Tropical Journal of Pharmaceutical Research October 2026; 25 (10): 1373-1385
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
https://dx.doi.org/10.4314/tjpr.v25i10.5

Original Research Article

Associations between prenatal *Moringa oleifera* supplementation, gestational stress, and renal electrolyte and morphological changes in Wistar rat offspring

Odochi O Chukwu[1,2]*, Anthony CU Ezimah[2], Nwaeze G Konyefom[2], Ima O Jimmy[3]

[1]Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, David Umahi Federal University of Health Sciences, Uburu, Ebonyi State, [2]Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Alex Ekwueme Federal University, Ndufu-Alike, Ikwo, Ebonyi State, [3]Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, College of Medical Sciences, Alex Ekwueme Federal University, Ndufu-Alike, Ikwo, Ebonyi State, Nigeria

For correspondence:* ***Email:* *chukwuoo46@gmail.com; chukwuoo@dufuhs.edu.ng;* ***Tel:*** *+2348109224709*

Sent for review: 22 January 2026 *Revised accepted: 21 October 2026*

Abstract

Purpose: *To evaluate whether maternal supplementation with methanolic Moringa oleifera leaf extract (MoLE) attenuates gestational stress-induced alterations in renal electrolytes and kidney morphology in rat offspring.*

Methods: *Twenty-five pregnant Wistar rats were randomized into five groups (n=5/group): control (no stress, standard chow/water), MoLE-L (5 mg/kg/day), MoLE-H (10 mg/kg/day), chronic unpredictable stress (CUS) + MoLE-L, and CUS + MoLE-H. CUS and oral MoLE were administered from gestational day 8 to 21. At puberty (postnatal days 42–49), five offspring per group (one per litter; n = 5 per group) were sacrificed under anesthesia; blood was collected via retro-orbital puncture for serum electrolyte analysis, and kidneys were processed for histology (hematoxylin and eosin [H&E] staining).*

Results: *CUS-exposed offspring showed significantly elevated serum sodium (CUS + MoLE-L: 150.00 ± 1.15; CUS + MoLE-H: 149.00 ± 0.58 mmol/L) and chloride (104.33 ± 2.03; 108.33 ± 0.88 mmol/L) versus controls (Na^+: 134.67 ± 2.03; Cl^-: 89.00 ± 1.15 mmol/L; $p < 0.05$), with tubular degeneration, hemorrhage, and inflammation on histology. However, the total renal lesion score was equal in the two CUS groups (4 vs. 4). CUS + MoLE-H showed lower inflammation (0 vs. 2) but greater tubular degeneration (1 vs. 0) and focal fatty change (1 vs. 0) than CUS + MoLE-L. High-dose MoLE did not attenuate electrolyte disturbances or reduce the total lesion score.*

Conclusion: *Gestational stress disrupts offspring renal electrolyte balance and structure. In this exploratory design, maternal MoLE supplementation did not show clear dose-dependent protection against stress-associated renal changes. The absence of a CUS-only group limits definitive conclusions.*

Keywords: *Electrolyte imbalance, Kidney histology, Moringa oleifera, Prenatal stress, Renal programming*

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Scopus, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

© 2026 The authors. This work is licensed under the Creative Commons Attribution 4.0 International License

INTRODUCTION

Pregnancy is a critical period of developmental plasticity wherein maternal conditions shape fetal organ development and postnatal health. Gestational stress heightens offspring risk of cardiometabolic and renal disorders through endocrine, metabolic, and inflammatory disruptions [1,2]. Gestational stress activates the maternal hypothalamic–pituitary–adrenal (HPA) axis [3], elevating circulating glucocorticoids that cross the placenta and modulate fetal tissue differentiation [1,4]. The developing kidney is especially vulnerable, as nephrogenesis, tubular differentiation, and ion transporter maturation occur predominantly in late gestation. Disruption of these processes may reduce nephron number, impair electrolyte handling, and compromise renal microvasculature, predisposing offspring to hypertension and chronic kidney disease [4,5].

Chronic unpredictable stress (CUS) models approximate human psychosocial stress [6]. CUS protocols link gestational stress to aberrant fetal growth, placental dysfunction, renal structural changes, and persistent cardiovascular and renal alterations in offspring [6]. These findings support the Developmental Origins of Health and Disease (DOHaD) hypothesis, which posits that suboptimal intrauterine environments shape long-term disease vulnerability [2]. Given the rising burden of psychosocial stress during pregnancy, especially in resource-limited settings, safe, accessible maternal interventions are needed. *Moringa oleifera* Lam. (Moringaceae), known as the drumstick tree, horseradish tree, or “Zogale” in northern Nigeria, is widely consumed across tropical regions as a dietary supplement. Its leaves, the most frequently consumed part, contain bioactive constituents with cytoprotective and anti-inflammatory properties [7,8]. Plant flavonoids and phenolic compounds are widely recognized for their pharmaceutical and medical potential, and emerging evidence indicates that Moringa oleifera leaf extract (MoLE) modulates stress-responsive pathways and preserves tissue integrity [9, 10]. However, its effects on gestational stress-related renal electrolyte disturbances and kidney morphology remain unexplored. This study evaluated the effects of maternal MoLE administration during gestation on renal function, electrolyte balance, and kidney histomorphology in offspring of CUS-exposed dams. We hypothesized that gestational stress would disrupt offspring renal electrolyte regulation and structure, and that maternal MoLE supplementation would confer dose-dependent attenuation.

