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Research Article

Moringa oleifera Leaf Extract Effects on Histology of Reproductive Organs, Heart and Liver of Rats Exposed to Chronic Unpredictable Stress

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Abstract

Background: Chronic and unpredictable psychosocial stressors produce endocrine, immune and structural organ changes that have broad consequences for health. *Moringa oleifera* (MO) leaf preparations are widely consumed for nutritional and medicinal purposes and are reported to possess antioxidant, hepatoprotective and anti-inflammatory properties; however, their effects when consumed during periods of chronic stress are incompletely characterized.

Objective: To evaluate the phytochemical constituents of methanolic *M. oleifera* leaf extract (MoLE) and to determine the histological effects of MoLE administration on the uterus, ovaries, heart and liver in female Wistar rats exposed to chronic unpredictable stress (CUS).

Methods: Methanolic MoLE GC-MS profiling has been previously published by our group. In brief, twenty mature virgin female Albino-Wistar rats were randomized into four groups (n = 5): (I) Control (no stress); (II) CUS only; (III) CUS + MoLE 200 $mg \cdot kg^{-1} \cdot day^{-1}$; (IV) CUS + MoLE 300 $mg \cdot kg^{-1} \cdot day^{-1}$. CUS was applied unpredictably for 14 days using validated stressors. MoLE was administered by oral gavage daily after stress exposure. At study end, reproductive organs, heart and liver were harvested for routine histology; lesions were described and semi-quantitatively graded.

Results: GC-MS identified 41 compounds in MoLE; the major constituents included hexadecanoic acid, methyl ester (26.18% TIC), methyl stearate (12.11% TIC), and dodecanoic acid methyl ester (6.01% TIC). Histological assessment showed that CUS alone induced marked degenerative changes across organs (uterine cystic degeneration and hemorrhage; ovarian fibrocystic change; myocardial inflammation/hemorrhage; severe hepatic fatty change and fibrosis). Administration of MoLE during CUS did not prevent these changes and, at the doses tested, was associated with persistent or, in some tissues, dose-related alterations (e.g. cystic uterine changes, ongoing hepatic steatosis and inflammatory aggregates). The presence of steroidal and fatty-acid methyl esters with potential estrogenic activity (as per our GC-MS and docking data) may contribute to organ-specific interactions observed.

Conclusion: Under the present experimental conditions, MoLE consumption during short-term CUS did not protect against and may have exacerbate stress-related histopathology in female rats. These findings underscore the need for careful evaluation of botanical supplements during periods of physiological stress and for mechanistic follow-up (endocrine profiling, receptor assays, oxidative stress markers).

Keywords: *Moringa oleifera; Chronic Unpredictable Stress; GC-MS; Histology; Uterus; Ovary; Liver; Heart*

Introduction

Chronic or repeated exposure to psychosocial and environmental stressors provokes integrated neuroendocrine, immune, and metabolic responses that can have lasting effects on organ systems [1,2]. Maternal and life-course stress exposures are associated with altered immune function in offspring and increased susceptibility to infectious and inflammatory disease [3-5]. Glucocorticoid and sympathoadrenal activation during stress produce systemic changes (e.g. cytokine modulation, altered metabolic fluxes) that can damage organs including the liver and heart and compromise reproductive function [1,6,7]. The chronic unpredictable stress (CUS) model reproduces many features of human psychosocial stress by delivering varied stressors in an unpredictable schedule, reliably eliciting behavioral, endocrine, and histopathological changes in rodents [8,9].

Moringa oleifera (MO) is widely cultivated and consumed in Nigeria for nutritional and medicinal uses. Leaf preparations are particularly popular and are reported to contain polyphenols, fatty-acid derivatives, and other bioactive constituents with putative antioxidant, hepatoprotective, anti-inflammatory, and metabolic benefits [10-12]. Nevertheless, plant extracts are complex mixtures whose net biological effect depends on dose, timing, and interactions with host endocrine and immune status. In our previous work, we reported the GC-MS profile of methanolic *M. oleifera* leaf extract and identified multiple fatty-acid methyl esters and steroidal derivatives, some of which have putative estrogenic activity [13]. Given the potential for endocrine-active phytochemicals to interact with stress-activated pathways (HPA/HPO axes), it is important to examine the histological consequences of MoLE consumption during stress exposure.

This study therefore combined phytochemical profiling (published data; Chukwu., *et al.* [13]) with an organ-level histopathological evaluation to determine whether MoLE administration during CUS ameliorates or worsens tissue injury in the uterus, ovary, heart, and liver of female rats.

Materials and Methods

Ethical statement

All procedures were performed in compliance with institutional and national guidelines for care and use of laboratory animals. The study protocol was 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).

Plant material collection and extraction

Fresh leaves of *Moringa oleifera* were harvested early in the morning from a cultivated garden in Abakaliki, Ebonyi State. Botanical authentication was carried out at the Herbarium Unit, Department of Biological Sciences, AE-FUNAI. Leaves were shade-dried at ambient temperature and milled to a uniform powder. Methanolic extraction was performed using a Soxhlet apparatus (200g powdered leaf, 500 mL methanol, 48h per batch, three replicates). Combined extracts were filtered (Whatman No.1) and concentrated under reduced pressure at 40°C using a rotary evaporator; concentrated extracts were stored at 4°C until use. Extraction and handling procedures follow those previously reported by our group and others for methanolic MoLE preparation [10,13]. To ensure safety for *in-vivo* administration, concentrated extracts were completely freed of residual methanol. Final traces of solvent were removed by extended drying under reduced pressure, confirming that no methanol remained in the MoLE preparation prior to use.

