FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 10 No. 02, January, 2026, pp 65 – 72
DOI: https://doi.org/10.33003/fjs-2026-1002-4358

NEPHROPROTECTIVE EFFECTS OF *Alstonia boonei* AGAINST ASPIRIN-INDUCED RENAL ALTERATIONS IN ALBINO RATS

[1]Ogeyemhe Blessing Emosho and [*2]Odigie Efosa Bolaji

[1]Histopathology Unit, Department of Medical Laboratory Science, University of Benin, Edo State, Nigeria.
[2]Department of Medical Laboratory Science, School of Basic Medical Science, University of Benin, Edo State, Nigeria.

*Corresponding authors' email: bolaji.odigie@uniben.edu
ORCID: https://orcid.org/0000-0002-1233-0491, https://orcid.org/0009-0002-9244-2131

ABSTRACT

The kidneys play a critical role in maintaining fluid and electrolyte balance and are vulnerable to pharmacological stress. Aspirin is widely used for its analgesic and anti-inflammatory properties; however, concerns regarding its renal safety persist *Alstonia boonei is a medicinal plant reported to possess antioxidant and organ-supportive properties*. This study evaluated the renal safety and supportive effects of *A. boonei* during aspirin administration in albino rats. Thirty rats were randomly assigned to six groups: control, aspirin only (10 mg/kg), *A. boonei* only (1000 mg/kg), and aspirin combined with *A. boonei* (1000, 1500, or 2000 mg/kg) for 14 days. Body weight, kidney weight, renal biochemical markers, and kidney histology were assessed. All groups exhibited normal weight gain, indicating absence of overt systemic toxicity. Aspirin administration was associated with reduced kidney weights relative to controls, suggesting mild structural sensitivity, while extract-treated and co-treated groups showed partial preservation of kidney mass, particularly at moderate extract doses. Renal biochemical indices remained stable, with serum urea ranging from 25.00 ± 10.82 to 38.00 ± 11.02 mg/dL, creatinine from 0.83 ± 0.15 to 1.05 ± 0.15 mg/dL, sodium from 137.33 ± 0.88 to 140.33 ± 1.45 mmol/L, and potassium from 3.90 ± 0.10 to 4.07 ± 0.15 mmol/L across groups, showing no statistically significant differences. Histology showed preserved glomerular and tubular architecture across groups. Low dose aspirin did not cause overt renal toxicity, while *A. boonei* was renally safe. Co-administration, particularly at moderate doses, supported renal structural and biochemical stability during aspirin exposure.

Keywords: Alstonia Boonei, Renal Function, Aspirin, Kidney Histology, Nephrotoxicity, Medicinal Plants

INTRODUCTION

The kidneys are highly specialized organs responsible for maintaining internal homeostasis through regulation of fluid and electrolyte balance, acid–base equilibrium, nitrogenous waste excretion, endocrine signaling, and blood pressure regulation. Their unique microvascular architecture, high metabolic activity, and constant exposure to circulating xenobiotics render them particularly vulnerable to pharmacological stress (Rapa et al., 2019; Chebotareva et al., 2022; Liu H. et al., 2025; Ahsan et al., 2025). Renal responses to drugs often begin within the nephron, where glomerular filtration, tubular reabsorption, and secretion processes may be subtly influenced by oxidative stress, mitochondrial activity, membrane dynamics, and altered hemodynamics (Kwiatkowska et al., 2021). Even in the absence of overt injury, mild biochemical fluctuations may reflect early nephron stress or adaptive physiological responses to pharmacological exposure.

Aspirin remains one of the most widely used nonsteroidal anti-inflammatory drugs due to its analgesic, antipyretic, and antiplatelet properties. Although generally considered safe at therapeutic doses, concerns persist regarding its renal effects under specific conditions, including prolonged use, higher doses, or compromised renal reserve (Arif & Aggarwal, 2023). Aspirin inhibits renal prostaglandin synthesis, a mechanism that may influence renal blood flow, glomerular filtration, and tubular function, particularly when renal compensatory mechanisms are challenged (Liu W. et al., 2025; Džidić-Krivić et al., 2024). Salicylate metabolites have also been reported to modulate mitochondrial activity, reactive oxygen species generation, and electrolyte handling within nephron segments (Hosohata, 2016; Lee et al., 2021; Lu et al., 2023; Razavi et al., 2025). While clinically significant nephrotoxicity is uncommon at low doses, evaluating renal safety and adaptive responses to aspirin remains important for understanding its pharmacological profile (George et al., 2017; Kim et al., 2023).

Alstonia boonei De Wild is a medicinal plant widely used in African ethnomedicine and is rich in phytochemicals such as indole alkaloids, flavonoids, phenolic acids, and triterpenoids. These bioactive compounds exhibit antioxidant, anti-inflammatory, membrane-stabilizing, and cytoprotective properties that may support renal physiological stability (Olanlokun et al., 2021; Sharma & Goyal, 2022). Previous studies on *A. boonei* and related medicinal plants have demonstrated their ability to modulate oxidative stress pathways, preserve tubular integrity, and support renal functional balance in experimental models exposed to pharmacological or environmental stressors (Taiwo et al., 2019; Uroko et al., 2020; Tienda-Vázquez et al., 2022). Such properties suggest a potential supportive role for *A. boonei* in maintaining renal homeostasis during drug exposure rather than reversing established injury (Natesan & Kim, 2025).

The increasing use of aspirin alongside growing interest in natural products for drug safety makes it necessary to evaluate renal responses during combined exposure. Repeated aspirin exposure, even at low therapeutic doses, may subtly affect glomerular filtration and tubular handling, particularly when renal adaptive capacity is limited (Sharma & Goyal, 2022). Such early alterations may occur in the absence of overt nephrotoxicity. Assessment of kidney weight, renal biochemical markers, and histological features therefore provides a sensitive means of detecting early renal stress and functional changes. This study investigated the renal safety and supportive effects of *Alstonia boonei* during aspirin administration in albino rats to determine its influence on renal functional stability and tissue integrity under mild pharmacological stress.