EXPERIMENTAL

Plant collection, identification, and extraction

Fresh leaves of *Moringa oleifera* Lam. were collected in the early morning (between 6:00 and 8:00 AM) from a local garden in Abakaliki, Ebonyi State, Nigeria (GPS coordinates: 6°19'27.6"N, 8°06'21.6"E) in March 2023. Botanical identification and authentication were carried out by a certified taxonomist at the Herbarium Unit, Department of Biological Sciences, Alex Ekwueme Federal University, Ndufu-Alike Ikwo (AE-FUNAI). A voucher specimen was deposited for reference (Herbarium No.: AE-FUNAI UH 504a). The leaves were shade-dried at ambient temperature for two weeks to minimize degradation of heat-sensitive constituents, after which they were pulverized into a fine powder using a laboratory mill. The powdered material was sieved to ensure uniform particle size. Two hundred grams (200 g) of the powder was extracted with 500 mL of methanol using a Soxhlet apparatus for 48 h. The extraction procedure was repeated three times to ensure complete extraction, and the combined filtrates were concentrated under reduced pressure at 40 °C using a rotary evaporator to obtain a thick methanolic extract. The extract was stored in airtight containers at 4 °C until use.

Qualitative chemical characterization of the extract

The chemical profile of the methanolic extract was confirmed by gas chromatography–mass spectrometry (GC-MS) analysis, as previously reported by Chukwu et al. [11]. Briefly, analysis was performed using an Agilent 6890 GC system equipped with an HP-88 capillary column (100 m × 0.25 mm i.d. × 0.20 µm film thickness), with a 70 eV ionization voltage, an oven temperature of 180–181 °C (1 min hold), 1 µL injection volume, and 15 min run time. Samples were methylated before analysis to convert free fatty acids to their methyl esters for enhanced detection. The analysis revealed fatty acid methyl esters, alcohols, amines, alkanes, and heterocyclic compounds. No flavonoids or phenolic acids were identified in this GC-MS dataset. The full 41-compound profile is reported in Chukwu et al. [11] and is reproduced in Tables 1–5 under CC BY 4.0.

A single batch of methanolic extract was prepared from a pooled sample of pulverized leaves, with the extraction procedure repeated three times to ensure complete extraction. The combined filtrates were pooled to obtain a

homogeneous extract batch used for all treatment groups.

Quantitative standardization of phytochemical markers was not performed in the present study. Chukwu et al. [11] did not report quantitative standardization of these markers; therefore, the extract was administered on a crude-weight basis. This is a limitation because batch-to-batch variation may affect reproducibility. This decision was guided by the exploratory nature of the study, the absence of validated reference standards for all identified compounds, and the aim to reflect traditional consumption practices wherein the plant material is used in its crude form. Future investigations should incorporate quantitative standardization of key bioactive markers.

Animal grouping

Twenty-five healthy, outbred virgin female albino Wistar rats (180–220 g) were obtained from the Animal House, Alex Ekwueme Federal University, Ndufu-Alike, Ebonyi State, Nigeria. The experiments were conducted in the Department of Physiology and Department of Anatomy laboratories, Alex Ekwueme Federal University, Ndufu-Alike. Animals were housed under standard laboratory conditions (22 ± 2 °C; 12 h light/dark cycle) with free access to water and standard rat chow (Vital Feed®, Nigeria). A two-week acclimatization period was allowed before the commencement of the experiment. All experimental procedures involving animals were approved by the Faculty of Basic Medical Sciences Research Ethics Committee, Alex Ekwueme Federal University Ndufu-Alike, Ebonyi State, Nigeria (Approval Code: FBMS/EC/AE/1983). They were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals (National Institutes of Health Guide for the Care and Use of Laboratory Animals, 8th edition).

Estrous cycles were monitored by daily vaginal smear examination, and all 25 females exhibiting two consecutive regular four-day cycles were selected for mating. During pro-estrus, one male rat was introduced to two females overnight. The presence of spermatozoa in vaginal smears the following morning was taken as confirmation of pregnancy and designated gestational day (GD) 0.

Design and treatment

Pregnant rats were randomly assigned to five groups (n = 5 dams per group):

Group I (Control): No stress exposure; standard chow and water *ad libitum*

Group II (Low Dose MoLE): MoLE 5 mg/kg/day

Group III (High Dose MoLE): MoLE 10 mg/kg/day

Group IV (CUS + Low Dose MoLE): Chronic unpredictable stress (CUS) + MoLE 5 mg/kg/day

Group V (CUS + High Dose MoLE): Chronic unpredictable stress (CUS) + MoLE 10 mg/kg/day

All treatments were administered orally by gavage once daily from gestational day 8 to 21, corresponding to a critical period of organogenesis and nephrogenesis in rats [12,14]. The selected doses represent two reference levels commonly used in experimental studies [12] and were not intended to establish a formal dose–response relationship. Instead, the study was designed to explore whether maternal supplementation at these doses is associated with differences in offspring renal outcomes under stress conditions.

No CUS-only group (stressed dams without MoLE treatment) was included, as the study prioritized evaluation of MoLE's dose-dependent protective effects over replication of established stress outcomes, while adhering to ethical constraints on animal use (3Rs principle; Replacement, Reduction, Refinement) and resource limitations [15]. This limitation is acknowledged and should be addressed in future studies.

Chronic unpredictable stress (CUS) protocol

Dams in the stress groups (Groups IV and V) were subjected to a chronic unpredictable stress paradigm from gestational day 8 to 21, adapted from previously validated models [12,13]. One stressor was applied daily in a randomized order to prevent habituation, with individual stressors lasting between 6 and 12 h. The stressors included wet bedding, cage tilting, overnight food restriction, predator exposure (visual contact with a caged cat), sleep deprivation using a pedestal surrounded by water, restraint stress in ventilated tubes, continuous overnight illumination, and social isolation. One stressor was applied daily on each of 14 consecutive days (GD 8–21). The eight stressors were applied in a randomized sequence; each stressor was used at least once, and six stressors were randomly selected for a second application, giving 14 daily stress sessions in total.

