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Development and Evaluation of a Poly Herbal Mixture Containing *Curcuma longa* and *Camellia sinensis* Against Fluoride-Induced Nephrotoxicity in Rats

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Abstract

Fluoride-induced nephrotoxicity, marked by oxidative stress, inflammation, and renal dysfunction, poses a significant health risk. This study assessed the nephroprotective potential of a polyherbal mixture comprising *Curcuma longa* (turmeric) and *Camellia sinensis* (green tea) in a rat model. Male Sprague Dawley rats were assigned to five groups: normal control, disease control (600 ppm fluoride), positive control (vitamin C, 10 mg/kg), and two treatment groups receiving the polyherbal mixture at 200 mg/kg and 400 mg/kg. Fluoride exposure led to notable renal dysfunction, evident through decreased body weight, altered urinary profiles, elevated serum creatinine and blood urea nitrogen (BUN), increased oxidative stress (elevated malondialdehyde and antioxidant enzyme activities), and heightened inflammation (increased TNF-α and IL-6). Histopathological analysis also confirmed kidney damage. Treatment with the polyherbal mixture, especially at the high dose, significantly ameliorated these alterations—normalising renal biomarkers, reducing oxidative and inflammatory markers, and preserving renal histology. The high-dose group exhibited more pronounced protective effects than the vitamin C-treated group. These findings highlight the multi-targeted nephroprotective action of *C. longa* and *C. sinensis*, attributed to their antioxidant, anti-inflammatory, and tissue-protective properties. The results support their therapeutic potential against fluoride-induced renal injury. Further studies, including human trials, are recommended to validate efficacy and determine optimal dosing for clinical applications.

Keywords: Fluoride Nephrotoxicity, *Curcuma longa*, *Camellia sinensis*, Oxidative Stress, Inflammation, Nephroprotection

1. Introduction

The kidneys are vital organs responsible for maintaining internal homeostasis through their roles in filtration, detoxification, electrolyte balance and hormone regulation. However, the continuous exposure of the kidneys to circulating toxins renders them highly susceptible to damage[1]. Nephrotoxicity, a pathological condition arising from exposure to toxic substances, disrupts renal function and can lead to acute or Chronic Kidney Disease (CKD). Among the environmental nephrotoxins, fluoride, a naturally occurring element found in water, soil, and various industrial products, has garnered significant attention owing to its potential to induce renal damage at elevated concentrations[2].

Fluoride toxicity predominantly affects the kidneys through mechanisms involving oxidative stress,

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inflammation, and structural damage to the renal tissues. Chronic exposure to high fluoride levels has been associated with reduced glomerular filtration, tubular necrosis, and interstitial inflammation, leading to impaired renal function. The increasing prevalence of environmental fluoride exposure underscores the urgent need for effective therapeutic interventions to mitigate its nephrotoxic effects[3,4].

Herbal medicines have emerged as promising candidates for nephroprotection because of their multifaceted pharmacological properties and low side effect profiles. *Curcuma longa* (turmeric) and *Camellia sinensis* (green tea) have demonstrated notable antioxidative and anti-inflammatory effects. Curcumin, the principal bioactive compound in *C. longa*, exhibits robust free radical-scavenging properties, inhibits pro-inflammatory pathways, and promotes cellular repair mechanisms[5–10]. Similarly, catechins in *C. sinensis* are potent antioxidants that enhance renal function by reducing oxidative stress and inflammation[11–16]. Although both herbs individually offer nephroprotective benefits, their combined effects in addressing fluoride-induced renal damage remain unexplored.

This study aims to evaluate the nephroprotective potential of a polyherbal mixture of *C. longa* and *C. sinensis* in a rat model of fluoride-induced nephrotoxicity. The effects of the mixture were assessed through a comprehensive analysis of renal function markers, oxidative stress parameters, inflammatory mediators, and histopathological changes. By leveraging the synergistic properties of these herbs, this study sought to provide insights into a natural, multi-targeted therapeutic approach for mitigating fluoride-induced kidney damage.

2. Methodology

2.1 Preparation of Poly-Herbal Mixture

A polyherbal mixture utilising *C. longa* (turmeric) and *C. sinensis* (green tea) requires consideration of the synergistic effects of these herbs, their active compounds, and the intended health benefits based on the available literature. 48% extract of each plant and 4% piperine were incorporated to enhance absorption[17,18]. Plant extracts, in their respective quantities, were formulated into a suspension using 0.5% Sodium Carboxymethyl cellulose (Na-CMC).

2.2 Animal Selection

Thirty male Sprague Dawley rats, aged 8-10 weeks and weighing–200-250 grams, were selected for the study due to their well-documented susceptibility to nephrotoxic agents, making them suitable for investigating nephroprotective interventions. The rats were housed in a controlled environment with a 12-hour light/dark cycle, maintained at a temperature of 22 ± 2°C and relative humidity of 50-60%. Standard laboratory chow and water were provided ad libitum to ensure that the nutritional needs of the animals were met throughout the study. All animal experiments were approved by IAEC 1523/PO/Re/S/11/CPCSEA.

2.3 Induction of Nephrotoxicity

Nephrotoxicity is induced by administering fluoride (sodium fluoride) at a concentration of 60 ppm in drinking water for one week. This concentration has been previously shown to induce significant renal damage without excessive mortality[19].

2.4 Experimental Design

The study involved the random allocation of rats into five experimental groups, each comprising six animals. The Normal Control Group received the vehicle control (distilled water) orally without any additional treatment. The Disease Control Group was orally administered fluoride water (600 ppm) to induce nephrotoxicity. The Positive Control Group was treated with vitamin C, a standard nephroprotective agent, administered orally at a dose of 10 mg/kg body weight. The low-dose polyherbal mixture group received the polyherbal mixture at a dose of 200 mg/kg body weight orally, while the high-dose polyherbal mixture group was administered the same mixture at a dose of 400 mg/kg body weight orally. This design enabled the evaluation of the nephroprotective potential of the polyherbal mixture at different doses compared to standard treatment and disease control.

