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Toxicity Evaluation of Aqueous Extract of *Vigna subterranea* Seeds in Male Wistar Rats

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Abstract

There has been a surge in the consumption of herbal medicines raising the needs for toxicological studies. The aim of this study is to evaluate the effects of the administration of aqueous extract of Vigna subterranea seeds on wistar rats as it relates to its liver and kidney functioning indices. Male wistar rats of good health condition, weighing 170.43 ± 4.67 g were divided into four groups (A-D) at random, each consisting of seven rats, and were administered distilled water, 100, 250, and 500 mg/kg of the

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extract, orally and respectively. Serum was collected at the conclusion of the experimentation, for use in biochemical tests. When compared to the control group, the concentrations of aspartate aminotransferase, alanine aminotransferase, total protein, albumin, globulin, total, and direct bilirubin were not significantly ($p>0.05$) changed after the extract was administered at all dose. With a dose of 100 mg/kg, there was a significant ($p<0.05$) decrease in serum creatinine concentration but a significant ($p<0.05$) rise in the concentrations of urea and uric acid in kidney function indices. When compared to the control group, the concentration of potassium and sodium ions in the serum did not change significantly ($p<0.05$) at any of the extract's dosages. The study findings revealed that the plant has no discernible harmful effects on the liver, pointing to a typical rate of biotransformation. Furthermore, concentrations of the uric acid and urea from the animals showed that the extract should be used cautiously if taken over an extended length of time, since the study found that the extract may have some adverse effects on the kidney.

Keywords: Kidney function indices, Liver function indices, Safety, Toxicity, *Vigna subterranea*

INTRODUCTION

Nigeria's rich cultural legacy and traditional healing practices have made herbal medicine an essential component of the country's healthcare system for generations. Herbal treatments have provided millions of people in a country where cultural variety is abundant with primary healthcare alternatives. They provide an alternative to conventional medicine, especially in rural and underprivileged regions (Eruaga *et al*., 2024). Users tend to overconsume it because they believe it has therapeutic benefits, which raises the risk to their health. In Nigeria, a number of products from various users enhanced with a variety of herbs and plant components, such *Vigna subterranea*, have become widely accepted despite the lack of knowledge regarding these plants' potential effects on the kidney and liver.

When there are abnormalities in the hepatorenal indices from testing for liver and kidney function, damage to the liver and kidney is often identified. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, creatinine, urea, and bilirubin are among these hepatorenal indicators (Maalman *et al*., 2024). However, there's a rising awareness of the necessity to protect and advance Nigeria's rich history of using herbal medicine while also making sure that its people are safe and well in a more complicated healthcare environment (Nzeh *et al*., 2023). This entails funding research investigations on the pharmacological characteristics, safety, and efficacy of medicinal plants in addition to clinical trials assessing how well they work to treat certain medical diseases (Shaito *et al*., 2020).

The legume known as "bambara groundnut" (*Vigna subterranea*) is a member of the Fabaceae and Faboidea families, respectively (Ibny *et al*., 2019). Regional names for the underutilized African legume *Vigna subterranea* are *Gurjiya* in Hausa, *Okpa* in Igbo, and *Epa Roro* in Yoruba. In English, it's sometimes referred to as Bambara nut (Mbosso *et al*., 2020).

Repackaging herbal bitters—a mixture of herbs, spices, roots, and seeds—as tonic and therapeutic items has resulted from increased awareness and acceptance of the use of herbs (such as *Vigna subterranea*) in the treatment of ailments (Adeyemi *et al*., 2022).

According to reports, *Vigna subterranea*, for example, has been shown to have anti-inflammatory (Alam *et al*., 2020), anti-hypertensive (Kgaogelo *et al*., 2020), antibacterial (Airaodion *et al*., 2021), antioxidant (Adedayo *et al*., 2021), antilipidemic (Megwas *et al*., 2021), and anti-inflammatory properties (Alam *et al*., 2020). The impact of *Vigna subterranea* seed aqueous extract on hepatorenal indicators has not been the subject of any research. Therefore,

in order to determine this medicinal plant's safety, it is essential to look at how it affects the liver and kidney functions in male Wistar rats.

MATERIALS AND METHODS

Materials

Plant Material and Authentication

The seeds of *Vigna subterranea* were bought in Ilorin, Kwara State, Nigeria, at the Mandate Market. A voucher sample (UILH/001/1791/2024) was placed at the University of Ilorin Herbarium, Department of Plant Biology, where the seeds were identified and verified.

Preparation of *Vigna subterranea* Seed Extracts

The process described by Ugwu *et al.* (2018) was used, however slightly modified. The traditional healer's procedures were followed during the extraction. The seeds were rinsed with water and then left to air dry before being ground into a fine powder. 2000 milliliters of distilled water were added to a beaker that had 200 grams of the powdered material. The mixture was properly mixed and let to rest for around 24 hours, shaking periodically, to maximize the extraction capacity. After soaking, the material was filtered using muslin fabric, and the filtrate was concentrated using a rotary evaporator. The extracted material was stored in an airtight sample vial in the refrigerator.
The yield was calculated mathematically to get the percentage (%).