Postnatal handling and offspring selection

After delivery, pups were reared by their respective dams under standard conditions until weaning on postnatal day 21. Male and female offspring were separated after weaning and maintained on standard feed and water ad libitum. Offspring retained their maternal treatment group designation. For biochemical and histological analyses, five offspring per group were used, one from each of the five litters per group (n = 5 per group). No more than one offspring per litter was used for a given analysis. The sex distribution was 3 males and 2 females per group. Because of the small sample size, sex was not included as a separate factor in the statistical analysis; offspring of both sexes were pooled. This is acknowledged as a limitation.

Sample collection and biochemical analysis

At the onset of puberty (postnatal days 42–49), approximately 2 mL of blood was collected from the five selected offspring per group (one per litter; n = 5 per group) via the retro-orbital plexus under light ether anesthesia using plain, non-heparinized capillary tubes. Samples were allowed to clot at room temperature and centrifuged at 3,000 rpm for 10 min to obtain serum. Serum levels of creatinine, urea, sodium, chloride, and bicarbonate were measured spectrophotometrically using standard biochemical methods to assess renal function and electrolyte balance.

Histological examination of kidney tissue

Following blood collection, offspring were humanely sacrificed by cervical dislocation under light ether anesthesia, and kidneys were excised, rinsed in ice-cold saline, and fixed in 10 % neutral buffered formalin for 48 h. Fixed tissues were processed using standard histological techniques according to established protocols [16]. The tissues were dehydrated through a graded ethanol series, cleared in xylene, embedded in paraffin wax, sectioned at 5–7 µm thickness using a microtome, and stained with hematoxylin and eosin (H&E). Sections were examined under a light microscope (Olympus CX43, Japan) for glomerular and tubular morphology, inflammatory cell infiltration, hemorrhage, and overall tissue architecture. For histology, the same five offspring per group (one per litter; n = 5 per group) were used. Three sections per kidney were scored by two observers blinded to group allocation. Scores were averaged per animal, and group values are presented as medians (range). Semi-quantitative scoring was: 0 = none; 1 = mild; 2 = moderate; 3 = severe. The total lesion score was the sum of individual parameter scores. No formal statistical testing was performed because the scores are ordinal and the sample size was small.

Statistical analysis

Data were analyzed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA) and expressed as mean ± standard error of the mean (SEM). The sample size was n = 5 offspring per group, each from a different litter. Group differences were assessed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Statistical significance was set at $p < 0.05$. No formal power calculation was performed; the sample size was limited by ethical and resource constraints. Given the exploratory nature of the study, the findings should be interpreted as preliminary and the p-values viewed as hypothesis-generating.

RESULTS

GC-MS analysis of MoLE

The GC-MS analysis of the methanolic extract of *Moringa oleifera* leaves revealed the presence of 41 compounds, representing various phytochemical classes including fatty acid methyl esters, alcohols, amines, alkanes, and heterocyclic compounds (Tables 1 to 5). The major constituents identified were hexadecanoic acid, methyl ester (26.182 %), methyl stearate (12.113 %), dodecanoic acid, methyl ester (6.008 %), and erythritol (6.842 %). Other notable compounds included various methyl esters of fatty acids (octanoic acid methyl ester, 2.299 %; decanoic acid methyl ester, 1.750 %; methyl tetradecanoate, 4.899 %; 9-octadecenoic acid methyl ester, 4.770 %), as well as 1-propanol and pyridine derivatives. The presence of these compounds is consistent with previously reported chemical profiles of *Moringa oleifera* leaves and suggests the extract contains a diverse array of bioactive constituents including flavonoids, phenolic acids, fatty acid derivatives, and other potentially bioactive compounds [11]. Many of the identified compounds possess documented biological activities including antioxidant, anti-inflammatory, and cytoprotective properties, which may contribute to the observed effects of MoLE supplementation.

Serum renal function indices and electrolyte levels in offspring

The effects of gestational exposure to CUS and maternal MoLE supplementation on serum renal function indices and electrolyte levels in offspring

are presented in Table 6 (n = 5 offspring per group, one per litter). Serum creatinine and urea concentrations did not differ significantly among the experimental groups ($p > 0.05$). Similarly, bicarbonate (HCO_3^-) levels were comparable across all groups. In contrast, significant alterations were observed in serum sodium (Na^+) and chloride (Cl^-) concentrations. Offspring born to stressed dams (CUS + Low Dose MoLE and CUS + High Dose MoLE groups) exhibited significantly higher Na^+ and Cl^- levels compared with the control group ($p < 0.05$). Elevated Na^+ and Cl^- concentrations were also observed in offspring from the High Dose MoLE group in the absence of stress, while the Low Dose MoLE group showed intermediate values. These differences indicate that gestational stress exposure was associated with marked disturbances in offspring electrolyte balance, particularly affecting sodium and chloride regulation.

