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Effects of Cricket-Based Diet on Renal Oxidative Stress, Biochemical Profile, and Histology in Protein-Malnourished Sprague-Dawley Weaned Rats

(Kesan Diet Berasaskan Cengkerik terhadap Tekanan Oksidatif Buah Pinggang, Profil Biokimia dan Histologi pada Tikus Sprague-Dawley yang Kekurangan Protein Selepas Cerai Susu)

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ABSTRACT

Protein malnutrition impairs kidney development during critical growth phases, increasing chronic kidney disease risk. Edible crickets, rich in high-quality protein, offer a sustainable nutritional intervention. This study evaluated a cricket-based diet (CBD) on renal oxidative stress, biochemical profiles, and histology in protein-deficient weaned Sprague-Dawley rats. Three diets were formulated: low protein diet (LPD; 6% protein), normal protein diet (NPD; 18% protein), and CBD (18% protein). Three-week-old rats (n=8/group/sex) were randomly assigned to different groups: male NPD (MN), male LPD (ML), male treatment (MT), male CBD (MC), and their respective female counterparts (FN, FL, FT, FC). MN and FN received NPD, while ML and FL were fed LPD for 10 weeks. MT and FT were given LPD for 3 weeks followed by NPD for 7 weeks, and MC and FC received LPD for 3 weeks before CBD for 7 weeks. No significant difference in body weight was observed between the MN and MC groups, though FC was significantly lighter than FN. Notably, FC exhibited significantly higher relative kidney weights compared to FN. Serum urea levels in MC were elevated compared to ML, while no differences in serum creatinine were found. ML demonstrated higher SOD activity than all other groups. Histological analysis showed that MC and FC had improved glomerular morphometry compared to ML and FL. Fibrosis persisted in both sexes. CBD treatment partially ameliorated renal function and structure following early-life protein malnutrition, with some sex-specific effects. Further investigation is needed to optimise its efficacy and fully elucidate its underlying mechanisms.

Keywords: Cricket-based diet; kidney; oxidative stress; protein malnutrition; sex difference

ABSTRAK

Kekurangan protein menjejaskan perkembangan buah pinggang semasa fasa pertumbuhan kritikal, sekali gus meningkatkan risiko penyakit buah pinggang kronik. Cengkerik yang kaya dengan protein berkualiti tinggi menawarkan intervensi pemakanan yang mampan. Kajian ini menilai kesan diet berasaskan cengkerik (CBD) terhadap tekanan oksidatif buah pinggang, profil biokimia dan histologi dalam tikus Sprague-Dawley yang kekurangan protein selepas cerai susu. Tiga jenis diet telah disediakan: diet rendah protein (LPD; 6% protein), diet protein normal (NPD; 18% protein) dan CBD (18% protein). Tikus berumur tiga minggu (n=8/kumpulan/jantina) diagihkan secara rawak kepada kumpulan berbeza: jantan NPD (MN), jantan LPD (ML), jantan rawatan (MT), jantan CBD (MC) serta pasangan betinanya (FN, FL, FT, FC). MN dan FN menerima NPD, manakala ML dan FL diberi LPD selama 10 minggu. MT dan FT diberi LPD selama 3 minggu diikuti NPD selama 7 minggu, manakala MC dan FC menerima LPD selama 3 minggu diikuti CBD selama 7 minggu. Tiada perbezaan signifikan dalam berat badan diperhatikan antara kumpulan MN dan MC, namun FC didapati lebih ringan secara signifikan berbanding FN. Menariknya, FC menunjukkan berat relatif buah pinggang yang lebih tinggi berbanding FN. Paras urea serum dalam MC meningkat berbanding ML, manakala tiada perbezaan pada kreatinin serum diperhatikan. Aktiviti SOD lebih tinggi dalam ML berbanding semua kumpulan lain. Analisis histologi menunjukkan MC dan FC mempunyai morfometri glomerulus yang lebih baik berbanding ML dan FL. Fibrosis masih kekal dalam kedua-dua jantina. Rawatan CBD sebahagiannya memperbaiki fungsi dan struktur buah pinggang selepas kekurangan protein awal hayat dengan beberapa kesan khusus jantina. Kajian lanjut diperlukan untuk mengoptimumkan keberkesanannya dan menjelaskan sepenuhnya mekanisme yang terlibat.

Kata kunci: Buah pinggang; diet berasaskan cengkerik; kekurangan protein; perbezaan jantina; tekanan oksidatif

INTRODUCTION

Protein-energy malnutrition (PEM) is a severe form of undernutrition commonly observed in children facing food shortages due to social, economic, and environmental factors (Dipasquale, Cucinotta & Romano 2020). Insufficient protein intake leads to systemic impairments, including muscle wasting, immunodeficiency, reduced bone density, and chronic inflammation (Yue et al. 2020). When occurring during critical growth phases, PEM can lead to stunted growth, neurological deficits, compromised organ development, and an increased risk of chronic diseases in adulthood (Bhutta et al. 2017; Grey et al. 2021).

Protein is essential for kidney development and maturation (Tain & Hsu 2017). In humans, renal maturation largely completes within the first 1000 days of life, with glomerular filtration rate (GFR) doubling in the first two weeks postnatally and reaching adult levels by age two (Hsu & Tain 2021). Inadequate protein intake reduces amino acid availability, impairing albumin synthesis and leading to hypoalbuminemia, decreased plasma oncotic pressure, and altered glomerular permeability, which compromises renal perfusion and GFR (Shah & Mandiga 2024). Additionally, protein deficits trigger muscle catabolism, generating reactive oxygen species (ROS) while diminishing antioxidant capacity, resulting in oxidative damage to renal tissues and sustained inflammation (Michael et al. 2022; Turkmen 2017).