% Yield = (Weight of Crude Extract (g)/Weight of Dried Sample (g)) ×100

Experimental Animals

Twenty eight male rats weighing 170.43 ± 4.67 g, apparently in good health were purchased. Every animal was kept in a standardised animal housing at room temperature, with cages. Guidelines for the care of laboratory animals were adhered to [National Institutes of Health (NIH), 2023].

Experimental Design

In this study, 28 male Wistar rats were randomised into four groups (I, II, III and IV) of 7 animals each and treated as described:
Group I - Distilled water only
Group II- 100 mg/kg body weight of aqueous extract of *Vigna subterrenea*
Group III- 250 mg/kg body weight of aqueous extract of *Vigna subterrenea*
Group IV- 500 mg/kg body weight of aqueous extract of *Vigna subterrenea*

Preparation of Serum

The method outlined by Nurudeen *et al.* (2023) was used to sacrifice the animals. In summary, once the rats lost consciousness when anesthetized with diethyl ether fumes, the jugular veins were cut and 5 milliliters of blood were collected into dry, clean centrifuge vials. Centrifuging the clotted blood samples at 1252 x g for 10 minutes resulted in the collection of clear serum, which was then refrigerated for before being utilized for further analysis.

Toxicity Testing

The safety profile of the aqueous extract on the liver and kidney tissues was evaluated using the methods described by Reitman and Frankel (1957) for the activity of alanine aminotransferase and aspartate aminotransferase. The method described by Jendrassik and Grof (1938) was used to measure Serum direct and total bilirubin concentration while Gornall

et al. (1949) method was used to determine the level of Serum total protein. The method described by Veniamin and Vakirtzi (1970) was used for the determination of Serum urea concentration. Bartels and Bohmer (1972) method was used to determine Serum creatinine concentration. The Procedure described by Tietz (1994) was used to assay for Serum sodium ion, potassium ion and uric acid concentration.

Data Analysis

The data generated from the study was presented as mean ± standard error of the mean of seven replicates it was then subjected to one-way Analysis of Variance (ANOVA). The data was considered statistically different at ($p < 0.05$) using GraphPad Prism version 8.01 (GraphPad Software, Inc., San Diego, California, United States).

RESULTS

The concentrations of AST (Figure 1), ALT (Figure 2), Serum direct bilirubin (DB) and total bilirubin (TB) (Figure 3,4), Serum total protein (Figure 5), albumin (Figure 6) and globulin (Figure 7) were not significantly ($p > 0.05$) altered following the administration of 100, 250 and 500 mg/kg body weight of the extract when compared with the control group.

After 100, 250, and 500 mg/kg body weight of the extract were administered, the concentrations of serum urea (Figure 8) and uric acid (Figure 9) were significantly ($p < 0.05$) higher than in the control group. The concentration of serum creatinine (Figure 10) was ($p < 0.05$) lower at 100 mg/kg body weight of the extract than it was at 250 and 500 mg/kg body weight of the extract as compared to the control group.

When compared to the control group, the concentration of serum sodium (Figure 11) and potassium ion (Figure 12) was not ($p < 0.05$) changed after the extract was administered at doses of 100, 250, and 500 mg/kg body weight.

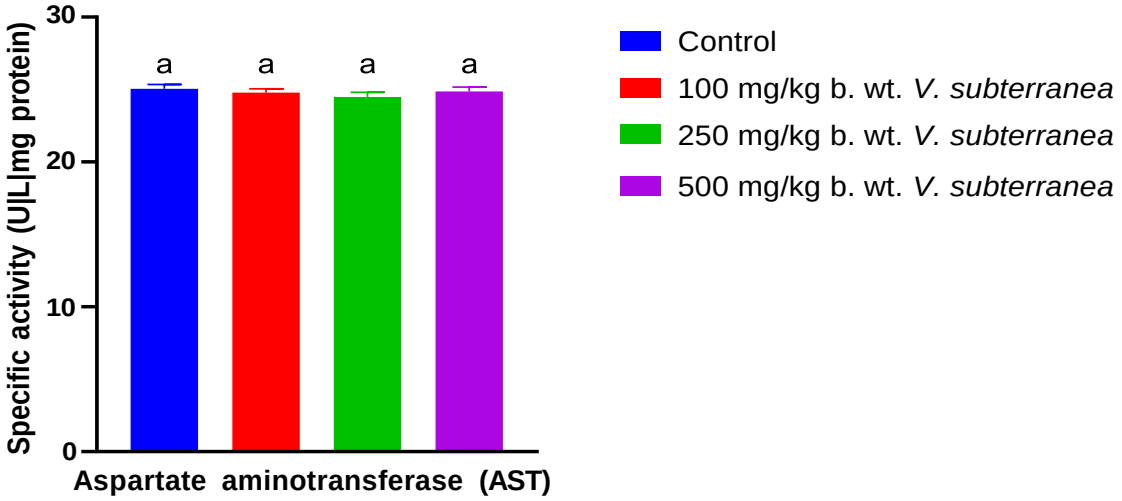

Figure 1: Specific activity of aspartate aminotransferase following administration of aqueous extract of *Vigna subterranea* seeds in male wistar rats