MATERIALS AND METHODS

Study Setting

The experiment was conducted in a controlled animal research laboratory within the Department of Medical Laboratory Science, University of Benin, Nigeria, under standardized environmental conditions appropriate for rodent physiology. Temperature stability, adequate ventilation, and minimal external stressors were maintained throughout the study period. All procedures adhered to internationally recognized ethical guidelines for laboratory animal care, including principles outlined in contemporary toxicology and nephrotoxicity research that emphasize humane handling and welfare protection (OECD, 2022; Mugale et al., 2024; Barnett & Cummings, 2018). Ethical approval for the study was obtained from the Medical and Allied Faculty of Science Animal Ethics Committee, University of Benin, Nigeria (Approval No. MAFSAEC-025-01/09/0003).

Collection and Preparation of Study Materials

Fresh leaves of *Alstonia boonei* De Wild. were collected and authenticated at the Herbarium Unit, Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria and a voucher specimen was deposited (UBH-A591). The leaves were rinsed to remove debris, air-dried at ambient temperature, and ground into a fine powder. Extraction was carried out by ethanol maceration for 72 hours with intermittent agitation. The filtrate was concentrated using a rotary evaporator under reduced temperature to preserve heat-sensitive phytochemicals. Extracts were stored in airtight containers and kept refrigerated until required. This extraction approach aligns with established phytochemical protocols used in evaluating nephroprotective medicinal plants and related species (Sharma & Goyal, 2022; Mishra et al., 2025; Uroko et al., 2020; Tienda-Vázquez et al., 2022).

Furthermore, aspirin tablets used in the study were obtained from a certified pharmaceutical company (Roche Pharmaceuticals, Basel, Switzerland). Aspirin solutions were freshly prepared daily at a dose of 10 mg/kg. This dosage is supported by previous work showing that NSAID exposure can induce measurable renal alterations while allowing evaluation of nephroprotective interventions (Lu et al., 2023; Razavi et al., 2025; Hosohata, 2016; Lee et al., 2021). Extract doses of 1000, 1500, and 2000 mg/kg were selected based on LD_{50} findings and previous literature confirming their safety and biological activity.

Acute Toxicity Study (LD_{50} Determination)

A preliminary acute toxicity test was performed using the OECD Up-and-Down Procedure, which recommends stepwise dose escalation with clinical monitoring over a 72-hour period to determine extract safety margins (OECD, 2022). No mortality or signs of distress were observed at the tested doses, indicating a high safety threshold and supporting the suitability of the extract for subsequent oral administration. Similar safety and preclinical toxicity findings for medicinal plant extracts and natural products have been reported in recent nephroprotective and kidney disease studies (Natesan & Kim, 2025; Mugale et al., 2024; Tienda-Vázquez et al., 2022).

Experimental Animals and Grouping

Thirty adult albino rats weighing 130 to 180 g were used for the study. They were housed in clean polypropylene cages under a 12-hour light and dark cycle, provided with standard vita feed, and allowed free access to water and feed. The animals underwent a two-week acclimatization period consistent with recommended practices for minimizing environmental stress and stabilizing physiological parameters before toxicological experiments (Chebotareva *et al.,* 2022; Ahsan *et al.,* 2025; Kwiatkowska *et al*., 2021). Rats were randomly assigned into six experimental groups of five animals each per group (n=5) to reduce selection bias and improve statistical validity (Griffin *et al*., 2019; Perazella and Rosner, 2022; Kwiatkowska *et al.,* 2021). The sample size was selected based on common practice in preliminary toxicological and nephroprotective studies and in line with ethical principles aimed at minimizing animal use. Group I served as the control and received no treatment. Group II was administered aspirin alone at a dose of 10 mg/kg body weight orally. Group III received *A. boonei* extract alone at a dose of 1000 mg/kg body weight orally. Group IV was treated concurrently with aspirin (10 mg/kg) and *A. boonei* extract (1000 mg/kg) orally. Group V received aspirin (10 mg/kg) combined with 1500 mg/kg of *A. boonei* extract orally, while Group VI was treated with aspirin (10 mg/kg) and 2000 mg/kg of *A. boonei* extract orally. All treatments were administered once daily for 14 consecutive days using using calibrated oral gavage needle.

Assessment of Renal Biochemical Markers

Following an overnight fast at the end of the treatment period, animals were sacrificed humanely and blood samples collected via cardiac puncture. Serum was separated by centrifugation and analyzed for renal biomarkers including urea, creatinine, sodium, potassium, chloride, and bicarbonate. These biochemical indices serve as sensitive markers of glomerular filtration, electrolyte handling, and early nephron injury, and are widely employed in evaluating drug-induced and NSAID-induced renal dysfunction (George *et al.,* 2017; Liu W. *et al.,* 2025; Džidić-Krivić *et al.,* 2024).

Histopathological Examination of Renal Tissue

Both kidneys were excised, rinsed gently in physiological saline, blotted dry, and weighed. They were fixed in 10% neutral buffered formalin to preserve cellular and structural integrity. Routine dehydration and paraffin embedding were performed, followed by sectioning at approximately 5 µm using a rotary microtome. Hematoxylin and eosin staining was utilized to visualize renal morphology. Histology slides of the kidney were examined using a Leica DM750 binocular microscope. Expert pathologists of which are blinded to the sample identities, independently evaluated the sections to ensure an unbiased assessment. Microscopic assessment included evaluation of glomerular architecture, tubular epithelial integrity, interstitial spaces, and vascular structures. Renal histology remains a key diagnostic approach for assessing nephrotoxicity and detecting early structural alterations induced by pharmacological agents and oxidative-inflammatory renal injury (Barnett & Cummings, 2018; Perazella & Rosner, 2022; Kwiatkowska *et al.,* 2021; Rapa *et al.,* 2019; Liu H. *et al.,* 2025).

Statistical Analysis

All data were expressed as mean ± standard error of the mean (SEM). Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Statistical analyses were conducted using GraphPad Prism version 9.0 (GraphPad Software Inc., San Diego, CA, USA). A p-value < 0.05 was considered statistically significant. A p-value < 0.05 was considered statistically significant. This statistical approach is consistent with current analytical practices employed in nephrotoxicity and nephroprotective

research, including studies of drug-induced kidney injury and plant-derived protective agents (Kwiatkowska *et al.,* 2021; Mugale *et al*., 2024).

