

## Original Research Article

# Alcoholic Bitters Produced Profound Renal Dysfunction when Compared with Sildenafil Citrate and Aqueous Extract of *Trigonella foenum-graecum* in Male Wistar Rat

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**Abstract:** Sexual-performance-enhancing products (aphrodisiacs) are widely used, yet their chronic and combined consumption may pose renal risks because the kidneys are central to drug metabolism and excretion. In Nigeria, concurrent use of prescription agents (e.g., sildenafil), herbal extracts (e.g., *Trigonella foenum-graecum*), and alcoholic bitters is common, but comparative experimental evidence on renal safety is limited. This study aimed to compare the effects of sildenafil citrate, aqueous *T. foenum-graecum* seed extract, and a selected alcoholic bitters—alone and in combination—on renal function in male Wistar rats. A randomized controlled experimental design was used: forty adult male rats were allocated into eight groups (control; low/high fenugreek; low/high sildenafil; low/high bitters; and combined low-dose of all test substances) and treated orally once daily for six weeks. Renal function indices (electrolytes/acid–base indices, urea, creatinine) were assessed, kidneys were examined histologically, and data were analysed using one-way ANOVA with Tukey’s HSD post hoc test ( $\alpha = 0.05$ ). There were significant between-group differences in bicarbonate ( $F(7,32) = 7.712, p = 0.000$ ), sodium ( $F(7,32) = 6.071, p = 0.000$ ), and urea ( $F(7,32) = 6.849, p = 0.000$ ). Pairwise comparisons showed significant differences involving the control group for bicarbonate (most pairings,  $p < 0.05$ ), and significant sodium differences including high-dose bitters versus several groups (e.g., Group 6 vs Group 8,  $p = 0.000$ ). Creatinine differed significantly across multiple treated groups versus control (e.g., control vs Groups 3–6,  $p \leq 0.038$ ). Histology showed dose-related renal injury, with alcoholic bitters demonstrating the most pronounced lesions (glomerulonephritis and myxoid degeneration), while fenugreek and sildenafil produced milder-to-moderate tubular/glomerular alterations. In conclusion, repeated exposure to these aphrodisiacs produced treatment- and dose-dependent renal dysfunction and structural damage, with alcoholic bitters showing the greatest nephrotoxic potential. Regulatory oversight, consumer education, and further mechanistic studies to identify toxic constituents and safe exposure limits are recommended.

**Keywords:** Aphrodisiacs, Renal Function, Sildenafil Citrate, *Trigonella Foenum-Graecum*, Alcoholic Bitters, Wistar Rats.

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## 1. INTRODUCTION

Sexual function constitutes an essential component of overall quality of life and subjective well-being in humans. Sexual dysfunction is widely prevalent and has been shown to adversely affect mood, self-esteem, and interpersonal relationships (Avasthi *et al.*, 2017). Across different cultures and historical periods, considerable effort has been devoted to identifying substances capable of enhancing sexual desire and performance, particularly in the management of male

erectile dysfunction (Wentzell, 2017). This long-standing quest has driven the development and widespread use of substances collectively referred to as aphrodisiacs.

Aphrodisiacs are defined as substances or foods that enhance sexual desire, arousal, performance, or pleasure. Individuals may seek aphrodisiacs for various reasons, including reduced libido, impaired sexual performance, or the desire to intensify sexual experiences (Kendra, 2021). These substances may be of

natural origin, such as plant extracts, or synthetic, such as pharmacological agents, and their consumption has increased substantially in recent years.

*Trigonella foenum-graecum* (fenugreek) is one of the oldest medicinal plants with a long history of use in traditional medicine. The plant is nutritionally rich, containing oleic, linolenic, and linoleic acids, nicotinic acid, dietary fibres, glycolipids, phospholipids, choline, and several vitamins, including vitamins A, B<sub>1</sub>, B<sub>2</sub>, C, and niacin (Ayah Syed, 2021). Owing to its phytochemical composition, fenugreek has been associated with various therapeutic effects and is commonly incorporated into herbal formulations used as sexual enhancers.

Sildenafil citrate, a phosphodiesterase-5 inhibitor, has been widely used for the treatment of erectile dysfunction since its approval by the United States Food and Drug Administration in 1998. Its efficacy has been demonstrated in diverse clinical populations, including individuals with diabetes, cardiovascular disease, mild depression, spinal cord injury, and multiple sclerosis (Puşcaşu *et al.*, 2023). Despite its established therapeutic benefit, concerns remain regarding its long-term safety, particularly when used indiscriminately or in combination with other substances.

In Nigeria, the consumption of herbal medicinal products formulated as alcoholic bitters with aphrodisiac claims is widespread. These products are commonly marketed in retail shops, open markets, and motor garages and are frequently used to enhance sexual performance, especially in urban settings where social pressures and heightened sexual expectations are prevalent (Dike *et al.*, 2024). Due to factors such as affordability, accessibility, and perceived efficacy, these herbal formulations are often consumed chronically and, in some cases, in combination with synthetic drugs.

The increasing and indiscriminate use of aphrodisiac substances raises concerns about their potential adverse effects on vital organs, particularly the kidneys, which play a central role in drug metabolism and excretion. Socio-economic challenges such as poverty, work-related stress, and lifestyle pressures have further encouraged the use of sexual performance-enhancing agents across different age groups in Nigeria (gyamfi *et al.*, 2025; dike *et al.*, 2024).

However, scientific data on the renal implications of prolonged or combined use of these substances remain limited.