Phytochemical profiling and GC-MS

Comprehensive GC-MS profiling of the same methanolic MoLE preparation used in this study was carried out and reported previously [13]. That analysis identified 41 compounds, with hexadecanoic acid, methyl ester (methyl palmitate) representing the largest single peak (26.18% TIC) and other methyl esters (e.g. methyl stearate, dodecanoic acid methyl ester) present at appreciable abundance (Table 1). For this manuscript the published GC-MS dataset [13] was used to interpret histological findings and hypothesize potential receptor interactions; recent in-silico docking work from our group supports interaction of selected MoLE constituents with estrogen receptor α [14].

Animals, housing and experimental groups

Twenty (20) mature, virgin, female Albino-Wistar rats (weight range at baseline consistent with previous work from our laboratory) were procured from the AE-FUNAI Animal House. Animals were housed in polypropylene cages under standard laboratory conditions (12h light/dark cycle, 23 ± 2°C, humidity 50-60%) with free access to standard feed (Vital Feed®, Nigeria) and tap water. Animals were acclimatized for one week prior to study commencement.

Rats were randomized into four groups (n = 5 per group):

- **Group I (Control):** no stress, vehicle only (distilled water).
- **Group II (CUS control):** subjected to CUS and received vehicle.
- **Group III (CUS + MoLE-Low):** CUS + MoLE 200 $mg \cdot kg^{-1} \cdot day^{-1}$ (oral gavage).
- **Group IV (CUS + MoLE-High):** CUS + MoLE 300 $mg \cdot kg^{-1} \cdot day^{-1}$ (oral gavage).

MoLE was freshly prepared in distilled water daily and administered by oral gavage following each day's stress exposure.

Chronic unpredictable stress (CUS) protocol

CUS was applied for 14 consecutive days using a set of validated, randomized stressors adapted from established paradigms [13]. Stressors were rotated unpredictably and included: wet bedding (300mL water mixed with sawdust), cage tilting (45°), overnight restricted access to food, exposure to a caged cat (psychological stress), sleep deprivation using a pedestal in shallow water, restraint stress in perforated 50 mL tubes (three sessions of 2h with 30-minutes intersession gaps), continuous overnight light, and social isolation for 6 - 12h. The unpredictable schedule and variety of stressors aim to model chronic psychosocial stress and its physiological sequelae [9,13].

Tissue collection and histology

Animals were humanely euthanized at study termination (method consistent with institutional guidelines). The uterus, ovaries, heart and liver were excised, rinsed in saline, blotted, and fixed in Bouin's fluid or 10% neutral-buffered formalin for 24 - 48 hours. Fixed tissues were processed routinely (graded alcohol dehydration, xylene clearing), embedded in paraffin, sectioned at 4-5 μm and stained with hematoxylin and eosin (H&E) following standard histological protocols [15,16]. Slides were examined under light microscopy and photomicrographs were captured for representative fields.

Lesion description and semi-quantitative scoring

Histopathological features were recorded descriptively and lesions (degeneration, inflammation, hemorrhage, fatty change, fibrosis, cystic change) were graded semi-quantitatively on a 0-3 scale (0 = absent, 1 = mild, 2 = moderate, 3 = severe). Scoring was performed independently by two blinded observers, and discrepancies were resolved by joint review and consensus [15].

Data handling and statistics

Lesion scores are presented as medians (interquartile range, IQR) to account for ordinal distribution. Group comparisons were analyzed by Kruskal-Wallis test followed by Dunn's multiple comparisons. Two-tailed p values < 0.05 were considered statistically significant. Analyses were performed with GraphPad Prism (version 9.0, GraphPad Software, San Diego, CA, USA).

Results

GC-MS phytochemical summary

GC-MS analysis of the methanolic *M. oleifera* leaf extract (MoLE) used in this study identified 41 compounds as reported previously by Chukwu., *et al.* [13] (Table 1). Major constituents (by % total ion chromatogram) included hexadecanoic acid, methyl ester (methyl palmitate; 26.18% TIC), methyl stearate (12.11% TIC), dodecanoic acid methyl ester (6.01% TIC), and erythritol (6.84% TIC) among others. Several fatty-acid methyl esters and steroidal derivatives of potential endocrine relevance were present [13].

Macroscopic observations and general health

All animals tolerated the procedures without overt acute toxicity. Bodyweight trends and clinical observations during the 14-day CUS/administration period (recorded but not shown here) suggested mild weight changes in stressed groups relative to controls; no treatment-related mortality occurred.

Histology of reproductive organs, heart and liver (Representative description)

Uterus: Control uteri exhibited normal endometrial architecture with preserved stratum basalis and stratum functionalis (Figure 1). CUS control animals (Group II) showed marked degenerative changes with large polycystic cavities and peri-uterine hemorrhage (Figure 2). In CUS + MoLE groups (Groups III-IV), cystic areas and vascular congestion persisted; the low-dose group showed focal inflammatory aggregates with vascular congestion (Figure 3), while the high-dose group exhibited mild cystic change and persistent vessel congestion (Figure 4). Semi-quantitative scoring indicated highest median lesion severity in Group II and persistent moderate scores in Groups III-IV.