RESULTS AND DISCUSSION

Changes in body and kidney weights provide an initial indication of systemic and organ-specific responses to drug exposure and protective interventions. As shown in Table 1, all experimental groups demonstrated increases in body weight between the initial and final measurements, indicating that none of the treatments caused overt systemic toxicity severe enough to impair growth. The control group showed steady physiological weight gain consistent with normal metabolic function. Rats administered aspirin alone exhibited a modest increase in final body weight, suggesting that short-term administration of aspirin at 10 mg/kg did not produce marked systemic stress capable of suppressing growth. However, animals treated with *A. boonei* either alone or in combination with aspirin showed comparatively higher weight gains, particularly in the extract-only and moderate co-treatment groups (see Table 1). Preservation of body weight gain in toxicological models is widely regarded as an indicator of maintained metabolic balance and reduced physiological burden, whereas weight loss or stagnation often reflects systemic toxicity and oxidative stress, with body weight change routinely monitored as a key endpoint in acute and sub-acute toxicity studies (Mugale *et al.,* 2024; Subramanian *et al.,* 2025). The observed weight trends therefore suggest that *A. boonei* did not disrupt normal metabolic processes and may have contributed to improved physiological stability during aspirin exposure, a pattern consistent with reports linking antioxidant-rich plant extracts to improved growth outcomes in nephrotoxic models (Chebotareva *et al.,* 2022; Ahsan *et al*., 2025; Kwiatkowska *et al.,* 2021).

Evaluation of kidney weights further supports the systemic observations. Table 1 further showed that both right and left kidney weights were significantly reduced in the aspirin-only group compared with the control group, indicating early renal structural compromise. Reductions in kidney mass are commonly associated with nephron loss, tubular atrophy, or reduced cellular integrity resulting from toxic or ischemic insults. Such changes have been documented in models of NSAID-induced nephrotoxicity, where prostaglandin inhibition leads to reduced renal perfusion and subsequent structural vulnerability (George *et al.,* 2017; Liu W. *et al*., 2025; Džidić-Krivić *et al.,* 2024). In contrast, rats administered *A. boonei* alone exhibited kidney weights comparable to those of the control group, indicating that the extract itself did not exert deleterious morphological effects. Co-treatment with aspirin and *A. boonei* resulted in partial restoration of kidney weights, with the most preserved renal mass observed in the groups receiving moderate extract doses. These findings suggest a dose-responsive protective effect of the extract on renal structure, likely mediated through preservation of nephron integrity and attenuation of tubular injury (Tienda-Vázquez *et al*., 2022; Natesan & Kim, 2025). The comparatively lower kidney weights observed in the lowest-dose co-treatment group indicate that insufficient phytochemical concentration may limit structural protection, whereas moderate dosing appears more effective in mitigating aspirin-induced renal mass loss, consistent with dose-dependent nephroprotective effects reported for plant-derived antioxidants in experimental kidney injury models (Tienda-Vázquez *et al*., 2022; Mugale *et al.,* 2024).

Table 1: Body and Organ Weight of Rats across all Groups

Parameter	Group A	Group B	Group C	Group D	Group E	Group F	F-value	p-value
Initial Body Weight (g)	101.67 ± 1.20^{a}	154.67 ± 18.28^{b}	137.33 ± 12.44^{bc}	119.33 ± 5.78^{ab}	135.00 ± 8.00^{bc}	106.33 ± 4.41^{a}	4.059	0.022
Final Body Weight (g)	126.00 ± 0.58^{ab}	146.67 ± 14.68^{bc}	154.00 ± 4.93^{c}	126.33 ± 5.70^{ab}	144.00 ± 8.08^{bc}	114.33 ± 5.67^{a}	3.766	0.028
Right Kidney Weight (g)	0.80 ± 0.03^{a}	0.58 ± 0.10^{b}	0.48 ± 0.05^{bc}	0.38 ± 0.02^{c}	0.41 ± 0.03^{bc}	0.48 ± 0.02^{a}	7.859	0.002
Left Kidney Weight (g)	0.86 ± 0.04^{a}	0.64 ± 0.11^{b}	0.54 ± 0.06^{bc}	0.43 ± 0.03^{c}	0.46 ± 0.04^{bc}	0.53 ± 0.03^{a}	8.112	0.001

Values are expressed as mean ± SEM (n = 5 per group). Groups: A = Control; B = Aspirin (10 mg/kg); C = *Alstonia boonei* extract (1000 mg/kg);
D = Aspirin (10 mg/kg) + *A. boonei* (1000 mg/kg); E = Aspirin (10 mg/kg) + *A. boonei* (1500 mg/kg); F = Aspirin (10 mg/kg) + *A. boonei* (2000 mg/kg).
Values with different superscript letters (a–c) within the same row are significantly different at $p < 0.05$.

Renal biochemical parameters provide functional insight into the structural trends observed. As presented in Table 2, administration of aspirin alone did not result in statistically significant alterations in serum urea, creatinine, or electrolyte levels when compared with the control group, although mild numerical variations were observed. While these changes did not reach statistical significance, such trends are consistent with early or subclinical renal stress, where functional compensation may initially preserve biochemical indices despite emerging structural vulnerability. Similar observations have been reported in early stages of NSAID-related renal injury, where biochemical markers may lag behind morphological alterations (Hosohata, 2016; Lu *et al.,* 2023; Razavi *et al*., 2025). The absence of marked biochemical derangement at this dose and duration suggests that aspirin-induced renal injury in this model was mild and developing, rather than fully established.

Rats treated with *A. boonei* alone showed biochemical values closely aligned with those of the control group, reinforcing the conclusion that the extract does not impair renal function under normal physiological conditions (see Table 2). In co-treated groups, urea and creatinine values remained within comparable ranges across extract doses, with bicarbonate levels showing a mild upward trend that approached but did not reach statistical significance. These biochemical patterns, illustrated graphically in Figure 1-6, indicate stabilization of glomerular filtration and electrolyte handling in extract-treated animals, as serum urea and creatinine are established indicators of glomerular filtration status, while bicarbonate and electrolytes reflect tubular handling and acid base regulation (George et al., 2017; Kwiatkowska et al., 2021; Perazella & Rosner, 2022). Although no statistically significant differences were detected across groups (Figure 1-6), the overall trend toward normalization in extract-treated rats aligns with documented antioxidant and membrane-

stabilizing properties of *A. boonei* phytoconstituents, including flavonoids, alkaloids, and phenolic acids. These compounds are known to reduce oxidative stress, preserve mitochondrial function, and support tubular transport mechanisms in nephrotoxic settings (Taiwo *et al*., 2019; Tienda-Vázquez et al., 2022; Natesan & Kim, 2025).