Early protein repletion is more effective for reversing these adaptations due to greater physiological plasticity in childhood (Bhutta et al. 2017). However, global food insecurity, driven by a population projected to reach 9.7 billion by 2050 (United Nations 2024), limits access to traditional proteins like poultry and fish. Conventional farming faces sustainability challenges from climate change, pollution, urbanisation, and resource depletion (Kousar et al. 2021). Edible crickets (*Acheta domesticus*) emerge as a sustainable alternative, providing high-quality protein with essential amino acids (Murugu et al. 2021). Crickets also offer antioxidant, anti-inflammatory, and anti-obesity benefits (Kemsawasd et al. 2022), with environmental advantages including rapid production cycles, efficient feed conversion, lower emissions, and minimal waste (Morales-Ramos et al. 2020).

Prior research suggests cricket-based diets (CBD) improve health outcomes in malnourished models (Agbemafle et al. 2019; Bergmans et al. 2020; Payne et al. 2016). However, the impact of CBD on renal health in contexts of protein malnutrition, including risks for chronic kidney disease, remains underexplored. This study investigated the effects of CBD on renal oxidative stress, biochemical profiles, and histological changes in weanling protein-deficient rats over a seven-week intervention, aiming to elucidate its potential in mitigating protein deficiency-induced renal damage.

MATERIALS AND METHODS

EXPERIMENTAL DIET PREPARATION

Three diets were formulated: low-protein diet (LPD; 6% protein), normal-protein diet (NPD; 18% protein), and CBD (18% protein) (Table 1). LPD and NPD were based on Teklad rodent diets TD.90016 and TD.96180 (Envigo, Madison, WI, USA). CBD incorporated 100% whole cricket powder (Ento, Kuala Lumpur, Malaysia). Ingredients were weighed, homogenised in a stainless-steel blender with distilled water to form dough, pelleted, oven-dried at 37 °C for 24 h, sealed, and stored at -80 °C.

ANIMALS AND EXPERIMENTAL DESIGN

The protocol was approved by the Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC;

TABLE 1: Composition of the experimental diets

Ingredients (g)	Low protein diet	Normal protein diet	Cricket-based diet
Whole cricket powder	-	-	350.00
Casein	207.00	69.00	-
DL-methionine	2.70	0.90	-
Sucrose	451.30	571.80	391.60
Corn starch	200.00	200.00	200.00
Corn oil	52.60	53.90	-
Cellulose	41.06	57.82	13.06
Vitamin mix, Teklad (40060)	10.00	10.00	10.00
Butylhydroquinone, antioxidant	0.01	0.01	0.01
Mineral mix, Ca-P deficient (79055)	13.37	13.37	13.37
Calcium phosphate, dibasic	17.36	21.60	17.36
Calcium carbonate	4.60	1.60	4.60

Reference No.: FSK/2022/SEE MENG/23-NOV./1288-NOV.-2022-AUG.-2025). Sixty-four three-week-old Sprague-Dawley rats (32 males, 32 females) were sourced from the Laboratory Animal Resource Unit, Universiti Kebangsaan Malaysia (Kuala Lumpur, Malaysia). Rats were housed under standard conditions (12-h light/dark cycle, 25 ± 2 °C) with *ad libitum* food and water. After a one-week acclimatisation period, rats were randomly assigned to eight groups (n = 8/sex/group): male NPD (MN), male LPD (ML), male treatment (MT), male CBD (MC), and their respective female counterparts (FN, FL, FT, FC). MN and FN groups received NPD for 10 weeks, whereas ML and FL groups were fed LPD for the same duration. MT and FT groups received LPD for the initial 3 weeks, followed by NPD for the remaining 7 weeks. MC and FC groups were similarly fed an LPD for 3 weeks before switching to CBD for 7 weeks. Body weights were recorded weekly.

BLOOD AND KIDNEY TISSUE COLLECTION

After 10 weeks, rats were anaesthetised with an overdose of ketamine/xylazine/Zoletil (0.1 mL/100 g body weight intraperitoneally). Euthanasia and blood collection were performed between 08:00 and 10:00 to mitigate the effects of circadian fluctuations. Animals were not fasted prior to sampling to avoid inducing additional metabolic stress in the rats. Blood was collected via cardiac puncture, allowed to clot, and then centrifuged (3000 rpm, 10 min, 4 °C) to obtain the serum. Serum was then stored at -80 °C prior to testing. To ensure analyte stability, all biochemical measurements were performed in a single batch, and samples were subjected to a single-thaw protocol to avoid freeze-thaw cycles. Kidneys were excised, rinsed in ice-cold phosphate-buffered saline (pH 7.4), and weighed. Right kidney homogenised for oxidative stress assays and stored at -80 °C while left kidney fixed in 10% neutral-buffered formalin for histology evaluation.

RENAL FUNCTION TEST

Serum urea and creatinine were measured using a Biolis 24i Premium automated chemistry analyser (Tokyo Boeki, Japan). Creatinine concentrations were determined using the kinetic Jaffé method without deproteinisation, based on the reaction of creatinine with alkaline picrate to form an orange-red complex. Serum urea was quantified using an enzymatic UV assay (urease–glutamate dehydrogenase [GLDH] method). All measurements were performed using commercially available reagents from Biorex Mannheim (Mannheim, Germany). Assays were conducted at 37 °C with bichromatic readings at 340/700 nm for urea and 505/570 nm for creatinine. The analyser was calibrated daily using a linear calibration model. Quality control was ensured by analysing standard control sera prior to each batch, with intra- and inter-assay precision maintained within the manufacturer's specified coefficients of variation (CV < 3.0% for urea and CV < 4.0% for creatinine).