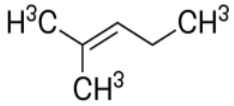
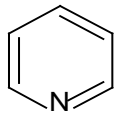
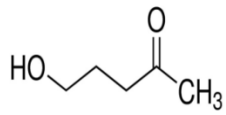
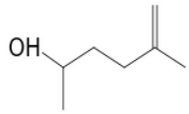
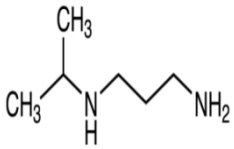
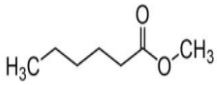
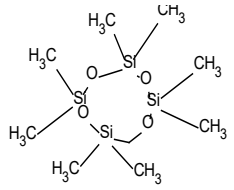
Ovary: Control ovaries demonstrated normal follicles at different stages and unremarkable stroma (Figure 5). CUS animals developed severe fibrocystic change with hemorrhagic cystic areas (Figure 6). MoLE-treated stressed groups showed dose-dependent attenuation of severity but residual polycystic changes and hemorrhagic foci were present (Groups III and IV; Figure 7 and 8).

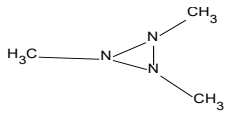
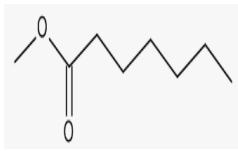
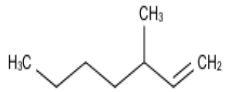
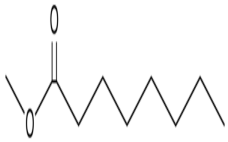
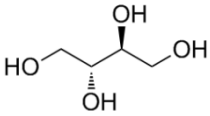
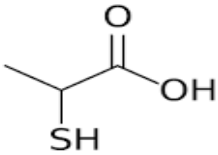
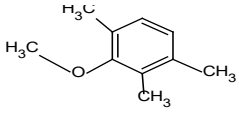
Heart: Control myocardium displayed normal cardiac fibers and architecture (Figure 9). CUS induced inflammatory aggregates, focal hemorrhage and degenerative/myonecrotic changes (Figure 10). MoLE administration did not fully prevent myocardial inflammation: both treated groups showed moderate inflammatory aggregates; the high-dose group displayed focal hemorrhage and localized tissue loss (Figure 11 and 13).

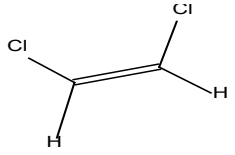
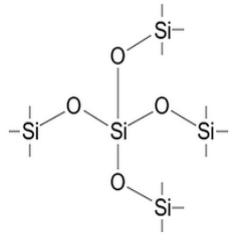
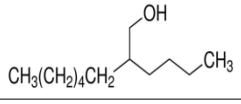
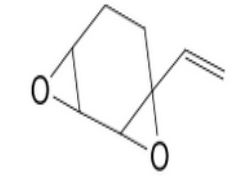
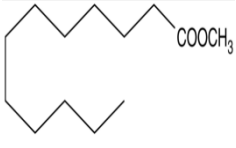
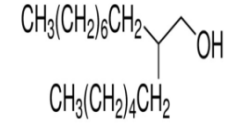
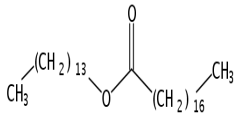
Liver: Normal hepatic architecture with central vein and portal triad was observed in controls (Figure 14). CUS caused severe fatty change, marked fibrosis, portal inflammatory aggregates and ground-glass cytoplasmic appearance (Figure 15). In MoLE-treated groups, hepatic lesions persisted: the low-dose group exhibited moderate steatosis and intrahepatic inflammation (Figure 12), while the high-dose group showed mild fatty change and portal inflammation (Figure 16). Overall, MoLE did not reverse CUS-induced hepatic pathology within the 14-day exposure.

Discussion

This study combined phytochemical profiling (previously published GC-MS data; Chukwu., *et al.* [13] with a focused histopathological evaluation to determine whether methanolic *Moringa oleifera* leaf extract (MoLE) modulates tissue injury during short-term chronic unpredictable stress (CUS) in female rats. Three principal findings emerged: (1) MoLE contains multiple fatty-acid methyl esters and steroidal derivatives, with hexadecanoic acid methyl ester identified as a dominant compound [13]; (2) CUS produced pronounced histopathological changes in reproductive organs, the heart, and the liver, consistent with stress-mediated injury; and (3) MoLE administration during CUS did not prevent these changes and, in some cases, was associated with persistent or dose-dependent alterations.

S/ No	Name of Compound	Mol. formular	Mol. Wt (g)	RT (min)	% TIC	Structure	Activity
1	1-Propanol, 3,3'-oxy-bis-	$C_6H_{14}O_3$	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		Anti-malarial 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		Flavouring agents
10	Cyclotetrasiloxane, octamethyl-	$C_8H_{24}O_4Si_4$	296	6.031	0.468		Pharmaceuticals, Polymers, Hair/Skin care products, Anti-perspirants and Deodorants, Lubricants, Sealants, Adhesives, Waxes and Coating.

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		Flavour and fragrance
13	Heptanoic acid, methyl ester	$C_8H_{16}O_2$	144	6.595	1.204		Human Metabolite, Flavouring 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		Flavour 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
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		Flavouring 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, Flavouring agents
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		Flavour and Fragrance agents