The graphical representation of renal markers in Figure 1-6 further reinforces the biochemical findings presented in Table 2. Across all panels, urea, creatinine, sodium, potassium, bicarbonate, and chloride levels remained largely comparable between treated and control groups, with only subtle fluctuations observed. The mild elevation in bicarbonate levels across treated groups suggests a possible adaptive response in acid-base regulation, although this trend did not achieve statistical significance. Such compensatory adjustments are commonly reported in early renal stress states, where tubular buffering mechanisms remain largely intact (Kwiatkowska *et al.,* 2021; Mugale *et al*., 2024). The concordance between Table 2 and Figure 1-6 confirms that *A. boonei* did not induce electrolyte imbalance and may have supported renal homeostasis during aspirin exposure.

Table 2: Effect of Aspirin and *A. Boonei* on Kidney Function Markers after Treatment across all Treatment Groups

Test	Group A	Group B	Group C	Group D	Group E	Group F	p-value
Urea	29.00 ± 1.00	25.00 ± 10.82	34.00 ± 6.93	38.00 ± 11.02	37.67 ± 5.78	33.50 ± 3.50	0.853
Creatinine	1.00 ± 0.10	0.83 ± 0.15	0.97 ± 0.03	0.97 ± 0.12	0.97 ± 0.07	1.05 ± 0.15	0.815
Na	138.00 ± 1.00	137.33 ± 0.88	140.33 ± 1.45	139.00 ± 1.53	138.00 ± 0.58	138.00 ± 2.00	0.593
K	3.90 ± 0.10	3.97 ± 0.20	4.07 ± 0.15	3.93 ± 0.17	4.00 ± 0.10	3.90 ± 0.10	0.968
HCO_3	17.00 ± 1.00	20.00 ± 1.15	21.33 ± 0.88	19.67 ± 0.67	18.67 ± 0.67	17.00 ± 1.00	0.051
Cl	105.00 ± 2.00	106.00 ± 0.58	106.00 ± 1.00	105.00 ± 1.53	106.33 ± 0.67	105.00 ± 2.00	0.930

Values are expressed as mean ± SEM (n = 5 per group). No statistically significant differences were observed among groups for urea, creatinine, sodium, potassium, bicarbonate, and chloride ($p > 0.05$, one-way ANOVA). Values are expressed as mean ± SEM (n = 5 per group). Groups: A = Control; B = Aspirin (10 mg/kg); C = *Alstonia boonei* extract (1000 mg/kg); D = Aspirin (10 mg/kg) + *A. boonei* (1000 mg/kg); E = Aspirin (10 mg/kg) + *A. boonei* (1500 mg/kg); F = Aspirin (10 mg/kg) + *A. boonei* (2000 mg/kg). Values with different superscript letters (a–c) within the same row are significantly different at $p < 0.05$.

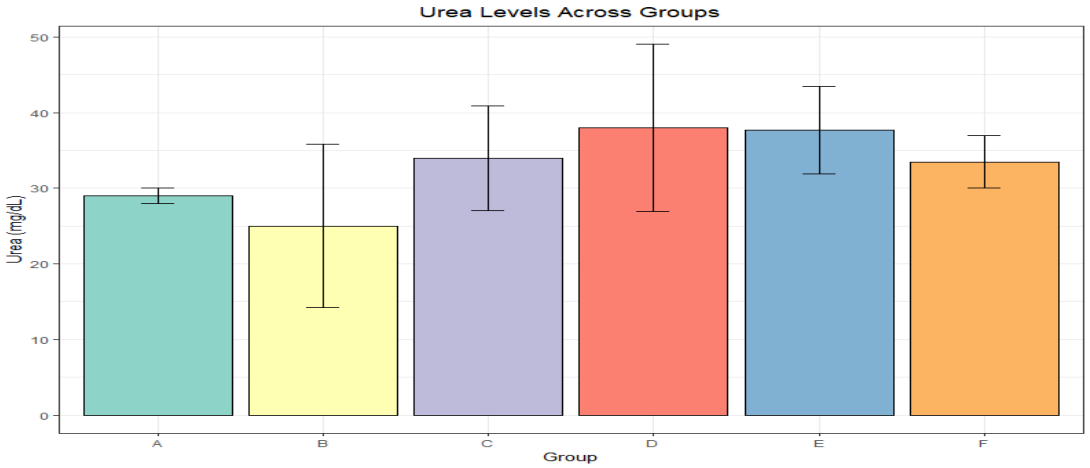

Figure 1: Urea Levels Across Experimental Groups

Mean serum urea concentrations in control and treated rats following aspirin and *A. boonei* administration. Data are expressed as mean ± SEM (n = 5). No significant differences were observed among groups ($p > 0.05$).

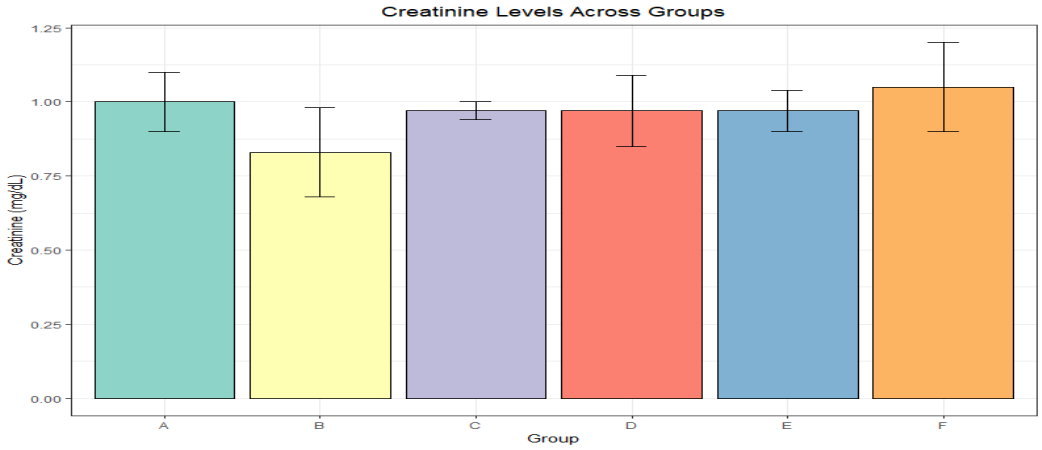

Figure 2: Creatinine Levels Across Experimental Groups

Mean serum creatinine concentrations in control and treated rats following aspirin and *Alstonia boonei* administration. Data are expressed as mean ± SEM (n = 5). No significant differences were observed among groups ($p > 0.05$).

