

Effect of *Zingiber Officinale* Ethanol Extract on Renal Functions of Male Wistar Albino Rats Induced with Inflammation

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Abstract: This study evaluated the effect of ethanol extract of *Zingiber officinale* on renal function markers in male Wistar albino rats subjected to albumin-induced inflammation. The animals were divided into five groups: blank control, negative control (inflammation without treatment), standard control (treated with a reference anti-inflammatory drug), and two treatment groups administered low and high doses of *Z. officinale* extract. Renal function was assessed through serum creatinine, urea, calcium (Ca), and chloride (Cl⁻) levels. The negative control group exhibited significantly elevated levels of creatinine (0.59±0.00 mg/dl), urea (12.22±0.001 mg/dl), and calcium (6.93±0.11 ppm), alongside a sharp reduction in chloride (4.42±0.00 ppm), compared to the blank control (p < 0.05), indicating renal impairment caused by inflammation. Treatment with high-dose *Z. officinale* extract significantly restored creatinine (0.52±0.00 mg/dl), urea (11.02±0.03 mg/dl), calcium (5.33±0.13 ppm), and chloride (7.54±0.11 ppm) levels to values comparable to the standard control and blank groups (p > 0.05), while the low-dose treatment showed only partial improvement. These findings demonstrate that *Zingiber officinale* ethanol extract, particularly at high doses, possesses nephroprotective properties, mitigating inflammation-induced renal dysfunction and restoring electrolyte balance.

Keywords: *Zingiber officinale*, inflammation, Albino rats, and Renal function.

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Introduction

Background of the Study

By controlling fluid balance, electrolyte levels, and waste excretion through urine production, the kidneys are vital organs that preserve homeostasis (Beckerman *et al.*, 2020). They are in charge of reabsorbing essential nutrients, filtering about 180 liters of blood every day, and eliminating waste materials including urea, creatinine, and extra salts. Changes in biochemical indicators including serum creatinine, blood urea nitrogen (BUN), calcium, and chloride levels are common signs of any impairment in kidney function (Beckerman *et al.*, 2020). Renal efficiency is frequently assessed using these indicators in both clinical and experimental nephrology.

The pathophysiology of renal failure has been linked to inflammation, a fundamental physiological reaction to injury and infection (Martínez-Klimova *et al.*, 2020). Prolonged inflammation impairs glomerular and tubular function by causing oxidative stress, immune cell infiltration, and structural damage to renal tissues. Elevated blood creatinine and urea levels as well as changed electrolyte concentrations, such as calcium and chloride, are biochemical manifestations of this injury (Sun *et al.*, 2021). Testing possible treatment approaches and investigating the pathogenesis of renal inflammation have both benefited from the use of animal models.

Zingiber officinale, is a well-known medicinal plant that has long been utilized in Chinese medicine, Ayurveda, and other ancient healing systems. Its bioactive ingredients, including zingerone, paradols, shogaols, and gingerols, have drawn a lot of

attention since they have strong antibacterial, anti-inflammatory, and antioxidant properties (Rahmani *et al.*, 2019). *Zingiber officinale* can lower oxidative stress, modify inflammatory pathways, and shield different organ systems from harmful assaults, according to recent study that supports traditional wisdom (Afzal *et al.*, 2020).

Because of its medicinal qualities, *Zingiber officinale* is a great option for treating renal failure brought on by inflammation. In animal models exposed to nephrotoxic substances as cisplatin, gentamicin, and lead, it has demonstrated promise in lowering renal oxidative stress and enhancing histological architecture (Al-Malki and Sayed, 2021). Reduction of reactive oxygen species (ROS), suppression of pro-inflammatory cytokines (e.g., IL-6, TNF- α), and restoration of endogenous antioxidant systems like glutathione and superoxide dismutase are hypothesized to be the mechanisms behind these effects.

Given that gingerols and shogaols are more soluble and bioavailable in alcohol than in water, using *Zingiber officinale* ethanol extract is particularly beneficial (Salehi *et al.*, 2021). Investigating safer and more affordable substitutes for synthetic medications is crucial given the increasing prevalence of kidney disorders, particularly those associated with oxidative stress and inflammation. A dependable method for researching the physiological and biochemical impacts of interventions on kidney health is to use animal models, such as male Wistar albino rats.

