaction, serum samples or standards were mixed with the reaction solution in microplate wells. After cooling and centrifugation, the absorbance of the supernatant was recorded at 593 nm using a spectrophotometer.

Malondialdehyde (MDA)

Lipid peroxidation was assessed by quantifying malondialdehyde (MDA) levels in serum using the MDA Content Assay Kit (Solarbio Life Sciences, China; Cat. No. BC0025). Blood samples were allowed to clot at room temperature and centrifuged at 3000 × g for 10 min to obtain clear serum. For the assay, serum samples

were mixed with the MDA working reagent (prepared by dissolving Reagent II in Reagent I according to the manufacturer's instructions) and Reagent III. The mixture was incubated at 100 °C for 60 min to allow condensation of MDA with thiobarbituric acid under acidic conditions. After cooling and centrifugation, the absorbance of the supernatant was recorded at 532 nm using a spectrophotometer.

Histopathological examination of kidneys

For histopathological assessment, the kidneys were carefully excised, longitudinally opened, and immediately fixed in 10% neutral-buffered formalin for 48 hours at room temperature (22–25 °C). The fixed tissues were then processed following standard histological procedures, which included graded dehydration through ascending concentrations of ethanol, clearing in xylene (two stages), and embedding in paraffin wax at 56 °C for approximately 2 hours. Paraffin blocks were sectioned at a thickness of 5 µm using a rotary microtome. The obtained sections were dewaxed, rehydrated, and stained with Harris hematoxylin and eosin (H&E). Finally, the stained slides were examined under a light microscope at magnifications of 4×, 10×, and 40× to evaluate renal histoarchitecture and pathological alterations.

Statistical Analysis

All data were analyzed using GraphPad Prism software, version 9 (GraphPad Software, San Diego, CA, USA). The Shapiro–Wilk test was applied to assess the normality of data distribution. Differences among experimental groups were evaluated using a mixed-model, repeated-measures one-way analysis of variance (ANOVA). A *p*-value of less than 0.05 was considered statistically significant. Results are expressed as the mean ± standard error of the mean (SEM).

3-Result

The Serum levels of TNF-α were significantly altered among the experimental groups (Figure 2). The CYP group exhibited a marked elevation in TNF-α concentration (104.56 ng/L) compared to the control group (CON, 60.20 ng/L; ****p < 0.0001). Administration of SYL I alone did not produce significant change compared with the control (62.76 ng/L; ns), indicating its lack of pro-inflammatory effect. Notably, co-treatment with Sylvestroside I CYP+SYL significantly reduced TNF-α levels (50.79 ng/L) compared with the CYP group (***p < 0.0001), bringing the values closer to normal and even slightly lower than the control group (**p < 0.01). These findings confirm that cyclophosphamide induces a strong pro-inflammatory response through TNF-α upregulation, while Sylvestroside I effectively attenuates this cytokine surge and demonstrates protective anti-inflammatory properties.

As shown in Figure 3, serum IL-6 levels varied significantly among the experimental groups. Cyclophosphamide administration led to a significant increase in IL-6 concentration (195.20 ng/ml) compared to the control group (CON, 175.59 ng/L; ****p < 0.0001). Treatment with Sylvestroside I alone (178.86 ng/L) showed no significant difference compared with the control (ns), indicating that Sylvestroside I did not provoke an inflammatory response when given independently. In contrast, co-administration of Sylvestroside I with cyclophosphamide CYP+SYL significantly reduced IL-6 levels (163.37 ng/L) compared to the CYP group (***p < 0.0001) and even showed a modest reduction compared with the control group (**p < 0.01). These findings suggest that while cyclophosphamide induces IL-6 upregulation, Sylvestroside I exhibits a potent modulatory effect by attenuating IL-6 expression, thus demonstrating its protective anti- inflammatory action.

As illustrated in Figure 4, IL-22 levels were significantly altered by cyclophosphamide treatment and Sylvestroside I administration. The control group (CON) exhibited baseline IL-22 concentrations (46.89 ng/ml). Cyclophosphamide administration (CYP) caused a pronounced reduction (23.07 ng/L; ****$p < 0.0001$ vs. CON), indicating suppression of this protective cytokine. Sylvestroside I alone (SYL) partially restored IL-22 levels (35.45 ng/L; ****$p < 0.0001$ vs. CYP, but still significantly lower than CON). Interestingly, the co-treatment group (CYP+SYL) demonstrated a substantial elevation (48.56 ng/L), which was significantly higher than the CYP group (****$p < 0.0001$) and comparable to the control group (ns). These findings suggest that Sylvestroside I not only mitigated the CYP-induced suppression of IL-22 but also restored its levels to near-normal, highlighting its role in promoting immune protection and tissue repair.