2.5 Assessment of Nephroprotective Activity

After the completion of the treatment period, the nephroprotective activity will be assessed using various methods, including blood collection, renal function parameter evaluation, histopathological examination, oxidative stress marker analysis and biochemical assessment.

2.5.1 Blood Collection

Upon completion of the treatment, the rats were anaesthetised using a combination of ketamine (70 mg/kg) and xylazine (10 mg/kg) administered via intraperitoneal injection. This anaesthetic regimen was chosen for its efficacy in providing sedation and analgesia while minimising stress to the animals. Following anaesthesia, blood was collected from the retro-orbital plexus using heparinised microcapillary tubes to avoid coagulation. The procedure will be performed with utmost care to ensure minimal distress and prevent injury to animals. The collected blood samples were allowed to clot at room temperature for 30 min, after which they were centrifuged at 3000 rpm for 10 min to separate the serum from the cellular components. The serum was stored at -20°C for subsequent biochemical analyses.

2.5.2 Renal Function Parameters

To assess renal function, serum samples were analysed for key parameters, including serum creatinine and Blood Urea Nitrogen (BUN). Serum creatinine will be measured using an enzymatic colourimetric assay that quantifies the level of creatinine in the blood, providing insights into kidney filtration capability. BUN will be determined using a urease /Berthelot reaction-based method, allowing for the assessment of protein metabolism and kidney function. Additionally, urine output will be collected over 24 hours before euthanasia using metabolic cages to measure the volume of urine produced by each rat, which is a crucial indicator of renal function.

2.5.3 Measurement of Oxidative Stress Markers

Oxidative stress levels in the kidneys were assessed by preparing homogenates of snap-frozen kidney tissues in ice-cold Phosphate-Buffered Saline (PBS). Malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities were assessed using an ELISA KITS (ELAB Science, USA). The assays were performed according to the manufacturer's instructions.

2.5.4 Inflammatory Mediators

In addition to oxidative stress markers, inflammatory markers will be analysed to assess the inflammatory response associated with nephrotoxicity. The levels of Tumour Necrosis Factor-alpha (TNF-α) and Interleukin-6 (IL-6) were quantified using Eenzyme-Linked Immunosorbent Assay (ELISA) kits (ELAB Science, USA) according to the manufacturer's instructions. These cytokines serve as indicators of inflammation and can provide insights into the underlying mechanisms of the nephroprotective effects.

2.5.5 Histopathological Examination

After blood collection, the rats were euthanised using CO_2 inhalation according to ethical guidelines. Kidneys were harvested immediately and rinsed in cold PBS to remove excess blood and debris. One kidney was fixed in 10% formalin for histological analysis, whereas the other was snap-frozen in liquid nitrogen for biochemical assessment. For histopathological examination, kidney tissues were embedded in paraffin and sectioned into 5µm slices using a microtome. The sections were stained with Hematoxylin and Eosin (H&E) for general morphological evaluation. A light microscope was used to assess histopathological changes, including tubular dilation, necrosis, and inflammatory cell infiltration, providing insights into the severity of renal injury.

2.5.6 Statistical Analysis

Data will be analysed using appropriate statistical methods to evaluate the significance of the findings. One-way ANOVA variance was used to compare the differences between the experimental groups. If significant differences were observed, post-hoc tests (Tukey's test) were conducted to determine which groups differed from each other. The results are presented as mean ± Standard Deviation (SD), and statistical significance was set at $p < 0.05$, allowing for a clear interpretation of the nephroprotective effects of the polyherbal mixture.

3. Results

3.1 Body Weight

The body weight results indicated the systemic impact of fluoride-induced nephrotoxicity and the protective effects of the treatments. The normal control group maintained stable body weight, reflecting good health. In contrast, the disease control group experienced weight reduction, indicating adverse effects of fluoride exposure. The positive control group displayed partial recovery, suggesting moderate protection from vitamin C. However, the low-dose polyherbal mixture group showed slight improvement, while the high-dose group

had weights similar to the normal group, indicating significant nephroprotective potential which is shown in Figure 1.

3.2 Physical Characteristics of Urine

The physical characteristics of urine provide insight into the renal health and function of test subjects across different treatment groups. The control group exhibited healthy urine output and a pale yellow colour, reflecting normal renal function. In contrast, the disease control group exposed to fluoride had significantly reduced urine output and a dark yellow colour, indicating impaired kidney function and dehydration. The decrease in urine volume ($p < 0.001$) compared to that in the normal group confirms the nephrotoxic effect of fluoride.

The positive control group, treated with vitamin C, showed an improvement in urine output and a lighter urine colour than the disease group ($p < 0.001$). This suggests that vitamin C partially mitigated nephrotoxic effects and supported better renal function. In the low-dose polyherbal mixture group, urine output moderately increased, with the colour remaining yellow. While not as pronounced as in the high-dose group, this indicates some degree of nephroprotection. The high-dose polyherbal mixture group demonstrated significant improvement, with urine output near that of the normal group and a pale-yellow colour, indicating restored kidney function and effective nephroprotection ($p < 0.001$ compared to the disease control) which is shown in Figure 2.

3.3 Urine Analysis

Urine analysis revealed significant differences in kidney function and systemic health among the experimental groups. The control group showed healthy urine characteristics, including standard specific gravity, minimal leukocytes, and negative results for nitrates, proteins, glucose, ketones, bilirubin, and blood cells. This indicates typical renal and systemic functions.

The disease control group exposed to fluoride exhibited notable deterioration in urine parameters, with significantly lower specific gravity ($p < 0.001$) and increased leukocyte count, indicative of inflammation and possible infection. The presence of nitrates, elevated protein levels ($p < 0.001$), glucose, and ketones suggests compromised kidney function and metabolic stress. The rise in urobilinogen levels ($p < 0.001$), positive bilirubin levels, and the presence of blood cells further confirmed severe nephrotoxicity and potential renal damage.