For each parameter, the groups that have superscripts distinct from the control group are statistically different ($p<0.05$). All values are given as the mean ± standard error of seven replicates.

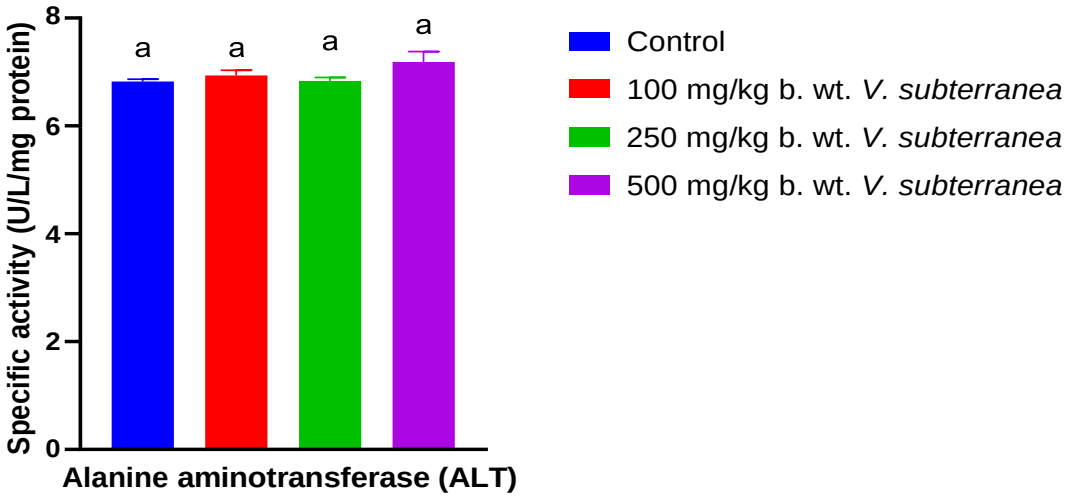

Figure 2: Specific activity of alanine aminotransferase following administration of aqueous extract of *Vigna subterranea* seeds in male wistar rats

For each parameter, the groups that have superscripts distinct from the control group are statistically different (p<0.05). All values are given as the mean ± standard error of seven replicates.

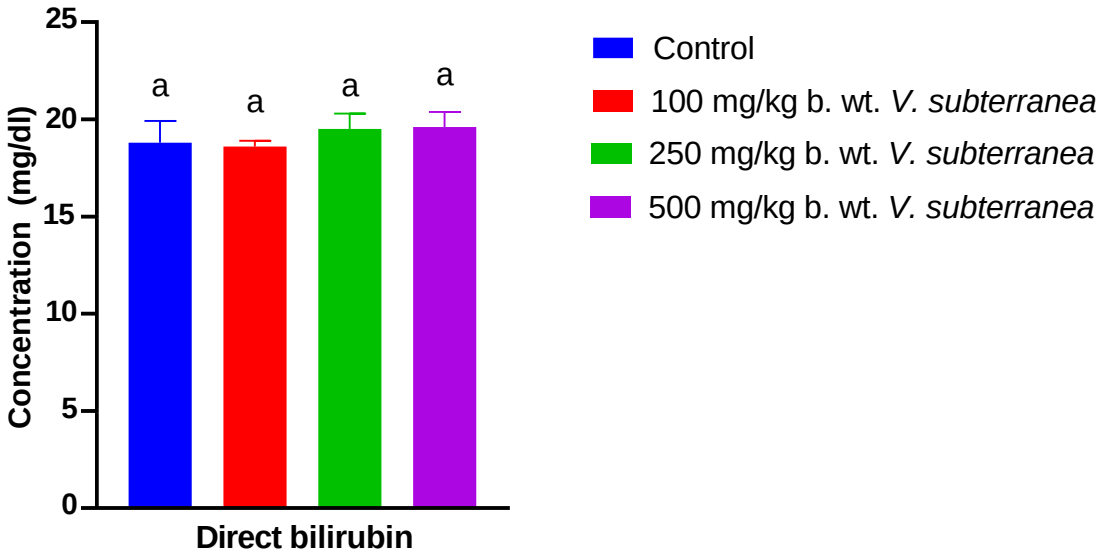

Figure 3: Concentrations of direct bilirubin after administration of aqueous extract of *Vigna subterranea* seeds in male wistar rats

For each parameter, the groups that have superscripts distinct from the control group are statistically different (p<0.05). All values are given as the mean ± standard error of seven replicates.