Therefore, the present study investigates the effects of *Trigonella foenum-graecum*, sildenafil citrate, and alcoholic bitters on renal function in male Wistar rats, with the aim of providing experimental evidence on their potential nephrotoxic or protective effects following repeated administration.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Animals

Forty (40) adult male Wistar rats weighing 100–150 g were obtained from the Animal House of the Department of Pharmacology, Rivers State University, Port Harcourt. The animals were housed in plastic cages with aluminium covers under standard laboratory conditions with natural light/dark cycles and adequate ventilation. They were provided with commercial rat pellets and water ad libitum and allowed to acclimatize for two weeks prior to the commencement of the experiment.

### 2.2 Study Design and Grouping

A randomized controlled experimental design was adopted. Following acclimatization, the animals were randomly assigned into eight groups (n = 7) as follows:

1. Low-dose *Trigonella foenum-graecum* extract (50 mg/kg)
2. High-dose *Trigonella foenum-graecum* extract (100 mg/kg)
3. High-dose sildenafil citrate (10 mg/kg)
4. Low-dose sildenafil citrate (5 mg/kg)
5. High-dose alcoholic bitters (50 mg/kg)
6. Low-dose alcoholic bitters (25 mg/kg)
7. Low doses of all test substances
8. Control (standard feed and water only)

All treatments were administered orally once daily using an oral gavage for a period of six weeks.

### 2.3 Preparation of Test Substances

#### 2.3.1 Sildenafil Citrate

Sildenafil citrate tablets (100 mg each) were pulverized into fine powder. A total of 1000 mg of the powdered drug was dissolved in 100 mL of distilled water and mixed thoroughly to obtain a homogenous solution for dosing.

#### 2.3.2 *Trigonella Foenum-Graecum*

Dried seeds of *Trigonella foenum-graecum* were purchased from Mile 3 Market, Diobu, Rivers State. The seeds were ground into fine powder using a mechanical grinder and taken to the Department of Pharmacology, College of Medical Sciences, Rivers State University for extraction.

**2.3.3 Alcoholic Bitters:** Commercially available alcoholic bitters were used at the manufacturer's stated concentration.

#### 2.4 Preparation of Extract

The powdered *Trigonella foenum graecum* seeds were stored in clean glass jars at room temperature. Extraction was carried out using distilled water obtained from the Department of Chemistry, Faculty of Sciences, Rivers State University. A total of 120 g of the powdered material was soaked in 500 mL of distilled water and allowed to stand for 12 hours. The mixture separated into two layers, and the supernatant was collected by decantation and filtered. The resulting yellowish extract was stored in a refrigerator at 4°C until use (Moomin *et al.*, 2023)

#### 2.5 Phytochemical Screening

Qualitative phytochemical analyses of the *Trigonella foenum-graecum* extract were conducted to determine the presence of bioactive compounds. Standard procedures were employed to test for alkaloids, reducing sugars, saponins, steroids (Salkowski test), cardiac glycosides, tannins, flavonoids, carbohydrates, and phenols. Positive reactions were indicated by characteristic colour changes or precipitate formation, confirming the presence of these phytochemicals.

#### 2.6 Dosage Determination

Dosages were calculated based on the mean body weight of the rats (0.16 kg). For sildenafil citrate, a

stock solution was prepared by dissolving 1000 mg in 100 mL of distilled water. Low and high doses corresponded to 5 mg/kg and 10 mg/kg, respectively. For *Trigonella foenum-graecum*, 120 g of extract in 500 mL of water yielded a concentration of 2.4 mg/mL. Low and high doses were calculated as 50 mg/kg and 100 mg/kg, respectively, and administered according to the calculated volume per body weight. Alcoholic bitters were administered at low and high doses of 25 mg/kg and 50 mg/kg, respectively, based on the corresponding volumes.

#### 2.7 Sample Collection

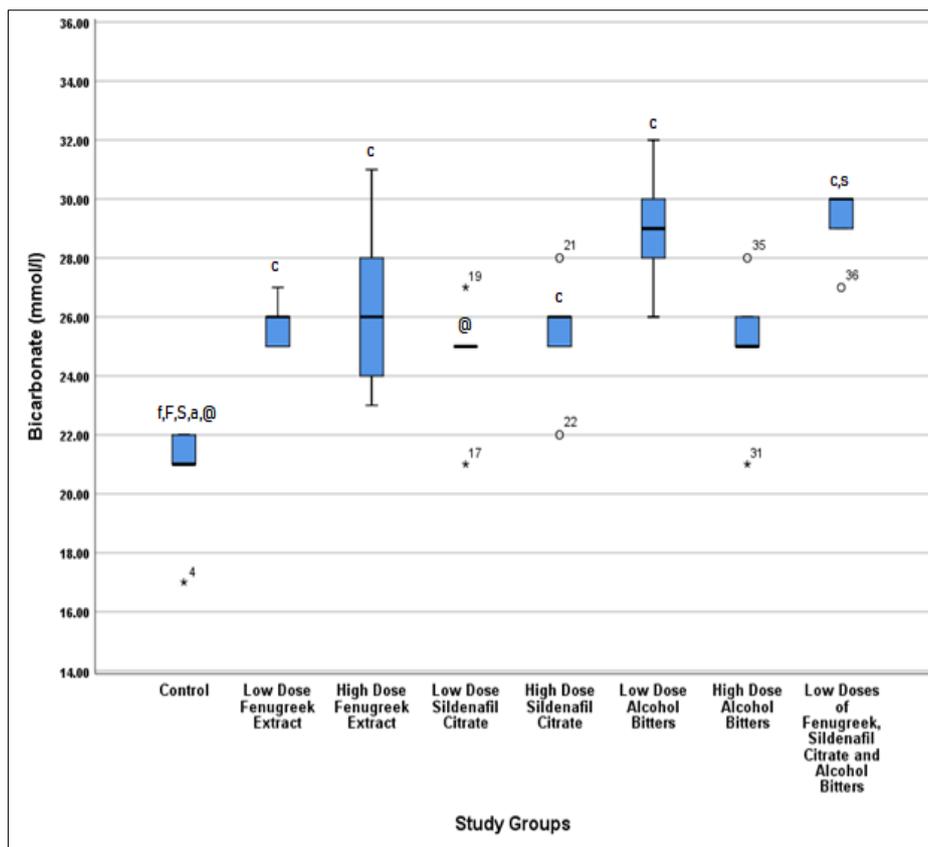
At the end of the six-week treatment period, the rats were sacrificed by cervical dislocation. Blood samples were collected into plain sample bottles for renal function analysis. The kidneys were carefully excised and preserved for histological processing.