The Blood MDA levels, an indicator of lipid peroxidation, did not show statistically significant differences among the experimental groups (Figure 5). The control group (CON) recorded a mean value of 1.55 nmol/mL, while the cyclophosphamide group (CYP) demonstrated a slight but non-significant reduction (1.25 nmol/mL). Rats treated with Sylvestroside I alone (SYL) showed levels similar to the control (1.50 nmol/mL), indicating no notable oxidative imbalance. Interestingly, the co-treatment group (CYP+SYL) displayed a mild elevation in MDA (1.71 nmol/mL), yet this change was also not statistically significant compared to either the control or CYP groups (ns). These findings suggest that, under the current experimental conditions, cyclophosphamide and Sylvestroside I did not significantly influence systemic lipid peroxidation as reflected in blood MDA levels.

As illustrated in Figure 6, total antioxidant capacity (TAC) levels varied significantly across the groups. The control group (CON) exhibited the highest TAC value (7.35 nmol/mL), while cyclophosphamide administration (CYP) resulted in a significant decline (6.48 nmol/mL; ****$p < 0.0001$ vs. CON), confirming oxidative stress induction. Treatment with Sylvestroside I alone (SYL) led to a marked reduction in TAC (2.24 nmol/mL; ****$p < 0.0001$ vs. CON and CYP), suggesting altered antioxidant balance under its administration. Similarly, the co-treatment group (CYP+SYL) displayed low TAC levels (2.48 nmol/mL), which were significantly reduced compared with both the control and CYP groups (***$p < 0.001$). No significant difference was detected between SYL and CYP+SYL groups (ns). These findings indicate that while cyclophosphamide reduced antioxidant capacity, Sylvestroside I—either alone or combined—did not restore TAC but rather maintained suppressed antioxidant levels in systemic circulation.

Histological examination of renal tissue from the control group (Figure 7-A) revealed a normal kidney architecture. The glomerulus appeared intact, with well-defined capillary tufts and normal mesangial cellularity. Bowman's capsule was preserved, showing no signs of thickening or distortion. The surrounding renal tubes maintained their typical morphology with no evidence of degeneration, necrosis, or inflammatory infiltration. These findings confirm the absence of pathological alterations, reflecting the normal histological features of healthy renal tissue. Renal sections from the cyclophosphamide-treated group showed (Figure 7-B) marked histopathological alterations compared to the control. The glomeruli exhibited shrinkage with widened Bowman's space and partial loss of normal architecture. There was evidence of mesangial cell proliferation and degeneration of glomerular capillary tufts. Tubular structures displayed vacuolar degeneration,

cytoplasmic swelling, and focal necrosis. Inflammatory cell infiltration was also observed in the interstitial areas. These changes are indicative of cyclophosphamide-induced nephrotoxicity, reflecting both glomerular and tubular injury. Kidney tissue from rats treated with Sylvestroside I alone demonstrated largely preserved renal histoarchitecture. The glomeruli appeared normal with intact capillary tufts and no apparent thickening of the Bowman's capsule. Mesangial cells were within normal limits, and the surrounding renal tubules maintained their typical structure with only minimal alterations. There was no significant evidence of necrosis, degeneration, or inflammatory infiltration. These findings indicate that Sylvestroside I, when administered alone, does not exert nephrotoxic effects and maintains normal renal morphology (Figure 7-C). Renal sections from rats co-treated with cyclophosphamide and Sylvestroside I (Figure 7-D) showed a marked improvement in kidney architecture compared to the cyclophosphamide group alone. The glomeruli appeared more preserved, with nearly normal capillary tufts and reduced mesangial proliferation. Bowman's space was less dilated, and tubular structures showed minimal degenerative changes. Evidence of necrosis and inflammatory infiltration was significantly attenuated. These findings indicate that Sylvestroside I exerted a protective effect against cyclophosphamide-induced nephrotoxicity, restoring glomerular and tubular morphology toward normal.