Histopathological findings in offspring kidneys

Histological examination of kidney sections from offspring revealed treatment-dependent structural alterations, as summarized in Table 7 and illustrated in Figure 1. Kidneys from the control group (Figure 1 A) exhibited normal renal histoarchitecture, characterized by intact glomeruli, well-defined Bowman's spaces, and preserved tubular organization. Offspring from the Low Dose MoLE group (Figure 1 B) showed mild tubular degeneration and mild inflammatory cell infiltration, with no hemorrhage or fatty change. In the High Dose MoLE group (Figure 1 C), kidneys displayed mild intrarenal hemorrhage and inflammatory changes, while overall structural organization remained largely preserved. More pronounced histopathological changes were observed in offspring of stressed dams. Kidneys from the CUS + Low Dose MoLE group (Figure 1 D) demonstrated moderate intrarenal hemorrhage, interstitial inflammation, and focal tubular disorganization. In the CUS + High Dose MoLE group (Figure 1 E), renal sections showed mild tubular degeneration, moderate hemorrhage, and focal fatty changes, with no inflammation. Semi-quantitative lesion scoring (Table 7; n = 5 offspring per group, one per litter) showed that total renal lesion scores were equal in the two CUS groups (4 vs. 4). Compared with CUS + Low Dose MoLE, CUS + High Dose MoLE showed lower inflammation (0 vs. 2) but greater tubular degeneration (1 vs. 0) and focal fatty change (1 vs. 0); hemorrhage scores were equal (2 vs. 2). Therefore, the histological data do not show a lower total lesion score or milder degeneration with high-dose MoLE.

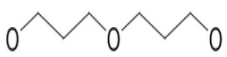
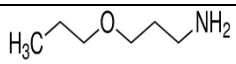
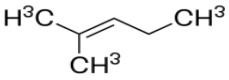
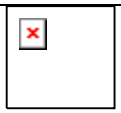
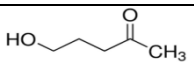
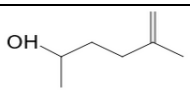
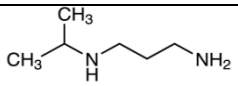
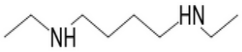
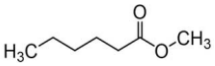
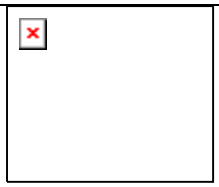
DISCUSSION

The present findings demonstrate that prenatal exposure to chronic unpredictable stress is associated with disturbances in offspring renal electrolyte balance and kidney architecture, reinforcing the concept that maternal stress during gestation influences developmental programming of renal function. Offspring of stressed dams treated with MoLE exhibited elevated serum sodium and chloride levels alongside histological evidence of tubular degeneration, hemorrhage, and inflammatory infiltration. These biochemical and structural alterations occurred in the absence of significant changes in serum creatinine and urea concentrations, suggesting that gestational stress predominantly affects tubular electrolyte handling rather than global glomerular filtration at this developmental stage.

The electrolyte disturbances observed align with earlier investigations linking impaired renal development to modifications in renal tubular transporter function and sodium-chloride homeostasis [4]. Chronic activation of the maternal HPA axis during pregnancy [3] may increase fetal glucocorticoid exposure, which is known to modulate nephrogenesis, renal vascular development, and epithelial ion channel expression [1,4,5]. Such perturbations may lead to diminished nephron endowment or impaired tubular reabsorptive capacity, thereby increasing susceptibility to electrolyte imbalances and subsequent cardiovascular dysfunction.

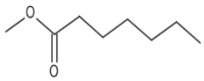
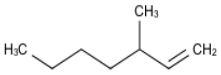
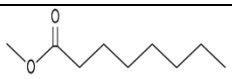
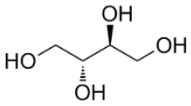
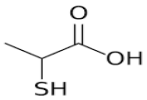
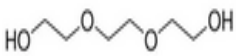
Histopathological examination corroborated the biochemical findings, revealing intrarenal hemorrhage, interstitial inflammation, and tubular disorganization in kidneys of offspring from stressed dams. These morphological changes are characteristic of renal injury in experimental models and may arise from vascular instability, inflammatory activation, and enhanced cellular vulnerability during critical developmental windows [4]. The persistence of these alterations through puberty indicates that gestational stress effects may extend beyond early postnatal life. Maternal MoLE administration did not show a clear dose-dependent protective effect in this study. Although CUS + High Dose MoLE offspring had lower inflammation scores than CUS + Low Dose MoLE offspring, they had greater tubular degeneration and focal fatty change, and the total lesion scores were equal (4 vs. 4).

Table 1: GC-MS analysis of MoLE. Reproduced from Chukwu et al. [11] under CC BY 4.0.

S/no	Name of compound	Mol. formula	Mol. Wt(g)	RT (min)	TIC (%)	Structure	Activity
1	1-Propanol, 3,3'-oxybis-	$C_6H_{14}O_3$	134.	3.045	1.521		Humectants (Food additive/Moisturizer)
2	1-Propanamine, 3-propoxy-	$C_5H_{12}NO$	117	3.327	0.803		Textile resins, Drugs, Pesticides
3	2-Pentene, 2-methyl-	C_6H_{12}	84	3.778	0.593		Photochemical and ozonolysis studies
4	Pyridine	C_5H_5N	79	4.285	4.763		Drugs, Vitamins, Food flavorings, Pesticides
5	2-Pentanone, 5-hydroxy-	$C_5H_{10}O_2$	102	4.820	0.630		Antimalarial drugs, Vitamin B1
6	5-Hexen-2-ol, 5-methyl-	$C_7H_{14}O$	114	5.102	0.887		Natural substances and Extractives
7	1,3-Propanediamine, N-(1-methylethyl)-	$C_6H_{16}N_2$	116	5.440	0.546		Useful research chemical compound
8	1,4-Butanediamine, N,N'-diethyl-	$C_8H_{20}N_2$	144	5.553	0.183		Unidentified
9	Hexanoic acid, methyl ester	$C_7H_{14}O_2$	130	5.722	0.843		Flavoring agents
10	Cyclotetrasiloxane, octamethyl-	$C_8H_{24}O_4Si_4$	296	6.031	0.468		Pharmaceuticals, Polymers, Hair/Skin care products, Antiperspirants and Deodorants, Lubricants, Sealants, Adhesives, Waxes and Coatings.