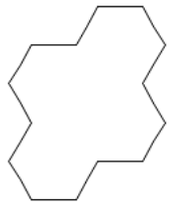
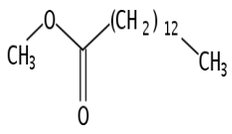
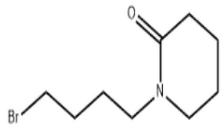
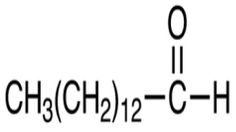
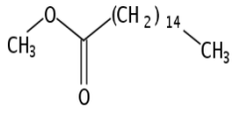
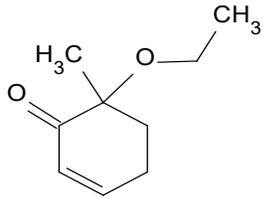
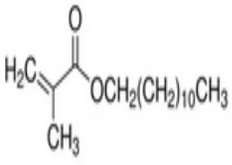
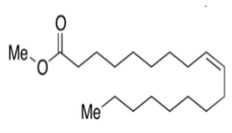
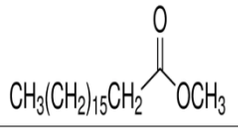
32	Cyclotetradecane	$C_{14}H_{28}$	196	15.468	1.427		Plant metabolite and a human metabolite
33	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	15.891	4.899		Plant metabolite, Flavouring agent and a Fragrance.
34	2-Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$	234	16.426	1.766		Antimicrobial
35	Tetradecanal	$C_{14}H_{28}O$	212	16.736	1.853		Human metabolite, Flavouring 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		Flavouring 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		Flavouring agents and Fragrance
41	Methyl stearate	$C_{19}H_{38}O_2$	298	18.736	12.113		Metabolites and Flavouring agents

Table 1: *GC-MS analysis of MOLE; An exempt from "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" by Chukwu., et al. 2024 [13].*

Organ /Tissue	Histological Lesion	Control (Group I)	CUS only (Group II)	CUS + MoLE 200 mg/kg (Group III)	CUS + MoLE 300 mg/kg (Group IV)
Uterus	Cystic degeneration	0	3	2	1
	Hemorrhage	0	3	2	2
	Inflammatory aggregates	0	2	2	1
Ovary	Fibrocystic change	0	3	2	2
	Hemorrhagic cysts	0	3	2	1
Heart	Inflammatory aggregates	0	2	2	2
	Hemorrhage	0	2	1	2
	Degenerative/myonecrotic change	0	3	2	2
Liver	Fatty change	0	3	2	1
	Fibrosis	0	3	2	2
	Inflammatory aggregates	0	3	2	2
	Ground-glass cytoplasm	0	2	1	1

Table 2: *Semi-quantitative scoring of histological lesions in reproductive organs, heart, and liver of rats exposed to chronic unpredictable stress (CUS) and treated with Moringa oleifera leaf extract (MoLE).*

Scoring scale: 0 = Absent; 1 = Mild; 2 = Moderate; 3 = Severe. Data represent median lesion severity per group (n = 5 animals per group, assessed by blinded histopathologists).

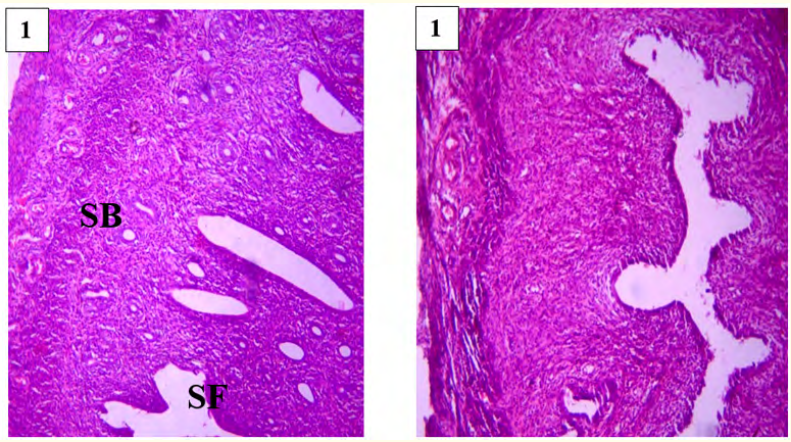


Figure 1: *Photomicrograph of group one (control) section of uterus (x400) (H/E) shows normal uterine tissue with stratum basal (SB), stratum functionalis (SF) and endometrial gland (EG). The stratum functionalis is lined by simple cuboidal epithelium (SCE) the overall features appear normal.*

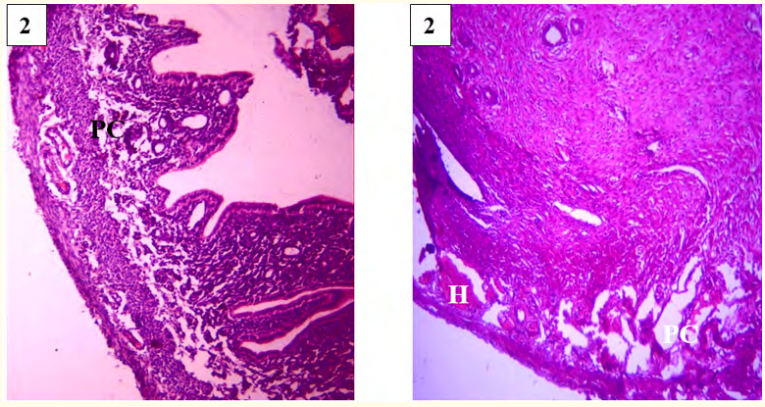


Figure 2: *Photomicrograph of group two (CUS control) section of uterus (x400) (H/E) shows severe degeneration of the uterine tissue, severe polycystic cavity (PC) with hemorrhage (H) within the perimetrium.*

