Bambara groundnut ameliorates kidney histology in female mice with protein deficiency

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ABSTRACT

BACKGROUND Protein deficiency (PD) can lead to kidney damage. Consuming plant-based proteins may improve this condition. Bambara groundnut *(Vigna subterranea)* has an essential amino acid score of 80%, which is higher than other legumes; thus, it is potent in overcoming malnutrition. This study aimed to determine the effect of Bambara groundnut supplementation on kidney histology in adult female mice with PD.

METHODS The study was conducted for 2 months in randomly selected female mice. These mice were grouped into the control, PD, and PD supplemented with Bambara groundnuts at 100, 200, and 300 g/kg of feed. 1 day after the last treatment, the kidneys of the mice were collected and processed histologically using the paraffin method (stained with hematoxylin and eosin and Masson's trichrome). Parameters for observation included histopathological scoring (glomerular and interstitial space fibrosis and tubular damage), kidney histomorphometry, and organ index. Semi-quantitative data were analyzed using the Kruskal–Wallis test, while quantitative data were analyzed using one-way ANOVA (followed by Tukey's test) and nested *t*-test. Statistical analysis was performed using SPSS software version 20 (IBM Corp., USA) ($p \leq 0.05$).

RESULTS PD caused cell sloughing (moderate level) and dilatation (severe level) of the kidney tubules. It also reduced glomerular diameter and area by approximately 17.66% and 29%, respectively. PD and Bambara groundnut administration had no significant effects on the glomerular number, cortex and medulla thickness, distal and proximal tubule diameter, and kidney organ index ($p > 0.05$).

CONCLUSIONS Bambara groundnut (*V. subterranea)* administration prevented damage to the kidney's histological structure of protein-deficient mice.

KEYWORDS female, histology, kidney, mice, protein deficiency, *Vigna subterranea*

Protein deficiency (PD) is reportedly experienced by approximately half of the world's population,[1] especially women. Women are vulnerable to malnutrition due to higher nutritional requirements during certain conditions, such as menstruation, pregnancy, and breastfeeding.[2] Inadequate protein intake in women can lead to malnutrition in their offspring.[3]

PD adversely reduces the effectiveness of the immune system and disturbs the endocrine, digestive, and circulatory systems. A lack of protein intake additionally impairs the function of the brain, lungs, stomach, intestines, and kidneys.[4,5] PD may cause structural damage to the glomeruli, tubules, blood vessels, or interstitial spaces. As a result, the kidneys cannot perform optimal blood filtration, as indicated by a decrease in glomerular filtration rate (GFR), renal blood flow, and renal vascular resistance, and inflammation arises.[6–9] Leukocyte infiltration, cast formation, fibrosis in the glomeruli and interstitial space, and tubular damage have been reported in the kidneys of protein-deficient mice.[10–12]

The consumption of legumes with high protein contents can improve malnutrition. Most residents of developing countries consume legumes, such as soybean, peanuts, peas, and chickpeas,[13] as an alternative protein source to replace expensive animal protein and meet their protein intake needs.[14] One legume with a complete nutrient content that remains underutilized for overcoming PD is the Bambara groundnut (*Vigna subterranea*). This groundnut contains 49–63.5% of carbohydrates, 4.5–7.4% of fat, 15–25% of protein, 5.2–6.4% of fiber, and 3.2–4.4% of ash.[13,15] Additionally, the Bambara groundnut has an essential amino acid score of 80%, which is higher than that of soybeans (*Glycine max*; 74%), peanuts (*Arachis hypogaea*; 65%), and cowpea (*Vigna unguiculata*; 64%).[16]

The Bambara groundnut has been primarily consumed as a roasted or boiled snack to ameliorate vision problems and joint pain, reduce nausea, diarrhea, and sexually transmitted infections, treat inflammation, and inhibit the development of cancer cells.[17–19] The Bambara groundnut is abundant and easily accessible in Indonesia, and is, therefore, a potential food supplement to overcome PD.[20] Hence, ascertaining the safety level of Bambara groundnut consumption for overcoming PD is essential. This study aimed to examine the effects of Bambara groundnut supplementation on the histological structure of the kidneys of female mice with PD.

METHODS

This animal experimental study was conducted in the Animal House, Pharmacology Laboratory, Biochemistry Laboratory of the Faculty of Medicine, Public Health, and Nursing, and the Animal Structure and Development Laboratory of the Faculty of Biology, Universitas Gadjah Mada, from December 2021 to June 2023.