#### 2.8 Statistical Analysis

Data obtained were subjected to statistical analysis using one-way analysis of variance (ANOVA). Results were expressed as mean  $\pm$  standard deviation, and statistical significance was set at  $p < 0.05$ .

### 3. RESULTS

#### 3.1 Effects of Extract on Bicarbonate Levels

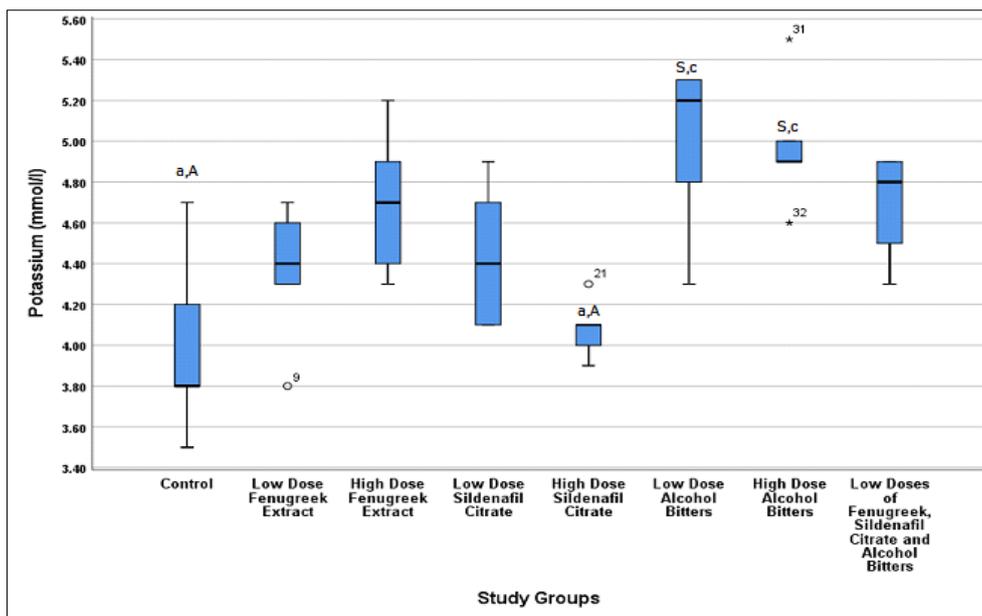


**Figure 3.1: A box plot showing levels of bicarbonate across the study groups**

Figure 3.1 shows the effects of different treatments on bicarbonate levels across the study groups. The analysis revealed that Group 8 consistently exhibited the highest bicarbonate levels, while other groups showed variable responses depending on the dose and type of extract. A one-way ANOVA indicated a statistically significant difference in bicarbonate levels across groups ( $F(7, 32) = 7.712, p = 0.000$ ). Post hoc analysis using Tukey’s HSD revealed significant

differences in all pairings involving Group 8, except with Groups 3 and 6. Additionally, comparison between Groups 3 and 7 demonstrated a significant difference ( $p = 0.040$ ). Other pairwise comparisons showed no significant differences ( $p > 0.05$ ), indicating that the extracts modulated bicarbonate levels in a group-specific manner.