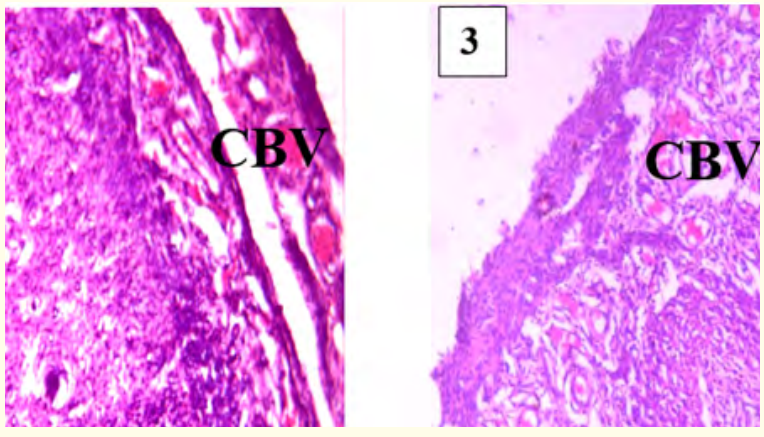


Figure 3: *Photomicrograph of group three (CUS + 200 mg/kg bwt MLE) section of uterus (x400) (H/E) shows mild aggregate of inflammatory cell (AIC) and congestion of blood vessel (CBV).*

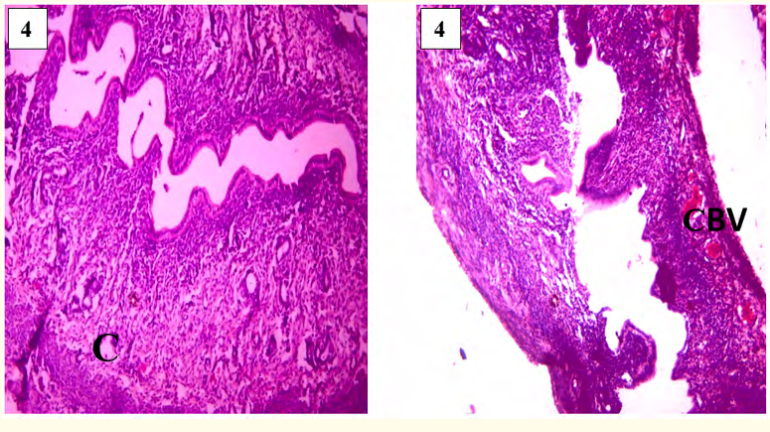


Figure 4: *Photomicrograph of group four (CUS + 300 mg/kg bwt MLE) section of uterus (x400) (H/E) shows mild cystic areas (CA) and congestion of blood vessel (CBV).*

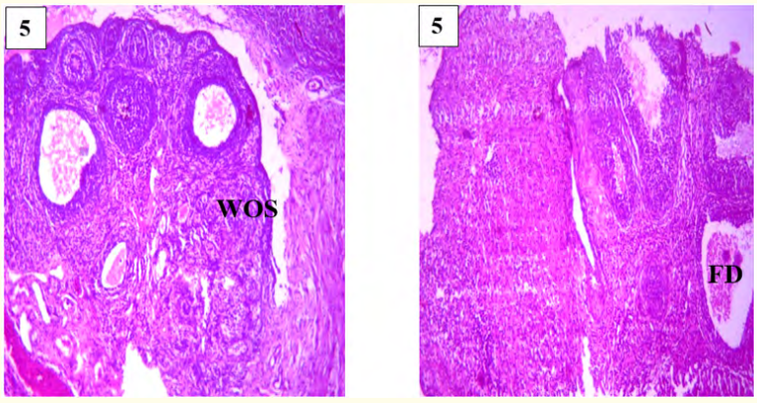


Figure 5: *Photomicrograph of group one (normal control) section of ovary (X100) (H/E) shows normal ovarian tissue with different stages of follicular development (FD), the whorl ovarian stroma (WOS) appear normal.*

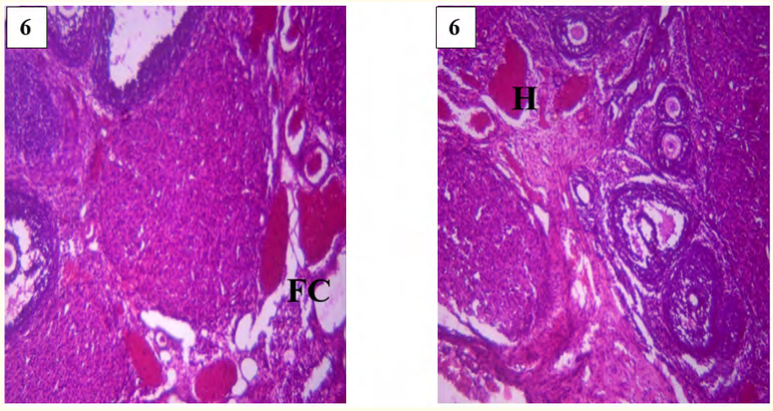


Figure 6: *Photomicrograph of group two (CUS control) section of ovary (X100) (H/E) shows severe degeneration of ovarian tissue with severe fibrocystic change (FC) within the whorled ovarian stroma (WOS) with areas of hemorrhage (H) within the cystic areas (CA).*

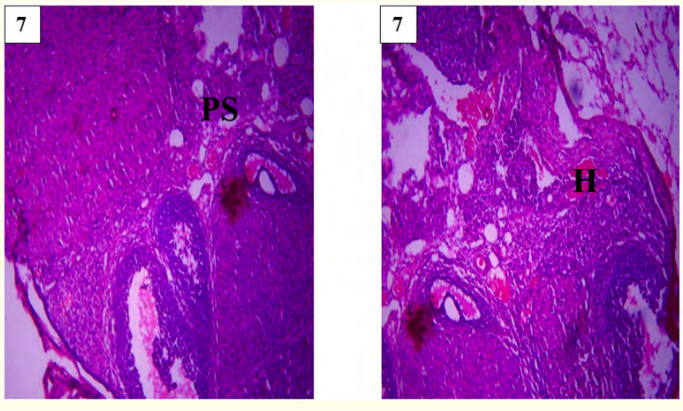


Figure 7: *Photomicrograph of group three (CUS + 200 mg/kg bwt MLE) section of ovary (X100) (H/E) shows moderate polycystic spaces (PS) and areas of hemorrhage (H).*