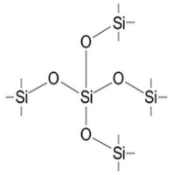
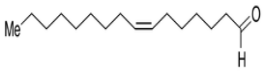
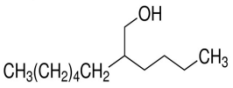
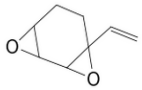
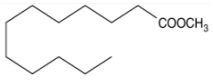
Mol. formula = Molecular formula; Mol. Wt = Molecular weight (g/mol); RT = Retention time; % TIC = Percentage of total ion current; Structure = Chemical structure

Table 2: GC-MS analysis of MoLE. Reproduced from Chukwu *et al.* [11] under CC BY 4.0. (continued)

S/no	Name of compound	Mol. formula	Mol. Wt(g)	RT (min)	% TIC	Structure	Activity
11	1,2,3-Trimethyldiaziridine	$C_4H_{10}N_2$	86	6.285	0.515		Unidentified
12	2-Hexyn-1-ol	$C_6H_{10}O$	98	6.426	0.212		Flavor and fragrance
13	Heptanoic acid, methyl ester	$C_8H_{16}O_2$	144	6.595	1.204		Human Metabolite, Flavoring agents, Fragrance,
14	1-Heptene, 3-methyl-	C_8H_{16}	112	6.905	0.608		Hydrocarbon
15	1-Fluorononane	$C_9H_{19}F$	146	7.158	0.459		Unidentified
16	Octanoic acid, methyl ester	$C_9H_{18}O_2$	158	7.440	2.299		Metabolite
17	Erythritol	$C_4H_{10}O_4$	122	7.834	6.842		Food additive and Sugar substitutes
18	2-Mercaptopropanoic acid	$C_3H_6O_2S$	106	8.313	2.240		Flavor and Fragrance agents
19	Triethylene glycol	$C_6H_{14}O_4$	150	9.243	1.079		Pesticides, Fragrance, Humectant, Disinfectant, Plasticizer for vinyl polymers
20	Decanoic acid, methyl ester	$C_{11}H_{22}O_2$	186	9.440	1.750		Biodiesel surrogate

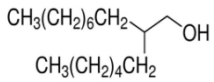
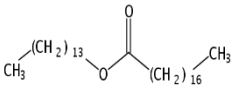
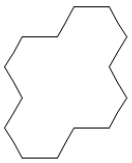
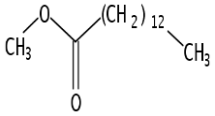
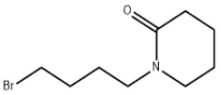
Mol. formula = Molecular formula; Mol. Wt = Molecular weight (g/mol); RT = Retention time; % TIC = Percentage of total ion current; Structure = Chemical structure

Table 3: GC-MS analysis of MoLE. Reproduced from Chukwu et al. [11] under CC BY 4.0. *(continued 2)*

S/no	Name of compound	Mol. formula	Mol. Wt (g)	RT (min)	% TIC	Structure	Activity
21	Benzene,2-methoxy-1,3,4-trimethyl	$C_{10}H_{14}O$	150	10.849	2.298		unidentified
22	Ethylene, 1,2-dichloro-, (Z)-	$C_2H_2Cl_2$	97	10.894	0.200		Pharmacology, Refrigerant, Degreaser, Adhesives, Lacquers, oils, and Resins
23	10-Undecenoic acid, methyl ester	$C_{12}H_{22}O_2$	198	11.102	0.275		Flavoring agents
24	Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl)oxy]-	$C_{12}H_{36}O_4Si_5$	385	11.581	0.360		Paints, Coatings, and Cosmetics, including some Personal care products
25	7-Hexadecenal, (Z)-	$C_{16}H_{30}O$	238	12.539	0.016		Derivative of essential oils with potential antibacterial activities.
26	1-Octanol, 2-butyl-	$C_{12}H_{26}O$	186	12.623	0.015		Human metabolite, Humectant
27	3,8-Dioxatricyclo[5.1.0.0(2,4)]octane, 4-ethenyl-	$C_8H_{10}O_2$	138	12.905	0.003		Undefined
28	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	214	13.356	6.008		Therapeutic uses, Flavoring agents

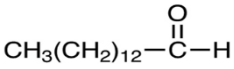
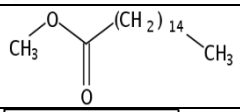
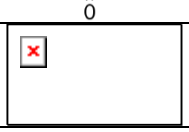
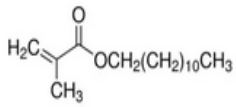
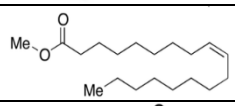
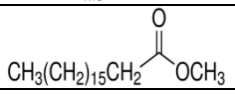
Mol. formula = Molecular formula; Mol. Wt = Molecular weight (g/mol); RT = Retention time; % TIC = Percentage of total ion current; Structure = Chemical structure

Table 4: GC-MS analysis of MoLE. Reproduced from Chukwu et al. [11] under CC BY 4.0. *(continued 3)*