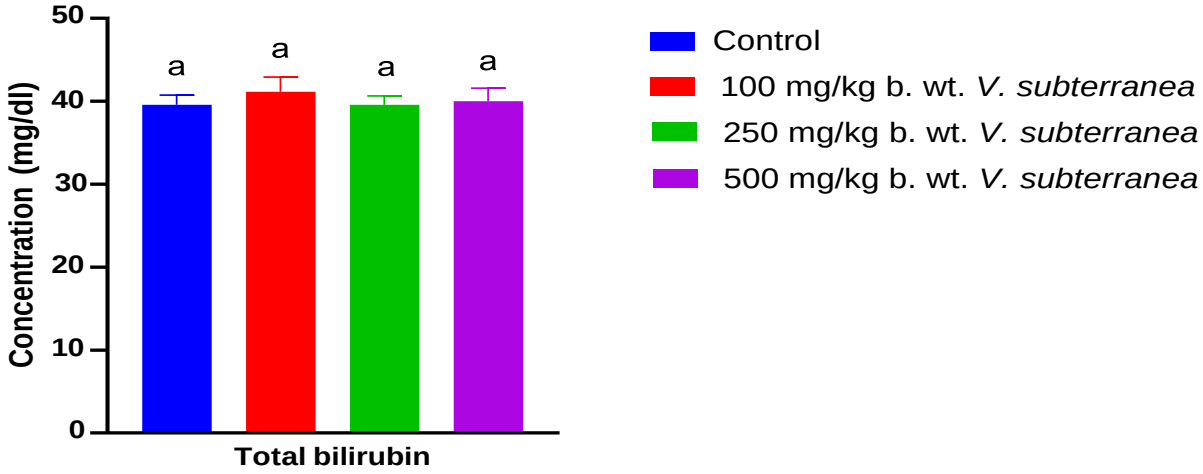

Figure 4: Concentrations of total bilirubin after administration of aqueous extract of *Vigna subterranea* seeds in male wistar rats

For each parameter, the groups that have superscripts distinct from the control group are statistically different (p<0.05). All values are given as the mean ± standard error of seven replicates.

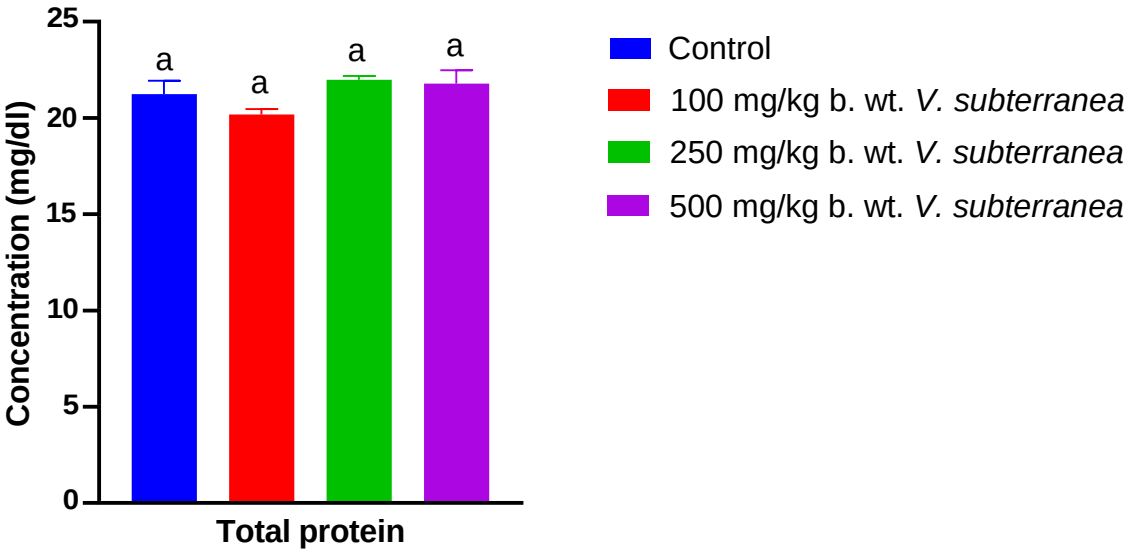

Figure 5: Concentrations of total protein after administration of aqueous extract of *Vigna subterranea* seeds in male wistar rats

For each parameter, the groups that have superscripts distinct from the control group are statistically different (p<0.05). All values are given as the mean ± standard error of seven replicates.