The goal of the current study is to assess how *Zingiber officinale* ethanol extract affects renal function in male Wistar

albino rats that have been inflamed. Important renal biomarkers that are indicators of nephrotoxicity and general kidney function, including serum creatinine, urea nitrogen, calcium, and chloride levels, are the focus of this study. The work may add to the expanding body of information on phytotherapy for renal diseases by elucidating ginger's restorative or protective capabilities in this setting.

Statement of the Problem

Particularly in low- and middle-income nations, renal dysfunction continues to be a major worldwide health concern, contributing significantly to morbidity and mortality (Yacoub and Habib, 2020). Even with advances in pharmacotherapy, many synthetic medications used to treat kidney disease have serious adverse effects, and long-term usage may hasten the degradation of the kidneys. Furthermore, because oxidative stress and inflammatory pathways are complex and contribute to kidney impairment, managing inflammation-induced nephropathy is extremely difficult.

Due to the rise in environmental pollutants, infections, and lifestyle-related diseases, which frequently go undetected in their early stages because they lack distinct symptoms, the burden of inflammation-induced kidney dysfunction has increased (Kang *et al.*, 2022). Serum creatinine and urea nitrogen levels are examples of traditional markers that are late predictors of kidney impairment; by the time spikes are noticed, substantial damage may have already taken place.

There is a lack of empirical evidence supporting the effectiveness of many plant-based treatments used in traditional medicine to treat kidney diseases, especially when it comes to modifying inflammatory processes at the renal level. *Zingiber officinale* is one of these medicinal herbs that is well known for its therapeutic versatility; nevertheless, little is known about how it protects the kidneys in inflammatory circumstances in experimental models.

Justification of the Study

The rising incidence of renal dysfunctions worldwide and the resulting socioeconomic burden serve as the rationale for this study. One of the main causes of both acute and chronic kidney impairment is inflammation, yet the methods used to treat it are ineffective and frequently have unfavorable side effects (Suresh *et al.*, 2021). This emphasizes how crucial it is to find safer, more efficient, and more reasonably priced substitutes.

Because of its diverse range of bioactive substances with nephron-protective, antioxidant, and anti-inflammatory qualities, *Zingiber officinale* makes a strong argument. Its effectiveness in reducing oxidative stress and regulating pro-inflammatory mediators has been validated by numerous *in vitro* and *in vivo* investigations (Rehman *et al.*, 2020). It has not yet been thoroughly determined how it might be used to treat inflammation-induced renal impairment, particularly by controlling important indicators including serum creatinine, blood urea nitrogen, calcium, and chloride.

Furthermore, the selection of ethanol extract is supported by science since it is a better solvent than aqueous extraction for phenolic and flavonoid components (Yeh *et al.*, 2022). Compared to their watery equivalents, ginger ethanol extracts have demonstrated superior pharmacological qualities, such as anti-inflammatory and antioxidant effects.

A consistent experimental paradigm with physiological traits that closely resemble human renal responses is provided by using male Wistar albino rats. It enables accurate measurement of renal function indices and regulated production of inflammation (Rehman *et al.*, 2020). Future clinical trials may be based on the data produced by this study, which may also help guide the creation of novel plant-based medicinal approaches.

Thus, this study contributes to both scientific knowledge and practical healthcare, especially in resource-constrained settings where access to modern pharmaceuticals is limited, and there is reliance on herbal medicine.

Aim of the Study

The primary aim of this study is to investigate the effect of ethanol extract of *Zingiber officinale* on renal function of male Wistar albino rats induced with inflammation.

Specific Objectives of the Study

The specific objective of this study were to:

- Determine the effect of *Zingiber officinale* ethanol extract on serum creatinine level of male Wistar albino rats induced with inflammation.
- Determine the effect of *Zingiber officinale* ethanol extract on serum urea nitrogen concentration of male Wistar albino rats induced with inflammation.
- Determine the effect of *Zingiber officinale* ethanol extract on calcium iron level of male Wistar albino rats induced with inflammation.
- Determine the effect of *Zingiber officinale* ethanol extract on chloride iron level of male Wistar albino rats induced with inflammation.