4-Discussion

The present study showed that Sylvestroside I co-treatment was associated with improvements in oxidative stress markers, antioxidant defenses, and cytokine profiles in a cyclophosphamide-induced nephrotoxicity model. Our results revealed that CYP administration led to marked renal injury characterized by histopathological damage, elevated serum creatinine and BUN, increased MDA, reduced TAC, and dysregulation of cytokines, notably elevated TNF-α and IL-6 with concomitant suppression of IL-22. Co-treatment with SYL significantly reversed these alterations, supporting its potential as a nephroprotective compound. The CYP nephrotoxicity is primarily mediated through its toxic metabolite acrolein, which induces reactive oxygen species (ROS) generation, lipid peroxidation, and activation of pro-inflammatory cascades(Ayza et al., 2022; Raoof et al., 2025). Consistent with our findings, several studies have reported increases in oxidative stress markers such as MDA and decreases in antioxidant enzymes (SOD, CAT, GSH) following CYP exposure ((Ghobadi et al., 2017b; Kara, 2024; Ma et al., 2021). The observed rise in TNF-α and IL-6 in the CYP group aligns with evidence that these cytokines are central mediators of CYP-induced renal inflammation via NF-κB and MAPK activation (Aladaileh et al., 2021; Bustani, G. S. & Alghetaa, 2025a). Interestingly, IL-22, a cytokine with renoprotective roles in tissue repair and inflammation resolution, was markedly suppressed in the CYP group, echoing reports that nephrotoxins attenuate IL-22 signaling and thereby compromise renal recovery (Dong, X. J. et al., 2021; Dong, Y. et al., 2021). Restoration of IL-22 by SYL indicates a potential role in balancing pro- and anti-inflammatory cytokine responses. These findings raise the possibility that SYL may contribute to regenerative pathways, similar to effects reported with other phytochemicals (Bustani, G. S. & Alghetaa, 2025b; Mombeini et al., 2022) and formononetin (Aladaileh et al., 2021; Gati & Bustani, 2022). The antioxidant potential of SYL, as evidenced by reduced MDA and improved TAC, is consistent with studies showing that natural iridoid glycosides and polyphenolic compounds counteract CYP-induced redox imbalance (Ayza et al., 2022; Zhang et al., 2021). Similar nephroprotection was observed

with synaptic acid (Raoof et al., 2025), thymoquinone (Mahmood et al., 2025), and cinnamaldehyde (Abd El Salam et al., 2023), all of which enhance Nrf2-mediated antioxidant responses and attenuate NF-κB-driven inflammation. Our histological findings further support the biochemical results, where CYP caused glomerular shrinkage, tubular necrosis, and interstitial infiltration, in line with previous reports of CYP-induced nephropathy (Ayza et al., 2022; Wanas et al., 2021). SYL administration mitigated these alterations, paralleling the effects of other phytochemicals such as berberine (Mombeini et al., 2022) and garlic extract (Ruan et al., 2021).

Based on the observed biochemical and cytokine changes, SYL may act through dual mechanisms: reducing oxidative stress and inflammatory mediators (e.g., TNF-α, IL-6), and enhancing protective cytokines such as IL-22. While our data cannot confirm signaling pathway involvement, these patterns are consistent with previous reports of phytochemicals acting on NF-κB, MAPK, and Nrf2 pathways (Zhang et al., 2021), and (ii) upregulation of protective cytokines like IL-22, which promotes renal epithelial regeneration and dampens chronic inflammation (Bustani, G. et al., 2025; Dong, Y. et al., 2021). This mechanistic profile aligns with other nephroprotective compounds that target the Nrf2/HO-1 and NF-κB/NLRP3 inflammasome pathways (Ayza et al., 2022; Hu, J. et al., 2022). Taking together, our findings suggest that phytochemicals such as SYL may mitigate CYP-induced toxicity by improving redox balance and modulating immune responses. These results warrant further mechanistic studies to clarify the pathways involved and to explore potential translational applications.