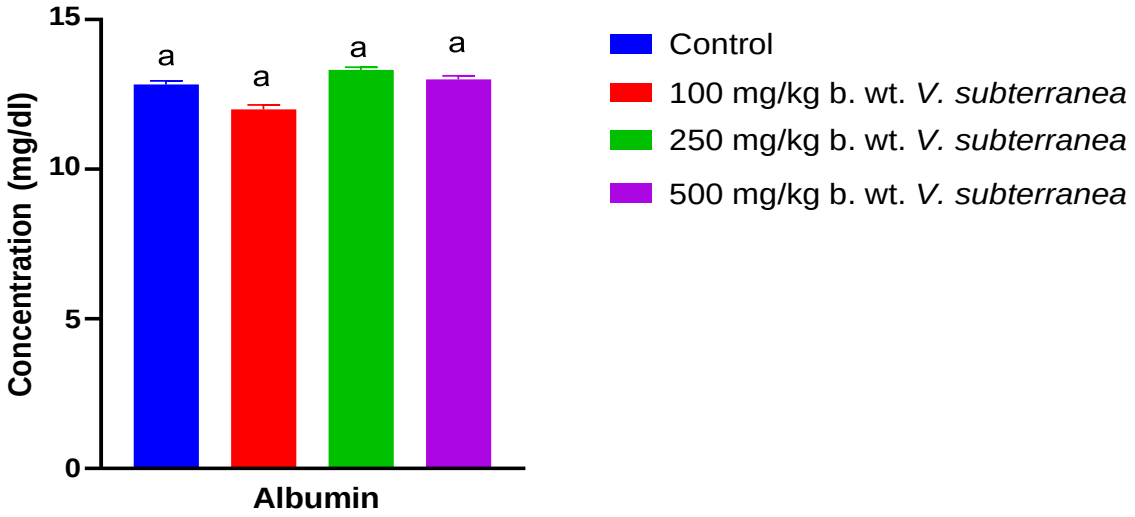

Figure 6: Concentrations of albumin after administration of aqueous extract of *Vigna subterranea* seeds in male wistar rats

For each parameter, the groups that have superscripts distinct from the control group are statistically different (p<0.05). All values are given as the mean ± standard error of seven replicates.

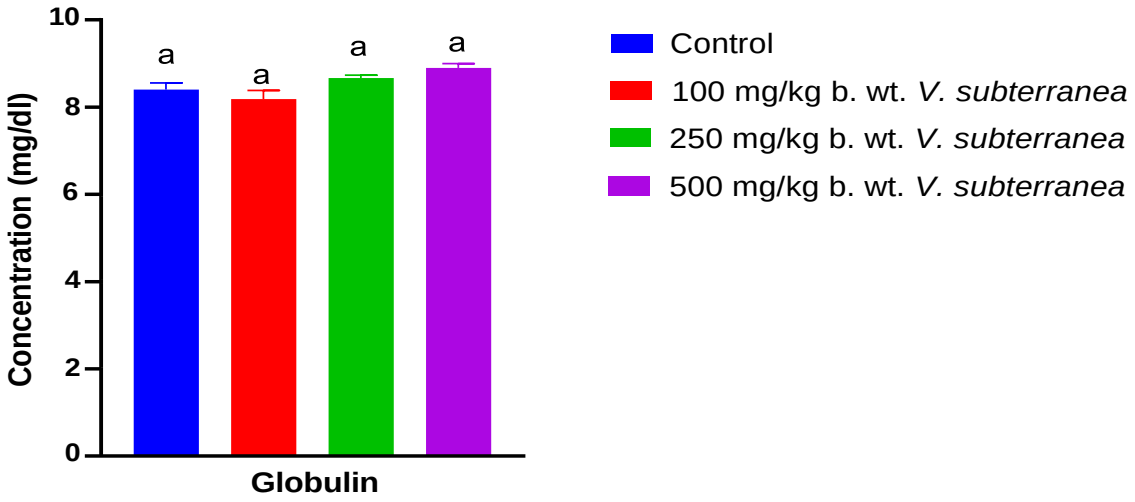

Figure 7: Concentrations of globulin after administration of aqueous extract of *Vigna subterranea* seeds in male wistar rats

For each parameter, the groups that have superscripts distinct from the control group are statistically different (p<0.05). All values are given as the mean ± standard error of seven replicates.