### 3.2 Effect of Extracts on Potassium Levels

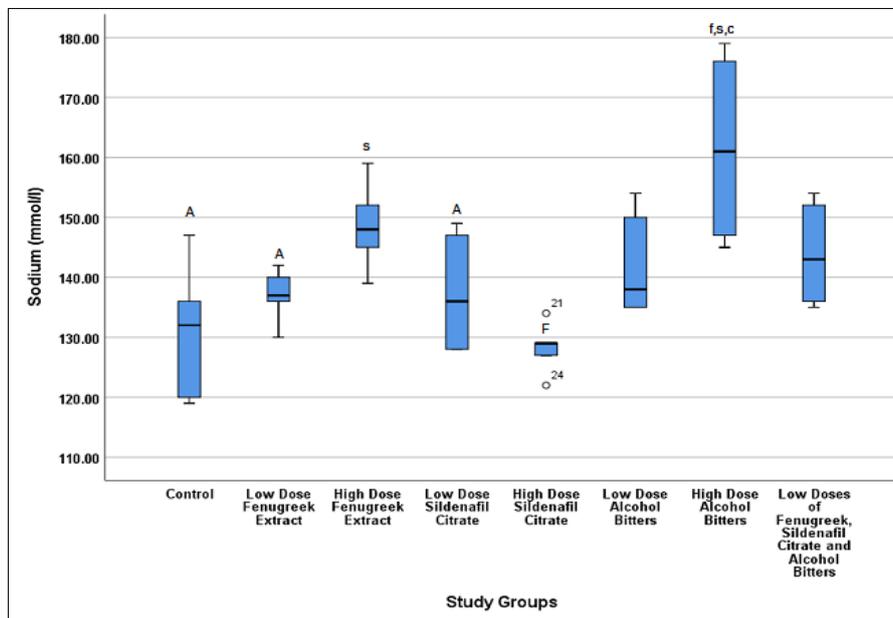


**Figure 3.2: A box plot showing levels of potassium across the study groups**

Figure 3.2 illustrates the impact of the extracts on potassium levels in the different study groups. Statistically significant differences were observed between Groups 4 and 5 ( $p = 0.007$ ), Groups 4 and 6 ( $p = 0.007$ ), Groups 8 and 5 ( $p = 0.002$ ), and Groups 8 and 6 ( $p = 0.002$ ). Other comparisons were not significant ( $p > 0.05$ ). The findings indicate that high doses of

sildenafil citrate and alcohol bitters, as well as the control, influenced potassium concentrations significantly, while low doses produced more moderate effects.

### 3.3 Effect of Extracts on Sodium

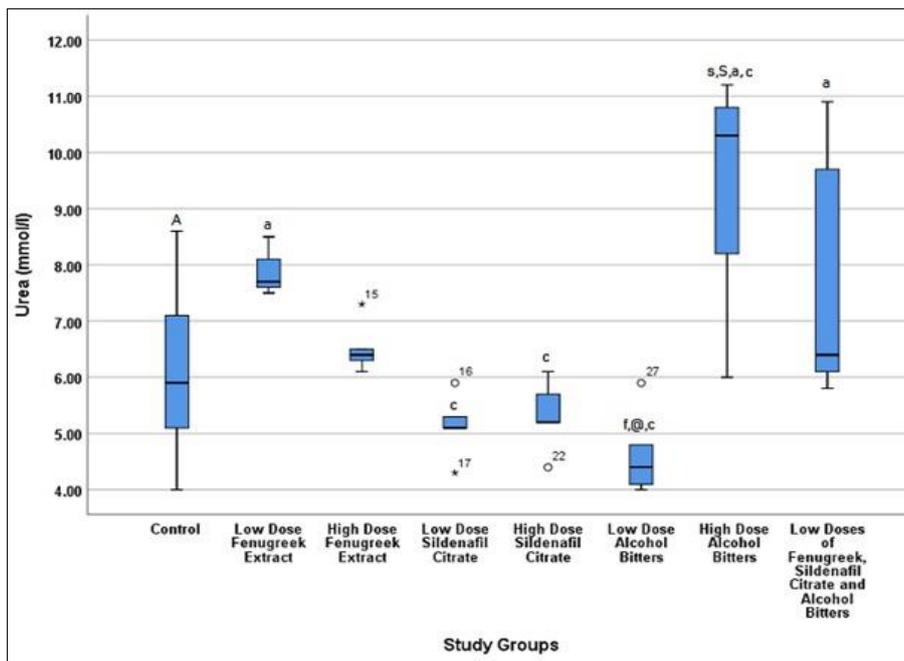


**Figure 3.3: A box plot showing levels of sodium across the study groups**

Figure 3.3 depicts the sodium levels among the treatment groups. A one-way ANOVA showed a statistically significant difference between at least two groups ( $F(7, 32) = 6.071, p = 0.000$ ). Tukey's HSD post hoc comparisons indicated significant differences between Groups 2 and 4 ( $p = 0.039$ ), Groups 6 and 1 ( $p$

$= 0.007$ ), Groups 6 and 3 ( $p = 0.009$ ), and Groups 6 and 8 ( $p = 0.000$ ). Other group comparisons were not significant ( $p > 0.05$ ). These results suggest that sodium levels were notably affected by high-dose fenugreek, sildenafil citrate, alcohol bitters, and the control group.

### 3.4 Effect of Extracts on Urea

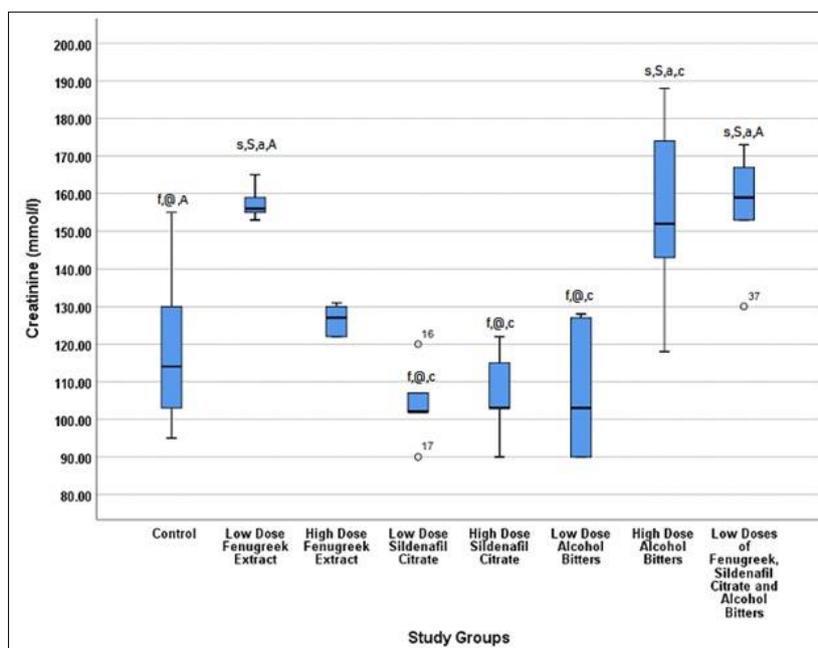


**Figure 3.4:** A box plot showing levels of urea across the study groups

Figure 3.4 shows the effects of the different treatments on urea levels. The ANOVA analysis revealed significant differences across groups ( $F(7, 32) = 6.849$ ,  $p = 0.000$ ). Tukey’s HSD post hoc test demonstrated significant differences between Groups 5 and 1 ( $p = 0.015$ ), Groups 5 and 7 ( $p = 0.020$ ), Groups 6 and 3 ( $p = 0.001$ ), Groups 6 and 4 ( $p = 0.002$ ), Groups 6 and 5 ( $p =$