Experimental animal preparation

This collaborative research was approved by the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, under the protocol KE/FK/0913/EC/2022 (amendment). Twenty female mice (*Mus musculus* L.) of the Swiss-Webster strain aged 1 month and weighing approximately 20 g were randomly selected. The sample size was determined based on a previous study by Arifin and Zahiruddin[21] and was calculated as 10, divided by the number of treatment groups, and added by one.

Mice acclimatization

All mice were acclimatized for 7 days to standard feed (2 g/20 g body weight [BW] of AIN-93M) prepared by the author (S) and drinking water *ad libitum*. The standard feed was obtained from Department of Biochemistry, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Indonesia. The mice were then randomly divided into five groups, and each group was maintained in a plastic cage (40 × 50 × 50 cm^3) containing husks. Cages were placed in a room with a 20–24°C temperature, 45–65% humidity, and 12-hour day/night periods.

Feed preparation and administration

PD feeds containing Bambara groundnuts were prepared by the author (S). The Bambara groundnuts used in this study were obtained from the Bambara Groundnut Research Center, Gresik, East Java. Five types of feed, differing in protein content, were employed, as follows: control (14% protein), PD (10% protein), PD supplemented with 100 g Bambara groundnuts (PD-100; 11.289% protein), PD supplemented with 200 g Bambara groundnuts (PD-200; 12.578% protein), and PD supplemented with 300 g Bambara groundnuts (PD-300; 13.867% protein). Feeding was performed for 2 months at a rate of 2 g/20 gBW.

Kidney collection

On the day after the last feeding, the 3-month-old female mice were euthanized via intraperitoneal ketamine (100 mg/kgBW) and xylazine injections (10 mg/kgBW), followed by neck dislocation. Twenty right and 20 left kidneys were collected and fixed in 10% neutral-buffered formalin.

Histological preparation

The kidneys were processed using the paraffin method to produce tissue sections with 6 μm thickness in cross-section (right kidney) and longitudinal section (left kidney). Staining of the preparations was performed with hematoxylin and eosin (Ehrlich hematoxylin [Merck, Germany] and eosin Y 1% [Merck]) to observe general tissue damage and Masson's trichrome (Ehrlich hematoxylin [Merck], acid fuchsin [Merck], phosphomolybdic acid [Merck], aniline blue [BDH Chemicals Ltd., United Kingdom], and glacial acetic acid [BDH Chemicals Ltd.]) to assess the presence of fibrosis.

Data analysis

The kidney tissues were observed under a light microscope (Leica ICC50 E [Leica Microsystems, Germany]). Tissue damage was observed in the longitudinal section of the kidney, while the number, diameter, area of the glomeruli, the thickness of the cortex and medulla, and the diameter of the distal convoluted tubule (DCT) and proximal convoluted tubule (PCT) were observed in the cross-section. ImageJ software (National Institutes of Health, USA) was used to measure the thickness, diameter, area, and glomerular number.

Tissue damage was evaluated by the authors (VAF and AN) using a modified ordinal scoring method,[22] as follows: 0 for none; 1 for ≤1% field of view (minimal); 2 for 1–5% field of view (mild); 3 for 6–10% field of view (moderate); and 4 for 11–15% field of view (severe). Semi-quantitative scoring data were analyzed using the Kruskal–Wallis test and Dunn's test of significant differences. Quantitative data were analyzed using one-way analysis of variance and nested *t*-test with Tukey's test for significant differences. Statistical analyses were performed using SPSS software version 20 (IBM Corp., USA) at a significance level of 5%.

RESULTS

The PD feed resulted in moderate tubular cell sloughing and severe tubular lumen dilatation (Figure 1). In contrast, Bambara groundnuts resulted in better kidney tissue by reducing cell sloughing and tubular lumen dilatation. Addition of 200 and 300 g of Bambara groundnuts to the protein-deficient feed prevented damage and ensured renal tissue close to normal conditions. However, the present study found no fibrosis in the glomerulus or interstitial space of the kidney after PD treatment. Similarly, administration of Bambara groundnuts did not result in the presence of collagen fibers in the kidney tissue (Figure 2a–e).