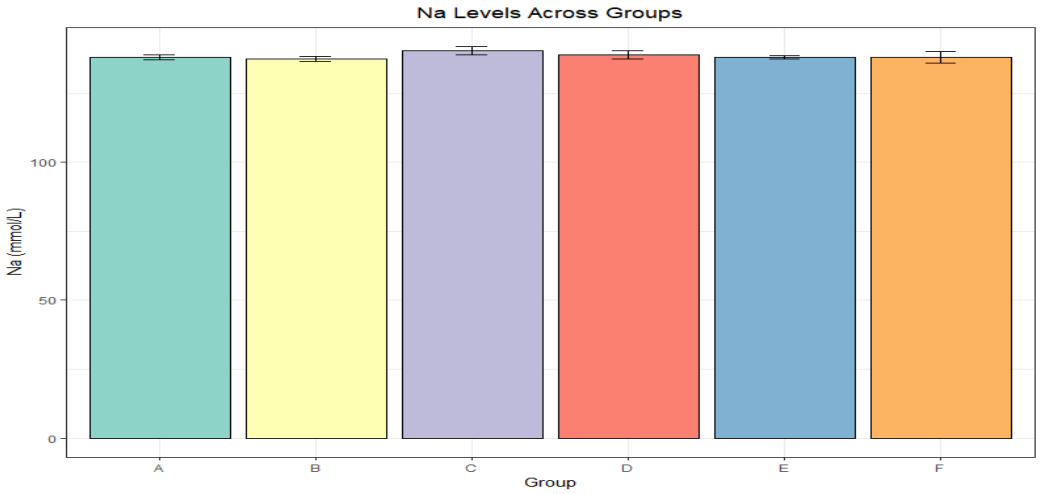

Figure 3: Sodium (Na^+) Levels Across Experimental Groups

Mean serum sodium concentrations across all groups. Data are expressed as mean ± SEM (n = 5). No significant differences were observed among groups ($p > 0.05$).

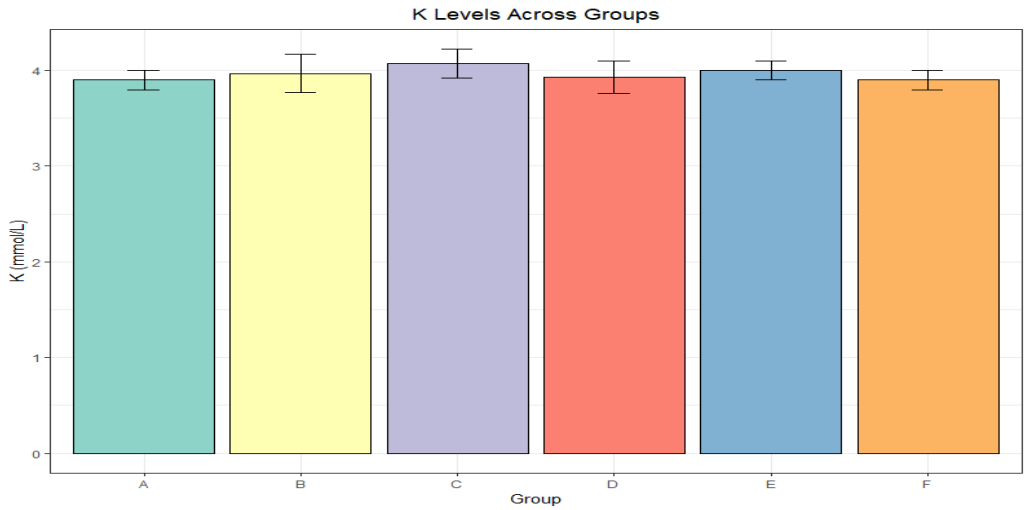

Figure 4: Potassium (K^+) Levels Across Experimental Groups

Mean serum potassium concentrations across all groups. Data are expressed as mean ± SEM (n = 5). No significant differences were observed among groups ($p > 0.05$).

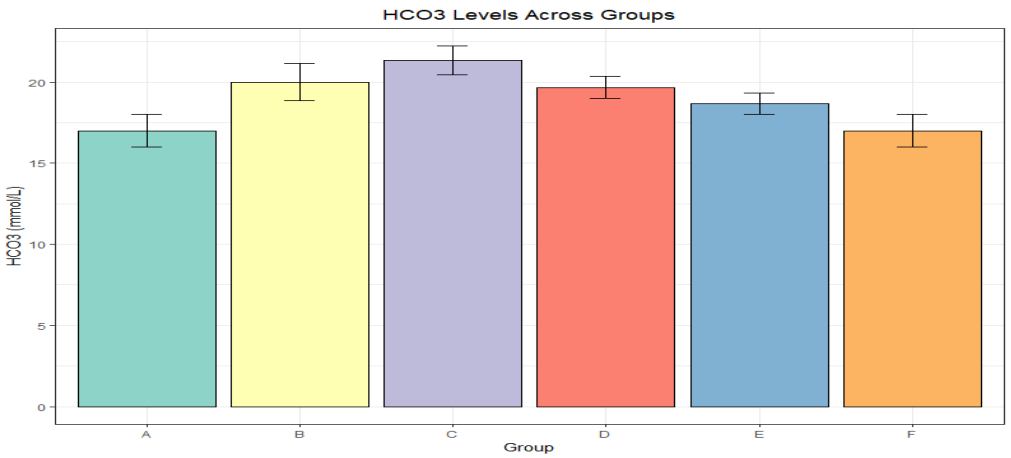

Figure 5: Bicarbonate (HCO_3^-) Levels Across Experimental Groups

Mean serum bicarbonate concentrations across all groups. Data are expressed as mean ± SEM (n = 5). A mild elevation was observed in treated groups but did not reach statistical significance (p = 0.051).