Materials and Methods

Plant Collection and Identification

Fresh rhizomes of *Zingiber officinale* were purchased from Nkpokiti Market, Enugu State. The plant material was authenticated by Prof. C. S. Eze in the Department of Applied Biology and Biotechnology at Enugu state University of Science and Technology.

Preparation of Ethanol Extract of *Zingiber officinale*

The rhizomes which weighed 76.4 grams were washed thoroughly with distilled water to remove dirt and dried using analytical oven 105°C. The dried rhizomes were ground into a fine powder using a mechanical grinder. The powdered sample was placed in a soxhlet apparatus, and 300 mL of pure ethanol was used as the solvent for extraction (Gao *et al.*, 2020). The soxhlet extraction process was carried out for 5 hours. The mixture was filtered using Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C to yield the crude ethanol extract.

Experimental Animals

A total of thirty (30) healthy male Wistar albino rats weighing 10–220 g were obtained from University of Nigeria Enugu Campus (UNEC) Animal House. The animals were housed in plastic cages under standard conditions of 12-hour light/dark cycle, temperature (25°C). Rats were acclimatized for two weeks before the commencement of the experiment and had standard rat feed and clean water.

Induction of Inflammation

Inflammation was induced by intraperitoneal injection of 0.5 ml of egg albumin solution and in the hind paw region. The inflammation peaked within 3-6 hours. The paw thickness was monitored using a Vernier caliper to confirm the presence and resolution of inflammation.

Experimental Design

The study was laid on a Complete Randomized Experimental Design (CRED), and the animals were sampled and grouped into 5 comprising six rats:

- Group A (Blank Control): were neither induced nor treated, but retained feed and, water *ad libitum*
- Group B (Negative Control): induced with concentrated egg albumin, but received no treatment.
- Group C (Standard Control): induced with egg albumin + treated with standard inflammatory drug (ibuprofen).
- Group D (Low Dose *Zingiber officinale* Extract): induced with egg albumin + treated with 50 mg/kg body weight of *Zingiber officinale* extract.
- Group E (High Dose *Zingiber officinale* Extract): induced with egg albumin + treated with 200 mg/kg body weight of *Zingiber officinale* extract.

Treatments were administered orally (via intubation) once daily for 3 consecutive weeks following egg albumin induction.

Blood Sample Collection

At the end of the treatment period, the rats were anesthetized using chloroform. Blood samples were collected via ocular puncture into plain bottles for biochemical analysis.

Biochemical Analyses

Serum Creatinine Level

Measured using the Jaffe reaction, where creatinine reacts with picric acid under alkaline conditions to form a red-colored complex detectable at 520 nm using a spectrophotometer (Ali *et al.*, 2022).

Serum Urea Nitrogen (BUN) Concentration

Determined using an enzymatic colorimetric assay involving urease, which breaks down urea to produce ammonia.

Table 1: Effect of *Zingiber officinale* ethanol extract on creatinine (mg/dl) of male wistar albino rats induced with inflammation

GROUPS	CREATININE (mg/dl)
A (Blank Control)	0.51±0.001 ^a
B (Negative Control)	0.59±0.00 ^b
C (Standard Control)	0.51±0.011 ^a
D (Low-Dose Treated Group)	0.58±0.004 ^b
E (High-Dose Treated Group)	0.52±0.00 ^a

The values are expressed as (mean ± SEM)

Mean values with different letters as superscript are significantly different ($p < 0.05$)

The ammonia reacts to form a measurable color complex (Muthuraman *et al.*, 2020).

Calcium Ion Level

Quantified using the Arsenazo III method, where calcium forms a purple-colored complex with Arsenazo III dye, and absorbance is read at 650 nm (Shah *et al.*, 2021).

Chloride Ion Level

Assessed by mercuric thiocyanate colorimetric method or ion-selective electrodes, which provide rapid and accurate measurement of serum chloride (Tiwari *et al.*, 2021).

Statistical Analysis

All the statistical analysis was processed using the Statistical Package of Social Science (SPSS) for the window (version 21). The values of the measured parameters were expressed as mean ± SEM. One-way Analysis of Variance (1-way ANOVA) was used to determine the effect of inflammation and *Zingiber officinale* ethanol extract on the parameters studied and the difference between means were separated using Duncan's multiple range test. Test for significance was at 0.05 probability level.