S/no	Name of compound	Mol. formula	Mol. Wt (g)	RT (min)	% TIC	Structure	Activity
29	1-Decanol, 2-hexyl-	$C_{16}H_{34}O$	242	14.342	0.497		Fungicidal properties, Inhibitor (*Candida glabrata*), suggesting useful for treating Skin cancer
30	Myristyl stearate	$C_{32}H_{64}O_2$	481	14.623	0.181		Skin conditioning and Deodorant
31	2-Tridecenal, (E)-	$C_{13}H_{24}O$	196	14.877	0.219		Flavor and Fragrance agents
32	Cyclotetradecane	$C_{14}H_{28}$	196	15.468	1.427		Plant metabolite and a human metabolite
33	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	15.891	4.899		Plant metabolite, Flavoring agent and a Fragrance.
34	2-Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$	234	16.426	1.766		Antimicrobial

Mol. formula = Molecular formula; Mol. Wt = Molecular weight (g/mol); RT = Retention time; % TIC = Percentage of total ion current; Structure = Chemical structure

Table 5: GC-MS analysis of MoLE. Reproduced from Chukwu et al. [11] under CC BY 4.0. *(continued 4)*

S/no	Name of compound	Mol. formula	Mol. Wt (g)	RT (min)	% TIC	Structure	Activity
35	Tetradecanal	$C_{14}H_{28}O$	212	16.736	1.853		Human metabolite, Flavoring agent and Fragrance
36	Ethanol, 2-(octadecyloxy)-	$C_{20}H_{42}O_2$	314	16.877	1.420		Surfactant
37	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	17.384	26.182		Metabolite
38	6-Ethoxy-6-methyl-2-cyclohexenone	$C_9H_{14}O_2$	154	17.778	3.234		Flavoring agent
39	n-Dodecyl methacrylate	$C_{16}H_{30}O_2$	254	18.004	3.814		Drug (Clinical trials), Metabolites, Fragrance, Pesticides etc.
40	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	18.567	4.770		Flavoring agents and Fragrance
41	Methyl stearate	$C_{19}H_{38}O_2$	298	18.736	12.113		Metabolites and Flavoring agents

Mol. formula = Molecular formula; Mol. Wt = Molecular weight (g/mol); RT = Retention time; % TIC = Percentage of total ion current; Structure = Chemical structure

Table 6: Serum biochemical parameters

Group	Creatinine (µmol/L)	Urea (mmol/L)	Na^+ (mmol/L)	Cl^- (mmol/L)	HCO_3^- (mmol/L)
Control	59.67 ± 4.98	5.13 ± 0.20	134.67 ± 2.03[a]	89.00 ± 1.15[a]	18.33 ± 1.20
Low Dose MoLE	74.00 ± 5.86	5.43 ± 0.23	138.67 ± 1.20[ac]	97.00 ± 0.58[ab]	24.00 ± 1.53
High Dose MoLE	74.67 ± 13.87	7.20 ± 0.50	146.67 ± 3.71[b]	107.33 ± 5.81[b]	25.67 ± 0.88
CUS + Low Dose	72.33 ± 1.45	6.37 ± 0.33	150.00 ± 1.15[b]	104.33 ± 2.03[b]	24.67 ± 1.45
CUS + High Dose	83.33 ± 7.33	6.23 ± 2.35	149.00 ± 0.58[b]	108.33 ± 0.88[b]	24.00 ± 0.58

Note: *Data are expressed as mean ± SEM (n = 5 offspring per group, one per litter). MoLE = Moringa oleifera leaf extract; CUS = chronic unpredictable stress. Superscript letters (a,b,c) indicate significant differences between groups (p < 0.05; one-way ANOVA, Tukey's post hoc test). Groups with different superscripts differ significantly; groups sharing the same superscript are not significantly different.*

Table 7: Histopathological findings in kidneys

Group	Degeneration	Inflammation	Hemorrhage	Fatty change	Total lesion score
Control	0	0	0	0	0
Low Dose MoLE	1	1	0	0	2
High Dose MoLE	0	1	1	0	2
CUS + Low Dose	0	2	2	0	4
CUS + High Dose	1	0	2	1	4

Note: *Values are medians (range) of n = 5 offspring per group, one per litter. Three sections per kidney were scored by two observers blinded to group allocation. Histopathological changes were scored semi-quantitatively: 0 = none; 1 = mild; 2 = moderate; 3 = severe. Total Lesion Score = sum of individual parameter scores. No formal statistical testing was performed because the scores are ordinal and the sample size was small. MoLE = Moringa oleifera leaf extract; CUS = chronic unpredictable stress.*