The PD feed resulted in mice renal cortex and medulla with the most negligible thickness, 1,433.91 (113.26) μm and 2,445.04 (591.01) μm, respectively, although there were no differences compared to the control group. However, the thickness of the renal cortex and medulla increased after Bambara groundnut administration. The PD-300 group had the thickest cortex (1,673.33 [122.80] μm), while the PD-200 group had the thickest medulla (2,916.87 [678.66] μm). Although differences in size occurred, the thicknesses of the renal cortex and medulla between the PD diet and Bambara groundnut supplementation groups were not significantly different (Figure 2f).

The PD feed resulted in a smaller proximal tubule diameter than all Bambara groundnut treatments. Meanwhile, the distal tubule diameter in the PD group was greater than that in the PD-200 group. Groups fed with the PD diet and administered Bambara groundnuts had larger distal and proximal tubule diameters than the control group. Nonetheless, the variation in diameter among all groups was not significantly different (Figure 2g).

The glomerular diameter decreased with the PD treatment, which represented a 17.66% reduction compared to the standard diameter in the control group. The PD group also showed the smallest glomerular area among all treatments, with a decrease of approximately 29% from normal conditions. The glomerular diameter and area in the PD group differed significantly from those in the control, PD-200, and -300 groups. The addition of Bambara groundnut to protein-deficient feed progressively increased the glomerular diameter and area. The PD-300 group had the greatest diameter and glomerular area. However, the glomerular diameter closest to normal condition was observed in the PD-200 group (Figure 2h), and the glomerular area closest to normal was found in the PD-100 group (Figure 2i).

The PD group showed an average number of kidney glomeruli within a 50,000 μm^2 field of view lower than that of the control group. The PD-100 and -200 groups had a lower number of glomeruli than the PD group. The PD-300 group had a higher number of glomeruli than that in the PD group, which is closest to that in the control group. However, the mean number of glomeruli in all PD groups was not significantly different (Figure 2j).

The PD group had a lower left kidney index value than the control, PD-300, -200, and -100 groups. Meanwhile, the PD group had a slightly higher right kidney index value than the control and PD-200 groups, with minimal differences of 0.01 and 0.04, respectively. In general, Bambara groundnut administration increased the right and left kidney organ indices