OXIDATIVE STRESS AND ANTIOXIDANT STATUS

The right kidney was homogenised in ice-cold phosphate-buffered saline (PBS, pH 7.4) to prepare a 10% (w/v) homogenate using a tissue homogeniser. The homogenate was centrifuged at 4000 rpm for 15 min at 4 °C, and the resulting supernatant was used for all assays. To ensure standardisation, all markers were normalised to total protein content determined via the Bradford assay. All measurements were performed in triplicate to ensure reproducibility, with intra-assay CV maintained below 5%. Superoxide dismutase (SOD) activity was assayed by nitroblue tetrazolium reduction inhibition (Beyer & Fridovich 1987). Reduced glutathione (GSH) was quantified via Ellman's method, measuring chromophore formation at 412 nm (Ellman 1959). Malondialdehyde (MDA), used as an indicator of lipid peroxidation, was assessed colourimetrically via the Thiobarbituric Acid Reactive Substances (TBARS) assay (Ledwożyw et al. 1986). Advanced oxidation protein products (AOPP) were measured spectrophotometrically under acidic conditions (Witko-Sarsat et al. 1996).

HISTOMORPHOMETRY MEASUREMENT

Formalin-fixed kidneys were processed in an automated tissue processor (Leica TP1020), embedded in paraffin, sectioned (3-5 µm; Leica RM2135), and stained with hematoxylin and eosin (H&E) for morphology and Masson's trichrome for fibrosis. Slides were examined at 100× and 400×. Ten non-overlapping fields (400×) per section were imaged (X-PAD 97 Rax Vision camera) and analysed with ImageJ (NIH, Bethesda, MD, USA). Glomerular tuft area (GA) was measured for 20-30 glomeruli/animal. Glomerular tuft volume (GV) was calculated as GV = (β/k) × GA^{1.5}, with β=1.38 (sphere shape coefficient) and k=1.1 (size distribution coefficient) (Weibel & Gomez 1962). Fibrosis was qualitatively assessed.

STATISTICAL ANALYSIS

Data were analysed using GraphPad Prism v9.0 (GraphPad Software, San Diego, CA, USA) and presented as mean ± standard error of the mean (SEM). Normality was assessed via Shapiro-Wilk test. A one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons was used. This approach was selected to facilitate clear, direct comparisons between dietary treatments and their respective controls within each sex, ensuring that the results for each specific group were accurately represented in the graphical data. Statistical significance was set at $p < 0.05$.

RESULTS

BODY WEIGHTS

Figure 1 presents the final body weights of male and female rats across all experimental groups. ML and FL had

significantly lower weights than MN and FN ($p < 0.01$). MC did not differ from the MN, but FC remained lower than FN ($p < 0.01$).

RELATIVE KIDNEY WEIGHTS

No differences in relative kidney weights occurred among males (Figure 2). In females, FN had significantly lower relative kidney weights than the other 3 groups ($p < 0.05$).

RENAL FUNCTION ANALYSIS

ML had lower serum urea than MN ($p < 0.0001$) but higher creatinine than MN ($p < 0.05$) (Figure 3). MT and MC urea levels were higher than ML ($p < 0.0001$). MT creatinine decreased compared to ML ($p < 0.05$). In females, urea showed no differences; FL creatinine was higher than FN ($p < 0.01$).

OXIDATIVE STRESS AND ANTIOXIDANT STATUS

No GSH differences occurred in males (Figure 4(A)). ML SOD activity was elevated compared to MN ($p < 0.0001$) (Figure 4(B)). MT and MC reduced SOD compared to ML($p < 0.001$). MDA was higher in ML ($p < 0.01$) and MT ($p < 0.001$) than MN (Figure 4(C)). In females, FT GSH was lower than FL ($p < 0.01$); FC matched FN. No female differences in SOD or MDA. AOPP showed no changes across groups (Figure 4(D)).

RENAL HISTOMORPHOLOGY

H&E staining (Figure 5) showed normal morphology of renal in MN and FN, with minor interstitial fibrosis on Masson's trichrome (Figure 6). ML maintained glomerular morphology but showed tubular irregularities, while FL exhibited widening of capsule Bowman and tubular irregularities. MT and MC improved glomeruli but showed tubular hypertrophy. Mesangial fibrosis was more severe in ML, MT, and MC than MN (Figure 6). FT improved glomeruli but retained tubular abnormalities; FC showed glomerular recovery but tubular dilation, widened lumina, and vacuolisation. Interstitial fibrosis was pronounced in FL, FT, and FC compared to FN, and glomerulus atrophy was also observed in these groups.

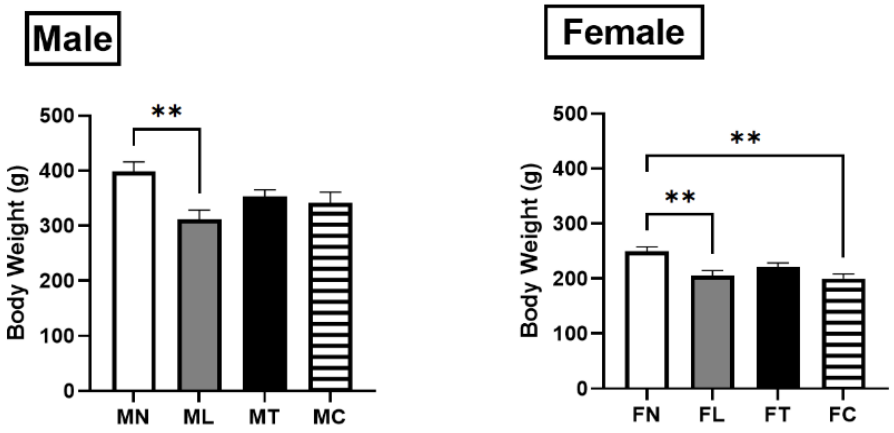
RENAL HISTOMORPHOMETRY

In males, MT GA and GV were larger than ML ($p < 0.05$) (Figure 7). In females, FL GA was smaller than FN ($p < 0.05$). FT GA and GV were larger than FL ($p < 0.05$).