Results

Creatinine Level

The table (Table 1) presents serum creatinine levels (mg/dl) across different experimental groups. Group A (Blank Control) and Group C (Standard Control) recorded identical or near-identical creatinine values of 0.51±0.001 mg/dl and 0.51±0.011 mg/dl, respectively, showing no significant difference between them ($p > 0.05$). Similarly, Group E (High-Dose Treated Group) showed a creatinine level of 0.52 ± 0.00 mg/dL, indicating that the high-dose treatment effectively preserved kidney function at levels comparable to those of the blank and standard controls ($p > 0.05$). In contrast, Group B (Negative Control) had an elevated creatinine concentration of 0.59 ± 0.00 mg/dL ($p < 0.05$), indicating significant renal stress or impairment due to the albumin-induced inflammation. Group D (Low-Dose Treated Group) also showed a significantly higher creatinine level of 0.58±0.004 mg/dl ($p < 0.05$), similar to the negative control, implying that the low dose of *Zingiber officinale* extract did not significantly mitigate renal dysfunction.

Urea Concentration

Group A (Blank Control) recorded a baseline urea level of 10.62±0.0001 mg/dl, while Group C (Standard Control) and Group E (High-Dose Treated Group) had similar values of 10.70±0.002 mg/dl and 11.02±0.03 mg/dl, respectively, with no significant differences among them (p > 0.05). This suggests that both the standard drug and the high dose of *Zingiber officinale* extract maintained urea levels within the normal physiological

range, showing effective protection against albumin-induced renal dysfunction. In contrast, Group B (Negative Control) exhibited a significantly elevated urea level of 12.22±0.001 mg/dl (p < 0.05), indicating renal stress or impaired kidney function due to inflammation. Group D (Low-Dose Treated Group) also showed a higher urea level of 11.92±0.02 mg/dl, with no significant difference to the negative control (p < 0.05), suggesting that the low dose of *Z. officinale* was not effective in restoring normal renal function (Table 2).

Table 2: Effect of *Zingiber officinale* ethanol extract on urea (mg/dl) of male wistar albino rats induced with inflammation

GROUPS	UREA (mg/dl)
A (Blank Control)	10.62±0.0001 ^a
B (Negative Control)	12.22±0.001 ^b
C (Standard Control)	10.70±0.002 ^a
D (Low-Dose Treated Group)	11.92±0.02 ^b
E (High-Dose Treated Group)	11.02±0.03 ^a

The values are expressed as (mean ± SEM)

Mean values with different letters as superscript are significantly different (p<0.05)

Calcium (Ca) Concentration

Group A (Blank Control) recorded a calcium level of 5.11±0.12 ppm, while Groups C (Standard Control), D (Low-Dose Treated Group), and E (High-Dose Treated Group) recorded 5.26±0.01 ppm, 5.27±0.12 ppm, and 5.33±0.13 ppm, respectively, with no significant difference among the four groups (p > 0.05). This suggests that both doses of *Zingiber officinale* extract, as well

as the standard treatment, effectively maintained calcium homeostasis close to normal levels. In contrast, Group B (Negative Control) exhibited a significantly elevated calcium level of 6.93±0.11 ppm, compared to the other groups (p < 0.05). The elevated calcium in the negative control may reflect inflammation-induced mineral imbalance or renal dysfunction (Table 3).

Table 3: Effect of *Zingiber officinale* ethanol extract on Ca (ppm) of male wistar albino rats induced with inflammation

GROUPS	Ca (ppm)
A (Blank Control)	5.11±0.12 ^a
B (Negative Control)	6.93±0.11 ^b
C (Standard Control)	5.26±0.01 ^a
D (Low-Dose Treated Group)	5.27±0.12 ^a
E (High-Dose Treated Group)	5.33±0.13 ^a

The values are expressed as (mean ± SEM)

Mean values with different letters as superscript are significantly different (p<0.05)

Chloride ion (Cl⁻) Concentration

Group A (Blank Control) recorded the highest chloride level at 10.45±0.03 ppm, representing the normal physiological range. In contrast, Group B (Negative Control) showed a significantly reduced chloride level of 4.42±0.00 ppm (p < 0.05). This marked decrease suggests that albumin-induced inflammation severely disrupted electrolyte balance, particularly chloride homeostasis. Groups C (Standard Control), D (Low-Dose Treated Group), and E

(High-Dose Treated Group) recorded chloride levels of 7.36±0.10 ppm, 7.18±0.02 ppm, and 7.54±0.11 ppm, respectively. Showing no significant differences among them (p > 0.05), but they are significantly different from both the blank control and negative control (p < 0.05). These values show that treatment with *Zingiber officinale* extract, at both doses, as well as the standard drug, significantly improved chloride levels compared to the negative control but did not fully restore them to normal (blank control) level (Table 4).