5-Conclusion

The present study demonstrated that cyclophosphamide induces profound nephrotoxicity characterized by oxidative stress, inflammatory cytokine imbalance, and histopathological alterations. Co-treatment with Sylvestroside I was associated with attenuation of these changes, including lower TNF-α and IL-6 levels, partial restoration of IL-22, improved antioxidant capacity, and better preservation of renal architecture. These findings suggest that Sylvestroside I may confer nephroprotective potential, possibly through modulation of oxidative stress and inflammatory pathways, although further mechanistic studies are required to confirm these effects.

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The legends

Figure 1. Chemical structure of Sylvestroside I

Figure 2. Serum TNF-α levels in experimental groups.

Cyclophosphamide administration (CYP) significantly increased TNF-α compared to the control (CON). Sylvestroside I alone (SYL) did not alter TNF-α levels compared with the control, whereas co-treatment (CYP+SYL) markedly reduced TNF-α compared to CYP, demonstrating an anti-inflammatory protective effect. Data are expressed as mean ± SEM; ns = non-significant, **p < 0.01, ****p < 0.0001.

Figure 3. Serum IL-6 levels in experimental groups.

Cyclophosphamide (CYP) induced a significant elevation in IL-6 compared with control (CON). Sylvestroside I alone (SYL) showed no significant difference from control, while co-treatment (CYP+SYL) significantly reduced IL-6 compared with CYP, indicating attenuation of inflammatory response. Data are expressed as mean ± SEM; ns = non-significant, **p < 0.01, ****p < 0.0001.

Figure 4. Serum IL-22 levels in experimental groups.

Cyclophosphamide administration (CYP) significantly reduced IL-22 levels compared with the control group (CON). Sylvestroside I treatment (SYL) maintained IL-22 values comparable to control, while co-treatment (CYP+SYL) significantly restored IL-22 levels relative to CYP, indicating a modulatory protective effect of Sylvestroside I. Data are expressed as mean ± SEM; statistical significance indicated as ns = non-significant, **p < 0.01, ****p < 0.0001.

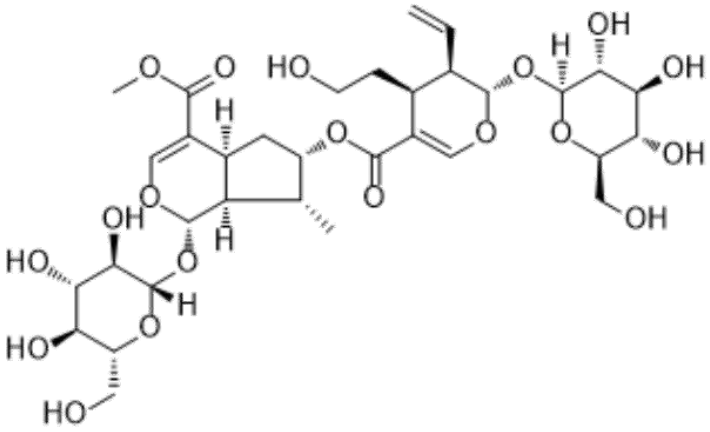
Figure 5. Blood malondialdehyde (MDA) levels in experimental groups.

No significant differences were observed in MDA levels among the control (CON), cyclophosphamide (CYP), Sylvestroside I (SYL), and co-treatment (CYP+SYL) groups, indicating that neither CYP nor Sylvestroside I markedly influenced systemic lipid peroxidation under these conditions. Data are expressed as mean ± SEM; ns = non-significant.

Figure 6. Blood total antioxidant capacity (TAC) in experimental groups.

Control rats (CON) exhibited the highest TAC values. Cyclophosphamide (CYP) significantly reduced TAC compared with control. Both Sylvestroside I alone (SYL) and co-treatment (CYP+SYL) showed further significant reductions in TAC compared with CON and CYP, with no significant difference between SYL and CYP+SYL. Data are expressed as mean ± SEM; ns = non-significant, ***p < 0.001, ****p < 0.0001.

Figure 7. Histopathological changes in kidney tissues of experimental groups (H&E staining, ×400). (A) **Control group (CON):** Normal renal histology with intact glomerular architecture, preserved Bowman's capsule, and well-organized renal tubules. (B) **Cyclophosphamide group (CYP):** Marked pathological alterations, including glomerular shrinkage, widened Bowman's space, tubular degeneration, and inflammatory infiltration, indicating nephrotoxicity. (C) **Sylvestroside I group (SYL):** Preserved kidney structure with intact glomeruli and tubules, showing no significant histological abnormalities. (D) **Cyclophosphamide + Sylvestroside I group (CYP+SYL):** Improved renal architecture with near-normal glomeruli, reduced tubular degeneration, and attenuated inflammatory changes, demonstrating the protective effect of Sylvestroside I against cyclophosphamide-induced injury.