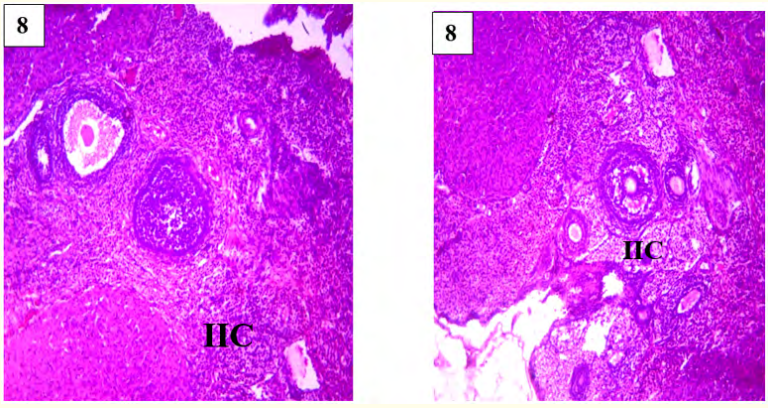


Figure 8: *Photomicrograph of group four (CUS + 300 mg/kg bwt MLE) section of ovary (X100) (H/E) shows mild Infiltration of inflammatory cell (IIC) within the whorl of ovarian stroma (WOS).*

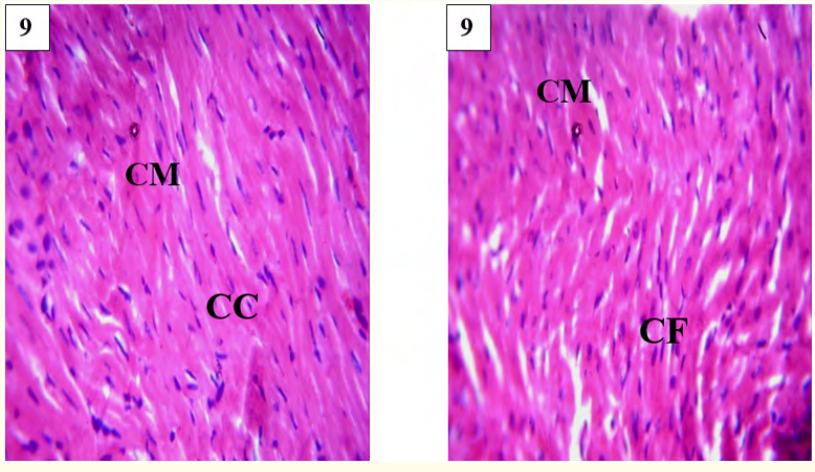


Figure 9: *Photomicrograph of group one (normal control) section of heart (X400) (H/E) shows normal cardiac tissue with cardiac cell (CC), cardiac fiber (CF), cardiac muscles (CM).*

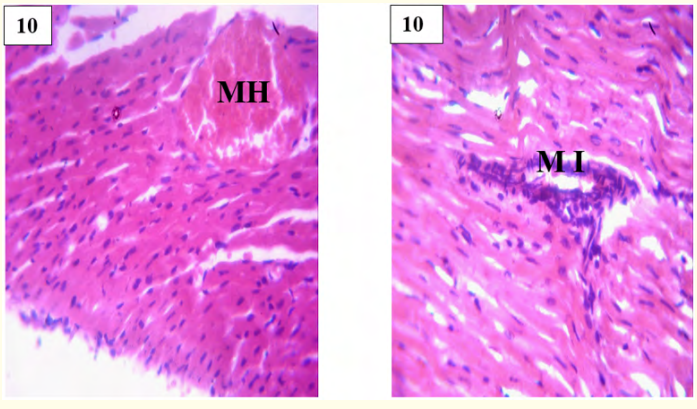


Figure 10: *Photomicrograph of group two (CUS control) heart section (X400) (H/E) shows moderate degeneration with aggregate of myocadiac inflammation (MI) and moderate myocardiac hemorrhage (MH).*

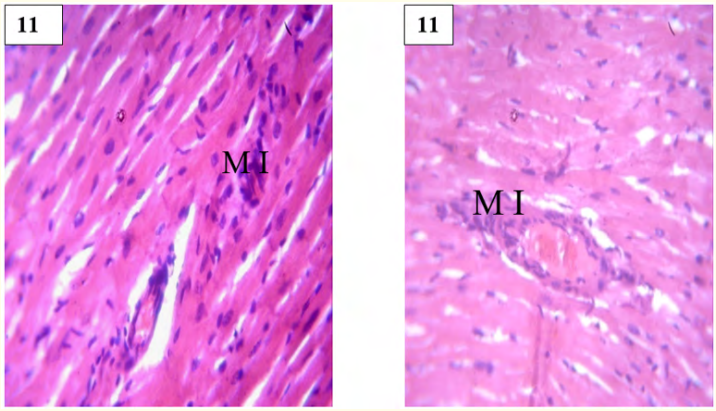


Figure 11: *Photomicrograph of group three (CUS + 200 mg/kg bwt MLE) heart section (X400) (H/E) shows moderate aggregate of myocardiac inflammation (MI).*