$0.000$ ), and Groups 8 and 6 ( $p = 0.019$ ). Multiple other pairings showed no significant differences ( $p > 0.05$ ). These results indicate that the extracts influenced urea metabolism in a dose- and treatment-dependent manner.

### 3.5 Effect of Extracts on Creatinine



**Figure 4.5:** A box plot showing levels of creatinine across the study groups

Figure 3.5 illustrates creatinine levels in the study groups. Significant differences were observed between Group 1 and Groups 3, 4, 5, and 6 ( $p = 0.000$ ,

$0.001$ ,  $0.001$ ,  $0.021$ , respectively); Group 7 and Groups 3, 4, 5, and 6 ( $p = 0.001$ ,  $0.001$ ,  $0.001$ ,  $0.028$ , respectively); and Group 8 and Groups 3, 4, 5, and 6 ( $p$

= 0.001, 0.002, 0.002, 0.038, respectively). Other comparisons were not significant ( $p > 0.05$ ). The data suggest that creatinine levels were markedly affected by

the treatments, with higher doses showing more pronounced changes.

### 3.6 Effect of Extracts on Chloride

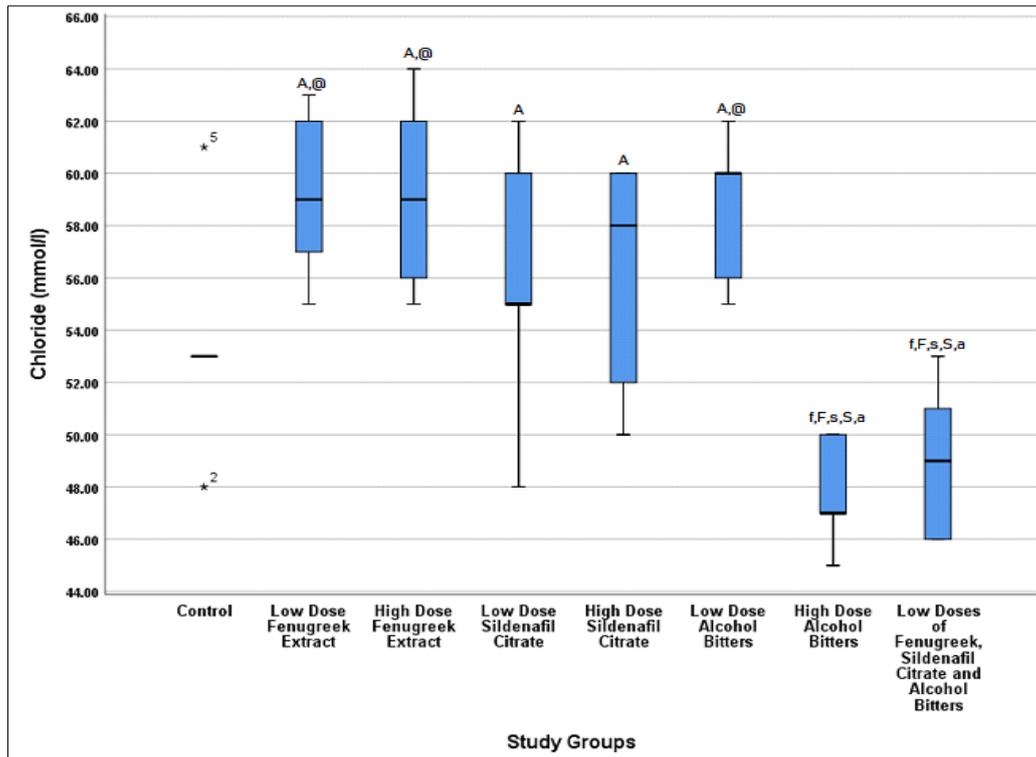


Figure 3.6: A box plot showing levels of chloride across the study groups

Figure 3.6 presents the chloride levels across the study groups. Statistical analysis revealed significant differences between Group 6 and Groups 1, 2, 3, 4, and 5 ( $p = 0.001, 0.001, 0.042, 0.042, 0.003$ , respectively), and between Group 7 and Groups 1, 2, and 5 ( $p = 0.005, 0.005, 0.010$ , respectively). Other pairwise comparisons

were not significant ( $p > 0.05$ ). The results indicate that chloride levels were sensitive to the administered extracts, particularly at higher doses and in combination treatments.