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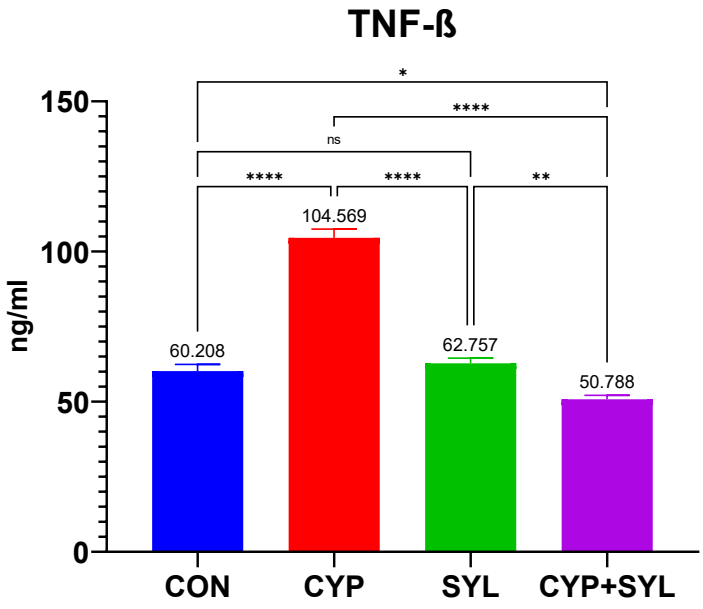

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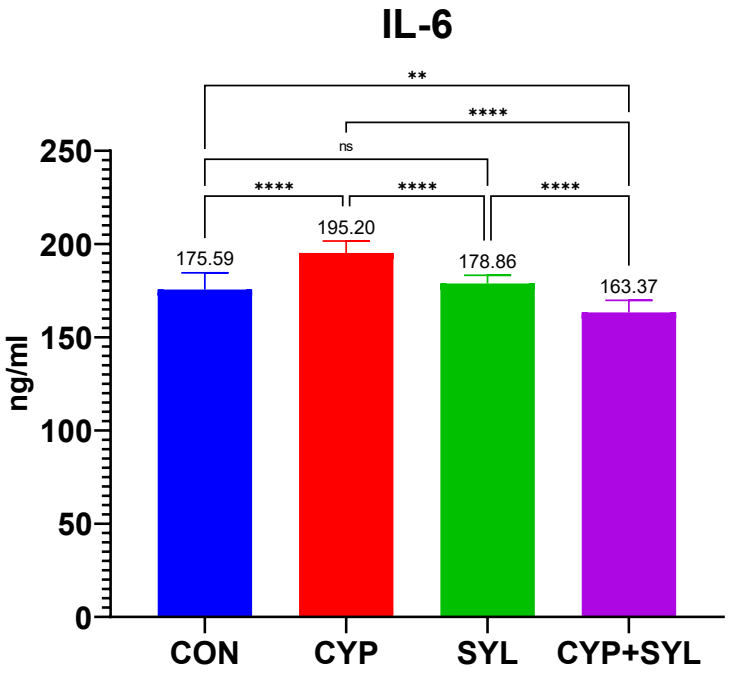