Table 4: Effect of *Zingiber officinale* ethanol extract on Cl⁻ (ppm) of male wistar albino rats induced with inflammation

GROUPS	Cl ⁻ (ppm)
A (Blank Control)	10.45±0.03 ^a
B (Negative Control)	4.42±0.00 ^b
C (Standard Control)	7.36±0.10 ^c

D (Low-Dose Treated Group)	7.18±0.02 ^c
E (High-Dose Treated Group)	7.54±0.11 ^c

The values are expressed as (mean ± SEM)

Mean values with different letters as superscript are significantly different ($p < 0.05$)

Discussion

This study examined the effects of *Zingiber officinale* ethanol extract on creatinine, urea, calcium, chloride, and renal function parameters in male Wistar albino rats with inflammation caused by albumin. The findings showed that inflammation markedly hampered renal function, as shown by decreased chloride levels and increased serum creatinine, urea, and calcium levels. Nevertheless, *Zingiber officinale* treatment, particularly at high dosages, markedly returned these parameters to normal, indicating a protective and regulating action of *Zingiber officinale* on kidney function.

Renal impairment brought on by inflammation was confirmed by elevated creatinine and urea levels in the negative control group. These results are consistent with those of Yaseen *et al.* (2021), who found that *Zingiber officinale* extract markedly reduced elevated creatinine and urea concentrations after induced nephrotoxicity. Similarly, *Zingiber officinale* extract dramatically reduced urea and creatinine levels in rats exposed to gentamicin-induced kidney damage, according to Mahmoud *et al.* (2019). The nephroprotective ability of *Z. officinale* was further supported in the current investigation when high-dose *Z. officinale* restored creatinine and urea levels to values statistically comparable to the blank and standard control groups ($p > 0.05$).

In terms of calcium regulation, the negative control group's inflammation caused a marked rise in calcium levels, which could be related to electrolyte imbalance or reduced renal excretion. The findings of Rashid *et al.* (2020), who documented calcium dysregulation in rats with renal injury, are consistent with this. In line with the findings of Musa *et al.* (2022), who discovered that *Z. officinale* administration restored mineral levels in nephrotoxic rats, treatment with *Z. officinale* kept calcium levels within the normal range. *Z. officinale's* antioxidant and anti-inflammatory phytochemicals, which stabilize renal tubular function, may be responsible for its capacity to control calcium.

The negative control group's chloride levels were considerably lower, which was indicative of changed electrolyte management brought on by inflammation-induced renal impairment. Nevertheless, ginger extract treatment especially at large doses partially brought chloride concentrations back to normal. The results of Ezejiolor *et al.* (2021), who found that ginger extract enhanced electrolyte profiles in rats with induced renal failure, are consistent with this. In a rat model of renal oxidative stress, Akpan *et al.* (2020) showed that supplementing with ginger prevented significant alterations in plasma electrolytes. By maintaining glomerular and tubular integrity, *Z. officinale* may help maintain electrolyte balance, as seen by the restored chloride levels in the treated groups.

Conclusion

This study concludes that ethanol extract of *Zingiber officinale* has a significant nephroprotective effect in male Wistar albino rats with albumin-induced inflammation. The high-dose treatment notably improved serum levels of creatinine, urea,

calcium, and chloride, indicating restoration of normal renal function and electrolyte balance. These findings support the therapeutic potential of ginger in managing inflammation-related kidney impairment.

Recommendations

We recommend that further studies should investigate the molecular mechanisms behind the nephroprotective effects of *Z. officinale*. Clinical trials are also recommended to validate the effectiveness of *Z. officinale* in human renal inflammatory conditions.

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