DISCUSSION

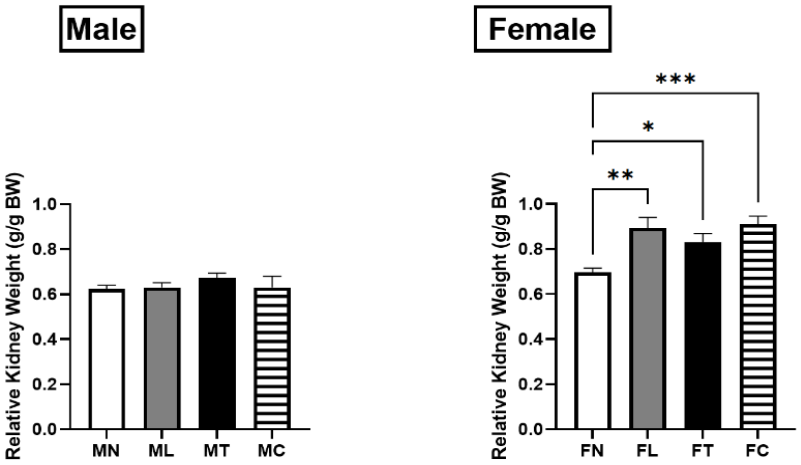
This study evaluated the therapeutic potential of CBD in ameliorating renal alterations induced by early-life protein malnutrition in weanling Sprague-Dawley rats, with a focus on renal function, oxidative stress markers, and histological outcomes. The findings show partial efficacy of CBD in promoting recovery, characterised by normalisation of select renal function and oxidative parameters, as well as glomerular morphometry, albeit with persistent sex-specific disparities and incomplete resolution of certain pathologies. These results underscore the potential of CBD as a sustainable nutritional intervention while highlighting the need for tailored approaches considering sexual dimorphism.

The observed reductions in body weight among LPD groups are consistent with established protein malnutrition models, where inadequate protein intake impairs growth through mechanisms such as reduced insulin-like growth factor-1 signalling and altered energy metabolism (Cupisti et al. 2020). In male rats, rehabilitation with either NPD



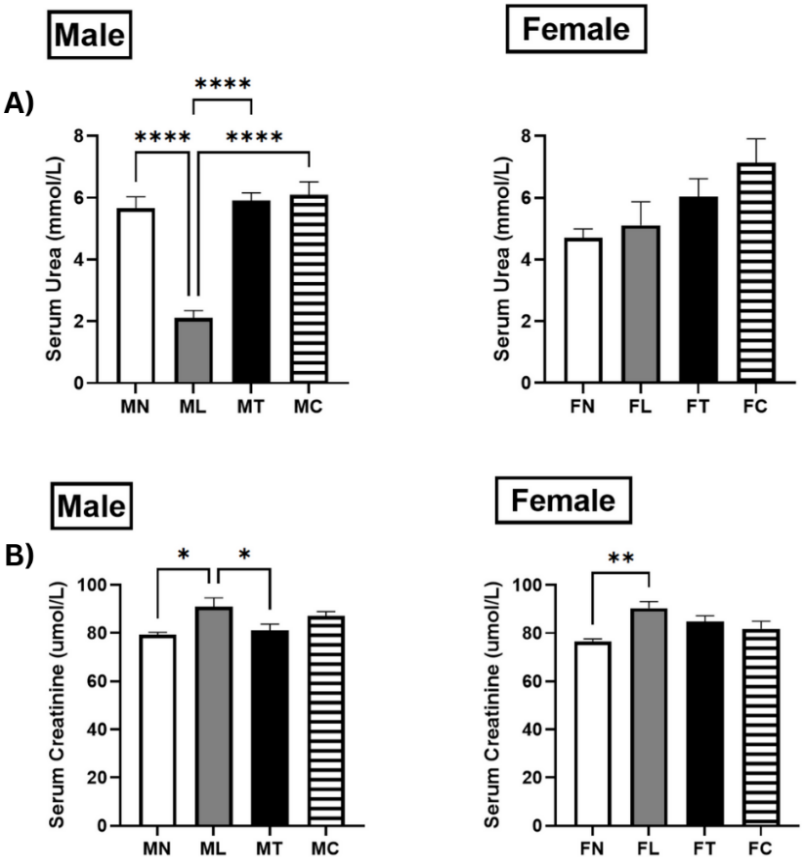
Data are presented as mean ± SEM (n = 8/sex/group). **p < 0.01. MN, male normal-protein diet; ML, male low-protein diet; MT, male treated with normal-protein diet; MC, male cricket-based diet; FN, female normal-protein diet; FL, female low-protein diet; FT, female treated with normal-protein diet; FC, female cricket-based diet