The positive control group showed improvements in urine parameters compared with the disease group, with a near-normal specific gravity and reduced leukocyte count

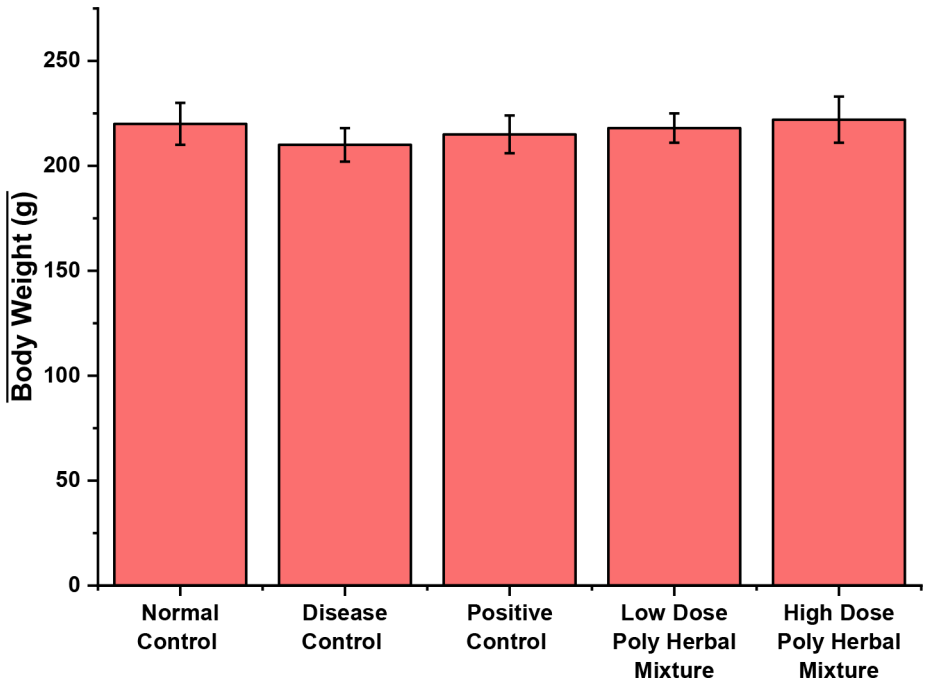


Figure 1. Body weight of the animals.
Data were analysed using one-way ANOVA followed by Tukey's multiple comparison post hoc test.

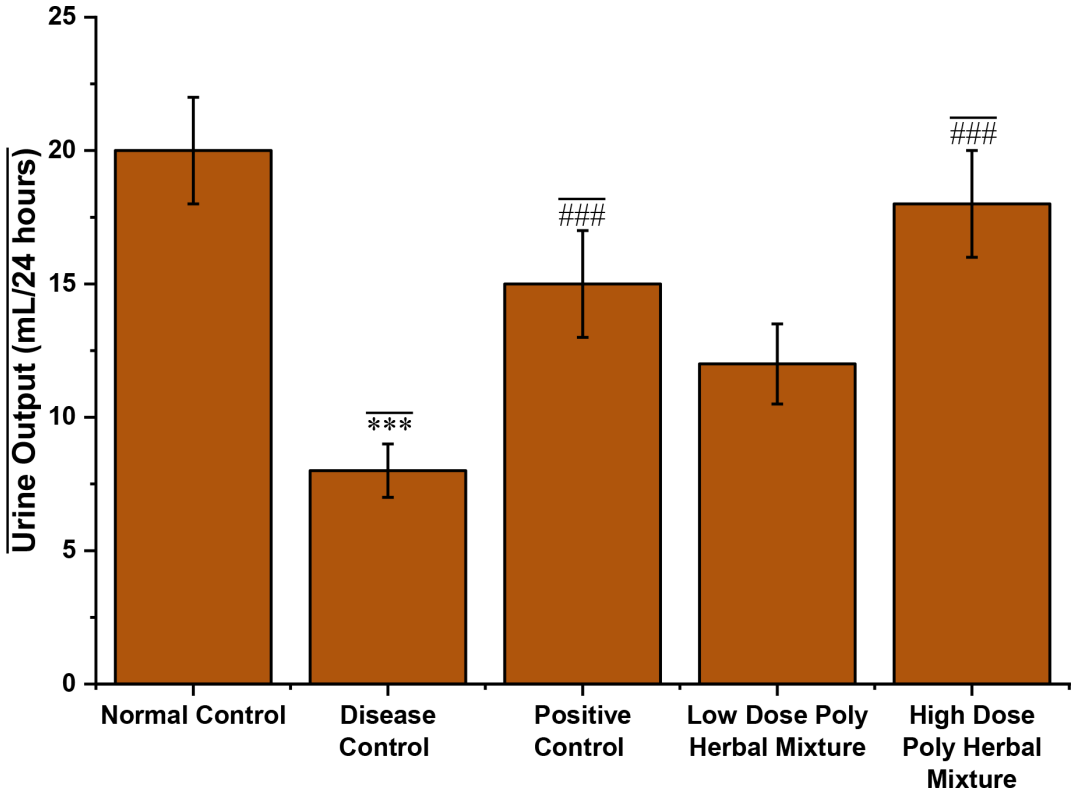


Figure 2. Urine output of the animals.

Data were analysed using one-way ANOVA followed by Tukey's multiple comparison post hoc test. ***p < 0.001 compared to the normal control group; ###p < 0.001 in contrast to the Disease Control group.

($p < 0.001$). The absence of nitrates and reduced protein levels ($p < 0.001$) indicated a partial restoration of kidney function. Glucose and ketone levels remained negative, and urobilinogen levels were controlled, indicating moderate nephroprotection.

The low-dose polyherbal mixture group demonstrated intermediate results, with specific gravity improving moderately and reduced leukocyte and protein levels ($p < 0.001$). This suggests that the 200 mg/kg dose protected against nephrotoxicity. However, urobilinogen levels were still slightly higher than the normal levels.