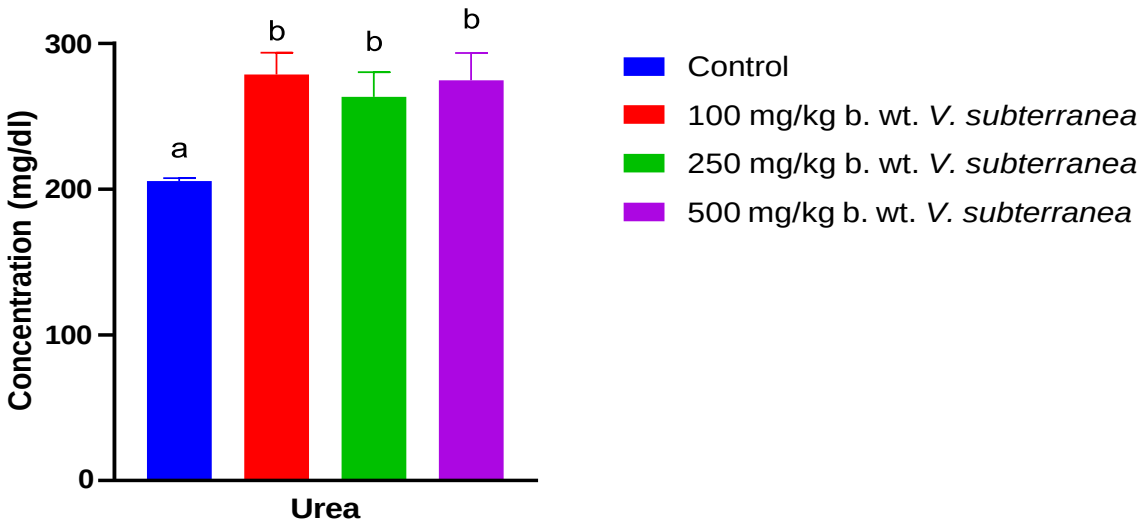

Figure 8: Urea concentrations after administration of aqueous extract of *Vigna subterranea* seeds in male wistar rats

For each parameter, the groups that have superscripts distinct from the control group are statistically different (p<0.05). All values are given as the mean ± standard error of seven replicates.

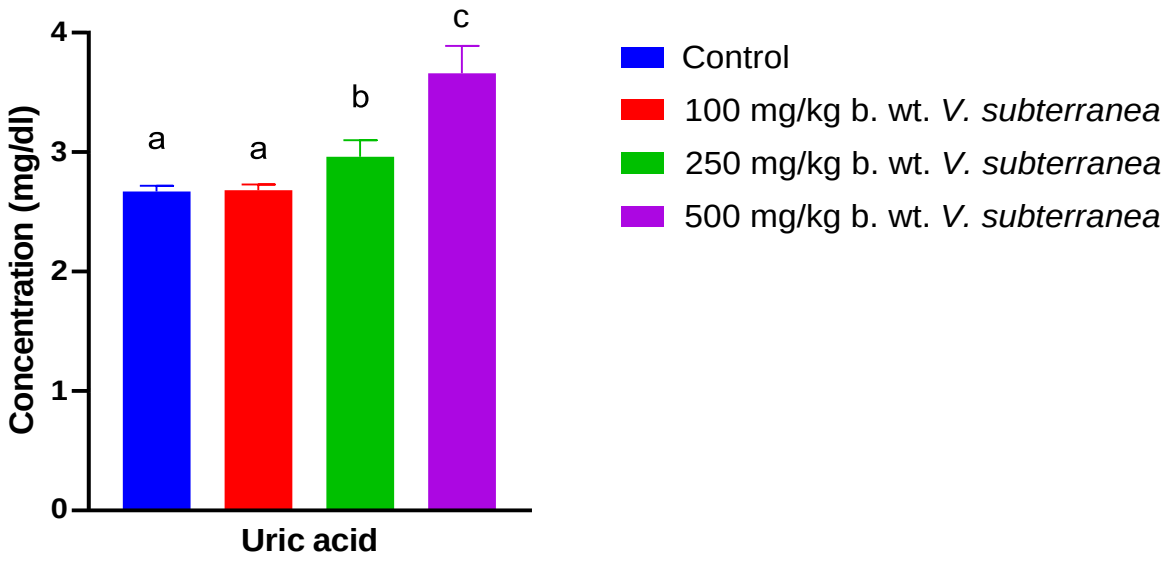

Figure 9: Uric acid concentrations after administration of aqueous extract of *Vigna subterranea* seeds in male wistar rats

For each parameter, the groups that have superscripts distinct from the control group are statistically different (p<0.05). All values are given as the mean ± standard error of seven replicates.