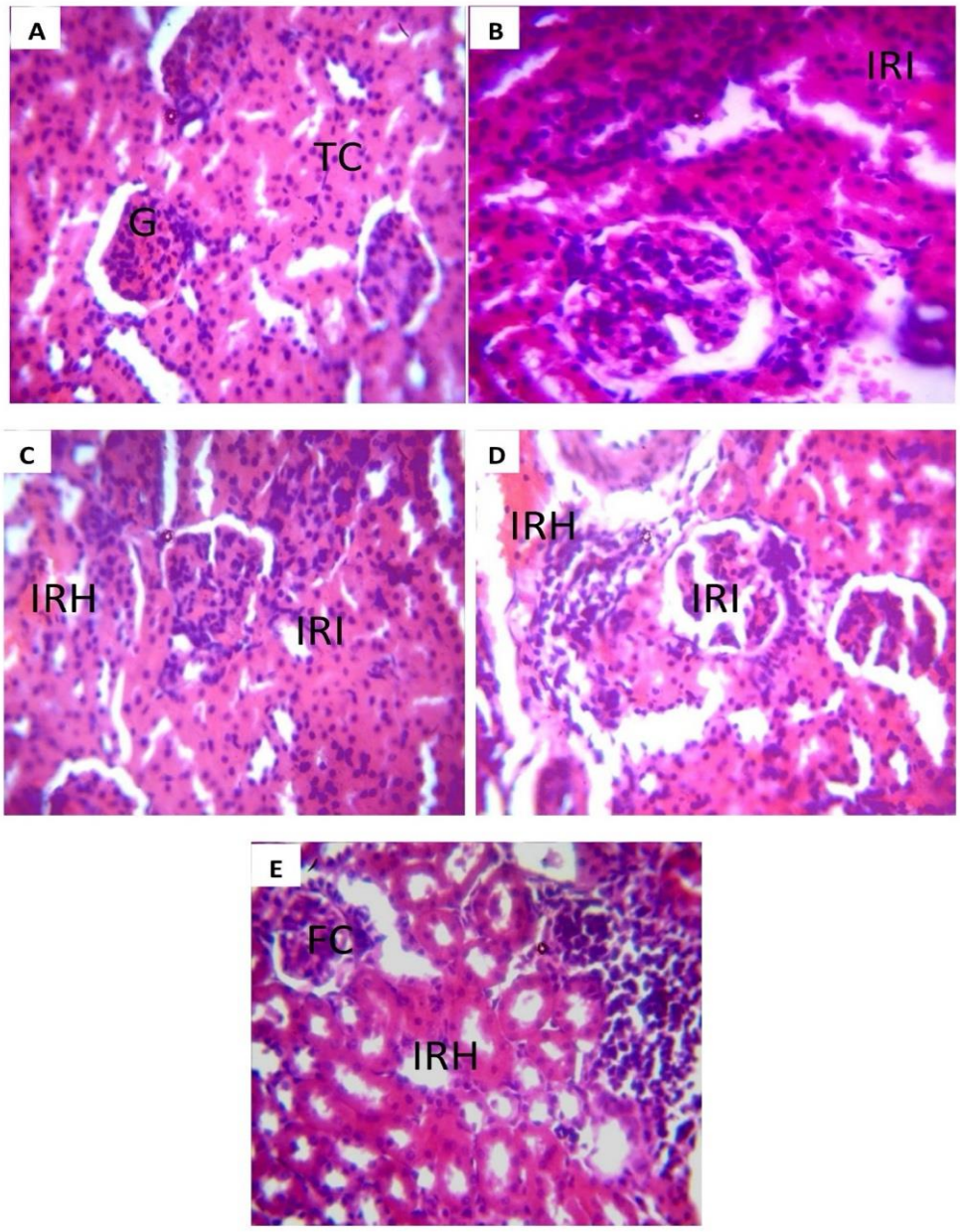


Figure 1: (A) Photomicrograph of Group I (Control) section of the offspring kidney (×400; H&E) shows renal tissue with well-outlined renal features showing glomeruli (G), Bowman's space (BS), renal tubules (RT) and tubular cells (TC). (B) Photomicrograph of Group II (Low Dose MoLE) section of the offspring kidney (×400; H&E) shows mild degeneration with intrarenal inflammation (IRI). (C) Photomicrograph of Group III (High Dose MoLE) section of the offspring kidney (×400; H&E) shows mild intrarenal hemorrhage (IRH) and intrarenal inflammation (IRI). (D) Photomicrograph of Group IV (CUS + Low Dose MoLE) section of the offspring kidney (×400; H&E) shows moderate intrarenal hemorrhage (IRH) and intrarenal inflammation (IRI). (E) Photomicrograph of Group V (CUS + High Dose MoLE) section of the offspring kidney (×400; H&E) shows mild degeneration with moderate intrarenal hemorrhage (IRH) and fatty change (FC)

In addition, Cl^- was slightly higher in CUS + High Dose MoLE (108.33 mmol/L) than in CUS + Low Dose MoLE (104.33 mmol/L), and Na^+ differed by only 1 mmol/L (149.00 vs. 150.00 mmol/L; same superscript, not significantly different). Thus, the biochemical and histological data do not support greater attenuation at the higher dose. The absence of a CUS-only control group remains a major limitation. These findings underscore the complexity of fetal programming and suggest that certain stress-induced alterations may be irreversible once critical developmental windows have elapsed.

Collectively, these findings support the DOHaD framework and indicate that maternal stress

during gestation may shape offspring renal physiology and morphology. Maternal MoLE supplementation may offer a supportive strategy for moderating, though not fully preventing, stress-associated renal developmental alterations. Future studies incorporating molecular analyses, extended dose ranges, standardized extracts, and long-term functional assessments are necessary to elucidate underlying mechanisms and establish translational relevance.

CONCLUSION

Prenatal chronic unpredictable stress exposure was associated with electrolyte disturbances and renal microstructural changes in offspring, consistent with stress-related developmental programming of kidney function. In this exploratory study, maternal MoLE supplementation did not show a clear dose-dependent protective effect. Total renal lesion scores were equal in the two CUS groups (4 vs. 4), and high-dose MoLE did not reduce electrolyte disturbances; Cl^- was slightly higher and Na^+ differed by only 1 mmol/L compared with CUS + Low Dose MoLE. The absence of a CUS-only group constitutes a significant limitation, precluding definitive attribution of observed effects to MoLE. Given the exploratory design, lack of extract standardization, and limited dose range, findings warrant cautious interpretation. Future investigations employing standardized extracts, expanded dose ranges, appropriate control groups (including CUS-only), mechanistic analyses, and longitudinal functional assessments are required to confirm these observations and establish clinical significance.