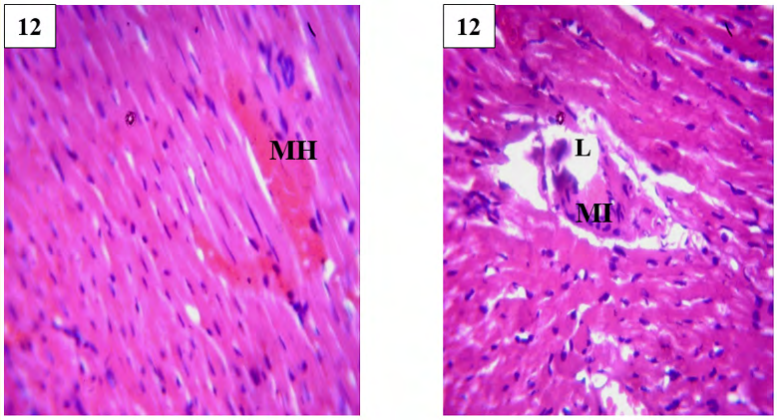


Figure 12: *Photomicrograph of group four (CUS + 300 mg/kg bwt MLE) heart section (X400)(H/E) moderate aggregate of myocardiac inflammation (MI) and focal myocardiac hemorrhage (MH) with loss (L) of cardiac tissue.*

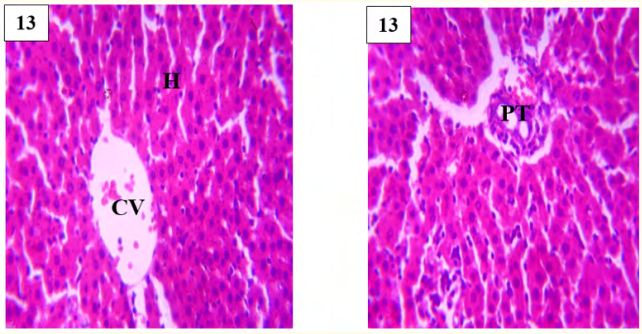


Figure 13: *Photomicrograph of group one (normal control) section of liver (x400) (H/E) shows normal hepatic architecture with portal triad (PT), central vein (CV) and hepatocyte (H).*

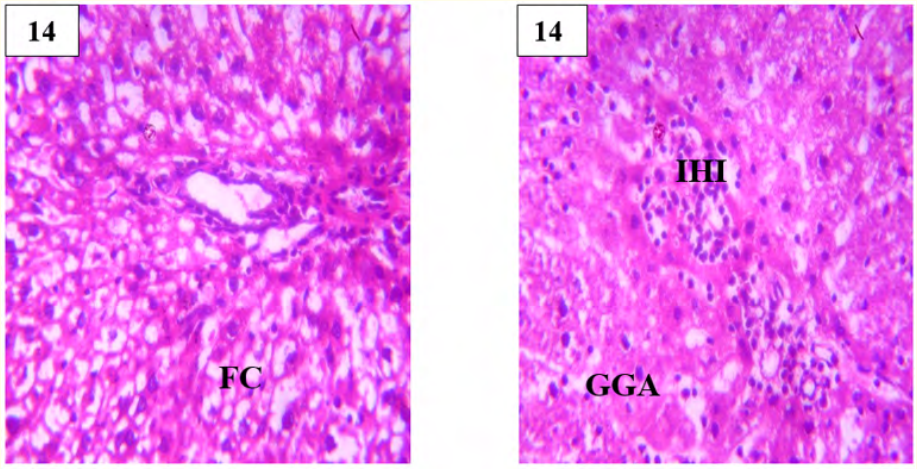


Figure 14: *Photomicrograph of group two (CUS control) section of liver (x400) (H/E) shows severe degeneration with severe fatty change (FC), severe fibrosis (F), portal aggregate of inflammation (PAI) and cytoplasm ground glass appearance (CGGA), and moderate intra hepatic inflammation (IHI).*

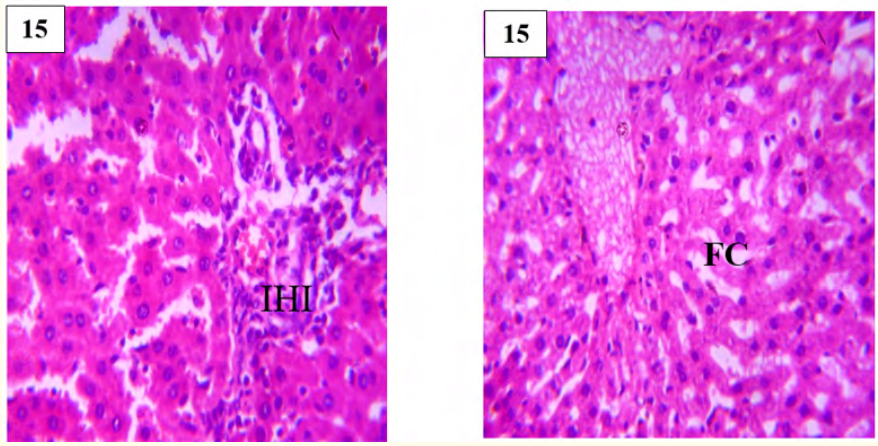


Figure 15: *Photomicrograph of group three (CUS + 200 mg/kg bwt MLE) section of the liver (x400) (H/E) shows moderate fatty change (FC) and moderate intra hepatic inflammation (IHI).*