### 3.7 Histology Results

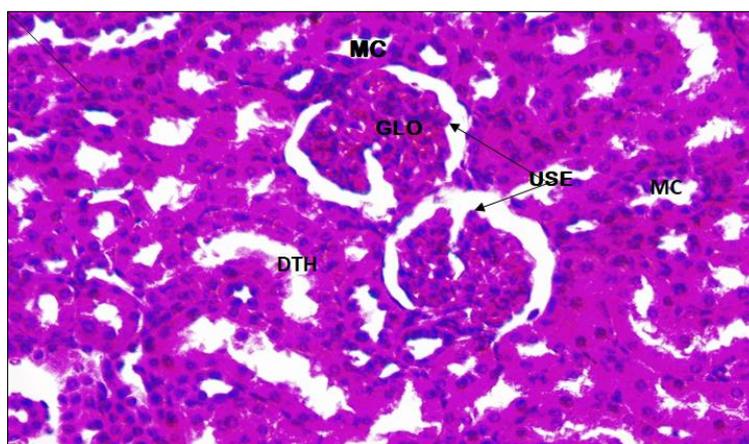
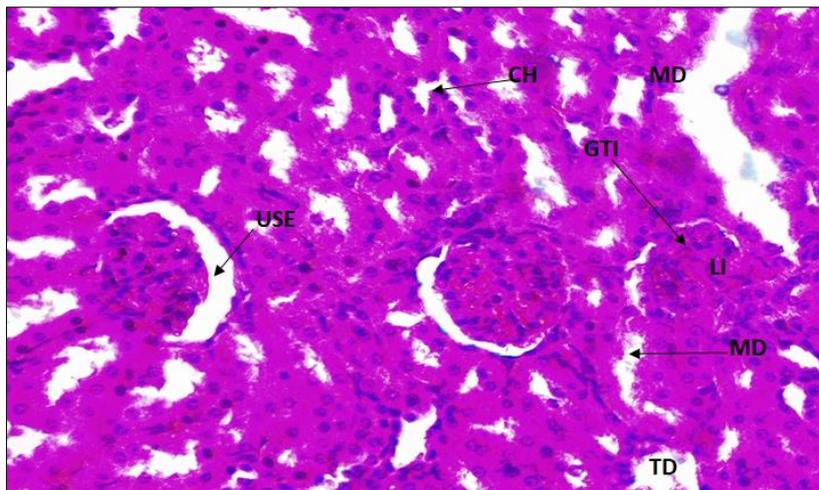
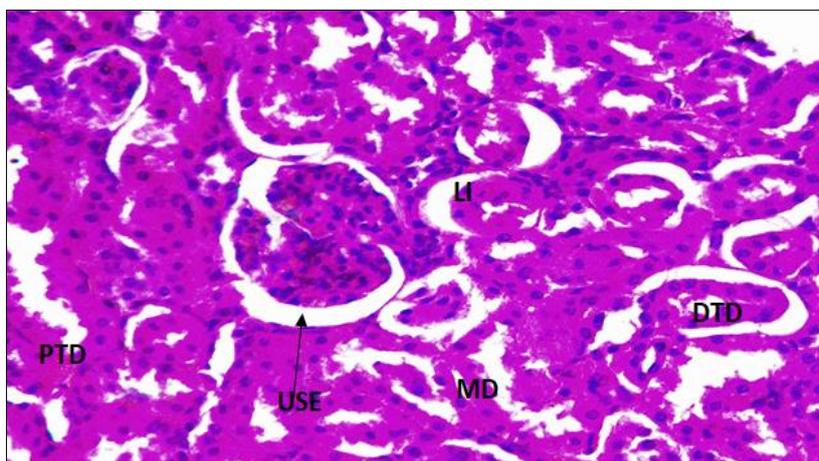


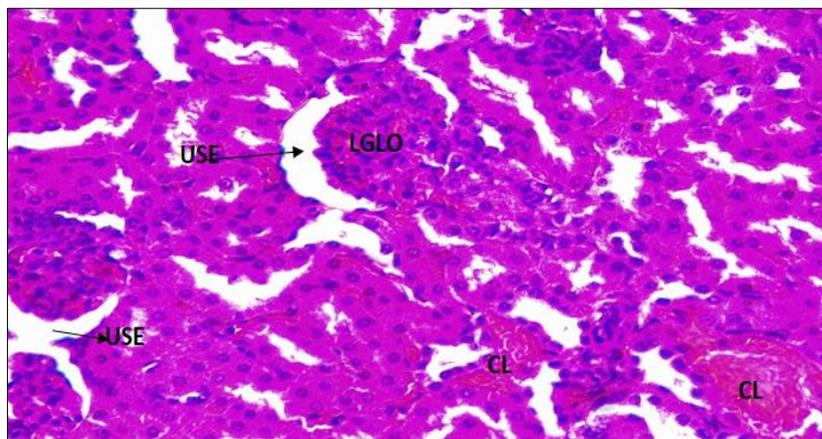
Plate 1: Photomicrograph section of rats' kidney tissue from Fenugreek low dose group. Section showed mild expansion of the urinary space of the glomerulus (USE). The proximal (MCH) and distal (DTH) tubules showed mild cellular hyperplasia. (MCH). H&E X100