The most pronounced improvement was observed in the high-dose polyherbal mixture group, where urine characteristics closely matched the normal control. Specific gravity was near typical levels, leukocyte counts were minimal, and protein presence was significantly lower ($p < 0.001$). All other parameters, including glucose, ketones, bilirubin, and blood cells, were negative, and the urobilinogen levels were restored to near-normal levels, which is shown in Figure 3.

3.4 Renal Biochemistry

The renal biochemistry results provided clear insights into the severity of nephrotoxicity and the protective effects of the treatments. The normal control group showed baseline levels of serum creatinine and BUN, indicative of healthy renal function. In contrast, the disease control group exhibited significantly elevated serum creatinine and BUN levels ($p < 0.001$), reflecting severe renal impairment caused by fluoride exposure.

The positive control group treated with vitamin C demonstrated notable improvement, with reduced serum creatinine and BUN levels ($p < 0.001$ compared to the disease group). This indicates partial nephroprotection and suggests that vitamin C mitigates fluoride-induced damage.

The low-dose polyherbal mixture group also showed improvement, with moderate decreases in serum creatinine and BUN levels ($p < 0.001$). Although not as effective as the high-dose group, this suggests a protective effect at the 200 mg/kg dose.

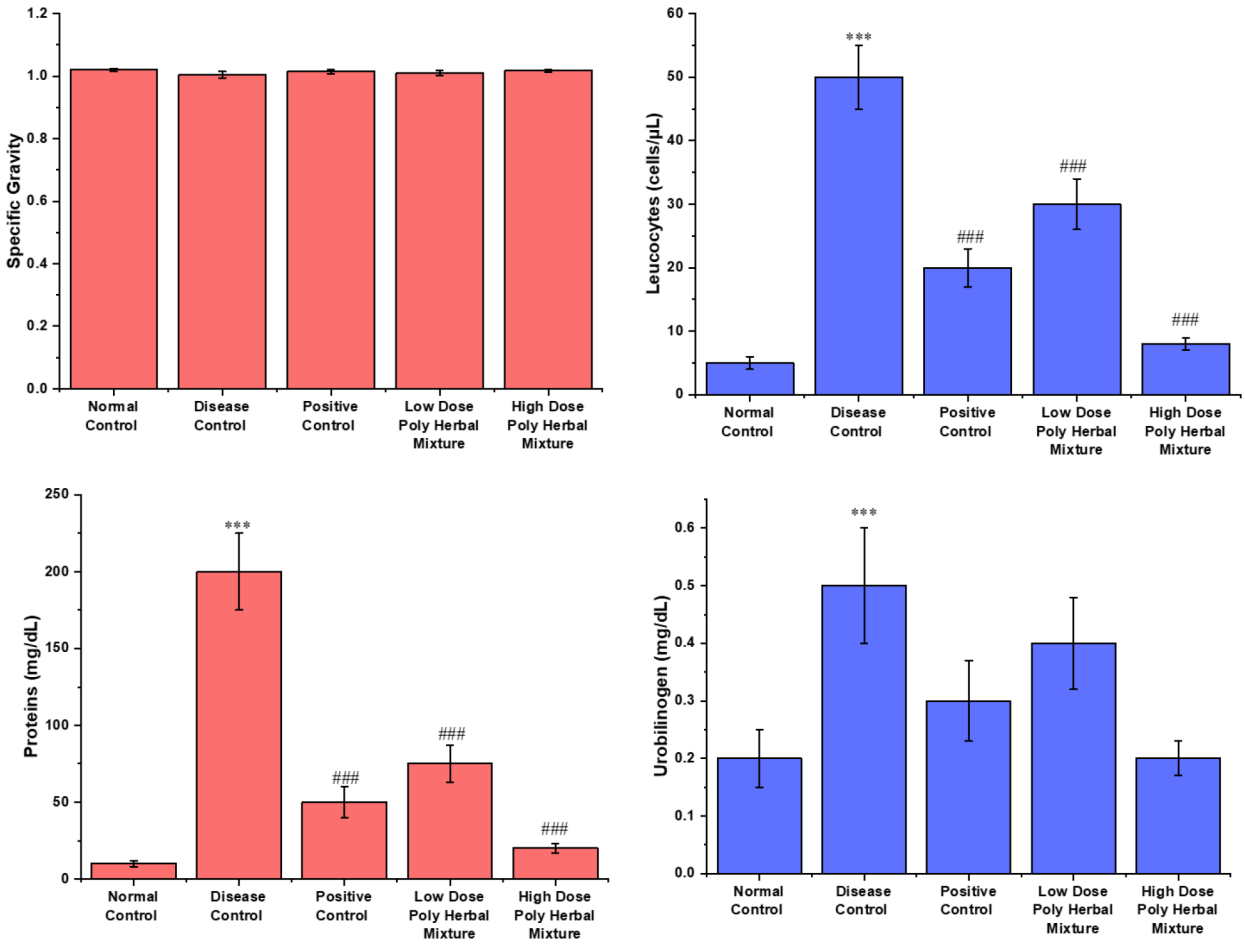


Figure 3. Urine analysis of the animals.

Table 1. Urine analysis of the animals

S. No	Treatment	Specific Gravity	Leucocytes (cells/µL)	Nitrates (mg/dL)	Proteins (mg/dL)	Glucose (mg/dL)	Ketones (mg/dL)	Urobilinogen (mg/dL)	Bilirubin (mg/dL)	Blood (cells/µL)
1	Normal Control	1.020 ± 0.005	5.0 ± 1.0	Negative	10.0 ± 2.0	Negative	Negative	0.2 ± 0.05	Negative	0.0 ± 0.0
2	Disease Control	1.005 ± 0.010	50.0 ± 5.0***	Positive	200.0 ± 25.0***	Positive	Positive	0.5 ± 0.1***	Positive	200.0 ± 30.0
3	Positive Control	1.015 ± 0.007	20.0 ± 3.0###	Negative	50.0 ± 10.0###	Negative	Negative	0.3 ± 0.07	Negative	20.0 ± 5.0
4	Low Dose Poly Herbal Mixture	1.010 ± 0.008	30.0 ± 4.0###	Negative	75.0 ± 12.0###	Negative	Negative	0.4 ± 0.08	Negative	10.0 ± 2.0
5	High Dose Poly Herbal Mixture	1.018 ± 0.004	8.0 ± 1.0###	Negative	20.0 ± 3.0###	Negative	Negative	0.2 ± 0.03	Negative	0.0 ± 0.0

Data were analysed using one-way ANOVA followed by Tukey's multiple comparison post hoc test. ***p < 0.001 compared to the normal control group; ###p < 0.001 in contrast to the Disease Control group.