FIGURE 1. Final body weight of rats after 10 weeks of dietary intervention



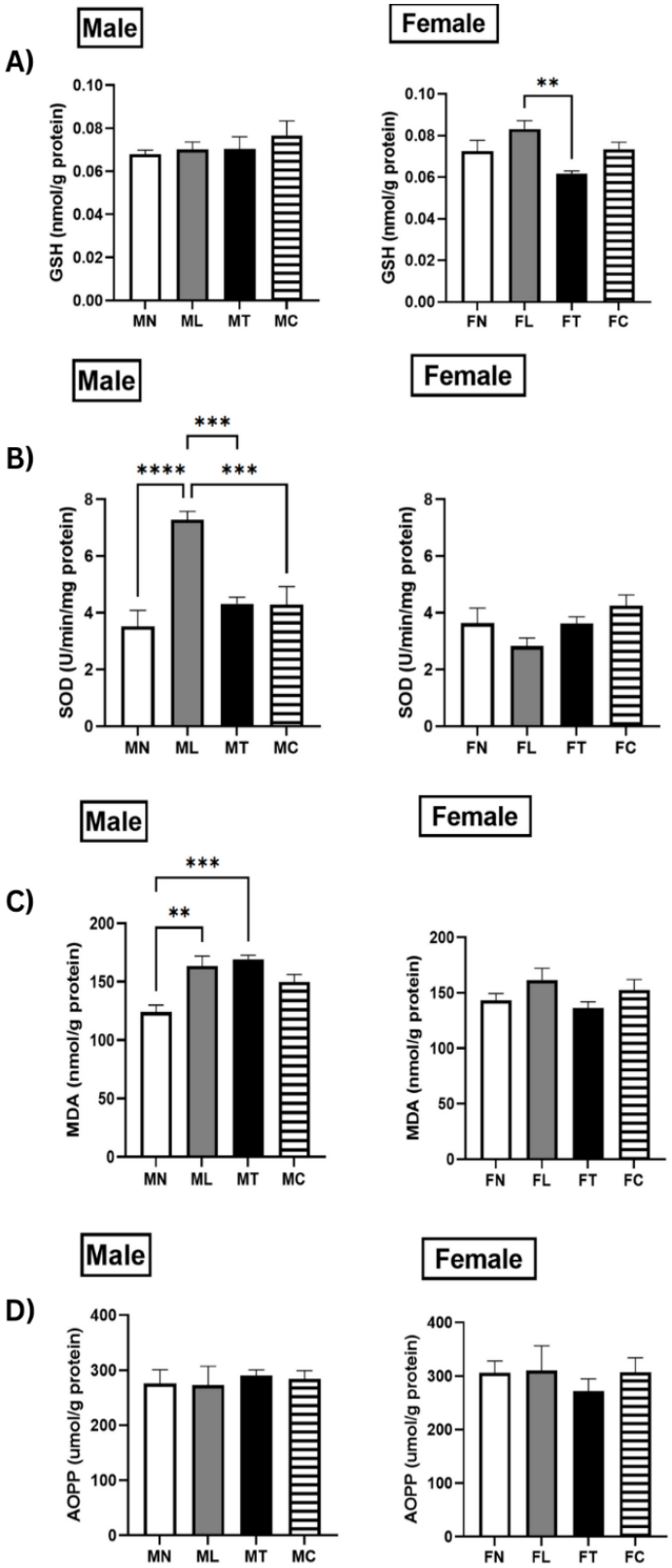
Data are presented as mean ± SEM (n = 8/sex/group). *p < 0.05; **p < 0.01; ***p < 0.001. BW, body weight; MN, male normal-protein diet; ML, male low-protein diet; MT, male treated with normal-protein diet; MC, male cricket-based diet; FN, female normal-protein diet; FL, female low-protein diet; FT, female treated with normal-protein diet; FC, female cricket-based diet

FIGURE 2. Relative kidney weight of rats after 10 weeks of dietary intervention



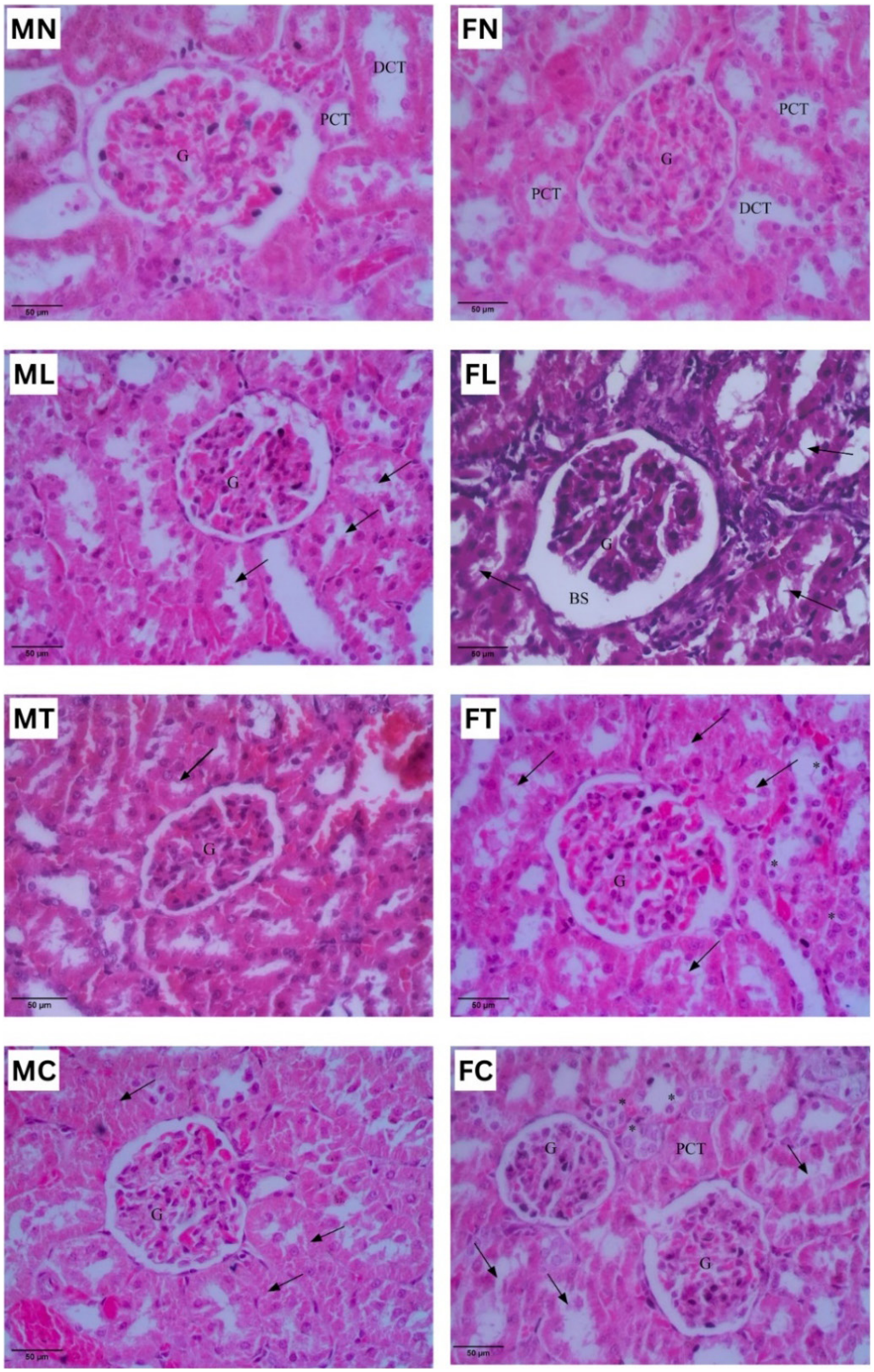
Data are presented as mean ± SEM (n = 8/sex/group). *p < 0.05; **p < 0.01; ****p < 0.0001. MN, male normal-protein diet; ML, male low-protein diet; MT, male treated with normal-protein diet; MC, male cricket-based diet; FN, female normal-protein diet; FL, female low-protein diet; FT, female treated with normal-protein diet; FC, female cricket-based diet