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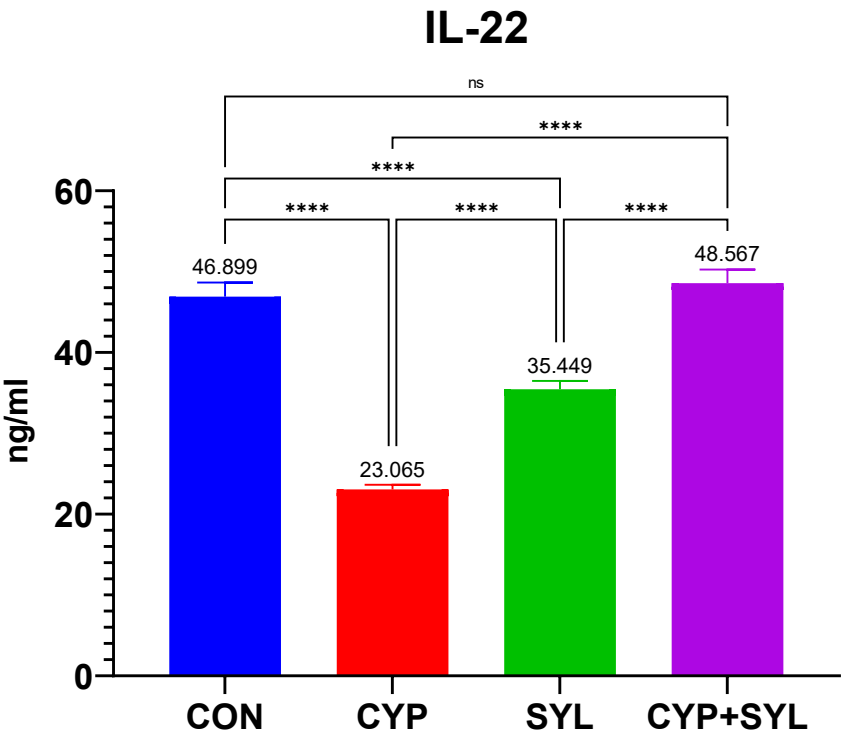
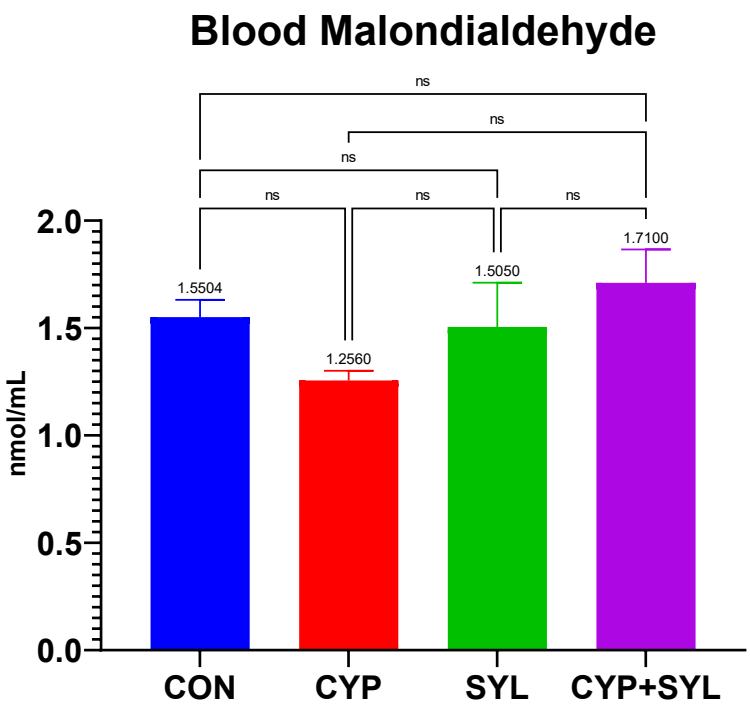


Figure 4. Serum IL-22 levels in experimental groups.



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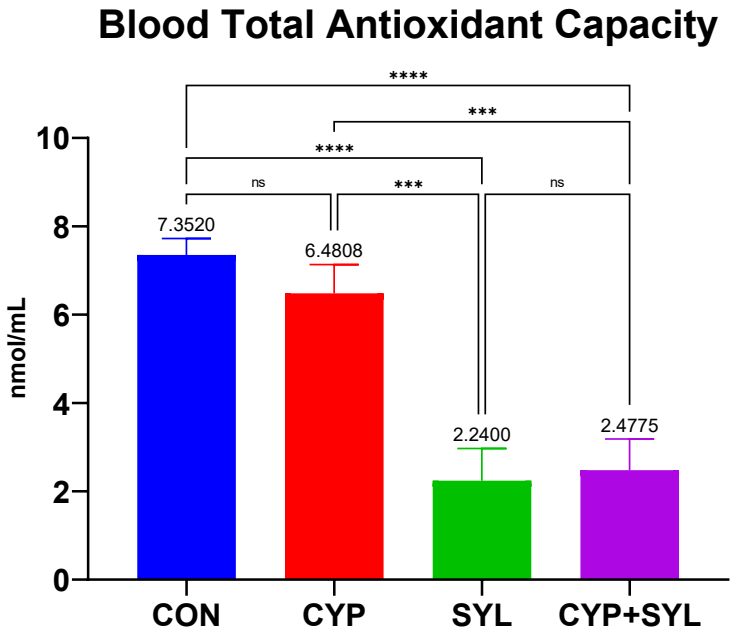