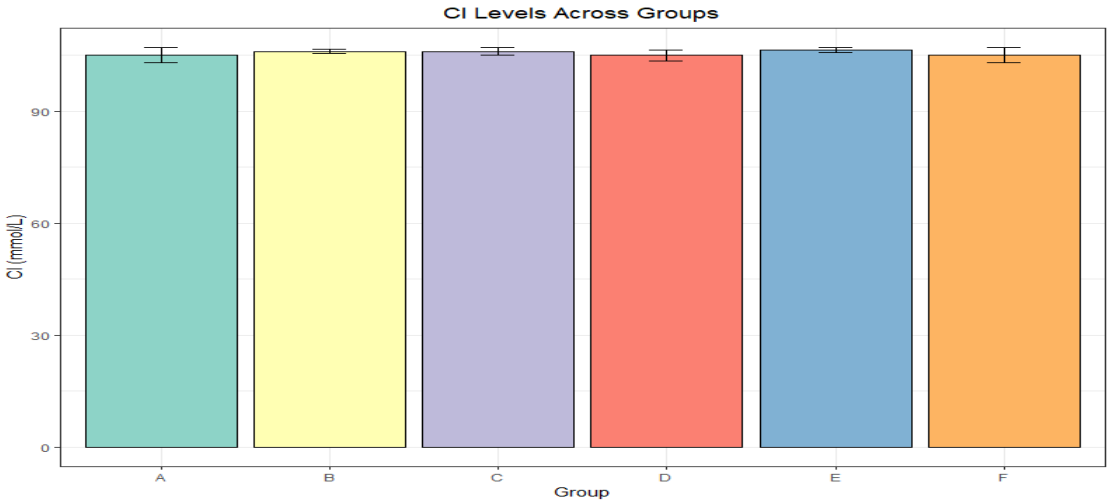

Figure 6: Chloride (Cl^-) Levels Across Experimental Groups

Mean serum chloride concentrations across all groups. Data are expressed as mean ± SEM (n = 5). No significant differences were observed among groups (p > 0.05).

Histopathological evaluation provided critical structural context for interpreting the biochemical and organ weight findings. Notably, kidney sections from rats treated with aspirin alone did not demonstrate deleterious histological alterations. As shown in Plate 1B, glomerular architecture remained intact, with preserved capillary tufts, normal Bowman's capsules, and well-organized tubular epithelium. The absence of tubular degeneration, interstitial inflammation, or glomerular distortion indicates that aspirin administration at 10 mg/kg for the study duration did not exert overt nephrotoxic effects. This observation supports the established safety profile of low-dose aspirin and reflects the kidney's capacity to maintain functional and structural integrity under therapeutic NSAID exposure. At low doses, aspirin-induced inhibition of cyclooxygenase enzymes does not sufficiently suppress renal prostaglandin synthesis to compromise renal blood flow or glomerular filtration, thereby preventing ischemic tubular injury and structural damage (Hosohata, 2016; Lu *et al*., 2023; Razavi *et al.,* 2025). Moreover, intact compensatory mechanisms within the nephron, including autoregulatory adjustments in afferent arteriolar tone and tubular transport processes, likely contributed to the preservation of renal morphology observed in this group (Kwiatkowska *et al.,* 2021).

The histological findings in the aspirin-only group are therefore consistent with the biochemical data presented in Table 2 and Figure 1, which showed no statistically significant alterations in urea, creatinine, or electrolyte levels. Together, these results indicate that aspirin exposure in this model induced, at most, mild and subclinical renal stress that did not progress to structural injury. Similar conclusions have been reported in earlier experimental and clinical studies, which demonstrate that short-term or low-dose aspirin administration is generally well tolerated by renal tissue in the absence of pre-existing kidney disease or additional nephrotoxic insults (George *et al*., 2017; Perazella & Rosner, 2022; Džidić-Krivić *et al.,* 2024).

Evaluation of kidney histology across the remaining experimental groups further reinforces the overall renal safety profile observed in this study. Kidney sections from animals treated with *Alstonia boonei* alone (Plate 1C) displayed normal glomeruli and tubules comparable to those of the control group, indicating that the extract did not adversely affect renal microarchitecture at the administered dose. Similarly, co-treated groups receiving aspirin in combination with *A. boonei* at 1000, 1500, and 2000 mg/kg (Plates 1D-F) exhibited preserved renal architecture, with no significant structural alterations in glomeruli or tubular compartments. While highly negligible gross differences were observed in some higher-dose groups, particularly in Plates 1B, 1D, and 1F, these changes did not meet criteria for pathological significance and were not accompanied by corresponding biochemical derangements.

The absence of meaningful histopathological damage across Plates 1B-F aligns with previous reports indicating that moderate doses of aspirin and medicinal plant extracts, including *Alstonia boonei*, do not induce severe nephrotoxicity when administered within therapeutic ranges. Plant-derived phytochemicals such as flavonoids, alkaloids, and phenolic compounds have been shown to exert antioxidant and membrane-stabilizing effects that protect renal epithelial cells from oxidative and inflammatory injury, thereby preserving nephron structure (Taiwo et al., 2019; Tienda-Vázquez et al., 2022; Natesan & Kim, 2025). In this study, *A. boonei* did not disrupt kidney architecture and may have contributed to maintaining renal structural stability during aspirin exposure, particularly by limiting subtle tubular stress that might otherwise progress to injury.