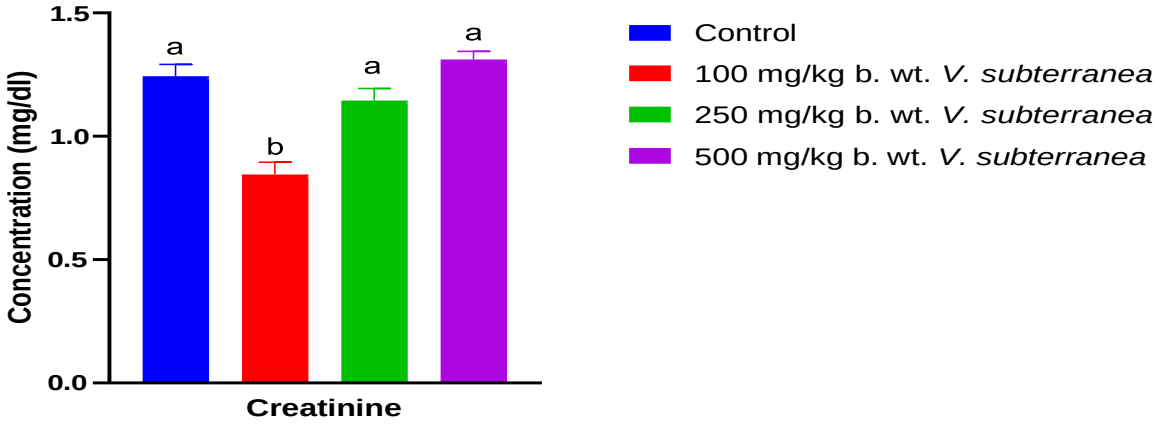

Figure 10: Concentrations of creatinine after administration of aqueous extract of *Vigna subterranea* seeds in male wistar rats

For each parameter, the groups that have superscripts distinct from the control group are statistically different (p<0.05). All values are given as the mean ± standard error of seven replicates.

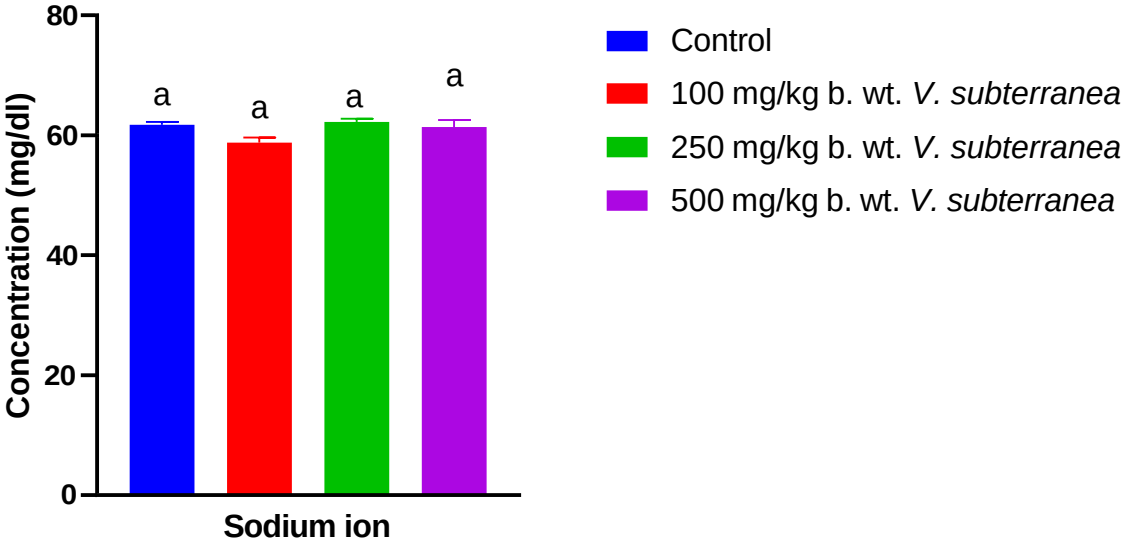

Figure 11: Sodium ion concentrations after administration of aqueous extract of *Vigna subterranea* seeds in male wistar rats

For each parameter, the groups that have superscripts distinct from the control group are statistically different (p<0.05). All values are given as the mean ± standard error of seven replicates.

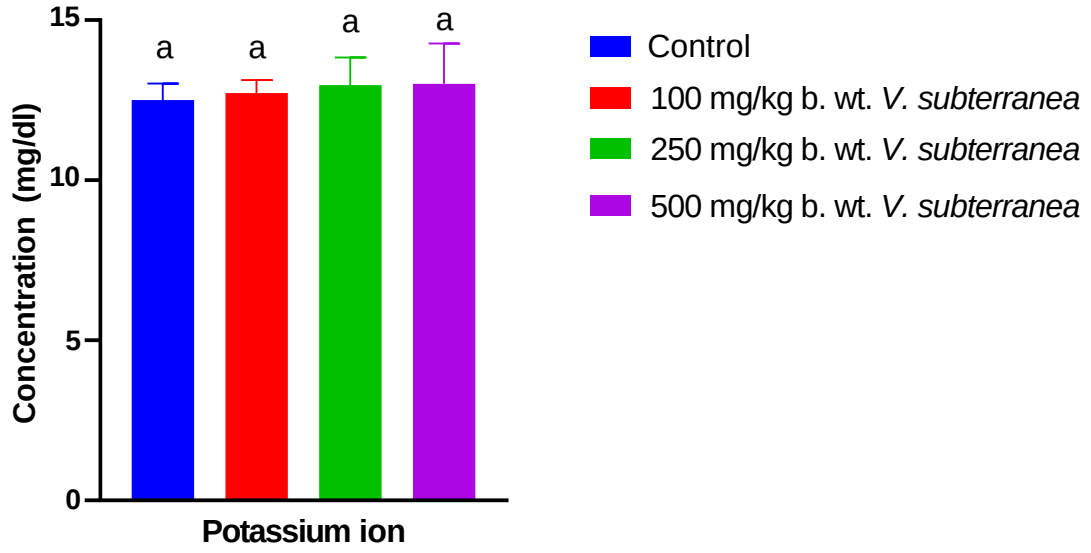

Figure 12: Potassium ion concentrations after administration of aqueous extract of *Vigna subterranea* seeds in male wistar rats