FIGURE 3. Serum urea level (A) and serum creatinine level (B) of rats after 10 weeks of dietary intervention



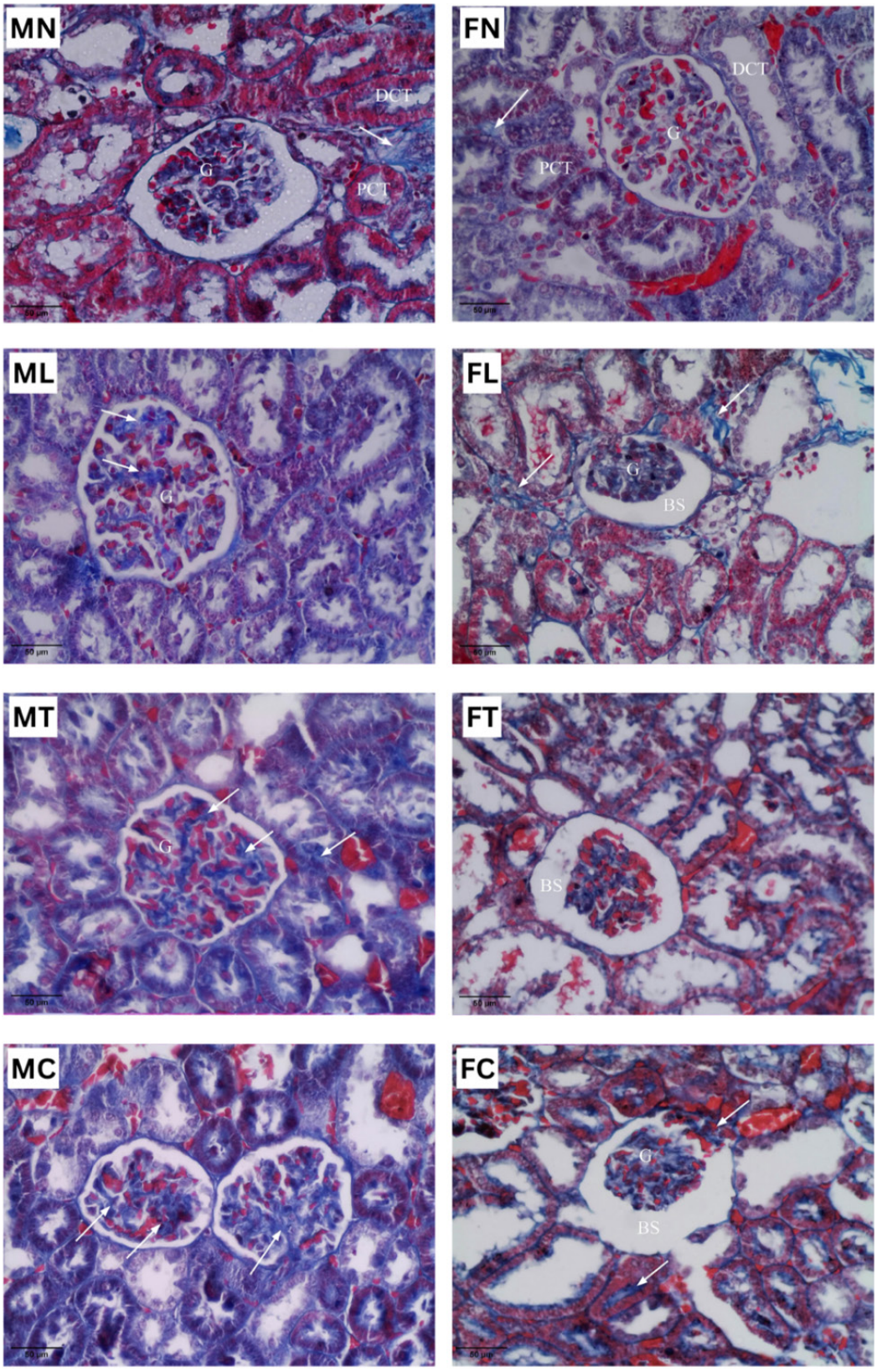
Data are presented as mean ± SEM (n = 8/sex/group). **p < 0.01; ***p < 0.001; ****p < 0.0001. MN, male normal-protein diet; ML, male low-protein diet; MT, male treated with normal-protein diet; MC, male cricket-based diet; FN, female normal-protein diet; FL, female low-protein diet; FT, female treated with normal-protein diet; FC, female cricket-based diet