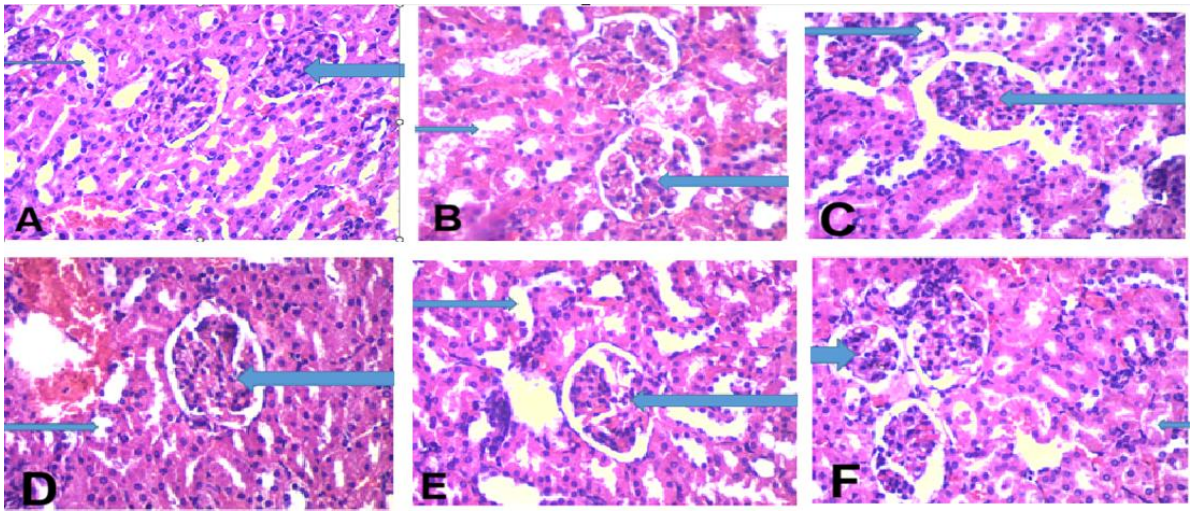

Figure 7: Showed Representative Photomicrographs of Kidney Sections from all Experimental Groups (A) Normal Renal Histoarchitecture, with Intact Glomerular Capillary Tufts, well-defined Bowman's Space, and Preserved Tubular Profiles. (B) Preserved Glomerular Structure and Tubular Organization, with no Evidence of Inflammatory Cell Infiltration, Tubular Necrosis, or Glomerular Distortion. (C) Normal Renal Morphology Comparable to the Control, with Intact Glomeruli and well-arranged Renal Tubules. (D) Preserved Glomerular Integrity and Tubular Architecture, with no Observable Degenerative or Inflammatory Changes. (E) Normal Renal Histology, with Intact Bowman's Capsules, Preserved Glomerular Tufts, and well-maintained Tubular Structures. (F) Preserved Renal Architecture, with no Evidence of Tubular Degeneration, Interstitial Inflammation, or Glomerular Injury (H&E stain, ×400).

Overall, the histological findings demonstrate that both aspirin at low dose and *A. boonei* at the administered concentrations are largely safe for renal tissue under the experimental conditions employed. The preserved glomerular and tubular morphology across all treatment groups supports the biochemical evidence of maintained renal function and underscores the importance of dose, duration, and physiological context in determining nephrotoxic risk. These findings further validate the suitability of *A. boonei* as a nephroprotective phytotherapeutic agent and reinforce existing evidence regarding the renal safety of therapeutic-dose aspirin.

CONCLUSION

This study indicates that renal structural integrity and functional indices remained stable following aspirin exposure, with co-treatment showing supportive preservation of kidney mass and nephron architecture. The absence of adverse changes in serum urea, creatinine, and electrolytes suggests maintained glomerular filtration and tubular handling under the experimental conditions. These findings support the renal safety of the treatments tested and suggest a potential supportive role of *A. boonei* in maintaining renal homeostasis during pharmacological stress. Further studies incorporating longer exposure periods, mechanistic biomarkers, and functional renal endpoints are warranted to clarify protective pathways and translational relevance.

DECLARATION

The authors declare that generative artificial intelligence tools, including ChatGPT (OpenAI), were employed only to support language editing and improve clarity and organization of the manuscript text. Artificial intelligence was not used for data generation, statistical analysis, result interpretation, or scientific decision-making, and responsibility for the content rests entirely with the authors.

REFERENCES

Ahsan, M. U., Ambreen, U., Javed, H., Noor, N., Jan, M., & Khan, M. N. (2025). NSAID-associated renal injury: Mechanisms, risks, and safer strategies. *Archives of Nephrology and Renal Studies*, *5*(1). https://doi.org/10.33696/nephrology.5.013

Arif, H., & Aggarwal, S. (2023, July 5). Salicylic acid (aspirin). In *StatPearls*. StatPearls Publishing. Retrieved [2024, December 6], from https://www.ncbi.nlm.nih.gov/books/NBK519032/

Barnett, L. M. A., & Cummings, B. S. (2018). Nephrotoxicity and Renal Pathophysiology: A Contemporary Perspective. *Toxicological sciences : an official journal of the Society of Toxicology*, *164*(2), 379–390. https://doi.org/10.1093/toxsci/kfy159

Chebotareva, N., Grechukhina, K., Mcdonnell, V., Zhukova, L., & Krasnova, T. (2022). Early biomarkers of nephrotoxicity associated with the use of anti-VEGF drugs. *Biomedical reports*, *16*(6), 46. https://doi.org/10.3892/br.2022.1529

Džidić-Krivić, A., Sher, E. K., Kusturica, J., Farhat, E. K., Nawaz, A., & Sher, F. (2024). Unveiling drug-induced nephrotoxicity using novel biomarkers and cutting-edge preventive strategies. *Chemico-Biological Interactions, 388*, Article 110838. https://doi.org/10.1016/j.cbi.2023.110838

George, B., You, D., Joy, M. S., & Aleksunes, L. M. (2017). Xenobiotic transporters and kidney injury. *Advanced drug delivery reviews*, *116*, 73–91. https://doi.org/10.1016/j.addr.2017.01.005

Griffin, B. R., Faubel, S., & Edelstein, C. L. (2019). Biomarkers of Drug-Induced Kidney Toxicity. *Therapeutic drug monitoring*, *41*(2), 213–226. https://doi.org/10.1097/FTD.0000000000000589

Hosohata K. (2016). Role of Oxidative Stress in Drug-Induced Kidney Injury. *International journal of molecular sciences*, *17*(11), 1826. https://doi.org/10.3390/ijms17111826

Kwiatkowska, E., Domański, L., Dziedziejko, V., Kajdy, A., Stefańska, K., & Kwiatkowski, S. (2021). The Mechanism of Drug Nephrotoxicity and the Methods for Preventing Kidney Damage. *International journal of molecular sciences*, *22*(11), 6109. https://doi.org/10.3390/ijms22116109

Lee, V. V., Muravlyova, L. E., Bakirova, R. Y., Kiziltunc, A., Turkhanova, Z. Z., & Ashirbekova, B. D. (2021). Molecular patterns of oxidative stress in drug-induced nephropathy. *Journal of Nephropathology*, *10*(3), e31. https://doi.org/10.34172/jnp.2021.31