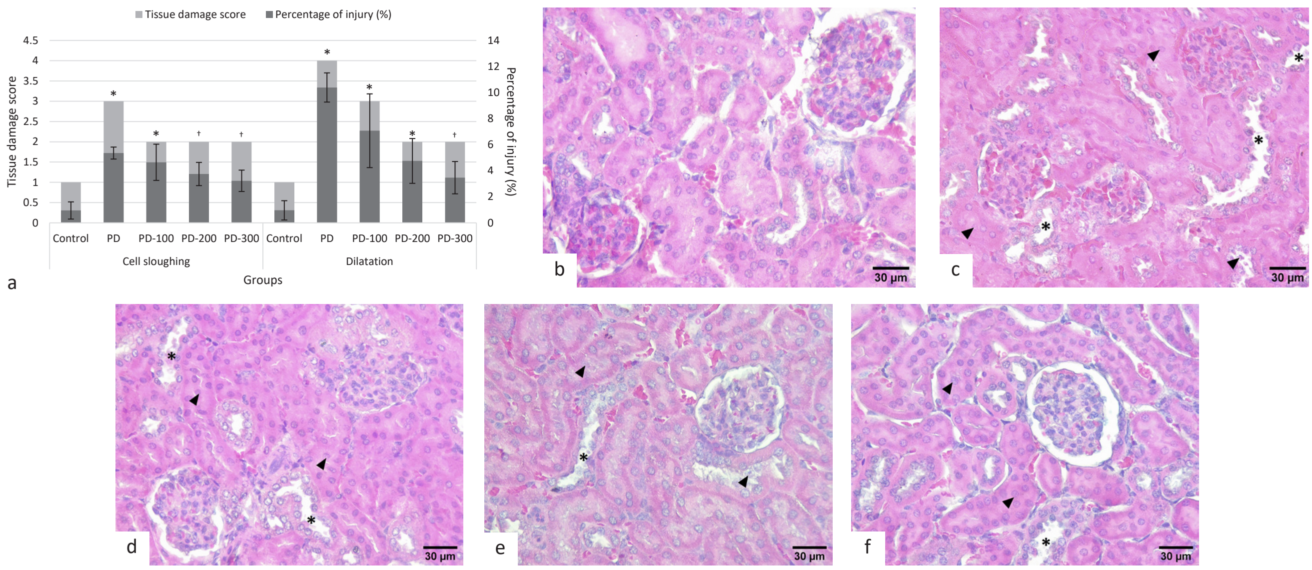

Figure 1. Kidney histological structure damage in female protein-deficient mice after Bambara groundnut supplementation. PD treatment resulted in damage to the kidney's histological structure (a) in the form of cell sloughing (arrowheads) and tubular lumen dilatation (asterisks) in the control (b), PD (c), PD-100 (d), PD-200 (e), and PD-300 groups (f) (H&E stain; scale bar 30 µm). Results are expressed as mean (SD). *$p \le 0.05$, compared to control; †$p \le 0.05$, compared to PD. H&E=hematoxylin and eosin; PD=protein deficiency; PD-100=protein deficiency supplemented with 100 g of Bambara groundnuts; PD-200=protein deficiency supplemented with 200 g of Bambara groundnuts; PD-300=protein deficiency supplemented with 300 g of Bambara groundnuts; SD=standard deviation