FIGURE 4. Effects of a 10-week dietary intervention on oxidative stress and antioxidant markers in rats: (A) reduced glutathione (GSH) levels, (B) superoxide dismutase (SOD) activity, (C) malondialdehyde (MDA) levels, and (D) advanced oxidation protein product (AOPP) levels



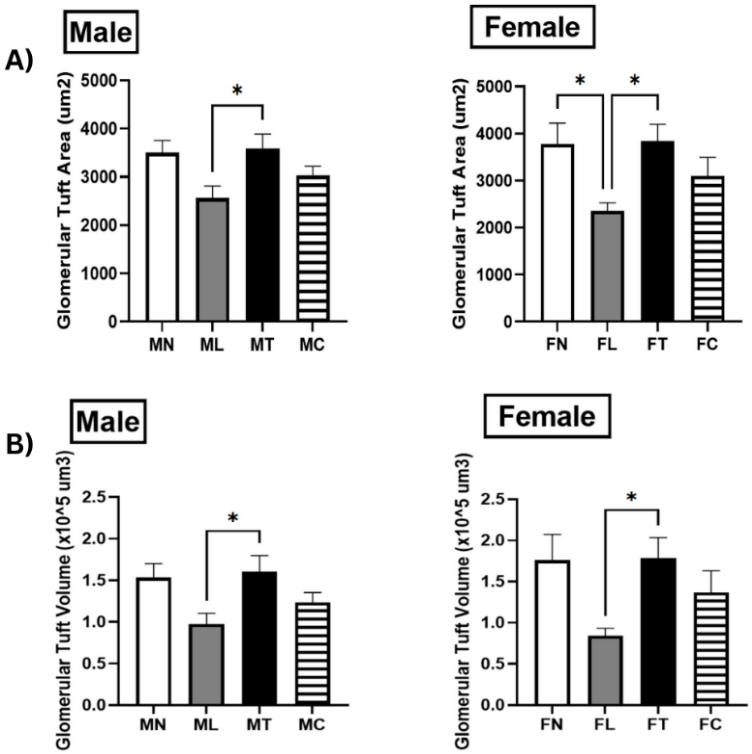
Arrow: tubular changes; *: cell vacuolization. BS: Bowman's capsule; DCT: Distal convoluted tubule; G: Glomerulus; PCT: Proximal convoluted tubule. MN, male normal-protein diet; ML, male low-protein diet; MT, male treated with normal-protein diet; MC, male cricket-based diet; FN, female normal-protein diet; FL, female low-protein diet; FT, female treated with normal-protein diet; FC, female cricket-based diet

FIGURE 5. Representative photomicrographs of the renal corpuscle and renal cortex stained with haematoxylin and eosin (H&E) at 400× magnification for each experimental group of rats



Arrow: fibrosis; BS: Bowman's capsule; DCT: Distal convoluted tubule; G: Glomerulus; PCT: Proximal convoluted tubule. MN, male normal-protein diet; ML, male low-protein diet; MT, male treated with normal-protein diet; MC, male cricket-based diet; FN, female normal-protein diet; FL, female low-protein diet; FT, female treated with normal-protein diet; FC, female cricket-based diet

FIGURE 6. Representative photomicrographs of the renal corpuscle and renal cortex stained with Masson's trichrome at 400× magnification for each experimental group of rats



Data are presented as mean ± SEM (n = 8/sex/group). *p<0.05. MN, male normal-protein diet; ML, male low-protein diet; MT, male treated with normal-protein diet; MC, male cricket-based diet; FN, female normal-protein diet; FL, female low-protein diet; FT, female treated with normal-protein diet; FC, female cricket-based diet

FIGURE 7. Glomerular tuft area (A) and glomerular tuft volume (B) in rats following a 10-week dietary intervention

or CBD facilitated somatic recovery, aligning with prior repletion studies demonstrating rapid catch-up growth upon protein restoration (Pertille et al. 2017). However, female CBD-fed rats exhibited incomplete body weight recovery, remaining significantly lighter than NPD controls. This sex-specific response may stem from inherent differences in feeding behaviour and metabolic adaptations to undernutrition. For instance, male rats typically consume more food and exhibit greater anabolic responses to protein repletion, which is influenced by testosterone's role in promoting muscle protein synthesis and stimulating appetite (Evans et al. 2005). In contrast, females may prioritise energy conservation or display altered satiety signals, potentially modulated by estrogen, leading to slower recovery trajectories (Martin et al. 2018).

The relative kidney weights increased significantly in female LPD, NPD-rehabilitated, and CBD-rehabilitated groups compared to controls. This hypertrophy relative to body mass may indicate compensatory mechanisms or underlying pathology, such as inflammation, edema, or early fibrotic changes, particularly in the context of persistent body weight deficits (Zafar & Naqvi 2010). The lack of similar changes in males further highlights sexual dimorphism in renal adaptive responses, where females might exhibit greater susceptibility to disproportionate organ growth under nutritional stress, possibly due to estrogen-mediated effects on renal hemodynamic and cellular proliferation (Colafella & Denton 2021).