The high-dose polyherbal mixture group had serum creatinine and BUN levels close to those of the normal control group, with significant reductions compared to the disease control group ($p < 0.001$). This demonstrates the strong nephroprotective potential and highlights the efficacy of a higher dose in preserving renal function. The results were shown in Figure 5.

3.5 Inflammatory Markers

The results for the inflammatory markers TNF-α and IL-6 reflect the extent of inflammation and the effectiveness of treatments in reducing inflammation. The normal control group maintained low levels of TNF-α and IL-6, which is indicative of minimal systemic inflammation. Conversely, the disease control group exhibited significantly elevated levels of both markers ($P < 0.001$), indicating a strong inflammatory response due to fluoride-induced nephrotoxicity.

The positive control group treated with vitamin C showed a substantial reduction in TNF-α and IL-6 levels ($p < 0.001$), suggesting that vitamin C effectively mitigated the inflammatory response. The low-dose polyherbal

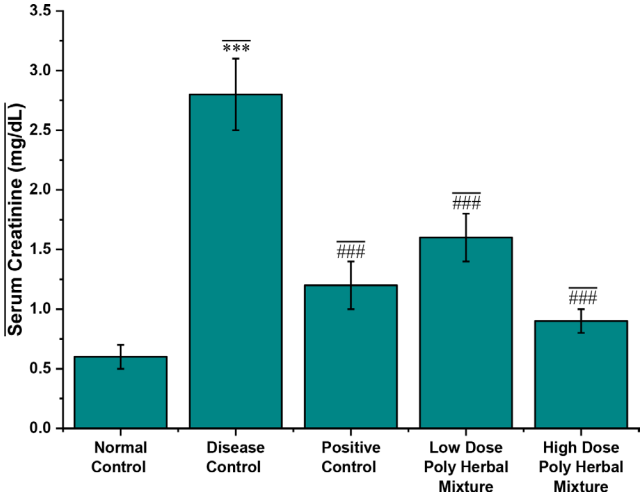


Figure 4. Levels of Serum Creatinine (mg/dL).
Data were analysed using one-way ANOVA followed by Tukey's multiple comparison post hoc test. ***p < 0.001 compared to the normal control group; ###p < 0.001 in contrast to the Disease Control group.

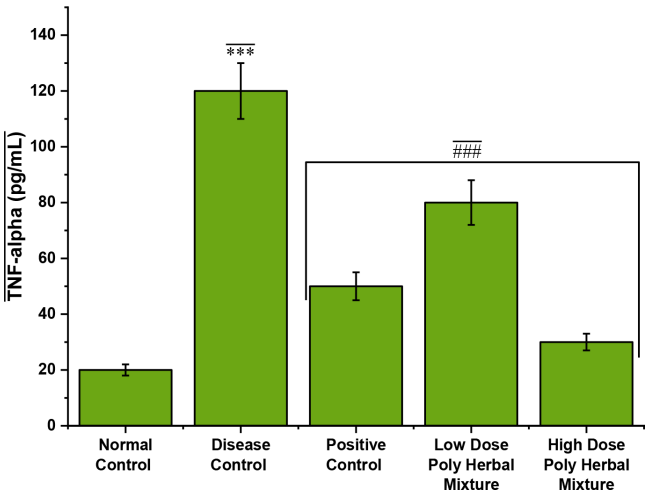


Figure 6. Levels of TNF-alpha (pg/mL)
Data were analysed using one-way ANOVA followed by Tukey's multiple comparison post hoc test. ***p < 0.001 compared to the normal control group; ###p < 0.001 in contrast to the Disease Control group.

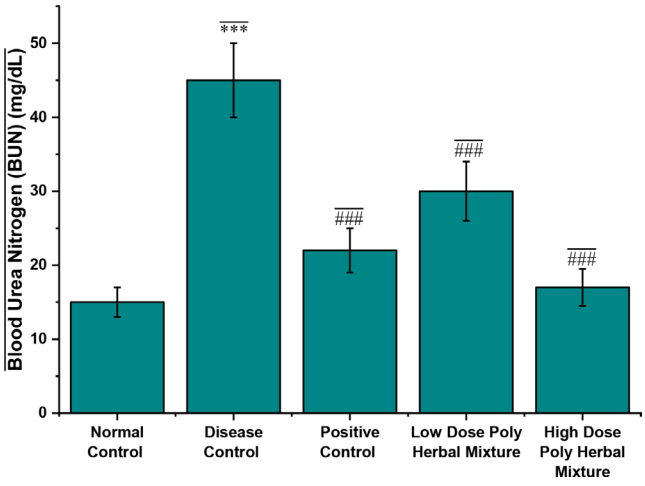


Figure 5. Levels of blood urea nitrogen (bun) (mg/dl).
Data were analysed using one-way ANOVA followed by Tukey's multiple comparison post hoc test. ***p < 0.001 compared to the normal control group; ###p < 0.001 in contrast to the Disease Control group.