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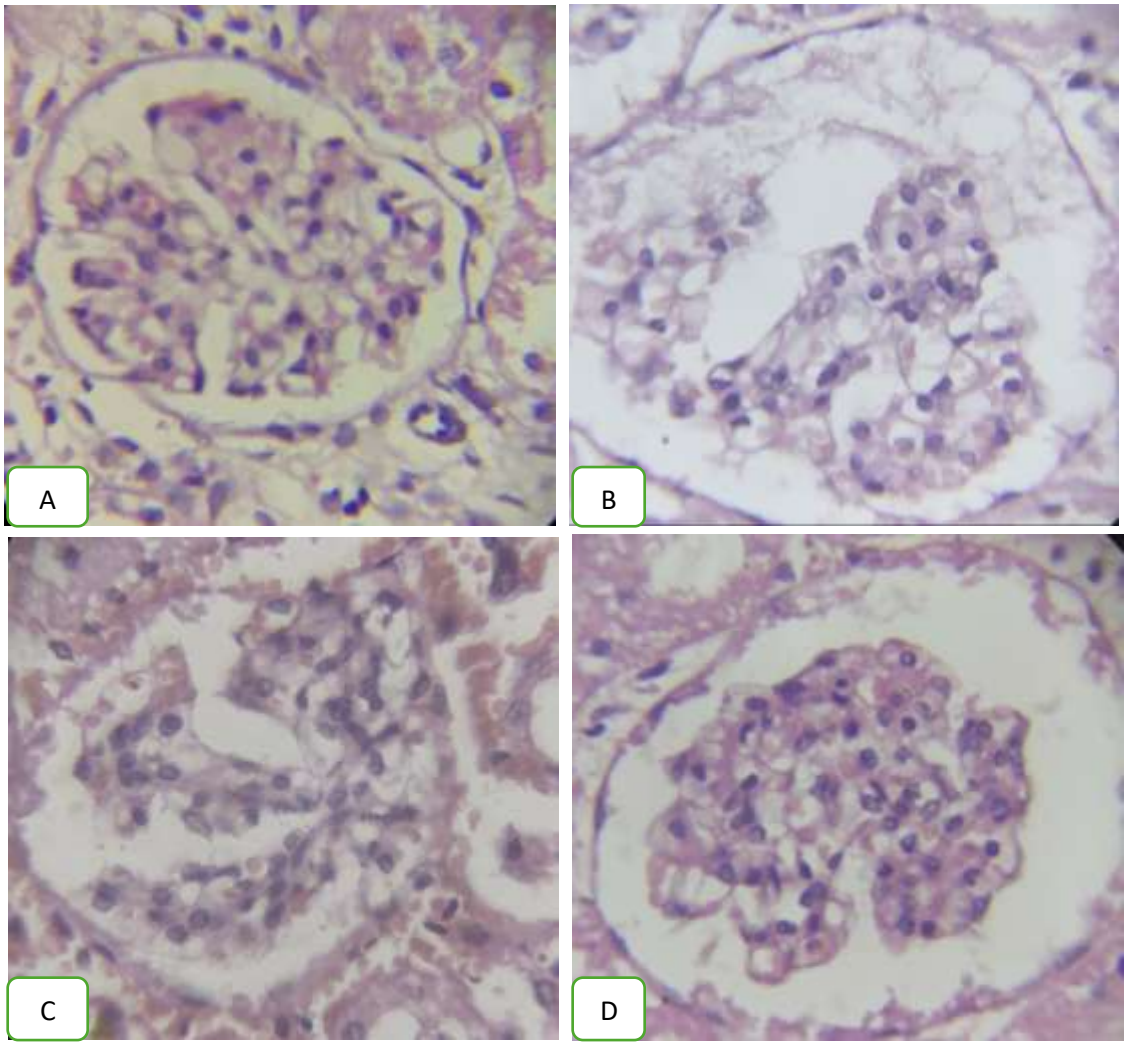

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