For each parameter, the groups that have superscripts distinct from the control group are statistically different (p<0.05). All values are given as the mean ± standard error of seven replicates.

DISCUSSION

The National Agency for Food and Drug Administration and Control (NAFDAC), the Traditional Medicine Development Division (TMDD) of the Ministry of Health, and state-level agencies are among the regulatory bodies in Nigeria that oversee the regulation of herbal medicine (Ojonugwa *et al*., 2021). The purpose of these regulatory frameworks is to guarantee adherence to quality, safety, and effectiveness requirements. Even while consumer protection has improved significantly (Odubo *et al*., 2023), there are still gaps in the field. For example, there is a dearth of thorough information on the efficacy and safety of herbal treatments, which makes it challenging to fairly weigh the advantages and hazards of using them (Faulkner *et al*., 2020). Therefore, the purpose of this study was to evaluate how aqueous extract of *Vigna subterranea* seeds affected the biochemical parameters of the liver and kidney in male Wistar rats.

Hepatopathy is characterized by the hepatic leakage of ALT, AST, protein (globulin and albumin), and bilirubin (total and direct), which are markers of liver injury (Joseph *et al.,* 2019). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are two examples of aminotransferases that can be used to detect liver damage and are useful indicators of liver cytolysis (Shahjahan *et al*., 2004). ALT and AST, two enzymes that are located in the liver and whose blood levels can rise in reaction to liver injury, are useful metrics for tracking and assessing liver function. When liver cells are injured, both of these enzymes are released into the circulation, and a straightforward blood test can determine each enzyme's quantity. Serum ALT and AST activity levels were not significantly altered following the administration of the *Vigna subterranea* aqueous extract, suggesting that the liver was not harmed.

The quantity of protein in the blood is measured by total protein tests; incorrect results might indicate a variety of diseases, including liver and kidney disease. Albumin is one of the numerous proteins found in the liver and accounts for a large amount of plasma. *Vigna*

subterranea aqueous extract does not seem to have a substantial effect on the liver's synthetic functions at any dosage, confirming the liver's functionality. This proves the extract's safety for liver health and shows that the plant has little to no effect on liver injury, which is why it is consumed. This outcome is consistent with research showing natural herbs have hepatoprotective effects on liver function (Dikwa *et al*., 2024).

Important indicators of the health and potential damage of the liver are the levels of total and direct bilirubin. Red blood cell destruction produces bilirubin, which is known to have some negative effects. The primary mechanism via which bilirubin is generated is the heme—a component found in red blood cells—degrading. It is taken up from the blood by the liver, which breaks it down and secretes it into the bile. In healthy individuals, blood frequently contains a tiny amount of bilirubin. The level of bilirubin in the blood may slightly increase as a result of conditions that promote increased bilirubin generation, such as the loss of red blood cells, or conditions that cause decreased bilirubin removal from the blood stream, such as liver dysfunction (Joseph *et al*., 2022; Simeon *et al.,* 2022). The investigation findings demonstrated that there was little variation in the level of total bilirubin. This further implies that the plant extract has little to no impact on any type of liver damage.

Toxins and waste products must be removed from the blood by the kidneys, and low renal function can cause dangerous compounds to build up in the body. In order to assess the possible toxicity of medications and plant extracts, kidney function indicators are essential. Renal indicators include serum creatinine, urea, uric acid, and electrolytes (potassium, sodium, chloride, and phosphate ions), the concentration of which is related to the health of the kidneys (El-Ishaq *et al*., 2021). The ability of the nephron to function at the glomerular and tubular levels is also disclosed by these markers. Urea and uric acid excretion are controlled by the kidney. Serum urea and uric acid concentrations significantly increased when the *Vigna subterranea* seed aqueous extract was added, whereas creatinine concentrations were decreased. This could be attributed to the extract's detrimental effects on renal function. Acute glomerulonephritis and other impaired renal functions are reflected in the distortion of creatinine, urea, and uric acid levels (Okpogba *et al.,* 2021).

CONCLUSION

Based on all liver functioning indicators, the study's findings indicate that the plant has no toxic or adverse effects on the liver. The concentrations of urea, creatinine, and uric acid, however, suggested that the extract may have some adverse effects on the kidney. For this reason, the plant should be taken with caution.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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