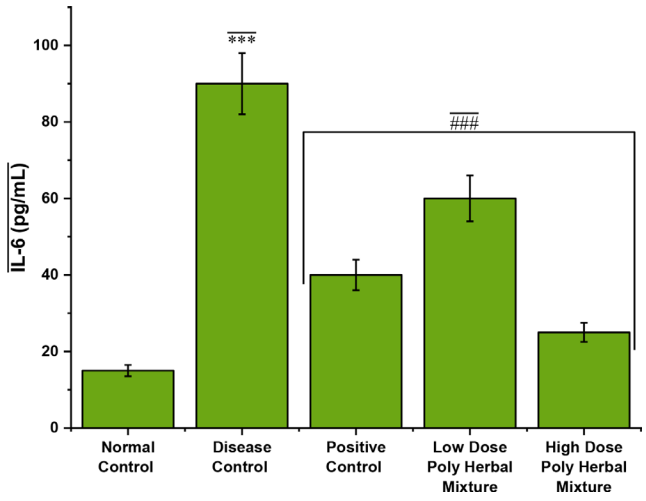


Figure 7. Levels of IL-6 (pg/mL)
Data were analysed using one-way ANOVA followed by Tukey's multiple comparison post hoc test. ***p < 0.001 compared to the normal control group; ###p < 0.001 in contrast to the Disease Control group.

mixture group demonstrated moderate reductions in these markers ($p < 0.001$ compared to the disease control), showing a partial anti-inflammatory effect.

The high-dose polyherbal mixture group exhibited the most significant decrease in TNF-α and IL-6 levels, close to those of the normal control group ($p < 0.001$). This indicated that the higher dose was highly effective in suppressing inflammation, aligning with improved renal function and reduced damage. The results were shown in Figure 6 & 7.

3.6 Anti-Oxidative Parameters

The anti-oxidative parameters showed significant changes across the treatment groups. The normal control group displayed balanced oxidative stress markers with low levels of MDA and high activities of SOD, CAT and GPx, indicating a healthy oxidative balance.

In the disease control group, there was a significant increase in MDA levels ($p < 0.001$), reflecting heightened lipid peroxidation and oxidative damage. Concurrently, there was a marked reduction in SOD, CAT, and GPx activities ($p < 0.001$) and signalling-depleted antioxidant defences due to fluoride-induced nephrotoxicity.

The positive control group showed considerable improvement in antioxidant markers. MDA levels were significantly reduced, and the activities of SOD, CAT, and GPx were substantially restored ($p < 0.001$ compared to the disease group), indicating a partial reversal of oxidative stress.

The low-dose polyherbal mixture group demonstrated moderate antioxidant protection, with reductions in MDA and improvements in antioxidant enzyme activities ($p < 0.001$ compared to the disease group). However, these levels did not reach those in the normal group.

The high-dose polyherbal mixture group displayed the most notable improvements, with MDA levels nearly matching the normal control and significant increases in SOD, CAT, and GPx activities ($p < 0.001$ compared to the

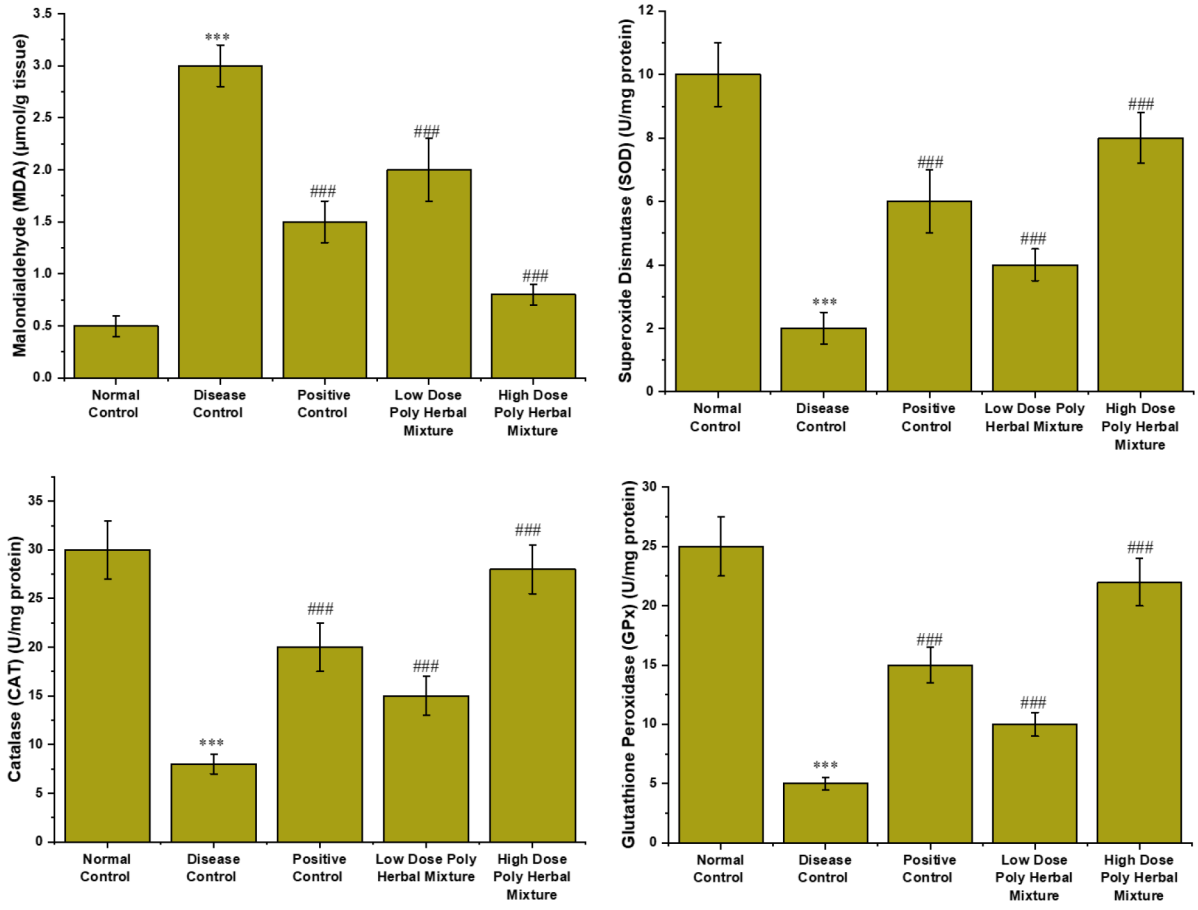


Figure 8. Oxidative parameters of the animals.