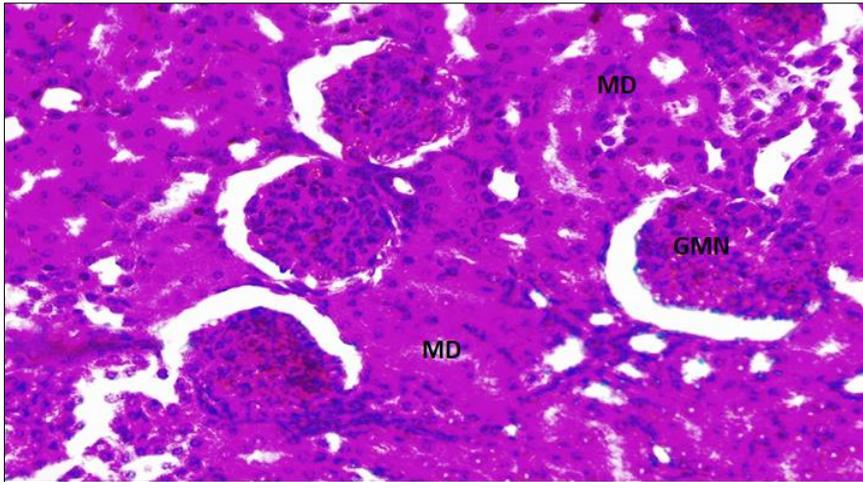
**Plate 2:** Photomicrograph section of kidney tissue from rats treated with high dose of fenugreek extract. Section showed inflammation of glomerulus cells (GTI) with lymphocytes infiltration (LI). The proximal and distal tubules showed no clear cellular differentiation (NCD), myxoid degeneration (MD) and tissues degeneration (TD). H&E X400



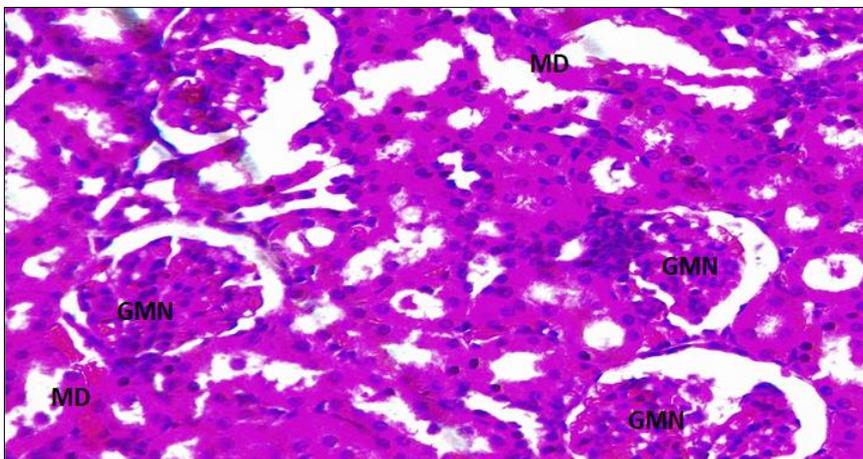
**Plate 3:** Photomicrograph section of kidney tissue from rats treated with high dose of sildenafil citrate. Section showed severe degenerated and distorted kidney tubules DTD and (PTD) and myxoid degeneration ((MD). There is expansion of the urinary space (USE) in the glomerulus (GLO) and lymphocytes infiltration (LI) H&E X100.



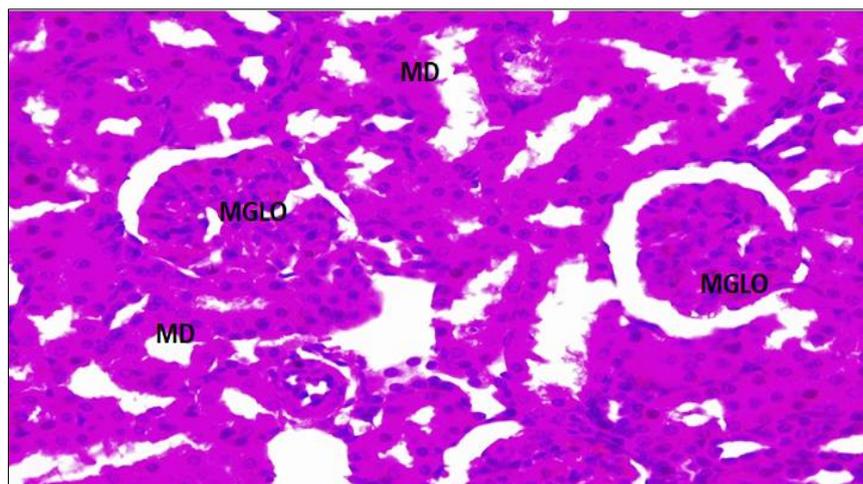
**Plate 4:** Photomicrograph of a section of kidney from rats treated with low dose sildenafil citrate alone showing lesion of glomerulus (LGLO) with expansion of the urinary space. The proximal and distal tubules showed cellular lesions. (CL). H&E X400



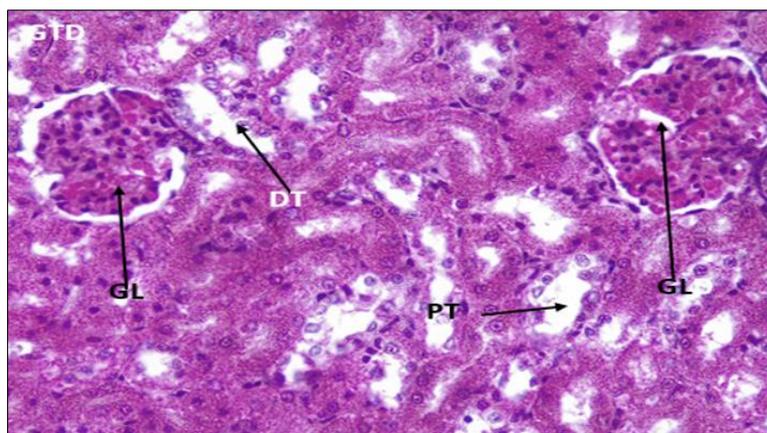
**Plate 5: Photomicrograph of a section of kidney tissues from rats treated with high dose Tombo bitters showed myxoid degeneration in the proximal and distal tubules (MD). There is glomerulonephritis (GMN) at the glomerulus. H&E X100**