DECLARATIONS

Acknowledgement/Funding

The authors acknowledge the Department of Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Alex Ekwueme Federal University Ndufu-Alike, Ebonyi State, Nigeria, for providing laboratory facilities and technical support for this study.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. O.O.C. conceived and designed the study, performed laboratory analyses, curated the data, and drafted the manuscript. N.G.K. contributed to investigation and manuscript review and editing. I.J. participated in laboratory analyses and methodological procedures. A.C.U.E. contributed to investigation, project administration, and supervision. All authors read and approved the final manuscript for publication.

Ethical Approval

All experimental procedures involving animals were approved by the Faculty of Basic Medical Sciences Research Ethics Committee, Alex Ekwueme Federal University Ndufu-Alike, Ebonyi State, Nigeria (approval no. FBMS/EC/AE/1983).

Availability of Data and Materials

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Use of Artificial Intelligence/Large Language Models

Artificial intelligence-based systems or large language models were not used in the design, data analysis, writing, editing, or referencing of this manuscript.

Use of Research Reporting Tool

This study was conducted and reported in accordance with the ARRIVE guidelines for reporting animal research.

REFERENCES

1. *Seckl JR, Holmes MC. Mechanisms of disease: glucocorticoids, their placental metabolism and fetal 'programming' of adult pathophysiology. Nat Clin Pract Endocrinol Metab 2007; 3(6): 479-488. doi:10.1038/ncpendmet0515.*
2. *Mandy M, Nyirenda M. Developmental origins of health and disease: the relevance to developing nations. Int Health 2018; 10(2): 66-70. doi:10.1093/inthealth/ihy006.*
3. *McEwen BS. Neurobiological and systemic effects of chronic stress. Chronic Stress (Thousand Oaks) 2017; 1: 2470547017692328. doi:10.1177/2470547017692328.*
4. *Luyckx VA, Bertram JF, Brenner BM, Fall C, Hoy WE, Ozanne SE, et al. Effect of fetal and child health on kidney development and long-term risk of hypertension and kidney disease. Lancet 2013; 382(9888): 273-283. doi:10.1016/S0140-6736(13)60311-6.*
5. *Singh RR, Cullen-McEwen LA, Kett MM, Boon WM, Dowling J, Bertram JF, et al. Prenatal corticosterone*

exposure results in altered AT1/AT2, nephron deficit and hypertension in the rat offspring. J Physiol 2007; 579(Pt 2): 503-513. doi:10.1113/jphysiol.2006.125773.

6. Frisbee JC, Brooks SD, Stanley SC, D'Audiffret AC. An unpredictable chronic mild stress protocol for instigating depressive symptoms, behavioral changes and negative health outcomes in rodents. J Vis Exp 2015; (106): 53109. doi:10.3791/53109.
7. Vergara-Jimenez M, Almatrafi MM, Fernandez ML. Bioactive components in Moringa oleifera leaves protect against chronic disease. Antioxidants (Basel) 2017; 6(4): 91. doi:10.3390/antiox6040091.
8. Adedapo AA, Falayi OA, Oyagbemi AA. Evaluation of the analgesic, anti-inflammatory, anti-oxidant, phytochemical and toxicological properties of the methanolic leaf extract of commercially processed Moringa oleifera in some laboratory animals. J Basic Clin Physiol Pharmacol 2015; 26(5): 491-499. doi:10.1515/jbcpp-2014-0105.
9. Onasanwo SA, Adamaigbo VO, Adebayo OG, Eleazer SE. Moringa oleifera-supplemented diet protects against cortico-hippocampal neuronal degeneration in scopolamine-induced spatial memory deficit in mice: role of oxido-inflammatory and cholinergic neurotransmission pathway. Metab Brain Dis 2021; 36(8): 2445-2460. doi:10.1007/s11011-021-00855-9.
10. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview. Medicines (Basel) 2018; 5(3): 93. doi:10.3390/medicines5030093.
11. Chukwu OO, Iyare CO, Emelike CU, Ezimah ACU, Asogwa NT, Konyefom NG. GC-MS analysis of Moringa oleifera leaf extract and effects of administration on histology of reproductive organs and liver of female rats exposed to chronic unpredictable stress. Food Chem Adv 2024; 4: 100661. doi:10.1016/j.focha.2024.100661.
12. Chukwu OO, Iyare CO, Okorocha AE, Konyefom NG, Emelike CU, Onyeji GN, et al. Moringa oleifera Lam. is a potential mitigator of neurodevelopmental defects caused by prenatal stress in Wistar rats. J Med Plants 2025; 24(93): 79-101. Available from: http://jmp.ir/article-1-3709-en.html
13. Chukwu OO, Iyare C, Ezimah ACU, Okorocha AE, Konyefom NG, Asogwa NT, et al. Moringa oleifera docking to estrogen receptor Œ± ameliorates placental and brain damage in stressed rats. Avicenna J Med Biotechnol 2025; 17(1): 14-23. doi:10.18502/ajmb.v17i1.17673.
14. Zoetis T, Hurtt ME. Species comparison of anatomical and functional renal development. Birth Defects Res B Dev Reprod Toxicol 2003; 68(2): 111-120. doi:10.1002/bdrb.10013.
15. Russell WMS, Burch RL. The Principles of Humane Experimental Technique. London: Methuen; 1959.
16. Suvarna SK, Layton C, Bancroft JD, editors. Bancroft's Theory and Practice of Histological Techniques. 8th ed. London: Elsevier; 2019.