Biochemically, the hypouremia in male LPD rats reflects diminished protein catabolism and urea cycle activity due to substrate limitation, while elevated creatinine levels in both sexes suggest impaired GFR or heightened muscle breakdown to supply amino acids (Bongers et al. 2018; Vukovic et al. 2023). CBD intervention normalised serum urea in males and maintained creatinine levels comparable to controls in females, indicating partial restoration of renal excretory function. This improvement may be attributed to the high-quality protein profile of crickets, rich in essential amino acids that support albumin synthesis and osmotic balance, thereby enhancing GFR (Murugu et al. 2021). Moreover, bioactive components in crickets, such as chitin-derived compounds and polyunsaturated fatty acids, may exert renoprotective effects by modulating inflammatory pathways and enhancing endothelial function, as observed in other insect protein studies (Kemsawasd et al. 2022).

Sex-specific patterns in oxidative stress responses were prominent. In males, LPD induced compensatory upregulation of SOD activity, likely as a defence against elevated ROS from muscle catabolism, accompanied by increased MDA levels indicative of lipid peroxidation (Turkmen 2017). CBD effectively attenuated SOD hyperactivity and normalised MDA, suggesting superior

antioxidant properties compared to NPD, possibly due to cricket-derived peptides and micronutrients like zinc and selenium that enhance enzymatic defences (Michael et al. 2022; Wang, Ahn & Asmis 2020). In females, LPD elicited minimal disruptions in SOD or MDA, with CBD preserving GSH levels, which may reflect inherent protections afforded by estrogen's antioxidant actions, including upregulation of glutathione peroxidase and reduced ROS production in renal tissues (Bhatia et al. 2012; Liu et al. 2005). The absence of changes in AOPP across groups implies that protein oxidation was not a dominant feature in this protein malnutrition model, or that the intervention duration limited its manifestation.

Histologically, protein malnutrition provoked widening of the capsule Bowman in females and tubular distortions in both sexes, consistent with malnutrition-induced disruptions in renal programming (Tain & Hsu 2017). CBD restored glomerular tuft area and volume to control levels, indicating effective support for glomerular maturation, potentially via improved amino acid availability and anti-inflammatory bioactive compounds in crickets that mitigate mesangial expansion (Morales-Ramos et al. 2020). However, tubular pathologies persisted, with hypertrophy in males and dilation with vacuolization in females, suggesting differential vulnerability where males may undergo hypermetabolic stress and females experience estrogen-modulated fluid imbalances. Mesangial fibrosis in males and interstitial fibrosis across female groups were not fully resolved, aligning with animal models where fibrosis regression requires etiology removal and extended timeframes, often exceeding 4-8 weeks in unilateral ureteral obstruction or nephrectomy models (Klawitter et al. 2020). The presence of baseline fibrosis in controls complicates attribution but may reflect strain-specific or housing-related factors.

These results have broader implications for human nutrition, particularly in combating protein malnutrition in resource-limited settings. Crickets offer a sustainable, nutrient-dense alternative to traditional proteins, with potential to alleviate malnutrition while providing anti-inflammatory and gut-modulating benefits that indirectly support kidney health (Stull et al. 2018). However, the high potential renal acid load (PRAL) of insect proteins warrants caution in individuals with compromised kidney function, as it could exacerbate acidosis, though balanced diets may mitigate this (Storz & Ronco 2023). In protein malnutrition contexts, CBD's role in enhancing protein status and reducing oxidative stress may prevent long-term renal programming defects, offering a viable strategy for vulnerable populations, such as children in developing countries (Bhutta et al. 2017).

Limitations of this study include the seven-week intervention period, which may be insufficient for complete fibrosis reversal or full catch-up growth, especially in females. Furthermore, the reliance on serum urea and creatinine as indicators of renal status represents a limitation, as in protein malnutrition and refeeding models these markers are substantially influenced by variations in dietary protein intake and muscle mass, and therefore may not accurately reflect GFR or intrinsic renal function. Additionally, the colourimetric TBARS assay used to assess MDA has limited specificity, and therefore, the results should be interpreted as indicative of overall renal lipid peroxidation rather than precise quantification of MDA. Regarding the statistical framework, while one-way ANOVA enabled direct comparisons across the groups, it did not formally test for sex-diet interactions. Thus, observed sex-specific trends are descriptive and warrant verification through two-way ANOVA in future studies. The study was also limited by baseline interstitial fibrosis in controls, possibly due to environmental factors, and the absence of broader profiling for inflammatory cytokines and cricket bioactives. Additionally, while the rat model recapitulates protein malnutrition aspects, translational gaps exist regarding human palatability and cultural acceptance of insects.

Future investigations should extend intervention durations and incorporate mechanistic analyses of cricket-derived compounds via proteomics. To strengthen renal assessment, future studies should evaluate direct renal perfusion metrics and utilise more specific indicators such as creatinine clearance (via urine collection), urine protein/albumin ratios, or urinary tubular injury markers like kidney injury molecule-1 (KIM-1) and neutrophil gelatinase-associated lipocalin (NGAL). Furthermore, exploring sex hormone manipulations will be essential to dissect the observed dimorphic responses. Long-term studies in diverse models, including those mimicking chronic kidney disease, could further validate CBD's efficacy and safety for human applications.

CONCLUSION

A CBD demonstrates potential in mitigating specific markers of oxidative stress and supporting partial normalisation of renal-related biomarkers and structure improvement following early-life protein malnutrition, with some sex-specific responses. CBD normalised glomerular morphometry, serum urea (males), serum creatinine (females), and certain oxidative stress parameters (MDA in males, GSH in females) to control levels. However, it did not fully resolve all malnutrition-induced alterations, notably female body weight and some renal tubular pathologies, within the seven-week study timeframe. These findings highlight that CBD may be a potential nutritional strategy, warranting further investigation to optimise its efficacy and fully elucidate its mechanisms in managing malnutrition-induced kidney injury.

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