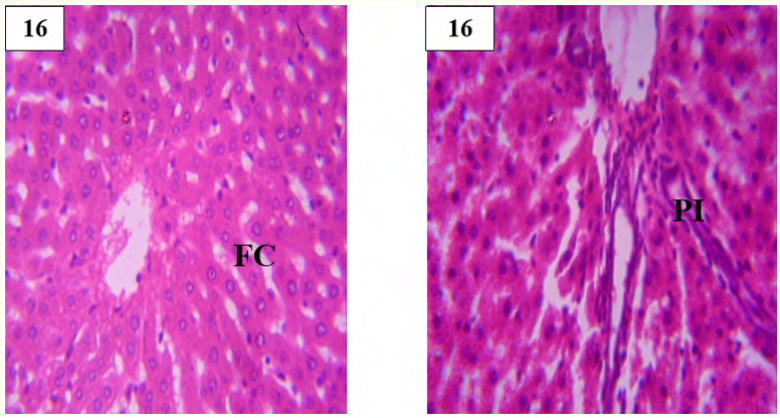


Figure 16: *Photomicrograph of group four (CUS + 300 mg/kg bwt MLE) section of the liver (x400) (H/E) shows mild fatty change (FC) and mild portal inflammation (PI).*

The organ changes observed after 14 days of CUS including inflammatory aggregates in myocardium, hepatic steatosis and fibrosis, and cystic degeneration of reproductive tissues are consistent with prior studies demonstrating that chronic stress provokes systemic inflammation, cytokine alterations, and organ-specific pathology [1,2,4]. Stress-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis and sympathoadrenal system elevates glucocorticoids and catecholamines, which in turn modulate immune responses and generate oxidative stress, contributing to hepatocellular injury and inflammatory remodeling [1,4]. Moreover, maternal and perinatal stress exposures have been associated with immune dysregulation and altered organ development in multiple species [3,7,17].

Previous studies have reported antioxidant and hepatoprotective properties of *M. oleifera* leaves under specific conditions of dose, route, and duration [10-12]. However, botanical extracts are complex mixtures that may contain constituents with opposing biological activities. Our GC-MS profile revealed a predominance of fatty-acid methyl esters and steroid-like compounds [13], and independent docking studies from our group have shown that certain MoLE constituents interact with estrogen receptor α [14]. These findings raise two hypotheses:

- **Endocrine interaction hypothesis:** Steroidal or fatty-acid derivatives in MoLE may exert estrogenic or endocrine-modulating effects that interact with stress-activated HPA and hypothalamic-pituitary-ovarian (HPO) axes, producing dysregulated tissue responses in estrogen-sensitive organs such as the uterus and ovary. Phytochemicals that bind nuclear receptors can act as agonists or antagonists depending on dose, receptor distribution, and hormonal milieu; under CUS, altered endogenous steroid levels may shift the net effect toward dysregulation.
- **Redox/metabolic context dependency:** Although MoLE contains antioxidant polyphenols, the dominant presence of methyl esters and other lipophilic constituents may alter hepatic lipid metabolism or interfere with phase I/II biotransformation under stress conditions, potentially exacerbating steatosis or inflammatory signaling.

Both mechanisms align with our observations that MoLE did not attenuate CUS-related inflammation in the myocardium and liver, and that certain lesions persisted or showed dose-related patterns. Notably, our prior mechanistic work demonstrated MoLE interactions with estrogenic pathways that modulated placental and brain outcomes in stressed animals [14], reinforcing the need for further mechanistic investigation.

Reports of protective MoLE effects often emphasize antioxidant endpoints, metabolic outcomes, or longer-term dosing in non-stressed animals [11,12]. Our findings indicate that MoLE's effects during active stress exposure may differ substantially, reflecting broader toxicological principles that context, dose, and timing determine biological outcomes. The CUS paradigm reliably produces immune and organ changes in rodents [8,9]. Our results extend these observations by showing that MoLE administered during stress does not mitigate histopathological changes and may interact with stress physiology to generate tissue-specific outcomes.

Limitations of the Study

Several limitations should be acknowledged. First, the exposure duration was relatively short (14 days), and group sizes were small (n = 5), limiting statistical power. Second, biochemical and endocrine assays (e.g. corticosterone, hepatic transaminases, oxidative stress indices, sex steroid levels) were not performed, which constrains mechanistic interpretation. Third, lesion scoring was semi-quantitative and primarily descriptive; although blinded evaluation enhances rigor, future work should incorporate morphometric analyses, immunohistochemistry for inflammatory and apoptotic markers, and quantitative lipid profiling. Future studies should expand exposure duration, explore dose-response relationships, and integrate receptor binding and reporter assays to test endocrine interactions suggested by GC-MS and docking analyses. Such work will be essential to clarify the safety profile of MoLE consumption during periods of physiological stress and to define conditions under which its bioactivities may be protective versus deleterious.

Conclusion

In this experimental model, administration of methanolic *Moringa oleifera* leaf extract during short-term chronic unpredictable stress did not prevent CUS-induced histopathological injury to reproductive organs, heart and liver; in some cases tissue alterations persisted or showed dose-associated patterns. The complex phytochemical profile of MoLE particularly the abundance of fatty-acid methyl esters and steroidal-like constituents may interact with stress-activated neuroendocrine pathways and underlie the observed effects. These findings advise caution regarding unregulated consumption of MoLE during periods of significant physiologic stress and support further mechanistic and toxicological evaluation prior to clinical recommendations.

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Author Contributions

O.O.C. conceived the study. O.O.C., A.C.U.E., N.I., and N.G.K. conducted the experiments, processed samples and analyzed histology. O.O.C. prepared the GC-MS dataset. All authors contributed to data interpretation and manuscript drafting. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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