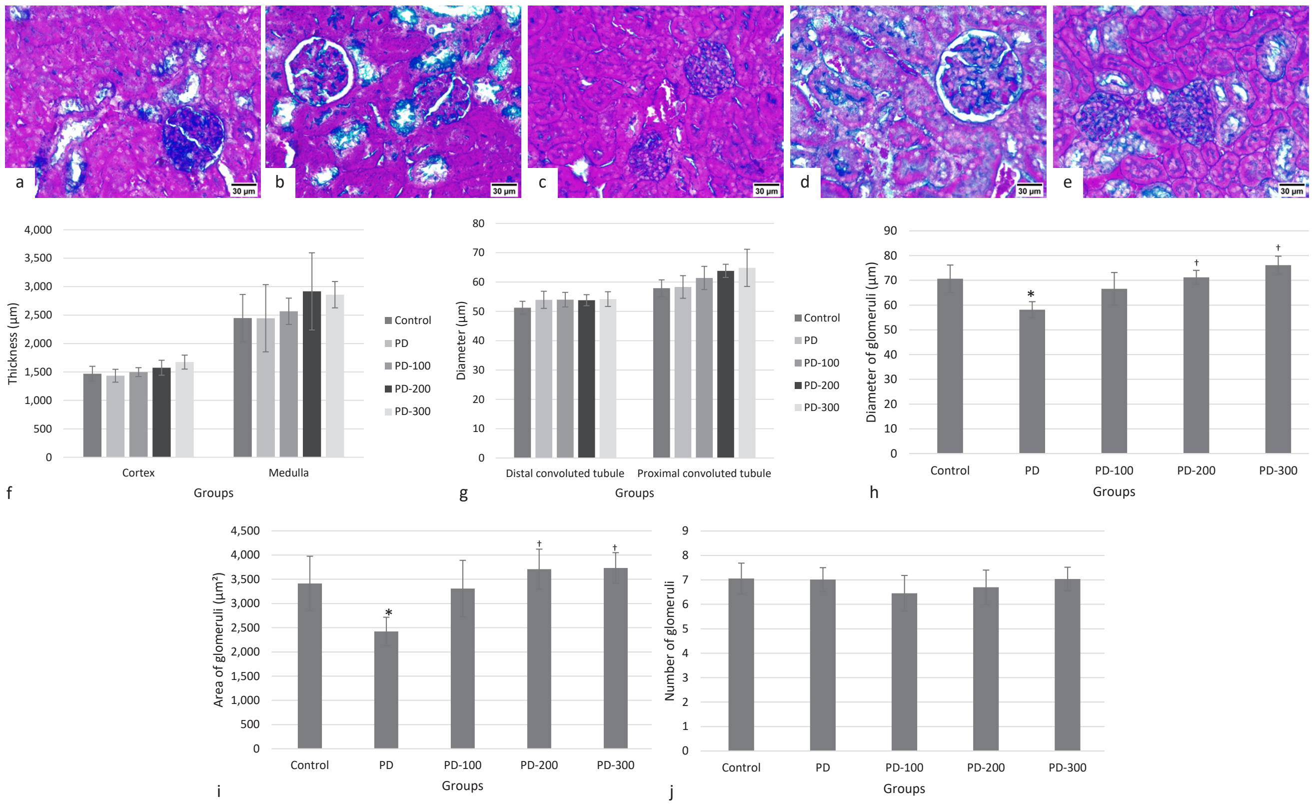

Figure 2. Effect of PD and Bambara groundnut supplementation on kidney histological structure, kidney's cortex and medulla thickness, urinary tubule diameter, and glomerular diameter, area, and number in female mice. Kidney histological structure appearance without glomerular and interstitial fibrosis in the control (a), PD (b), PD-100 (c), PD-200 (d), and PD-300 groups (e) (Masson's trichrome stain; scale bar 30 µm). No effect on thickness of cortex and medulla (f) and diameter of DCT and PCT (g). There were significant differences in glomerular diameter (h) and area (i) without significant difference in glomerular number (j). Results are expressed as mean (SD). *$p \le 0.05$, compared to control; †$p \le 0.05$, compared to PD. DCT=distal convoluted tubule; PCT=proximal convoluted tubule; PD=protein deficiency; PD-100= protein deficiency supplemented with 100 g of Bambara groundnuts; PD-200=protein deficiency supplemented with 200 g of Bambara groundnuts; PD-300=protein deficiency supplemented with 300 g of Bambara groundnuts; SD=standard deviation

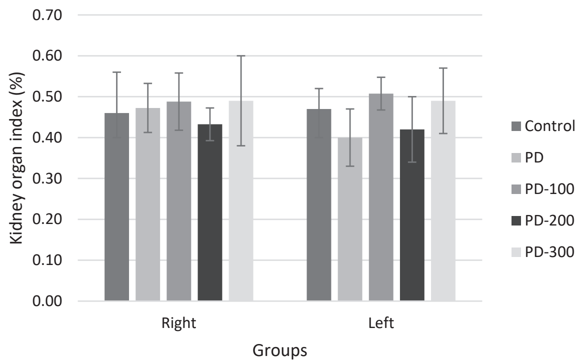

Figure 3. Bambara groundnut supplementation in female mice with PD showing no effect on the organ index of the right and left kidneys ($p>0.05$). Results are expressed as mean (SD). PD=protein deficiency; PD-100=protein deficiency supplemented with 100 g Bambara groundnuts; PD-200=protein deficiency supplemented with 200 g Bambara groundnuts; PD-300=protein deficiency supplemented with 300 g Bambara groundnuts; SD=standard deviation

compared to PD conditions, but not significantly different (Figure 3).

DISCUSSION

Proteins are vital providers of the amino acids needed to execute all biological processes in the body. Adequate amounts of protein are required to support the smooth functioning of organs such as the kidneys,[5] indicating that a diet's protein content should be precisely considered.[23] A lack of protein intake results in the disrupted regulation of physiological renal processes. PD decreases the GFR, which is in line with the decreased concentration of the dissolved Klotho protein. Consequently, reactive oxygen species (ROS) accumulate in the kidney, and the oxidative stress caused by ROS accumulation damages renal tubular cells.[24]

Cell sloughing occurs with the loss of polarity of tubular epithelial cells, causing their detachment from the basement membrane into the lumen.[25] Proximal tubule dilatation, followed by epithelium thinning shows the loss of the brush border, resulting in the tubular epithelium appearing flat with a clear lumen.[26] In the present study, the tissue damage in the PD group was the most severe among all groups. Bambara groundnuts contain phenolic compounds with high antioxidant activities, such as flavonoids, phenolic acids, and anthocyanins. Antioxidant compounds can protect cells from oxidative damage.[15,19] As a result, adding Bambara groundnuts to feed composition improved the tissular conditions. According to previous studies, legume supplementation can repair damage to the histological structure of the kidneys. Soy protein, for instance, can prevent glomerular hypertrophy in obese rats[27] and alleviate basement membrane changes in the renal tissues of diabetic rats.[28]

No fibrotic damage was identified in the glomeruli or interstitial space of the renal tissue of mice in the PD, PD-300, -200, and -100 groups (Figure 2b–e). This occurred because fibrosis appears solely after severe kidney damage.[5] The present study employed a low protein dose (10%), which is still not classified as chronic PD. Chronic PD with 8% protein content does not cause fibrosis yet induces an increased production of inflammatory cytokines.[5] Fibrosis, in turn, can quickly develop in environments with a high concentration of inflammatory cytokines.[29] The administration of legumes in previous studies showed that soy protein has a protective effect on renal tissue by preventing inflammation through suppression of the production of inflammatory cytokines (nuclear factor-κB) and induction of the expression of anti-inflammatory cytokines, such as interleukin-6 and tumor necrosis factor alpha.[28,30]