**Plate 6: Photomicrograph of a section of kidney tissues from testes of low dose Tombo bitters treatment showing glomerulonephritis (GMN) and myxoid degeneration at the tubules. (MD)**



**Plate 7: Photomicrograph of a section of kidney tissue from rats administered Low dose Fenugreek, low dose Tombo bitters and low dose sildenafil citrate showing mild cellular lesions at the glomerulus (MGLO) and myxoid degeneration of the tubules (MD). H&E X400**



**Plate 8: Photomicrograph section of kidney tissue from rats from normal control Group showing normal kidney glomerulus (GL), with proximal tubules (PT) and distal tubules (DT). H&E X400.**

#### 4. DISCUSSION

The kidney is seen to be the site of accumulation of chemicals; hence urea and creatinine are sensitive and reliable biochemical indices for evaluation of renal function. Based on the results, figure 4.1 showed that there was no statistically difference in the Bicarbonate level of the experimental rats when compared to the control group and each other, the Bicarbonate level was within the normal range value.

In figure 4.2, the Potassium levels of all experimental groups and the control group had no statistically significant difference. In figure 4.3, the Sodium levels were within the normal range value except for high dose alcohol bitters which came up with a statistically significant increase when compared with the control group and other groups. This increase in Sodium could be as a result of kidney dysfunction.

Figure 4.4 and 4.5 showed the urea and creatinine levels of the rats respectively in different groups, there was seen here a statistically significant increase in the high dose of alcoholic bitters group when compared to the control group and other experimental groups, this significant increase could be as a result of kidney dysfunction which could likely depend on the dosage consumption of the bitters.

The histology results when compared showed myxoid degeneration in all experimental groups, which can be due to the administration of these substances for a long term. Plate 5 and 6 which were administered high and low dose of alcohol bitters respectively were seen to have glomerulonephritis, which may likely be due to the alcohol level in the alcoholic bitters.

Based on the results, this study conforms with Nwachuku *et al.*, (2019) which said the bitters toxicity to the kidney may likely depend on the dosage and length

of consumption as seen in the increase in urea and creatinine level of the high dose bitters group and also the glomerular inflammation seen in the kidney tissues of the alcohol groups (both groups). The glomerulonephritis may likely be due to the alcohol level of the alcohol bitters.

Also, in the study carried out by Kemzi *et al.*, (2019), there was an indication that consumption of action bitters altered the renal indices as an increased creatinine value was also observed. This corroborates with this study where we observed an increased creatinine level of the group given high dose bitters hence a misuse or overdose might complicate the renal functions if intake is not properly guided.

#### 5. CONCLUSION AND RECOMMENDATIONS

##### 5.1. Conclusion

This study demonstrated that the chronic use of aphrodisiac substances can adversely affect renal function in male Wistar rats, with the extent of toxicity largely dependent on dosage and duration of exposure. Administration of aqueous *Trigonella foenum-graecum* seed extract, sildenafil citrate, and alcoholic bitters produced varying effects on renal biochemical and histological parameters. Among the tested substances, alcoholic bitters exhibited the most pronounced nephrotoxic effects, particularly at high doses, as evidenced by elevated renal function markers and structural alterations in kidney tissues. The findings further indicate that prolonged and high-dose consumption of alcoholic bitters poses a significant risk factor for nephrotoxicity compared with *Trigonella foenum-graecum* and sildenafil citrate. In contrast, *Trigonella foenum-graecum* and sildenafil citrate demonstrated relatively lower adverse effects on renal function when administered at moderate doses. Overall, this study highlights that aphrodisiac-induced nephrotoxicity is dose- and duration-dependent and

underscores the need for caution in the indiscriminate use of these substances, especially alcoholic bitters, to prevent potential kidney damage.

## 5.2 Recommendations

### For Researchers and Academic Institutions

Researchers should conduct detailed toxicological and mechanistic studies on alcoholic bitters to identify the specific constituents responsible for nephrotoxicity, as the present study demonstrated pronounced renal damage following high-dose exposure. Such studies should incorporate phytochemical profiling, oxidative stress markers, and histopathological evaluation to clarify the pathways of kidney injury. This will yield better outcomes in understanding and mitigating the renal risks associated with these widely consumed products.

Further studies should also evaluate the effects of *Trigonella foenum-graecum* and sildenafil citrate at higher doses and over longer durations, as the current findings suggest relatively lower renal toxicity at moderate doses. Long-term exposure models, larger sample sizes, and molecular analyses should be included to determine whether chronic use could precipitate delayed or cumulative kidney damage. This will yield better outcomes in establishing safe dosage limits and duration of use.

### For Policy Makers and Government Agencies

Government agencies, particularly ministries of health and regulatory authorities, should design and implement public awareness programmes highlighting the potential health risks associated with chronic and high-dose consumption of alcoholic bitters, as the present study identified these products as a significant risk factor for nephrotoxicity. This will yield better outcomes in reducing indiscriminate use and preventing avoidable kidney damage at the population level. Regulatory bodies should also strengthen quality control, labeling, and dosage regulation of herbal bitters and related products to ensure consumer safety. This will yield better outcomes in promoting responsible production, distribution, and use of herbal medicinal products.

### For Community Members and Consumers

Individuals who consume aphrodisiac substances, including herbal bitters, *Trigonella foenum-graecum*, and sildenafil citrate, should adhere strictly to recommended dosages and avoid prolonged or abusive use. As demonstrated in this study, nephrotoxicity is dose- and duration-dependent. This will yield better outcomes in preserving renal health and preventing long-

term complications associated with misuse of these substances.

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