Data were analysed using one-way ANOVA followed by Tukey's multiple comparison post hoc test. ***$p < 0.001$ compared to the normal control group; ###$p < 0.001$ in contrast to the Disease Control group.

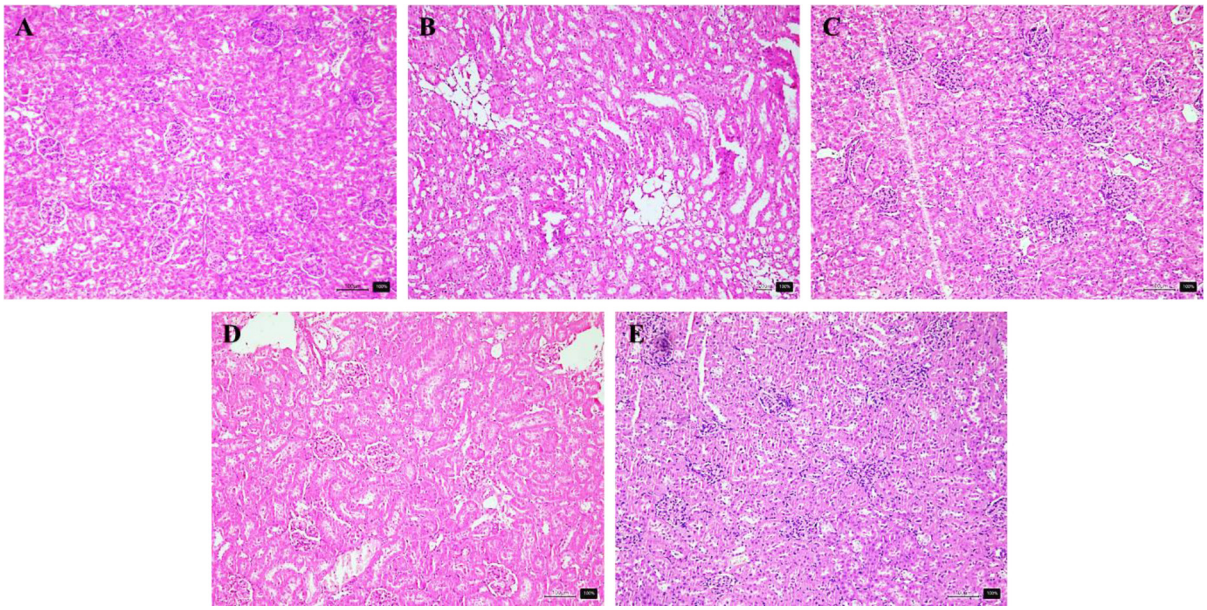


Figure 9. Histopathology of kidneys at 100X magnification A. Normal Control, **B.** Disease Control, **C.** Positive Control, **D.** Low Dose Poly Herbal Mixture, **E.** High Dose Poly Herbal Mixture.

disease group). These results suggest that the high dose effectively restored the antioxidant balance, contributing to nephroprotection as shown in the Figure 8.

3.7 Histopathology

Histopathological analysis of the kidney tissues confirmed the biochemical and oxidative stress results, highlighting significant differences among the treatment groups. The normal control group exhibited intact renal architecture with well-defined glomeruli and tubular structures, indicative of normal kidney function. In contrast, the disease control group subjected to fluoride exposure displayed pronounced renal damage, including glomerular congestion, tubular cell degeneration, and interstitial inflammation, which is consistent with severe nephrotoxicity. The positive control group treated with vitamin C showed partial structural recovery, as evidenced by reduced tubular damage, fewer inflammatory cells, and improved glomerular structure, thus underscoring the protective effects of vitamin C. The low-dose polyherbal mixture group demonstrated moderate improvements, with decreased tubular damage and less inflammation compared to the disease control group; however, there were still some signs of residual damage. Most notably, the high-dose polyherbal mixture group showed substantial histological preservation, with renal tissues appearing similar to those of the normal control group. The glomeruli were largely intact, and the tubular structures showed minimal degeneration and reduced inflammatory infiltration. These results suggest that the polyherbal mixture, particularly at higher doses, offers significant protection against fluoride-induced renal damage and supports its potential as an effective nephroprotective agent. The results were shown in Figure 9.

4. Discussion

The present study explored the nephroprotective effects of a polyherbal mixture containing *C. longa* (turmeric) and *C. sinensis* (green tea) against fluoride-induced nephrotoxicity in Sprague-Dawley rats. The study findings demonstrated significant improvements in all evaluated parameters, including renal function markers, oxidative stress, inflammatory cytokines, and histopathological outcomes, particularly with the high-dose mixture.

Body weight is a systemic indicator of overall health and toxicity. Fluoride exposure in the disease control group led to significant weight loss, reflecting systemic toxicity, consistent with the results of previous studies. Fluoride-induced oxidative stress and metabolic disturbances impair energy metabolism and contribute to weight reduction[20]. Restoration of body weight in the high-dose polyherbal mixture group aligns with findings

showing that antioxidants and anti-inflammatory agents, such as curcumin and green tea catechins, counteract metabolic dysregulation and toxicity, allowing for normalized weight gain[11,21].

The weight-maintaining effects of the polyherbal mixture may be attributed to its ability to mitigate systemic oxidative stress and inflammation while improving nutrient metabolism. These outcomes underscore the systemic protective effects of the mixture, likely through the synergistic action of bioactive compounds such as curcuminoids and catechins.

Urine volume and colour are critical markers of kidney function. Fluoride exposure in the disease control group caused a significant reduction in urine output and a dark yellow colour, indicating compromised filtration and dehydration. These findings are consistent with the literature, which reports that fluoride-induced nephrotoxicity results in renal tubular damage and impaired urinary concentration ability[22].