PD also causes renal angiotensin system disturbances through increased interactions between angiotensin II and angiotensin II receptor type 1 in renal blood vessels. As a result, the functional surface area of the glomeruli for filtration decreases, with a consequent decrease in GFR.[7] Similarly, the present study observed that the decrease in glomerular size due to PD showed improvement with an increased protein intake through Bambara groundnut supplementation. The raw Bambara groundnut used in this study contained the amino acids lysine, leucine, arginine, histidine, threonine, tryptophan, valine, and methionine, which can inhibit the angiotensin-I-converting enzyme. The inhibited enzyme activity reduces the production of angiotensin II and maintains the concentration of the vasodilator bradykinin, preventing the continuous contraction of the renal vessels.[31]

This study showed that the glomeruli number was not affected by the amount of protein consumed. The final glomeruli number in the mouse kidneys depends on the successful nephrogenesis during the gestational phase. Improper nephrogenesis due to inadequate nutrient intake during pregnancy causes a decrease in glomeruli.[32] The thickness of the cortex and medulla is determined by nephrogenesis and is influenced by the amount of protein intake. High levels of protein intake can increase the thickness of the cortex and medulla to compensate for the lower water concentration.[33] Administration of additional protein from Bambara groundnuts in this study led to increased thickness of the cortex and medulla and greater distal and proximal tubules diameters, yet not significant. This may have occurred because of an insufficient protein content in the Bambara groundnut feed, resulting in an absence of significant difference in the outcomes. The feed can be categorized as high in protein if it contains more than 25% protein.[23]

In line with the results from the present study, a previous report showed no significant differences in kidney cortex volumes, as well as glomeruli number, in rats with low protein intake (≤0.88 g/kgBW) compared to rats with regular protein intake (0.89–1.13 g/kgBW).[34] In another study, rats with a low protein intake (6%) showed a significant difference in cortical thickness compared to rats fed a normal diet (20% protein intake). However, the diameters of the glomeruli and proximal renal tubules were not significantly different between protein-deficient and normal mice.[35]

The low-protein diet and Bambara groundnut supplementation did not affect the right and left kidney indices in mice. The kidney index was obtained from the organ and BW ratio. Notably, BW positively correlates with kidney size; therefore, an increased BW impacts kidney weight gain.[36] Mice with PD caused by consuming feed with a 6% protein content had a higher energy intake, thereby increasing the total fat weight and reducing lean body mass compared to normal mice consuming 20% protein.[37,38] A lack of protein intake causes increased hunger, resulting in a tendency to consume large amounts of food. Such occurs because proteins can create a longer-lasting feeling of fullness than carbohydrates and fats.[39]

In this study, the protein content of Bambara groundnuts did not significantly affect the ratio of kidney weight to BW of female mice. This can be explained by the presence of tannins, phytic acid, and antitrypsin compounds in the feed, which act as anti-nutritional factors. Such compounds can inhibit the activity of protein-breaking enzymes, hampering protein digestion. Therefore, the high protein and essential amino acid contents of Bambara groundnuts cannot be optimally utilized.[15]

In the present study, a PD diet caused cell sloughing, tubular dilatation, and decreased glomerular diameter and area in the histological structure of the renal tissue of mice. Nevertheless, PD treatment did not affect the glomerular number or renal morphometry, namely, the thickness of the cortex and medulla, and the diameter of the DCT and PCT. Bambara groundnut supplementation prevented such damage and the decrease in glomerular size.

This study had several limitations. Treatment with a protein-deficient diet was conducted in a subchronic manner to enable the cells to retain their adaptability to stress conditions. Consequently, damage to the histological structure of the kidney remained minimal and likely reversible. At this stage, gross morphometry, including the thicknesses of the renal cortex and medulla, were not yet affected. Additionally, the anti-nutritional factors of Bambara groundnuts may impede its use as a dietary supplement. Therefore, a long-term study of the effect of Bambara groundnut supplementation on the histological structure of the kidney in protein-deficient mice is recommended. Furthermore, determining the levels of anti-nutritional factors in Bambara groundnuts is necessary to guarantee their suitability for consumption.

In conclusion, Bambara groundnut *(Vigna subterranea)* administration prevented damage to the kidney histological structure in protein-deficient mice with respect to cellular injuries (cell sloughing and dilatation) and reduction in the diameter and area of renal glomeruli.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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