Liu, H., Xiang, X., Shi, C., Guo, J., Ran, T., Lin, J., Dong, F., Yang, J., & Miao, H. (2025). Oxidative stress and inflammation in renal fibrosis: Novel molecular mechanisms and therapeutic targets. *Chemico-Biological Interactions*, *421*, Article 111784. https://doi.org/10.1016/j.cbi.2025.111784

Liu, W., Tang, Q., Zhang, W., Hua, A., & Ye, X. (2025). Acute kidney injury: A new category for understanding the mechanisms of drug-induced kidney injury. *Renal Failure*, *47*(1), Article 2540563. https://doi.org/10.1080/0886022X.2025.2540563

Lu, J. L., Shrestha, P., Streja, E., Kalantar-Zadeh, K., & Kovesdy, C. P. (2023). Association of long-term aspirin use with kidney disease progression. *Frontiers in medicine*, *10*, 1283385. https://doi.org/10.3389/fmed.2023.1283385

Mishra, N. M., Chouhan, K., Prasad, S., Ghatuary, S., & More, P. (2025). A comprehensive review on phytochemical and pharmacological properties of *Alstonia scholaris*. *International Journal of Innovations in Science, Engineering and Management*, *4*(3), 401–407. https://doi.org/10.69968/ijisem.2025v4i3401-407

Mugale, M. N., Dev, K., More, B. S., Mishra, V. S., Washimkar, K. R., Singh, K., Maurya, R., Rath, S. K., Chattopadhyay, D., & Chattopadhyay, N. (2024). A comprehensive review on preclinical safety and toxicity of medicinal plants. Clinical Complementary Medicine and Pharmacology, 4(1), Article 100129. https://doi.org/10.1016/j.ccmp.2024.100129

Natesan, V., & Kim, S. J. (2025). Natural Compounds in Kidney Disease: Therapeutic Potential and Drug Development. *Biomolecules & therapeutics*, *33*(1), 39–53. https://doi.org/10.4062/biomolther.2024.142

Organisation for Economic Co-operation and Development (OECD). (2022, June 30). *Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure*. OECD Guidelines for the Testing of Chemicals, Section 4. https://www.oecd.org/en/publications/2022/06/test-no-425-acute-oral-toxicity-up-and-down-procedure_g1gh2953.html

Olanlokun, J. O., Olowofolahan, A. O., Bodede, O., Adegbuyi, A. T., Prinsloo, G., Steenkamp, P., & Olorunsogo, O. O. (2021). Anti-inflammatory potentials of the n-hexane fraction of *Alstonia boonei* stem bark in renal inflammation models. *Journal of Inflammation Research*, *14*, 3905–3920. https://doi.org/10.2147/JIR.S304076

Perazella, M. A., & Rosner, M. H. (2022). Drug-Induced Acute Kidney Injury. *Clinical journal of the American Society of Nephrology : CJASN*, *17*(8), 1220–1233. https://doi.org/10.2215/CJN.11290821

Rapa, S. F., Di Iorio, B. R., Campiglia, P., Heidland, A., & Marzocco, S. (2019). Inflammation and Oxidative Stress in Chronic Kidney Disease-Potential Therapeutic Role of Minerals, Vitamins and Plant-Derived Metabolites. *International journal of molecular sciences*, *21*(1), 263. https://doi.org/10.3390/ijms21010263

Razavi, A. C., Chen, J., Yang, W., Sha, D., Dzaye, O., Bhatia, H. S., Tsimikas, S., Lash, J. P., Kansal, M., Rincon-Choles, H., Vaccarino, V., Jacobson, T. A., Budoff, M. J., Blumenthal, R. S., Whelton, S. P., Sperling, L. S., Blaha, M. J., & He, J. (2025). Primary prevention aspirin, lipoprotein(a), and cardiorenal outcomes in chronic kidney disease: Chronic renal insufficiency cohort. *JACC: Advances*, *4*(9), Article 102068. https://doi.org/10.1016/j.jacadv.2025.102068

Sharma, S., & Goyal, S. (2022). A comprehensive review on phytochemical and pharmacological profile of *Alstonia scholaris*. *International Journal of Pharmaceutical Research and Applications*, *7*(2), 574–579. https://doi.org/10.35629/7781-0702574579

Subramanian, A., Tamilanban, T., Abdullah, A. D. I., Chitra, V., Sekar, M., Swaminathan, G., Yadav, I., Manimaran, V., Rajakumari, V., & Subramaniyan, V. (2025). Safety assessment of resveratrol surrogate molecule 5 (RSM5): Acute and sub-acute oral toxicity studies in BALB/c mice. *Toxicology Reports*, 14, 101956. https://doi.org/10.1016/j.toxrep.2025.101956

Taiwo, J. E., Shemishere, U. B., & Omoregie, E. S. (2019). Assessment of the nephroprotective effect of *Alstonia boonei* leaf extracts in isoniazid and rifampicin co-treated rats. *World Journal of Biomedicine and Pharmaceutical Sciences*, *3*, 1–6. http://www.brsfoundation.org/wjbps

Tienda-Vázquez, M. A., Morreeuw, Z. P., Sosa-Hernández, J. E., Cardador-Martínez, A., Sabath, E., Melchor-Martínez, E. M., Iqbal, H. M. N., & Parra-Saldívar, R. (2022). Nephroprotective Plants: A Review on the Use in Pre-Renal and Post-Renal Diseases. *Plants (Basel, Switzerland)*, *11*(6), 818. https://doi.org/10.3390/plants11060818

Visagie, J. L., Aruwajoye, G. S., & van der Sluis, R. (2024). Pharmacokinetics of aspirin: Evaluating shortcomings in the literature. *Expert Opinion on Drug Metabolism & Toxicology*, 20(8), 727-740. https://doi.org/10.1080/17425255.2024.2386368

Uroko, R., Sangodare, R., Onyeabo, C., Agbafor, A., Uchenna, O., Nwuke, C., & Asadu, C. (2020). Investigation of antioxidant compositions and antioxidative activities of ethanol extract of *Alstonia boonei* stem bark. *Nigerian Journal of Pharmaceutical Research*, *16*(1), 71-80. https://doi.org/10.4314/njpr.v16i1.8