The significant improvement in urine output and normalisation of urine colour in the high-dose mixture group indicated the restoration of renal tubular function. The polyherbal mixture likely mitigates tubular damage by reducing oxidative stress and inflammation, mechanisms previously attributed to curcumin and green tea catechins[12,23].

Urine analysis revealed substantial abnormalities in the disease control group, including low specific gravity, proteinuria and elevated leukocyte and urobilinogen levels, consistent with renal dysfunction and inflammation. These findings mirror those of earlier reports that fluoride induces protein leakage and renal inflammation due to glomerular and tubular damage[22,24].

Treatment with the high-dose polyherbal mixture significantly normalised these parameters, highlighting its potent nephroprotective effects. Curcumin and green tea polyphenols, both known for their anti-inflammatory and antioxidant properties, likely contribute to the reduction of proteinuria and leukocyte infiltration by preserving glomerular integrity and mitigating oxidative damage[11,19,25].

Elevated serum creatinine and BUN levels in the disease control group confirmed the fluoride-induced renal impairment. These parameters are well-established indicators of kidney function, and their increase reflects reduced glomerular filtration and kidney damage[20]. The marked reduction in creatinine and BUN levels in the high-dose mixture group suggests preserved renal function, consistent with previous studies showing the nephroprotective effects of curcumin and green tea catechins[12,26].

The reduction in serum creatinine and BUN by the polyherbal mixture indicates its potential to enhance renal filtration capacity, possibly through improved mitochondrial function and reduction of oxidative stress in renal tissues[23].

The substantial elevation of TNF-α and IL-6 levels in the disease control group reflects the inflammatory response triggered by fluoride exposure, a well-documented mechanism of nephrotoxicity[3]. Chronic inflammation exacerbates renal damage by promoting fibrosis and tubular atrophy.

The high-dose mixture significantly reduced the TNF-α and IL-6 levels, demonstrating its potent anti-inflammatory effects. Curcumin, a well-known NF-κB inhibitor, and green tea catechins, which modulate proinflammatory pathways, likely work synergistically to suppress inflammation[11,27]. This reduction in inflammation is critical for preventing the progression of kidney damage and for promoting recovery.

Fluoride-induced nephrotoxicity involves heightened oxidative stress, as evidenced by increased MDA levels and decreased antioxidant enzyme activity in the disease control group. These findings align with previous studies that attributed the nephrotoxic effects of fluoride to its ability to generate Reactive Oxygen Species (ROS) and deplete endogenous antioxidants[3,24].

The high-dose polyherbal mixture significantly reduced MDA levels while restoring SOD, CAT, and GPx activities, highlighting its antioxidative properties. Curcumin and green tea catechins are potent ROS scavengers that have been shown to enhance endogenous antioxidant defences[11,23]. By reducing oxidative stress, the mixture not only mitigates cellular damage but also supports renal tissue repair.

Histological examination of renal tissues corroborated these biochemical findings. Severe tubular degeneration, glomerular congestion, and inflammatory infiltration in the disease control group confirmed fluoride-induced structural damage. These observations are consistent with those of prior histopathological studies on fluoride nephrotoxicity[21,28].

The high-dose mixture group showed significant preservation of the renal architecture, with minimal tubular damage and reduced inflammation. This histological protection is consistent with the ability of curcumin and green tea catechins to prevent apoptosis and fibrosis by modulating oxidative and inflammatory pathways[12,23]. These findings highlight the ability of the mixture to protect the renal tissues from structural damage and promote healing.

Interestingly, this study included a positive control group treated with vitamin C, a well-known antioxidant.

While this group showed improvements in most parameters, the effects were generally less pronounced than those observed in the high-dose polyherbal mixture group. This comparison highlights the potential superiority of the mixture over single-compound antioxidant treatment. The enhanced efficacy of the polyherbal mixture may be attributed to the synergistic effects of the multiple bioactive compounds present in *C. longa* and *C. sinensis*, which could target multiple pathways involved in fluoride-induced nephrotoxicity.

The dose-dependent effects observed in this study were noteworthy. While both low and high doses of the polyherbal mixture showed beneficial effects, the high-dose group consistently demonstrated superior outcomes across all measured parameters. This dose-response relationship not only provides strong evidence for the efficacy of the mixture but also suggests that higher doses may offer more comprehensive protection against nephrotoxicity. However, further research is needed to determine the optimal dosage for maximal nephroprotection while ensuring safety.

The multi-targeted approach to this polyherbal mixture represents a significant advancement in the field of nephroprotection. By simultaneously addressing oxidative stress, inflammation, and renal function, this mixture offers a more comprehensive solution to fluoride-induced kidney damage than single-compound treatments. This holistic approach may be particularly valuable in real-world scenarios where environmental toxins often exert their damaging effects through multiple mechanisms.

Although these results are highly promising, it is important to note that this study was conducted in a rat model, and further research is needed to fully elucidate the potential of this polyherbal mixture in human subjects. Long-term studies would be valuable to assess the efficacy of this mixture in preventing chronic kidney disease resulting from prolonged fluoride exposure. Additionally, clinical trials are necessary to determine the appropriate dosing, safety profile, and efficacy in human populations exposed to high fluoride levels.

5. Conclusion

In conclusion, this study provides robust evidence of the nephroprotective effects of a polyherbal mixture containing *Curcuma longa* and *Camellia sinensis* against fluoride-induced toxicity. The mixture's multi-faceted mechanism of action, encompassing antioxidant, anti-inflammatory, and renal function-preserving effects, offers a promising approach for mitigating kidney damage caused by environmental toxins. These findings have important implications for the development of natural and comprehensive strategies to protect renal health in populations exposed to high levels of fluoride or other nephrotoxic agents. As research in this area continues, this polyherbal mixture may pave the way for more effective nature-inspired solutions to combat the growing global burden of kidney diseases.

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